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(54) Title: USES OF ANTI-BCMA CHIMERIC ANTIGEN RECEPTORS

FIG. 1

(57) Abstract: Provided herein are uses of anti-B cell maturation antigen (BCMA) chimeric antigen receptors (CARs) for treating B-cell related conditions, such as BCMA-expressing cancers.

WO 2021/091978 A1

USES OF ANTI-BCMA CHIMERIC ANTIGEN RECEPTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 62/931,077, filed November 5, 2019, U.S. Provisional Patent Application No. 62/944,938, filed December 6, 2019, U.S. Provisional Patent Application No. 62/952,186, filed December 20, 2019, U.S. Provisional Patent Application No. 63/024,252, filed May 13, 2020, and U.S. Provisional Patent Application No. 63/037,471, filed June 10, 2020, each of which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

This application incorporates by reference a Sequence Listing submitted with this application as an ASCII text file, entitled 14247-549-228_SEQ_LISTING.txt, created on October 27, 2020 having a size of 27,620 bytes.

1. BACKGROUND

1.1. Technical Field

[0001] The disclosure presented herein relates to methods for treating B cell related conditions. More particularly, the disclosure relates to improved chimeric antigen receptors (CARs) comprising anti-BCMA antibodies or antigen binding fragments thereof, and immune effector cells genetically modified to express these CARs, and use of these compositions to effectively treat B cell related conditions. The disclosure also relates to methods for treating B cell related conditions using chimeric antigen receptors (CARs) comprising anti-BCMA antibodies or antigen binding fragments thereof, and immune effector cells genetically modified to express these CARs in combination with BCMA-based treatment modalities.

1.2. Description of the Related Art

[0002] Several significant diseases involve B lymphocytes, *i.e.*, B cells. Abnormal B cell physiology can also lead to development of autoimmune diseases including, but not limited to systemic lupus erythematosus (SLE). Malignant transformation of B cells leads to cancers including, but not limited to, lymphomas, *e.g.*, multiple myeloma and non-Hodgkins' lymphoma.

[0003] The large majority of patients having B cell malignancies, including non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM), are significant contributors to cancer mortality. The response of B cell malignancies to various forms of treatment is mixed. Traditional methods of treating B cell malignancies, including chemotherapy and radiotherapy, have limited utility due to toxic side effects. Immunotherapy with anti-CD19, anti-CD20, anti-CD22, anti-CD23, anti-CD52, anti-CD80, and anti-HLA-DR therapeutic antibodies have provided limited success, due in part to poor pharmacokinetic profiles, rapid elimination of antibodies by serum proteases and filtration at the glomerulus, and limited penetration into the tumor site and expression levels of the target antigen on cancer cells. Attempts to use genetically modified cells expressing chimeric antigen receptors (CARs) have also met with limited success. In addition, the therapeutic efficacy of a given antigen binding domain used in a CAR is unpredictable: if the antigen binding domain binds too strongly, the CAR T cells induce massive cytokine release resulting in a potentially fatal immune reaction deemed a "cytokine storm," and if the antigen binding domain binds too weakly, the CAR T cells do not display sufficient therapeutic efficacy in clearing cancer cells.

2. BRIEF SUMMARY

[0004] The present disclsoure generally provides improved methods of treating B-cell-related diseases, e.g, multiple myeloma.

[0005] In one aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and then determining a second level of soluble BCMA in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently provided a non-CAR T cell therapy to treat said disease. Also provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells); and (c) determining that a second level of soluble BCMA in a tissue sample from the subject is greater than 30% of said first level, and on the basis of the

determination in step c, subsequently providing a non-CAR T cell therapy to the subject. In a specific embodiment of either of the above embodiments, if said second level of sBCMA is greater than 40% of said first level, the subject is provided a non-CAR T cell therapy to treat said disease. In another specific embodiment, said second level of sBCMA is determined at 25-35 days after said administering. In another specific embodiment, said second level of sBCMA is determined at 28-31 days after said administering. In more specific embodiments, the subject is provided a non-CAR T cell therapy within three months, two months, or one month after said determining a second level of sBCMA. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0006] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a non-CAR T cell therapy, wherein the patient has previously been administered immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) and wherein a tissue sample from the patient subsequent to said administration contained a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0007] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a non-CAR T cell therapy after treatment with immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) in a tissue sample from the patient, wherein the patient has previously been administered the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if the level of sBCMA in the tissue sample is greater than 20%, 25%, 30%, 35%, 40%, 45% or 50% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the non-CAR T cell therapy. In a specific embodiment, the method further

comprises administering the non-CAR T cell therapy to the candidate for the non-CAR T cell therapy. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0008] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a non-CAR T cell therapy after treatment with immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) in a tissue sample from the patient, wherein the patient has previously been administered the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the non-CAR T cell therapy. In a specific embodiment, the method further comprises administering the non-CAR T cell therapy to the candidate for the non-CAR T cell therapy. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells.In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0009] In particular embodiments, wherein if the level of sBCMA in the tissue sample is greater than about 20%, 25%, 30%, 35%, 40%, 45% or 50% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the non-CAR T cell therapy. Optionally, the method may further comprises administering the non-CAR T cell therapy to the candidate for the non-CAR T cell therapy.

[0010] In another embodiment provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and determining a level of soluble BCMA (sBCMA) in a tissue sample from the subject; wherein, if said level of sBCMA is greater than 4000 ng/L, the subject is subsequently provided a non-CAR T cell therapy to treat said disease. In a specific embodiment, said level of sBCMA is determined at 50-70 days after said administering. In another specific embodiment, said level of sBCMA is determined at 55-65 days after said

administering. In another specific embodiment, said of sBCMA is determined at 58-62 days after said administering. In a specific embodiment of the preceding embodiments, the subject is provided said non-CAR T cell therapy within three months, two months, or one month after said determining a level of sBCMA. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0011] In another embodiment, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα) or both in a tissue sample from the subject; administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and subsequently determining a second level of IL-6, TNFα or both in a tissue sample from the subject; wherein, if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, respectively, then the subject is subsequently provided a non-CAR T cell therapy to treat said disease. In a specific embodiment, said first level is determined on the day of said administering to the subject said immune cells expressing a CAR directed to BCMA, and said second level is determined 1-4 days after said administering. In another specific embodiment, said second level is determined two days after said administering. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0012] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA)-expressing cells in a subject in need thereof, comprising: administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and determining a level of ferritin in a tissue sample from the subject; wherein, if said level of ferritin is greater than 1500 picomoles per liter, the subject is subsequently provided a therapy to treat cytokine release syndrome (CRS). In certain embodiments, said determining is performed within 0-4 days prior to said administering. In a specific embodiment, said determining is performed on the same day as said administering. In another specific embodiment, said therapy to treat CRS is first provided to said subject 0-5 days

after said administering. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0013] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) determining a second level of sBCMA and/or a second level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 20%, 25%, 30%, 35%, 40%, 45% or 50% of said first level of sBCMA and/or if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, the subject is subsequently provided a non-CAR T cell therapy to treat said disease. In certain embodiments, if said second level of IL-6, TNFα or both is not greater than about 80%, 90%, 95%, 100%, 110%, 120%, or 150% of said first level of IL-6, TNFα or both, the subject is subsequently provided a non-CAR T cell therapy to treat said disease. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0014] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) determining a second level of sBCMA and/or a second level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA and/or if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, the subject is subsequently provided a non-CAR T cell therapy to treat said

disease. In certain embodiments, if said second level of IL-6, TNFα or both is not greater than about 80%, 90%, 95%, 100%, 110%, 120%, or 150% of said first level of IL-6, TNFα or both, the subject is subsequently provided a non-CAR T cell therapy to treat said disease. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0015] In certain embodiments, if i) said second level of sBCMA is greater than about 20%, 25%, 30%, 35%, 40%, 45% or 50% of said first level of sBCMA, and ii) if said second level of IL-6, TNFα or both is not about 10%, 15%, 20%, 25%, 30%, 35%, 40% 45% or 50% greater than said first level of IL-6, TNFα or both, the subject is subsequently provided a non-CAR T cell therapy to treat said disease. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0016] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), (c) determining that a second level of sBCMA in a tissue sample from the subject is greater than 20%, 25%, 30%, 35%, 40%, 45% or 50% of said first level of sBCMA and/or a second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, and (d) on the basis of the determination in step c, subsequently providing a non-CAR T cell therapy to the subject. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0017] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b)

administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), (c) determining that a second level of sBCMA in a tissue sample from the subject is greater than 30% of said first level of sBCMA and/or a second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, and (d) on the basis of the determination in step c, subsequently providing a non-CAR T cell therapy to the subject. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0018] In certain embodiments the method comprises: (c) determining that a second level of IL-6, TNFα or both is not greater than about 80%, 90%, 95%, 100%, 110%, 120%, or 150% of said first level of IL-6, TNFα or both, and (d) on the basis of the determination in step (c), subsequently providing a non-CAR T cell therapy to the subject. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0019] In certain embodiments the method comprises: (c) determining that a second level of sBCMA is greater than about 20%, 25%, 30%, 35%, 40%, 45% or 50% of said first level of sBCMA, and determining that a second level of IL-6, TNFα or both is not greater than about 80%, 90%, 95%, 100%, 110%, 120%, or 150% of said first level of IL-6, TNFα or both, and (d) on the basis of the determination in step (c), subsequently providing a non-CAR T cell therapy to the subject. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0020] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a non-CAR T cell therapy, wherein the patient has previously been administered immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) and wherein a tissue sample from the patient subsequent to said administration contained (i) a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) a level of IL-6, TNFα or both not greater than a level of IL-6, TNFα or both found in a tissue

sample obtained from the patient prior to said administration. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0021] In certain embodiments the method comprises administering to the patient a non-CAR T cell therapy, wherein a tissue sample from the patient subsequent to said administration contained (i) a level of soluble BCMA (sBCMA) greater than about 20%, 25%, 30%, 35%, 40%, 45% or 50% of said first of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) a level of IL-6, TNFα or both not greater than about 20%, 25%, 30%, 35%, 40%, 45% or 50% of a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0022] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a non-CAR T cell therapy after treatment with immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) and/or a level of IL-6, TNFα or both in a tissue sample from the patient, wherein the patient has previously been administered the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if (i) the level of sBCMA in the tissue sample is greater than 20%, 25%, 30%, 35%, 40%, 45% or 50% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) the level of IL-6, TNFα or both is not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the non-CAR T cell therapy. In a specific embodiment, the method further comprises administering the non-CAR T cell therapy to the candidate for the non-CAR T cell therapy. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0023] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a non-CAR T cell therapy after treatment with immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) and/or a level of IL-6, TNFα or both in a tissue sample from the patient, wherein the patient has previously been administered the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if (i) the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) the level of IL-6, TNFα or both is not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the non-CAR T cell therapy. In a specific embodiment, the method further comprises administering the non-CAR T cell therapy to the candidate for the non-CAR T cell therapy. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0024] In certain embodiments of the method, if (i) the level of sBCMA in the tissue sample is greater than about 20%, 25%, 30%, 35%, 40%, 45% or 50% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) the level of IL-6, TNFα or both is not greater than about 20%, 25%, 30%, 35%, 40%, 45% or 50% of a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the non-CAR T cell therapy. In a specific embodiment, the method further comprises administering the non-CAR T cell therapy to the candidate for the non-CAR T cell therapy. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0025] In a specific embodiments of any of the above aspects or embodiments, said tissue sample is blood, plasma or serum. In another specific embodiments of any of the above aspects or embodiments, said disease caused by BCMA-expressing cells is multiple myeloma, chronic lymphocytic leukemia, or a non-Hodgkins lymphoma (*e.g.*, Burkitt's lymphoma, chronic

lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma). In specific embodiments, the disease is multiple myeloma, *e.g.*, high-risk multiple myeloma or relapsed and refractory multiple myeloma. In other specific embodiments, the high risk multiple myeloma is R-ISS stage III disease and/or a disease characterized by early relapse (e.g., progressive disease within 12 months since the date of last treatment regimen, such as last treatment regimen with a proteasome inhibitor, an immunomodulatory agent and/or dexamethasone). In specific embodiments, said disease caused by BCMA-expressing cells is a non-Hodgkins lymphoma, and wherein the non-Hodgkins lymphoma is selected from the group consisting of: Burkitt's lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma. In one embodiment, before the administration of the T cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA), the subject having a tumor has been assessed for expression of BCMA by the tumor.

[0026] In specific embodiments of any of the above aspects or embodiments, the immune cells are T cells, *e.g.*, CD4+ T cells, CD8+ T cells or cytocoxic T lymphocytes (CTLs), T killer cells, or natural killer (NK) cells. In another specific embodiment specific embodiment, the immune cells are administered in a dosage of from 150 x 10^6 cells to 450 x 10^6 cells.

[0027] In a specific embodiment of any of the above embodiments, the non-CAR T cell therapy comprises a proteasome inhibitor, lenalidomide, pomalidomide, thalidomide, bortezomib, dexamethasone, cyclophosphamide, doxorubicin, carfilzomib, ixazomib, cisplatin, doxorubicin, etoposide, an anti-CD38 antibody panobinostat, or elotuzumab. In more specific embodiments, before said administering said subject has received one or more lines of prior therapy comprising: daratumumab, pomalidomide, and dexamethasone (DPd); daratumumab, bortezomib, and dexamethasone (DVd); ixazomib, lenalidomide, and dexamethasone (IRd); daratumumab, lenalidomide and dexamethasone; bortezomib, lenalidomide and dexamethasone (RVd); bortezomib, cyclophosphamide and dexamethasone (BCd); bortezomib, doxorubicin and dexamethasone; carfilzomib, lenalidomide and dexamethasone (CRd); bortezomib and dexamethasone; bortezomib, thalidomide and dexamethasone; lenalidomide and dexamethasone; dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide and

bortezomib (VTD-PACE); lenalidomide and low-dose dexamethasone; bortezomib, cyclophosphamide and dexamethasone; carfilzomib and dexamethasone; lenalidomide alone; bortezomib alone; daratumumab alone; elotuzumab, lenalidomide, and dexamethasone; elotuzumab, lenalidomide and dexamethasone; bendamustine, bortezomib and dexamethasone; bendamustine, lenalidomide, and dexamethasone; pomalidomide and dexamethasone; pomalidomide, bortezomib and dexamethasone; pomalidomide, carfilzomib and dexamethasone; bortezomib and liposomal doxorubicin; cyclophosphamide, lenalidomide, and dexamethasone; elotuzumab, bortezomib and dexamethasone; ixazomib and dexamethasone; panobinostat, bortezomib and dexamethasone; panobinostat and carfilzomib; or pomalidomide, cyclophosphamide and dexamethasone; or any one of the other therapeutic agents listed in Section 5.9, below. In a more specific embodiment, the patient has not received said non-CAR T cell therapy prior to administration of CAR T cells.

[0028] In another specific embodiment of any of the above aspects or embodiments, before said administering said subject has received three or more lines of prior therapy, or one or more lines of prior therapy. In more specific embodiments, said lines of prior therapy comprise a proteasome inhibitor, lenalidomide, pomalidomide, thalidomide, bortezomib, dexamethasone, cyclophosphamide, doxorubicin, carfilzomib, ixazomib, cisplatin, doxorubicin, etoposide, an anti-CD38 antibody panobinostat, or elotuzumab. In more specific embodiments, before said administering said subject has received one or more lines of prior therapy comprising: daratumumab, pomalidomide, and dexamethasone (DPd); daratumumab, bortezomib, and dexamethasone (DVd); ixazomib, lenalidomide, and dexamethasone (IRd); daratumumab, lenalidomide and dexamethasone; bortezomib, lenalidomide and dexamethasone (RVd); bortezomib, cyclophosphamide and dexamethasone (BCd); bortezomib, doxorubicin and dexamethasone; carfilzomib, lenalidomide and dexamethasone (CRd); bortezomib and dexamethasone; bortezomib, thalidomide and dexamethasone; lenalidomide and dexamethasone; dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide and bortezomib (VTD-PACE); lenalidomide and low-dose dexamethasone; bortezomib, cyclophosphamide and dexamethasone; carfilzomib and dexamethasone; lenalidomide alone; bortezomib alone; daratumumab alone; elotuzumab, lenalidomide, and dexamethasone; elotuzumab, lenalidomide and dexamethasone; bendamustine, bortezomib and dexamethasone; bendamustine, lenalidomide, and dexamethasone; pomalidomide and dexamethasone;

pomalidomide, bortezomib and dexamethasone; pomalidomide, carfilzomib and dexamethasone; bortezomib and liposomal doxorubicin; cyclophosphamide, lenalidomide, and dexamethasone; elotuzumab, bortezomib and dexamethasone; ixazomib and dexamethasone; panobinostat, bortezomib and dexamethasone; panobinostat and carfilzomib; or pomalidomide, cyclophosphamide and dexamethasone. In various more specific embodiments, said subject has received two, three, four, five, six, seven or more of said lines of prior therapy; no more than three of said lines of prior therapy; no more than two of said lines of prior therapy; or no more than one of said lines of prior therapy.

[0029] In specific embodiments of any of the above aspects or embodiments, the immune cells are administered at a dose ranging from 150 x 10^6 cells to 450 x 10^6 cells, 300 x 10^6 cells to 600 x 10^6 cells, 350 x 10^6 cells to 600 x 10^6 cells, 350 x 10^6 cells to 550 x 10^6 cells, 400 x 10^6 cells to 600 x 10^6 cells, 150 x 10^6 cells to 300 x 10^6 cells, or 400 x 10^6 cells to 500 x 10^6 cells. In some embodiments, the immune cells are administered at a dose of about 150 x 10^6 cells, about 200 x 10^6 cells, about 250 x 10^6 cells, about 300 x 10^6 cells, about 350 x 10^6 cells, about 400 x 10^6 cells, about 450 x 10^6 cells, about 500 x 10^6 cells, or about 550 x 10^6 cells. In one embodiment, the immune cells are administered at a dose of about 450 x 10^6 cells. In some embodiments, the subject is administered one infusion of the immune cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA). In some embodiments, the administration of the immune cells expressing a CAR directed to BCMA is repeated (e.g., a second dose of immune cells is administered to the subject).

[0030] In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 150 x 10^6 cells to about 300 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administererd in a dosage of from about 350 x 10^6 cells to about 550 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administererd in a dosage of from about 400 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 150 x 10^6 cells to about 250 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 10^6 cells

to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 350 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administererd in a dosage of from about 250 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 10^6 cells to about 600 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 350 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 400 x 10^6 cells to about 600 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 400 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 10^6 cells to about 400 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 10^6 cells to about 350 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 10^6 cells to about 300 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 450 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 10^6 cells to about 400 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are

administered in a dosage of from about 250 x 10^6 cells to about 350 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells are T cells (e.g., autologous T cells). In specific embodiments of any of the embodiments described herein, the subjects being treated undergo a leukapharesis procedure to collect autologous immune cells for the manufacture of the immune cells expressing a CAR directed to BCMA prior to their administration to the subject. In specific embodiments of any of the embodiments described herein, the immune cells (e.g., T cells) are administered by an intravenous infusion.

[0031] In specific embodiments of any of the aspects or embodiments disclosed herein, before administration of immune cells expressing a CAR directed to BCMA, the subject being treated is administered a lymphodepleting (LD) chemotherapy. In specific embodiments, LD chemotherapy comprises fludarabine and/or cyclophosphamide. In specific embodiments, LD chemotherapy comprises fludarabine (e.g., about 30 mg/m^2 for intravenous administration) and cyclophosphamide (e.g., about 300 mg/m^2 for intravenous administration) for a duration of 1, 2, 3, 4, 5, 6, or 7 days (*e.g.*, 3 days). In other specific embodiments, LD chemotherapy comprises any of the chemotherapeutic agents described in Section 5.9. In specific embodiments, the subject is administered immune cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA) 1, 2, 3, 4, 5, 6, or 7 days after the administration of the LD chemotherapy (e.g., 2 or 3 days after the administration of the LD chemotherapy). In specific embodiments, the subject has not received any therapy prior to the initiation of the LD chemotherapy for at least or more than 1 week, at least or more than 2 weeks (at least or more than 14 days), at least or more than 3 weeks, at least or more than 4 weeks, at least or more than 5 weeks, or at least or more than 6 weeks. In specific embodiments of any of the embodiments disclosed herein, before administration of immune cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA), the subject being treated has received only a single prior treatment regimen.

[0032] For any of the above embodiments, the subject undergoes apheresis to collect and isolate said immune cells, *e.g.*, T cells. In a specific embodiment of any of the above embodiments, said subject exhibits at the time of said apheresis: M-protein (serum protein electrophoresis [sPEP] or urine protein electrophoresis [uPEP]): sPEP $\geq$ 0.5 g/dL or uPEP $\geq$ 200 mg/24 hours; light chain

multiple myeloma without measurable disease in the serum or urine, with serum immunoglobulin free light chain ≥ 10 mg/dL and abnormal serum immunoglobulin kappa lambda free light chain ratio; and/or Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1. In a more specific embodiment, said subject at the time of apheresis additionally: has received at least three of said lines of prior treatment, including prior treatment with a proteasome inhibitor, an immunomodulatory agent (lenalidomide or pomalidomide) and an anti-CD38 antibody; has undergone at least 2 consecutive cycles of treatment for each of said at least three lines of prior treatment, unless progressive disease was the best response to a line of treatment; has evidence of progressive disease on or within 60 days of the most recent line of prior treatment; and/or has achieved a response (minimal response or better) to at least one of said prior lines of treatment. In a specific embodiment of any of the above embodiments, said subject exhibits at the time of said administration: M-protein (serum protein electrophoresis [sPEP] or urine protein electrophoresis [uPEP]): sPEP ≥ 0.5 g/dL or uPEP ≥ 200 mg/24 hours; light chain multiple myeloma without measurable disease in the serum or urine, with serum immunoglobulin free light chain ≥ 10 mg/dL and abnormal serum immunoglobulin kappa lambda free light chain ratio; and/or Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1. In another more specific embodiment, said subject additionally: has received only one prior anti-myeloma treatment regimen; has the following high risk factors: R-ISS stage III, and early relapse, defined as (i) if the subject has undergone induction plus a stem cell transplant, progressive disease (PD) less than 12 months since date of first transplant; or (ii) if the subject has received only induction, PD < 12 months since date of last treatment regimen which must contain at minimum, a proteasome inhibitor, an immunomodulatory agent and dexamethasone.

[0033] In a specific embodiment of any of any of the above aspects or embodiments, said CAR comprises an antibody or antibody fragment that targets BCMA. In a more specific embodiment. said CAR comprises a single chain Fv antibody fragment (scFv). In a more specific embodiment, said CAR comprises a BCMA02 scFv. In a specific embodiment of any of the above aspects or embodiments, said immune cells are idecabtagene vicleucel cells. In one embodiment, the chimeric antigen receptor comprises a murine single chain Fv antibody fragment that targets BCMA, *e.g.,* human BCMA. In one embodiment, the chimeric antigen receptor comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide, a hinge domain comprising a CD8α polypeptide, a CD8α transmembrane domain, a CD137 (4-

1BB) intracellular co-stimulatory signaling domain, and a CD3ζ primary signaling domain. In one embodiment, the chimeric antigen receptor comprises a murine scFv that targets BCMA, *e.g.*, human BCMA, wherein the scFV is that of anti-BCMA02 CAR of SEQ ID NO: 9. In one embodiment, the chimeric antigen receptor is or comprises SEQ ID NO: 9. In a more specific embodiment of any embodiment herein, said immune cells are idecabtagene vicleucel cells. In one embodiment, the immune cells comprise a chimeric antigen receptor which comprises a murine single chain Fv antibody fragment that targets BCMA, *e.g.*, human BCMA. In one embodiment, the immune cells comprise a chimeric antigen receptor which comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, human BCMA, a hinge domain comprising a CD8α polypeptide, a CD8α transmembrane domain, a CD137 (4-1BB) intracellular co-stimulatory signaling domain, and a CD3ζ primary signaling domain. In one embodiment, the immune cells comprise a chimeric antigen receptor which is or comprises SEQ ID NO: 9.

[0034] In one aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) determining a second level of sBCMA and/or a second level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA and/or if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, the subject is subsequently provided a non-CAR T cell therapy to treat said disease.

[0035] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), (c) determining that a second level of sBCMA in a tissue sample from the subject is greater than 30% of said first level of sBCMA and/or a second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, and (d) on the

basis of the determination in step c, subsequently providing a non-CAR T cell therapy to the subject. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0036] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a non-CAR T cell therapy, wherein the patient has previously been administered immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) and wherein a tissue sample from the patient subsequent to said administration contained (i) a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) a level of IL-6, TNFα or both not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0037] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a non-CAR T cell therapy after treatment with immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) and/or a level of IL-6, TNFα or both in a tissue sample from the patient, wherein the patient has previously been administered the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if (i) the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) the level of IL-6, TNFα or both is not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the non-CAR T cell therapy. In a specific embodiment, the method further comprises administering the non-CAR T cell therapy to the candidate for the non-CAR T cell therapy. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T

cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0038] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) determining a second level of sBCMA in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently administered lenalidomide to treat said disease. In a specific embodiment, the lenalidomide is administered at a dosage of about 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg, or 25 mg. In another specific embodiment, the lenalidomide is administered at a dosage of about 25 mg daily orally on days 1-21 of a 28-day cycle. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0039] In a specific embodiment of the preceding embodiments, the disease is Multiple Myeloma (MM). In particular embodiments, the disease is relapsed and refractory multiple myeloma.

[0040] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) determining a second level of sBCMA in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently administered pomalidomide to treat said disease. In a specific embodiment, the pomalidomide is administered at a dosage of about 1 mg, 2 mg, 3 mg, or 4 mg once daily. In another specific embodiment, the pomalidomide is administered at a dosage of about 4 mg per day taken orally on days 1-21 of repeated 28-day cycles until disease progression. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In a specific

embodiment, the disease is Multiple Myeloma (MM). In particular embodiments, the disease is relapsed and refractory multiple myeloma.

[0041] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) determining a second level of sBCMA in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently administered CC-220 to treat said disease. In a specific embodiment, the CC-220 is administered at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg. In a specific embodiment, the CC-220 is administered orally at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily on days 1-21 of a 28-day cycle. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In a specific embodiment, the disease is Multiple Myeloma (MM). In particular embodiments, the disease is relapsed and refractory multiple myeloma.

[0042] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) determining a second level of sBCMA in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently administered CC-220 and dexamethasone to treat said disease. In a specific embodiment, the CC-220 is administered at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg. In a specific embodiment, the dexamethasone is administered at a dosage of about 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg. In a specific embodiment, the CC-220 is administered orally at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily on days 1-21 of a 28-day cycle. In a specific embodiment, the dexamethasone is administered orally at a dosage of about 20 mg, 25 mg, 30 mg, 35 mg, 40 mg,

45 mg, 50 mg, 55 mg, or 60 mg on days 1, 8, 15, and 22 of a 28-day cycle. In specific embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In a specific embodiment, the disease is Multiple Myeloma (MM). In particular embodiments, the disease is relapsed and refractory multiple myeloma.

[0043] In one aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) determining a second level of sBCMA in a tissue sample from the subject, wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease, wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0044] Also provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), (c) determining that a second level of sBCMA in a tissue sample from the subject is greater than 30% of said first level of sBCMA, and (d) on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject,wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0045] In a specific embodiment of the above embodiments, if said second level of sBCMA is greater than 40% of said first level of sBCMA, the subject is provided a second BCMA-based treatment modality to treat said disease. In another specific embodiment, said second level of sBCMA is determined at 25-35 days after said administering. In another specific embodiment, said second level of sBCMA is determined at 28-31 days after said administering. In other specific embodiments, the subject is provided a second BCMA-based treatment modality within three months, two months, or one month after said determining the second level of sBCMA. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0046] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a second BCMA-based treatment modality, wherein the patient has previously been administered a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, and wherein a tissue sample from the patient subsequent to said administration contained a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0047] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a second BCMA-based treatment modality after treatment with a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, comprising determining a level of soluble BCMA (sBCMA) in a tissue sample from

the patient, wherein the patient has previously been administered the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if the level of sBCMA in the tissue sample is greater than 20%, 25%, 30%, 35%, 40%, 45% or 50% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the second BCMA-based treatment modality. In a specific embodiment, the method further comprises administering the second BCMA-based treatment modality to the candidate for the second BCMA-based treatment modality. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0048] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a second BCMA-based treatment modality after treatment with a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, comprising determining a level of soluble BCMA (sBCMA) in a tissue sample from the patient, wherein the patient has previously been administered the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the second BCMA-based treatment modality. In a specific embodiment, the method further comprises administering the second BCMA-based treatment modality to the candidate for the second BCMA-based treatment modality. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0049] In particular embodiments, wherein if the level of sBCMA in the tissue sample is greater than about 20%, 25%, 30%, 35%, 40%, 45% or 50% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the second BCMA based treatment modality. Optionally, the method may further comprises administering the second BCMA based treatment modality to the candidate for the second BCMA based treatment modality.

[0050] In another embodiment provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (b) determining a level of soluble BCMA (sBCMA) in a tissue sample from the subject, wherein, if said level of sBCMA is greater than 4000 ng/L, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a specific embodiment, said level of sBCMA is determined at 50-70 days after said administering. In another specific embodiment, said level of sBCMA is determined at 55-65 days after said administering. In another specific embodiment, said level of sBCMA is determined at 58-62 days after said administering. In a specific embodiment of the preceding embodiments, the subject is provided said second BCMA-based treatment modality within three months, two months, or one month after said determining a level of sBCMA. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0051] In another embodiment, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα) or both in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) subsequently determining a second level of IL-6, TNFα or both in a tissue sample from the subject; wherein, if said second level of IL-6, TNFα or both is

not greater than said first level of IL-6, TNFα or both, then the subject is subsequently provided a second BCMA-based treatment modality to treat said disease, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a specific embodiment, said first level is determined on the day of said administering to the subject the first BCMA-based treatment modality comprising immune cells expressing a CAR directed to BCMA, and said second level is determined 1-4 days after said administering. In another specific embodiment, said second level is determined two days after said administering. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0052] In specific embodiments of any of the above aspects or embodiments, said disease caused by BCMA-expressing cells is multiple myeloma, chronic lymphocytic leukemia, or a non-Hodgkins lymphoma (e.g., Burkitt's lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma). In specific embodiments, the disease is multiple myeloma, e.g., high-risk multiple myeloma or relapsed and refractory multiple myeloma. In other specific embodiments, the high risk multiple myeloma is R-ISS stage III disease and/or a disease characterized by early relapse (e.g., progressive disease within 12 months since the date of last treatment regimen, such as last treatment regimen with a proteasome inhibitor, an immunomodulatory agent and/or dexamethasone). In specific embodiments, said disease caused by BCMA-expressing cells is a non-Hodgkins lymphoma, and wherein the non-Hodgkins lymphoma is selected from the group consisting of: Burkitt's lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma. In one embodiment, before the administration of the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), the subject having a tumor has been assessed for expression of BCMA by the tumor.

[0053] In specific embodiments of any of the above aspects or embodiments, the immune cells are T cells, *e.g.*, CD4+ T cells, CD8+ T cells or cytocoxic T lymphocytes (CTLs), T killer cells,

or natural killer (NK) cells. In another specific embodiment specific embodiment, the immune cells are administered in a dosage of from 150 x 10^6 cells to 450 x 10^6 cells.

[0054] In another specific embodiment of any of the above aspects or embodiments, before said administering said subject has received three or more lines of prior therapy, or one or more lines of prior therapy. In more specific embodiments, said lines of prior therapy comprise a proteasome inhibitor, lenalidomide, pomalidomide, thalidomide, bortezomib, dexamethasone, cyclophosphamide, doxorubicin, carfilzomib, ixazomib, cisplatin, doxorubicin, etoposide, an anti-CD38 antibody panobinostat, or elotuzumab. In more specific embodiments, before said administering said subject has received one or more lines of prior therapy comprising: daratumumab, pomalidomide, and dexamethasone (DPd); daratumumab, bortezomib, and dexamethasone (DVd); ixazomib, lenalidomide, and dexamethasone (IRd); daratumumab, lenalidomide and dexamethasone; bortezomib, lenalidomide and dexamethasone (RVd); bortezomib, cyclophosphamide and dexamethasone (BCd); bortezomib, doxorubicin and dexamethasone; carfilzomib, lenalidomide and dexamethasone (CRd); bortezomib and dexamethasone; bortezomib, thalidomide and dexamethasone; lenalidomide and dexamethasone; dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide and bortezomib (VTD-PACE); lenalidomide and low-dose dexamethasone; bortezomib, cyclophosphamide and dexamethasone; carfilzomib and dexamethasone; lenalidomide alone; bortezomib alone; daratumumab alone; elotuzumab, lenalidomide, and dexamethasone; elotuzumab, lenalidomide and dexamethasone; bendamustine, bortezomib and dexamethasone; bendamustine, lenalidomide, and dexamethasone; pomalidomide and dexamethasone; pomalidomide, bortezomib and dexamethasone; pomalidomide, carfilzomib and dexamethasone; bortezomib and liposomal doxorubicin; cyclophosphamide, lenalidomide, and dexamethasone; elotuzumab, bortezomib and dexamethasone; ixazomib and dexamethasone; panobinostat, bortezomib and dexamethasone; panobinostat and carfilzomib; or pomalidomide, cyclophosphamide and dexamethasone. In various more specific embodiments, said subject has received two, three, four, five, six, seven or more of said lines of prior therapy; no more than three of said lines of prior therapy; no more than two of said lines of prior therapy; or no more than one of said lines of prior therapy.

[0055] In specific embodiments of any of the above aspects or embodiments, the immune cells are administered at a dose ranging from 150 x 10^6 cells to 450 x 10^6 cells, 300 x 10^6 cells to 600

x 10^6 cells, 350 x 10^6 cells to 600 x 10^6 cells, 350 x 10^6 cells to 550 x 10^6 cells, 400 x 10^6 cells to 600 x 10^6 cells, 150 x 10^6 cells to 300 x 10^6 cells, or 400 x 10^6 cells to 500 x 10^6 cells. In some embodiments, the immune cells are administered at a dose of about 150 x 10^6 cells, about 200 x 10^6 cells, about 250 x 10^6 cells, about 300 x 10^6 cells, about 350 x 10^6 cells, about 400 x 10^6 cells, about 450 x 10^6 cells, about 500 x 10^6 cells, or about 550 x 10^6 cells. In one embodiment, the immune cells are administered at a dose of about 450 x 10^6 cells. In some embodiments, the subject is administered one infusion of the immune cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA). In some embodiments, the administration of the immune cells expressing a CAR directed to BCMA is repeated (e.g., a second dose of immune cells is administered to the subject).

[0056] In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 150 x 106 cells to about 300 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 350 x 106 cells to about 550 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 400 x 106 cells to about 500 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 150 x 106 cells to about 250 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 106 cells to about 500 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 350 x 106 cells to about 450 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 106 cells to about 450 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 106 cells to about 450 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 106 cells to about 600 x 106 cells. In specific embodiments of any of the embodiments described herein,

the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 106 cells to about 500 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 350 x 106 cells to about 500 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 400 x 106 cells to about 600 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 400 x 106 cells to about 450 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 106 cells to about 400 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 106 cells to about 350 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 106 cells to about 300 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 450 x 106 cells to about 500 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 106 cells to about 400 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 106 cells to about 350 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of about 450 x 106 cells. In specific embodiments of any of the embodiments described herein, the immune cells are T cells (e.g., autologous T cells). In specific embodiments of any of the embodiments described herein, the subjects being treated undergo a leukapharesis procedure to collect autologous immune cells for the manufacture of the immune cells expressing a CAR directed to BCMA prior to their administration to the subject. In specific embodiments of any of the embodiments described herein, the immune cells (e.g., T cells) are administered by an intravenous infusion.

[0057] In a specific embodiment of any of any of the above aspects or embodiments, said CAR comprises an antibody or antibody fragment that targets BCMA. In a more specific embodiment, said CAR comprises a single chain Fv antibody fragment (scFv). In a more specific embodiment, said CAR comprises a BCMA02 scFv. In a specific embodiment of any of the above aspects or embodiments, said immune cells are idecabtagene vicleucel cells. In one embodiment, the chimeric antigen receptor comprises a murine single chain Fv antibody fragment that targets BCMA, e.g., human BCMA. In one embodiment, the chimeric antigen receptor comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, e.g., a human BCMA polypeptide, a hinge domain comprising a CD8α polypeptide, a CD8α transmembrane domain, a CD137 (4-1BB) intracellular co-stimulatory signaling domain, and a CD3ζ primary signaling domain. In one embodiment, the chimeric antigen receptor comprises a murine scFv that targets BCMA, e.g., human BCMA, wherein the scFV is that of anti-BCMA02 CAR of SEQ ID NO: 9. In one embodiment, the chimeric antigen receptor is or comprises SEQ ID NO: 9. In a more specific embodiment of any embodiment herein, said immune cells are idecabtagene vicleucel cells. In one embodiment, the immune cells comprise a chimeric antigen receptor which comprises a murine single chain Fv antibody fragment that targets BCMA, e.g., human BCMA. In one embodiment, the immune cells comprise a chimeric antigen receptor which comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, e.g., human BCMA, a hinge domain comprising a CD8α polypeptide, a CD8α transmembrane domain, a CD137 (4-1BB) intracellular co-stimulatory signaling domain, and a CD3ζ primary signaling domain. In one embodiment, the immune cells comprise a chimeric antigen receptor which is or comprises SEQ ID NO: 9.

[0058] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) determining a second level of sBCMA and/or a second level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA and/or if said second level of

IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0059] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), (c) determining that a second level of sBCMA in a tissue sample from the subject is greater than 30% of said first level of sBCMA and/or a second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, and (d) on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject, wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0060] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a second BCMA-based treatment modality, wherein the patient has previously been administered a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, and wherein a tissue sample from the patient subsequent to said administration contained (i) a level of soluble BCMA (sBCMA) greater than 30% of a level

of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) a level of IL-6, TNFα or both not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0061] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a second BCMA-based treatment modality after treatment with a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) and/or a level of IL-6, TNFα or both in a tissue sample from the patient, wherein the patient has previously been administered the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein if (i) the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) the level of IL-6, TNFα or both is not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the second BCMA-based treatment modality, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a specific embodiment, the method further comprises administering the second BCMA-based treatment modality to the candidate for the second BCMA-based treatment modality. In specific embodiments, the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells. In particular embodiments, the disease is multiple myeloma, e.g., relapsed and refractory multiple myeloma.

[0062] In specific embodiments of any of the embodiments described herein, the second BCMA-based treatment modality comprises a BCMA-Antibody-Drug Conjugate (ADC), a bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA), a natural killer (NK) cell engager (NKCEs) that targets B-cell maturation antigen (BCMA), or immune cells expressing a

chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In a specific embodiment, the second BCMA-based treatment modality comprises a BCMA-Antibody-Drug Conjugate (ADC). In a specific embodiment, the BCMA-Antibody-Drug Conjugate (ADC) comprises CC99712 or GSK2857916 (belantamab mafodotin). In a specific embodiment, the second BCMA-based treatment modality comprises a bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA). In a specific embodiment, the bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA) comprises CC-93269, AMG 420, JNJ-64007957, AMG 701, PF-06863135, REGN5458, REGN5459, or TNB-383B. In a specific embodiment, the second BCMA-based treatment modality comprises a natural killer (NK) cell engager (NKCEs) that targets B-cell maturation antigen (BCMA). In a specific embodiment, the natural killer (NK) cell engager (NKCEs) that targets B-cell maturation antigen (BCMA) comprises DF3001, AFM26, CTX-4419, or CTX-8573. In a specific embodiment, the second BCMA-based treatment modality comprises immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In a specific embodiment, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) comprise JCARH125, KITE-585, P-BCMA-101, LCAR-B38M, CT053, anti-CD19/BCMA CAR-T cells, and CTX120.

[0063] In specific embodiments of any of the embodiments described herein, the immune cells in the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells.

[0064] In specific embodiments of any of the embodiments described herein, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

3. BRIEF DESCRIPTION OF THE DRAWINGS

[0065] **Figure 1** shows a schematic of a B cell maturation antigen (BCMA) CAR construct (anti-BCMA02 CAR).

[0066] **Figures 2A and 2B** show post-BCMA02 CAR T cell infusion profiles of IL-6 (Fig. 2A) and TNF-α (Fig. 2B). "TNF" = TNFα, "INTLK6" = interleukin-6. Non-responders are indicated by heavy lines, and responders (patients who achieved a PR or better) are indicated by lighter lines. The fold-changes at Days 1 – 9, particularly at Day 2, significantly differentiate these two groups ($p < .02$). "Scr" = date of screening.

[0067] **Figure 3** shows percent soluble BCMA reduction from infusion to Month 1 alongside best overall response for individuals from the Phase I trial with both efficacy end points and soluble BCMA measurements at both time points (n = 66).

[0068] **Figure 4** shows post-infusion profiles of soluble BCMA levels in the serum. Exquisite responder (defined as those who remain progression-free for at least 18 months after infusion with ide-cel) profiles are indicated by heavy lines, patients who progressed or died before 18 months are indicated by lighter lines. The month 2 levels of soluble BCMA are significantly lower in the exquisite responders (p = .0016).

[0069] **Figures 5A and 5B** show that the fold change (FC) in sBCMA at day 7 (Fig. 5A) and month 1 (Fig. 5B) correlated with overall response. PR, partial response.

[0070] **Figure 6** shows sBCMA levels in Durable (progression-free survival (PFS) ≥ 18 mo) and Nondurable (PFS < 18 mo) Responders at month 2 post-infusion. sBCMA levels are shown in ng/L.

[0071] **Figures 7A and 7B** show fold changes in IL-6 (Fig. 7A) and TNF-α (Fig. 7B) at day 2 post-infusion in Responders (≥ PR) and Nonresponders (< PR).

[0072] **Figure 8** shows partial dependent plots with respect to progression-free survival (PFS). For the normalized IL-2 plot, the normalized amounts of IL-2 (x-axis) are shown in pg/ml per $1x10^6$ CAR T cells. For the CD3+, CD8+, CAR+, CD25+ plot, the x-axis shows the percent CD25 positive cells in CD8+ CAR T cells. For both plots, the y-axis shows Accumulated Local Effect, Survival Probability Change.

[0073] **Figures 9A and 9B** show a Forest Plot of Univariate Cox-PH Model for selected variables with respect to progression-free survival (PFS) (Fig. 9A) and Box Plots of Univariate Cox-PH Model for selected variables with respect to CRS (Fig. 9B). "PD-Secreted IL-2" refers to Drug Product secreted IL-2.

[0074] **Figure 10** shows that tumor BCMA expression was observed in all evaluable patients prior to infusion. Each dot represents the percentage and average intensity score for an individual patient. The overlapping dots are offset around each intensity score. Abbreviations: BCMA, B-cell maturation antigen; ide-cel, idecabtagene vicleucel; IHC, immunohistochemistry; NE, not evaluable.

[0075] **Figures 11A-11D** show that higher BCMA receptor density, rather than percentage of BCMA-positive cells, was associated with a deeper tumor response. Abbreviations: BCMA, B-

cell maturation antigen; BOR, best overall response; CR, complete response; HR, hazard ratio; IHC, immunohistochemistry; NR, no response; PFS, progression-free survival; PR, partial response; R, response; sCR, stringent complete response; VGPR, very good partial response.

[0076] **Figures 12A and 12B** show BCMA IHC staining and VDJ clone tracking in a patient with suspected antigen loss. Figure 12A: BCMA IHC staining from patient with suspected antigen loss. Figure 12B: VDJ clone tracking illustrates return of initial and potential emergent clones at relapse in a multiple myeloma patient with suspected antigen loss. Abbreviations: BCMA, B-cell maturation antigen; BL, baseline; IHC, immunohistochemistry; M, month; MRD, minimal residual disease; PD, progressive disease; Scrn, screening; VDJ, variable, diversity, and joining.

[0077] **Figures 13A-13C** show that the magnitude of postinfusion cytokine C_{max} was associated with CAR T cell activation and expansion, and tumor response. Figure 13A: time course of ide-cel expansion and contraction. Figure 13A (continued): ide-cel expansion by clinical response. Figure 13B: the magnitude of pro-inflammatory cytokine induction by dose level. Figure 13C: the magnitude of pro-inflammatory cytokine induction (C_{max}) by clinical response. Abbreviations: AUC, area under the curve; BL, baseline; CAR, chimeric antigen receptor; Cmax, maximum concentration; CRP, C-reactive protein; D, day; IFN, interferon; IL, interleukin; M, month; ORR, overall response rate.

[0078] **Figures 14A-14C** show that the magnitude of postinfusion cytokine induction (cytokine C_{max}) rather than baseline levels was associated with higher grade CRS and investigator-identified NT. Figure 14A: the magnitude of pro-inflammatory cytokine induction and grade of CRS. Figure 14B: the magnitude of pro-inflammatory cytokine induction and grade of investigator-identified NT. Figure 14C: baseline angiopoietin-1 and -2 levels and grade of investigator-identified NT. In each graph shown in Figures 14A, 14B, and 14C, each rectangle with error bars is shown from left to right in the order of Grade 0, Grades 1+2, and Grade ≥ 3 (one rectangle with error bars per grade in each graph). Abbreviations: Ang, angiopoietin; CRP, C-reactive protein; IFN, interferon; NT, neurotoxicity; IL, interleukin.

[0079] **Figures 15A-15C** show that sBCMA clearance postinfusion was independent of baseline tumor burden or EMP involvement. Figure 15A (upper and lower panels): the correlation between baseline sBCMA and tumor burden. Figure 15B (upper and lower panels): the correlation between baseline sBCMA and presence of EMP. Figure 15C (upper and lower

panels): Correlation between baseline sBCMA and sBCMA clearance. Abbreviations: EMP, extramedullary plasmacytoma; LLOQ, lower limit of quantitation; sBCMA, soluble B-cell maturation antigen.

[0080] **Figures 16A-16C** show that sBCMA clearance occurred rapidly in responding patients and the time to sBCMA rebound was associated with depth of tumor response. Figure 16A: median sBCMA stratified by best overall response post ide-cel infusion. Figure 16B: the proportion of patients achieving sBCMA nadir <LLOQ by best overall response. Figure 16C: the time to rebound of sBCMA to detectable levels. Abbreviations: BL, baseline; CR, complete response; D, day; LLOQ, lower limit of quantitation; M, month; NPC, normal plasma cell; NR, no response; PD, progressive disease; PR, partial response; sBCMA, soluble B-cell maturation antigen; VGPR, very good partial response.

[0081] **Figures 17A and 17B** show that sBCMA response trajectories were consistent with sensitive MRD assessment by NGS and traditional serum markers of myeloma disease burden. Figure 17A: MRD (as determined using next-generation sequencing, measured in cells/million) and levels of sBCMA, M-protein, and FLC in non-responders, responders (progressed), and responders (ongoing) (responses were characterized as nonresponders (<partial response), responders who relapsed at time of data cut (responders, progressed), and responders who were still in response at the data cut (responders, ongoing)). Figure 17B: Detectable Biomarkers. Abbreviations: BL, baseline; FLC, free light chain; M, month; MFC; multi-color flow cytometry; M-prot, monoclonal protein; MRD, minimal residual disease; NGS, next-generation sequencing; sBCMA, soluble B-cell maturation antigen; NonR, Nonresponders; R, prog, Responders, progressed; R, ong, Responders, ongoing.

4. BRIEF DESCRIPTION OF THE SEQUENCE IDENTIFIERS

[0082] **SEQ ID NOs: 1-3** set forth amino acid sequences of exemplary light chain CDR sequences for BCMA CARs contemplated herein.

[0083] **SEQ ID NOs: 4-6** set forth amino acid sequences of exemplary heavy chain CDR sequences for BCMA CARs contemplated herein.

[0084] **SEQ ID NO: 7** sets forth an amino acid sequence of an exemplary light chain sequence for BCMA CARs contemplated herein.

[0085] **SEQ ID NO: 8** sets forth an amino acid sequence of an exemplary heavy chain sequence for BCMA CARs contemplated herein.

[0086] **SEQ ID NO: 9** sets forth an amino acid sequence of an exemplary BCMA CAR contemplated herein.

[0087] **SEQ ID NO: 10** sets forth a polynucleotide sequence that encodes an exemplary BCMA CAR contemplated herein.

[0088] **SEQ ID NO: 11** sets forth the amino acid sequence of human BCMA.

[0089] **SEQ ID NO: 12-22** set forth the amino acid sequences of various linkers.

[0090] **SEQ ID NOs: 23-35** set forth the amino acid sequences of protease cleavage sites and self-cleaving polypeptide cleavage sites.

[0091] **SEQ ID NO: 36** sets forth the polynucleotide sequence of a vector encoding a BCMA CAR. *See* Table 1.

[0092] Table 1: Listing of Sequences:

SEQ ID NO.	Sequence
1	RASESVTILGSHLIH
2	LASNVQT
3	LQSRTIPRT
4	DYSIN
5	WINTETREPAYAYDFRG
6	DYSYAMDY
7	DIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQKPGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTKLEIK
8	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDYWGQGTSVTVSS
9	MALPVTALLLPLALLLHAARPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQKPGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTKLEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKWMGWINTETREPAYAYDFRGRFAFSLETSA

SEQ ID NO.	Sequence
	STAYLQINNLKYEDTATYFCALDYSYAMDYWGQGTSVTVSSAA ATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD IYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTT QEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA LPPR
	atggcactccccgtcaccgcccttctcttgcccctcgccctgctgctgcatgctgccaggcccgacattg tgctcactcagtcacctcccagcctggccatgagcctgggaaaaagggccaccatctcctgtagagcc agtgagtccgtcacaatcttggggagccatcttattcactggtatcagcagaagcccgggcagcctcca acccttcttattcagctcgcgtcaaacgtccagacgggtgtacctgccagattttctggtagcgggtcccg cactgattttacactgaccatagatccagtggaagaagacgatgtggccgtgtattattgtctgcagagca gaacgattcctcgcacatttggtgggggtactaagctggagattaagggaagcacgtccggctcaggg aagccgggctccggcgagggaagcacgaaggggcaaattcagctggtccagagcggacctgagct gaaaaaacccggcgagactgttaagatcagttgtaaagcatctggctataccttcaccgactacagcata aattgggtgaaacgggcccctggaaagggcctcaaatggatgggttggatcaataccgaaactaggg agcctgcttatgcatatgacttccgcgggagattcgccttttcactcgagacatctgcctctactgcttacct ccaaataaacaacctcaagtatgaagatacagccacttacttttgcgccctcgactatagttacgccatgg actactggggacagggaacctccgttaccgtcagttccgcggccgcaaccacaacacctgctccaag gcccccccacacccgctccaactatagccagccaaccattgagcctcagacctgaagcttgcaggcccg cagcaggaggcgccgtccatacgcgaggcctggacttcgcgtgtgatatttatatttgggcccctttggc cggaacatgtggggtgttgcttctctcccttgtgatcactctgtattgtaagcgcgggagaaagaagctcc tgtacatcttcaagcagccttttatgcgacctgtgcaaaccactcaggaagaagatgggtgttcatgccg cttccccgaggaggaagaaggagggtgtgaactgagggtgaaattttctagaagcgccgatgctcccg catatcagcagggtcagaatcagctctacaatgaattgaatctcggcaggcgagaagagtacgatgttct ggacaagagacggggcagggatcccgagatgggggaaagccccggagaaaaaatcctcaggag gggttgtacaatgagctgcagaaggacaagatggctgaagcctatagcgagatcggaatgaaaggcg aaagacgcagaggcaaggggcatgacggtctgtaccagggtctctctacagccaccaaggacacttat

SEQ ID NO.	Sequence
	gatgcgttgcatatgcaagccttgccaccccgctaatga
11	MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNAS VTNSVKGTNAILWTCLGLSLIISLAVFVLMFLLRKINSEPLKDEFK NTGSGLLGMANIDLEKSRTGDEIILPRGLEYTVEECTCEDCIKSKP KVDSDHCFPLPAMEEGATILVTTKTNDYCKSLPAALSATEIEKSIS AR
12	DGGGS
13	TGEKP
14	GGRR
15	GGGGS
16	EGKSSGSGSESKVD
17	KESGSVSSEQLAQFRSLD
18	GGRRGGGS
19	LRQRDGERP
20	LRQKDGGGSERP
21	LRQKDGGGSGGGSERP
22	GSTSGSGKPGSGEGSTKG
23	$EX_1X_2YX_3QX_4$ X_1 is Any amino acid X_2 is Any amino acid X_3 is Any amino acid X_4 is Gly or Ser
24	ENLYFQG
25	ENLYFQS
26	LLNFDLLKLAGDVESNPGP
27	TLNFDLLKLAGDVESNPGP
28	LLKLAGDVESNPGP

SEQ ID NO.	Sequence
29	NFDLLKLAGDVESNPGP
30	QLLNFDLLKLAGDVESNPGP
31	APVKQTLNFDLLKLAGDVESNPGP
32	VTELLYRMKRAETYCPRPLLAIHPTEARHKQKIVAPVKQT
33	LNFDLLKLAGDVESNPGP
34	LLAIHPTEARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP
35	EARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP
36	tcgcgcgtttcggtgatgacggtgaaaacctctgacacatgcagctcccggagacggtcacagcttgtc tgtaagcggatgccgggagcagacaagcccgtcagggcgcgtcagcgggtgttggcgggtgtcggg gctggcttaactatgcggcatcagagcagattgtactgagagtgcaccatcatatgccagcctatggtga cattgattattgactagttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccg cgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaat aatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggt aaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacgg taaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacgt attagtcatcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatagcggtttgactc acggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggac tttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggaggtc tatataagcagagctcgtttagtgaaccgggtctctctggttagaccagatctgagcctgggagctctctg gctaactagggaacccactgcttaagcctcaataaagcttgccttgagtgctcaaagtagtgtgtgcccg tctgttgtgtgactctggtaactagagatccctcagaccctttttagtcagtgtggaaaatctctagcagtgg cgcccgaacagggacttgaaagcgaaagtaaagccagaggagatctctcgacgcaggactcggcttg ctgaagcgcgcacggcaagaggcgaggggcggcgactggtgagtacgccaaaaattttgactagcg gaggctagaaggagagagtagggtgcgagagcgtcggtattaagcgggggagaattagataaatgg gaaaaaattcggttaaggccagggggaaagaaacaatataaactaaaacatatagttagggcaagcag ggagctagaacgattcgcagttaatcctggccttttagagacatcagaaggctgtagacaaatactggg acagctacaaccatcccttcagacaggatcagaagaacttagatcattatataatacaatagcagtcctct attgtgtgcatcaaaggatagatgtaaaagacaccaaggaagccttagataagatagaggaagagcaa

SEQ ID NO.	Sequence
	aacaaaagtaagaaaaaggcacagcaagcagcagctgacacaggaaacaacagccaggtcagcca aaattaccctatagtgcagaacctccaggggcaaatggtacatcaggccatatcacctagaactttaaatt aagacagcagtacaaatggcagtattcatccacaattttaaaagaaaagggggggattgggggtacagt gcaggggaaagaatagtagacataatagcaacagacatacaaactaaagaattacaaaaacaaattac aaaaattcaaaattttcgggtttattacagggacagcagagatccagtttggaaaggaccagcaaagctc ctctggaaaggtgaaggggcagtagtaatacaagataatagtgacataaaagtagtgccaagaagaaa agcaaagatcatcagggattatggaaaacagatggcaggtgatgattgtgtggcaagtagacaggatg aggattaacacatggaaaagattagtaaaacaccatagctctagagcgatcccgatcttcagacctgga ggaggagatatgagggacaattggagaagtgaattatataaatataaagtagtaaaaattgaaccattag gagtagcacccaccaaggcaaagagaagagtggtgcagagagaaaaaagagcagtgggaatagga gctttgttccttgggttcttgggagcagcaggaagcactatgggcgcagcgtcaatgacgctgacggta caggccagacaattattgtctggtatagtgcagcagcagaacaatttgctgagggctattgaggcgcaa cagcatctgttgcaactcacagtctggggcatcaagcagctccaggcaagaatcctggctgtggaaag atacctaaaggatcaacagctcctggggatttggggttgctctggaaaactcatttgcaccactgctgtgc cttggaatgctagttggagtaataaatctctggaacagatttggaatcacacgacctggatggagtggga cagagaaattaacaattacacaagcttggtaggtttaagaatagtttttgctgtactttctatagtgaataga gttaggcagggatattcaccattatcgtttcagacccacctcccaaccccgaggggacccgacaggcc cgaaggaatagaagaagaaggtggagagagagacagagacagatccattcgattagtgaacggatc catctcgacggaatgaaagaccccacctgtaggtttggcaagctaggatcaaggttaggaacagagag acagcagaatatgggccaaacaggatatctgtggtaagcagttcctgccccggctcagggccaagaac agttggaacagcagaatatgggccaaacaggatatctgtggtaagcagttcctgccccggctcagggc caagaacagatggtccccagatgcggtcccgccctcagcagtttctagagaaccatcagatgtttccag ggtgccccaaggacctgaaatgaccctgtgccttatttgaactaaccaatcagttcgcttctcgcttctgtt cgcgcgcttctgctccccgagctcaataaaagagcccacaacccctcactcggcgcgattcacctgac gcgtctacgccaccatggcactccccgtcaccgcccttctcttgcccctcgccctgctgctgcatgctgc caggcccgacattgtgctcactcagtcacctcccagcctggccatgagcctgggaaaaagggccacc atctcctgtagagccagtgagtccgtcacaatcttggggagccatcttattcactggtatcagcagaagc ccgggcagcctccaacccttcttattcagctcgcgtcaaacgtccagacgggtgtacctgccagattttct

SEQ ID NO.	Sequence
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SEQ ID NO.	Sequence
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SEQ ID NO.	Sequence
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5. DETAILED DESCRIPTION

5.1. PREDICTING CAR T CELL EFFICACY AND DEVELOPMENT OF CRS USING SOLUBLE BCMA AND CYTOKINES

[0093] The disclosure presented herein generally relates to improved compositions and methods for treating B cell related conditions. As used herein, the term "B cell related conditions" relates to conditions involving inappropriate B cell activity and B cell malignancies.

[0094] Particular embodiments, presented herein relate to improved adoptive cell therapy of B cell related conditions using genetically modified immune effector cells. Genetic approaches offer a potential means to enhance immune recognition and elimination of cancer cells. One promising strategy is to genetically engineer immune effector cells to express chimeric antigen receptors (CAR) that redirect cytotoxicity toward cancer cells. However, existing adoptive cell immunotherapies for treating B cell disorders present a serious risk of compromising humoral immunity because the cells target antigens expressed on all of, or the majority of, B cells. Accordingly, such therapies are not clinically desirable and thus, a need in the art remains for more efficient therapies for B cell related conditions that spare humoral immunity.

[0095] The improved compositions and methods of adoptive cell therapy disclosed herein, provide genetically modified immune effector cells that can readily be expanded, exhibit long-term persistence *in vivo*, and reduce impairment of humoral immunity by targeting B cells expressing B cell maturation antigen (BCMA, also known as CD269 or tumor necrosis factor receptor superfamily, member 17; TNFRSF17). The disclosure also relates to methods for treating B cell related conditions using chimeric antigen receptors (CARs) comprising anti-BCMA antibodies or antigen binding fragments thereof, and immune effector cells genetically modified to express these CARs in combination with BCMA-based treatment modalities This disclosure also relates to methods for treating B cell related conditions using a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) and a second BCMA-based treatment modality,

wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities.

[0096] BCMA is a member of the tumor necrosis factor receptor superfamily (see, *e.g.*, Thompson *et al., J. Exp. Medicine*, 192(1): 129-135, 2000, and Mackay *et al., Annu. Rev. Immunol*, 21: 231-264, 2003. BCMA binds B-cell activating factor (BAFF) and a proliferation inducing ligand (APRIL) (*see, e.g.,* Mackay *et al.*, 2003 and Kalled *et al., Immunological Reviews*, 204: 43-54, 2005). Among nonmalignant cells, BCMA has been reported to be expressed mostly in plasma cells and subsets of mature B-cells (*see, e.g.,* Laabi *et al.*, *EMBO J.*, 77(1): 3897-3904, 1992; Laabi *et al., Nucleic Acids Res.*, 22(7): 1147-1154,, 1994; Kalled *et al.*, 2005; O'Connor *et al., J. Exp. Medicine,* 199(1): 91-97, 2004; and Ng *et al., J. Immunol.*, 73(2): 807-817, 2004. Mice deficient in BCMA are healthy and have normal numbers of B cells, but the survival of long-lived plasma cells is impaired (*see, e.g.,* O'Connor *et al.*, 2004; Xu *et al., Mol. Cell. Biol.,* 21(12): 4067-4074, 2001; and Schiemann *et al., Science,* 293(5537): 2 111-21 14, 2001). BCMA RNA has been detected universally in multiple myeloma cells and in other lymphomas, and BCMA protein has been detected on the surface of plasma cells from multiple myeloma patients by several investigators (*see, e.g.,* Novak *et al., Blood*, 103(2): 689-694, 2004; Neri e*t al., Clinical Cancer Research*, 73(19): 5903-5909, 2007; Bellucci *et al., Blood*, 105(10): 3945-3950, 2005; and Moreaux *et al., Blood*, 703(8): 3148-3157, 2004.

[0097] The amount of soluble (*i.e.*, non-membrane-bound) BCMA (sBCMA) after administration of a CAR T cell therapy, *e.g.*, an anti-BCMA CAR T cell therapy, can be used to determine whether a subject can be expected to respond to the CAR T cell therapy appropriately, or whether the subject should be administered a different anticancer therapy. A greater drop in sBCMA levels in a tissue sample (*e.g.*, serum, plasma, lymph, or blood) after administration of a CAR T cell therapy is correlated with a more clinically beneficial outcome (*e.g.*, very good partial response, complete response or stringent complete response). In one aspect, for example, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and then determining a second level of soluble BCMA in a tissue sample from the subject wherein, if said second level of sBCMA is greater than about 30% of said first level of sBCMA, the subject

is subsequently provided a non-CAR T cell therapy to treat said disease. Also provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells); and (c) determining that a second level of soluble BCMA in a tissue sample from the subject is greater than about 30% of said first level, and on the basis of the determination in step c, subsequently providing a non-CAR T cell therapy to the subject. In a specific embodiment of either of the above embodiments, if said second level of sBCMA is greater than 40% of said first level, the subject is provided a non-CAR T cell therapy to treat said disease. In a specific embodiment of either of the above embodiments, if said second level of sBCMA is greater than about 20%, 25%, 30%, 35%, 40%, 45%, or 50% of said first level, the subject is provided a non-CAR T cell therapy to treat said disease. In another specific embodiment, said second level of sBCMA is determined at 25-35 days after said administering. In another specific embodiment, said second level of sBCMA is determined at 23-35, 24-35, 25-36, 25-37, 23-35, or 25-37 days after said administering. In another specific embodiment, said second level of sBCMA is determined at 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, or 37 days after said administering. In another specific embodiment, said second level of sBCMA is determined at 28-31 days after said administering. In another specific embodiment, said second level of sBCMA is determined at 26-31, 27-31, 28-32, 28-33, 26-31, or 27-33 days after said administering. In another specific embodiment, said second level of sBCMA is determined at 26, 27, 28, 29, 30, 31, 32, or 33 days after said administering. In more specific embodiments, the subject is provided a non-CAR T cell therapy within three months, two months, or one month after said determining a second level of sBCMA.

[0098] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a non-CAR T cell therapy, wherein the patient has previously been administered immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) and wherein a tissue sample from the patient subsequent to said administration contained a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration.

[0099] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a non-CAR T cell therapy after treatment with immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) in a tissue sample from the patient, wherein the patient has previously been administered the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the non-CAR T cell therapy. In a specific embodiment, the method further comprises administering the non-CAR T cell therapy to the candidate for the non-CAR T cell therapy.

[0100] The absolute level of sBCMA in a tissue sample (*e.g.*, plasma, serum, lymph or blood) may also be used to determine whether a person administered a CAR T cell therapy, *e.g.*, a BCMA CAR T cell therapy will appropriately benefit from that therapy, or should be administered adifferent anticancer therapy. Thus, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and determining a level of soluble BCMA (sBCMA) in a tissue sample from the subject; wherein, if said level of sBCMA is greater than 4000 ng/L, the subject is subsequently provided a non-CAR T cell therapy to treat said disease. In a specific embodiment of either of the above embodiments, if said level of sBCMA is greater than about 3000 ng/L, 3500 ng/L, 4000 ng/L, 4500 ng/L, or 5000 ng/L the subject is subsequently provided a non-CAR T cell therapy to treat said disease. In a specific embodiment, said first level of sBCMA is determined at 50-70 days after said administering. In a specific embodiment, said first level of sBCMA is determined at 45-70, 46-70, 47-70, 48-70, 49-70, 50-70, 50-71, 50-72, 50-73, or 50-75 days after said administering. In a specific embodiment, said first level of sBCMA is determined at 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 days after said administering. In another specific embodiment, said first level of sBCMA is determined at 55-65 days after said administering. In another specific embodiment, said first level of sBCMA is determined at 50-65, 51-65, 52-65, 53-65, 54-65, 55-64, 55-63, 55-62, or 55-61 days after said administering. In another specific embodiment, said

first level of sBCMA is determined at 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, or 65 days after said administering. In another specific embodiment, said level of sBCMA is determined at 58-62 days after said administering. In another specific embodiment, said level of sBCMA is determined at 53-62, 54-62, 55-62, 56-62, 57-62, 58-68, 58-67, 58-66, 58-65, 58-64, or 58-63 days after said administering. In another specific embodiment, said level of sBCMA is determined at 58, 59, 60, 61, or 62 days after said administering. In a specific embodiment of the preceding embodiments, the subject is provided said non-CAR T cell therapy within three months, two months, or one month after said determining a first level of sBCMA.

[0101] The levels of certain cytokines, *e.g.*, interleukin-6 (IL-6) and/or tumor necrosis factor alpha (TNFα) can also be used to determine whether a person administered a CAR T cell therapy, *e.g.*, a BCMA CAR T cell therapy will appropriately benefit from that therapy, or should be administered a different anticancer therapy. Thus, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα) or both in a tissue sample from the subject; administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and subsequently determining a second level of IL-6, TNFα or both in a tissue sample from the subject; wherein, if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, respectively, then the subject is subsequently provided a non-CAR T cell therapy to treat said disease. In a specific embodiment, said first level is determined on the day of said administering to the subject said immune cells expressing a CAR directed to BCMA, and said second level is determined 1-4 days after said administering. In another specific embodiment, said second level is determined one day after said administering. In another specific embodiment, said second level is determined two days after said administering. In another specific embodiment, said second level is determined three days after said administering. In another specific embodiment, said second level is determined four days after said administering.

[0102] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA)-expressing cells in a subject in need thereof, comprising: administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and determining a level of ferritin in a tissue sample from the

subject; wherein, if said level of ferritin is greater than 1500 picomoles per liter, the subject is subsequently provided a therapy to treat cytokine release syndrome (CRS). In certain embodiments, said determining is performed within 0-4 days prior to said administering. In a specific embodiment, said determining is performed on the same day as said administering. In another specific embodiment, said therapy to treat CRS is first provided to said subject 0-5 days after said administering.

[0103] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) determining a second level of sBCMA and/or a second level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA and/or if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, the subject is subsequently provided a non-CAR T cell therapy to treat said disease.

[0104] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), (c) determining that a second level of sBCMA in a tissue sample from the subject is greater than 30% of said first level of sBCMA and/or a second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, and (d) on the basis of the determination in step c, subsequently providing a non-CAR T cell therapy to the subject.

[0105] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a non-CAR T cell therapy, wherein the patient has previously been administered immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA

(BCMA CAR T cells) and wherein a tissue sample from the patient subsequent to said administration contained (i) a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) a level of IL-6, TNFα or both not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration.

[0106] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a non-CAR T cell therapy after treatment with immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) and/or a level of IL-6, TNFα or both in a tissue sample from the patient, wherein the patient has previously been administered the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if (i) the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) the level of IL-6, TNFα or both is not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the non-CAR T cell therapy. In a specific embodiment, the method further comprises administering the non-CAR T cell therapy to the candidate for the non-CAR T cell therapy.

[0107] In specific embodiments of any of the above aspects or embodiments, said CAR T cell therapy (e.g., immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), e.g., idecabtagene vicleucel (ide-cel) cells) comprises a population of cells that comprises about 10%, 5%, 3%, 2%, or 1% activated CAR T-cells, for example, activated CD8 CAR T-cells (CD3+/CD8+/CAR+/CD25+).

[0108] In specific embodiments of any of the above aspects or embodiments, said CAR T cell therapy (e.g., immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), e.g., idecabtagene vicleucel (ide-cel) cells) comprises a population of cells that comprises 10%, 5%, 3%, 2%, or 1% senescence population of CAR T-cells, for example, CD4 CAR T-cells (CD3+/CD4+/CAR+/CD57+).In a specific embodiments of any of the above aspects or embodiments, said tissue sample is blood, plasma or serum. In another specific embodiments of any of the above aspects or embodiments, said disease caused by BCMA-expressing cells is multiple myeloma, chronic lymphocytic leukemia, or a non-Hodgkins

lymphoma (*e.g.*, Burkitt's lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma). In specific embodiments, the disease is multiple myeloma, *e.g.*, high-risk multiple myeloma or relapsed and refractory multiple myeloma. In other specific embodiments, the high risk multiple myeloma is R-ISS stage III disease and/or a disease characterized by early relapse (e.g., progressive disease within 12 months since the date of last treatment regimen, such as last treatment regimen with a proteasome inhibitor, an immunomodulatory agent and/or dexamethasone). In specific embodiments, said disease caused by BCMA-expressing cells is a non-Hodgkins lymphoma, and wherein the non-Hodgkins lymphoma is selected from the group consisting of: Burkitt's lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma.

[0109] In one embodiment, before the administration of the T cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA), the subject having a tumor has been assessed for expression of BCMA by the tumor.

[0110] In specific embodiments of any of the above aspects or embodiments, the immune cells are T cells, *e.g.*, CD4+ T cells, CD8+ T cells or cytocoxic T lymphocytes (CTLs), T killer cells, or natural killer (NK) cells. In another specific embodiment specific embodiment, the immune cells are administered in a dosage of from 150 x 10^6 cells to 450 x 10^6 cells.

[0111] In a specific embodiment of any of the above embodiments, the non-CAR T cell therapy comprises a proteasome inhibitor, lenalidomide, pomalidomide, thalidomide, bortezomib, dexamethasone, cyclophosphamide, doxorubicin, carfilzomib, ixazomib, cisplatin, doxorubicin, etoposide, an anti-CD38 antibody panobinostat, or elotuzumab. In more specific embodiments, before said administering said subject has received one or more lines of prior therapy comprising: daratumumab, pomalidomide, and dexamethasone (DPd); daratumumab, bortezomib, and dexamethasone (DVd); ixazomib, lenalidomide, and dexamethasone (IRd); daratumumab, lenalidomide and dexamethasone; bortezomib, lenalidomide and dexamethasone (RVd); bortezomib, cyclophosphamide and dexamethasone (BCd); bortezomib, doxorubicin and dexamethasone; carfilzomib, lenalidomide and dexamethasone (CRd); bortezomib and dexamethasone; bortezomib, thalidomide and dexamethasone; lenalidomide and dexamethasone;

dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide and bortezomib (VTD-PACE); lenalidomide and low-dose dexamethasone; bortezomib, cyclophosphamide and dexamethasone; carfilzomib and dexamethasone; lenalidomide alone; bortezomib alone; daratumumab alone; elotuzumab, lenalidomide, and dexamethasone; elotuzumab, lenalidomide and dexamethasone; bendamustine, bortezomib and dexamethasone; bendamustine, lenalidomide, and dexamethasone; pomalidomide and dexamethasone; pomalidomide, bortezomib and dexamethasone; pomalidomide, carfilzomib and dexamethasone; bortezomib and liposomal doxorubicin; cyclophosphamide, lenalidomide, and dexamethasone; elotuzumab, bortezomib and dexamethasone; ixazomib and dexamethasone; panobinostat, bortezomib and dexamethasone; panobinostat and carfilzomib; or pomalidomide, cyclophosphamide and dexamethasone; or any one of the other therapeutic agents listed in Section 5.9, below. In a more specific embodiment, the patient has not received said non-CAR T cell therapy prior to administration of CAR T cells.

[0112] In a specific embodiment of any of the above embodiments, the non-CAR T cell therapy comprises lenalidomide. In certain embodiments, the lenalidomide is administered to a subject as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered at a dosage of about 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg, or 25 mg. In certain embodiments, the lenalidomide may be administered at a dosage of about 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg, or 25 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 25 mg once daily orally on Days 1-21 of repeated 28-day cycles. In certain embodiments, the lenalidomide may be administered at a dosage of about 25 mg once daily orally on Days 1-21 of repeated 28-day cycles to a subject for treating Multiple Myeloma (MM). In certain embodiments, the lenalidomide may be administered at a dosage of about 10 mg once daily

continuously on Days 1-28 of repeated 28-day cycles. In certain embodiments, the lenalidomide may be administered at a dosage of about 2.5 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 5 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 10 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 15 mg every other day. In certain embodiments, the lenalidomide may be administered at a dosage of about 25 mg once daily orally on Days 1-21 of repeated 28-day cycles. In certain embodiments, the lenalidomide may be administered at a dosage of about 20 mg once daily orally on Days 1-21 of repeated 28-day cycles for up to 12 cycles. In a certain embodiment, lenalidomide maintenance therapy is recommended for all patients. In a certain embodiment, lenalidomide maintenance therapy should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0113] In a specific embodiment of any of the above embodiments, the non-CAR T cell therapy comprises pomalidomide. In certain embodiments, the pomalidomide is administered to a subject as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered at a dosage of about 1 mg, 2 mg, 3 mg, or 4 mg. In certain embodiments, the pomalidomide may be administered at a dosage of about 1 mg, 2 mg, 3 mg, or 4 mg once daily. In certain embodiments, the pomalidomide may be administered at a dosage of about 4 mg per day taken orally on days 1-21 of repeated 28-day cycles until disease progression. In certain embodiments, the pomalidomide may be administered at a dosage of about 4 mg per day taken orally on days 1-21 of repeated 28-day cycles until disease progression to a subject for treating Multiple Myeloma (MM). In a certain embodiment, pomalidomide maintenance therapy is recommended for all patients. In a certain embodiment, pomalidomide maintenance therapy

should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0114] In a specific embodiment of any of the above embodiments, the non-CAR T cell therapy comprises CC-220 (iberdomide; *see*, e.g., Bjorkland, C.C. *et al.*, 2019, *Leukemia*, doi: 10.1038/s41375-019-0620-8; U.S. Patent No. 9,828,361). In certain embodiments, the CC-220 is administered to a subject as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg. In certain embodiments, the CC-220 may be administered orally. In certain embodiments, the CC-220 may be administered orally at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily for 21 days of a 28-day cycle, e.g., daily on days 1-21 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the CC-220 may be administered to a subject for treating Multiple Myeloma (MM). In a certain embodiment, CC-220 maintenance therapy is recommended for all patients. In a certain embodiment, the CC-220 maintenance therapy should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0115] In a specific embodiment of any of the above embodiments, the non-CAR T cell therapy comprises CC-220 (iberdomide) and dexamethasone. In certain embodiments, the CC-220 and dexamethasone are administered to a subject as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 and dexamethasone may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the dexamethasone may be

administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 and dexamethasone may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the dexamethasone may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 and dexamethasone may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the dexamethasone may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg. In certain embodiments, the dexamethasone may be administered at a dosage of about 20 mg, 25 mg., 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg. In certain embodiments, the dexamethasone may be administered at a dosage of about 40 mg. In certain embodiments, the CC-220 may be administered orally. In certain embodiments, the CC-220 may be administered orally at a dosage of about 15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily for 21 days of a 28-day cycle, e.g., daily on days 1-21 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the dexamethasone may be administered orally. In certain embodiments, the dexamethasone may be administered at a dose of about 20-60 mgs. In certain embodiments, the dexamethasone may be administered orally at a dosage of about 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg on days 1, 8, 15, and 22 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the CC-220 may be administered orally at a dosage of about 15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg,

1.0 mg, 1.1 mg, or 1.2 mg daily for 21 days of a 28-day cycle, e.g., daily on days 1-21 of a 28-day cycle, with the 28-day cycles repeated as needed, and the dexamethasone may be administered orally at a dosage of about 20 mg, 25 mg., 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg on days 1, 8, 15, and 22 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the CC-220 and dexamethasone may be administered to a subject for treating Multiple Myeloma (MM). In a certain embodiment, CC-220 and dexamethasone maintenance therapy is recommended for all patients. In a certain embodiment, the CC-220 and dexamethasone maintenance therapy should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0116] In another specific embodiment of any of the above aspects or embodiments, before said administering said subject has received three or more lines of prior therapy, or one or more lines of prior therapy. In more specific embodiments, said lines of prior therapy comprise a proteasome inhibitor, lenalidomide, pomalidomide, thalidomide, bortezomib, dexamethasone, cyclophosphamide, doxorubicin, carfilzomib, ixazomib, cisplatin, doxorubicin, etoposide, an anti-CD38 antibody panobinostat, or elotuzumab. In more specific embodiments, before said administering said subject has received one or more lines of prior therapy comprising: daratumumab, pomalidomide, and dexamethasone (DPd); daratumumab, bortezomib, and dexamethasone (DVd); ixazomib, lenalidomide, and dexamethasone (IRd); daratumumab, lenalidomide and dexamethasone; bortezomib, lenalidomide and dexamethasone (RVd); bortezomib, cyclophosphamide and dexamethasone (BCd); bortezomib, doxorubicin and dexamethasone; carfilzomib, lenalidomide and dexamethasone (CRd); bortezomib and dexamethasone; bortezomib, thalidomide and dexamethasone; lenalidomide and dexamethasone; dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide and bortezomib (VTD-PACE); lenalidomide and low-dose dexamethasone; bortezomib, cyclophosphamide and dexamethasone; carfilzomib and dexamethasone; lenalidomide alone; bortezomib alone; daratumumab alone; elotuzumab, lenalidomide, and dexamethasone; elotuzumab, lenalidomide and dexamethasone; bendamustine, bortezomib and dexamethasone; bendamustine, lenalidomide, and dexamethasone; pomalidomide and dexamethasone; pomalidomide, bortezomib and dexamethasone; pomalidomide, carfilzomib and dexamethasone; bortezomib and liposomal doxorubicin; cyclophosphamide, lenalidomide, and dexamethasone; elotuzumab, bortezomib and dexamethasone; ixazomib and dexamethasone; panobinostat,

bortezomib and dexamethasone; panobinostat and carfilzomib; or pomalidomide, cyclophosphamide and dexamethasone. In various more specific embodiments, said subject has received two, three, four, five, six, seven or more of said lines of prior therapy; no more than three of said lines of prior therapy; no more than two of said lines of prior therapy; or no more than one of said lines of prior therapy.

[0117] In specific embodiments of any of the above aspects or embodiments, the immune cells are administered at a dose ranging from 150 x 10^6 cells to 450 x 10^6 cells, 300 x 10^6 cells to 600 x 10^6 cells, 350 x 10^6 cells to 600 x 10^6 cells, 350 x 10^6 cells to 550 x 10^6 cells, 400 x 10^6 cells to 600 x 10^6 cells, 150 x 10^6 cells to 300 x 10^6 cells, or 400 x 10^6 cells to 500 x 10^6 cells. In some embodiments, the immune cells are administered at a dose of about 150 x 10^6 cells, about 200 x 10^6 cells, about 250 x 10^6 cells, about 300 x 10^6 cells, about 350 x 10^6 cells, about 400 x 10^6 cells, about 450 x 10^6 cells, about 500 x 10^6 cells, or about 550 x 10^6 cells. In one embodiment, the immune cells are administered at a dose of about 450 x 10^6 cells. In some embodiments, the subject is administered one infusion of the immune cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA). In some embodiments, the administration of the immune cells expressing a CAR directed to BCMA is repeated (e.g., a second dose of immune cells is administered to the subject).

[0118] In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 150 x 10^6 cells to about 300 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administererd in a dosage of from about 350 x 10^6 cells to about 550 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administererd in a dosage of from about 400 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 150 x 10^6 cells to about 250 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 350 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the

embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administererd in a dosage of from about 250 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 10^6 cells to about 600 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 350 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 400 x 10^6 cells to about 600 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 400 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 10^6 cells to about 400 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 10^6 cells to about 350 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 10^6 cells to about 300 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 450 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 10^6 cells to about 400 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 10^6 cells to about 350 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of about 450 x 10^6 cells. In specific

embodiments of any of the embodiments described herein, the immune cells are T cells (e.g., autologous T cells). In specific embodiments of any of the embodiments described herein, the subjects being treated undergo a leukapharesis procedure to collect autologous immune cells for the manufacture of the immune cells expressing a CAR directed to BCMA prior to their administration to the subject. In specific embodiments of any of the embodiments described herein, the immune cells (e.g., T cells) are administered by an intravenous infusion.

[0119] In specific embodiments of any of the aspects or embodiments disclosed herein, before administration of immune cells expressing a CAR directed to BCMA, the subject being treated is administered a lymphodepleting (LD) chemotherapy. In specific embodiments, LD chemotherapy comprises fludarabine and/or cyclophosphamide. In specific embodiments, LD chemotherapy comprises fludarabine (e.g., about 30 mg/m^2 for intravenous administration) and cyclophosphamide (e.g., about 300 mg/m^2 for intravenous administration) for a duration of 1, 2, 3, 4, 5, 6, or 7 days (*e.g.*, 3 days). In other specific embodiments, LD chemotherapy comprises any of the chemotherapeutic agents described in Section 5.9. In specific embodiments, the subject is administered immune cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA) 1, 2, 3, 4, 5, 6, or 7 days after the administration of the LD chemotherapy (e.g., 2 or 3 days after the administration of the LD chemotherapy). In specific embodiments, the subject has not received any therapy prior to the initiation of the LD chemotherapy for at least or more than 1 week, at least or more than 2 weeks (at least or more than 14 days), at least or more than 3 weeks, at least or more than 4 weeks, at least or more than 5 weeks, or at least or more than 6 weeks. In specific embodiments of any of the embodiments disclosed herein, before administration of immune cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA), the subject being treated has received only a single prior treatment regimen.

[0120] For any of the above embodiments, the subject undergoes apheresis to collect and isolate said immune cells, *e.g.*, T cells. In a specific embodiment of any of the above embodiments, said subject exhibits at the time of said apheresis: M-protein (serum protein electrophoresis [sPEP] or urine protein electrophoresis [uPEP]): sPEP $\geq$ 0.5 g/dL or uPEP $\geq$ 200 mg/24 hours; light chain multiple myeloma without measurable disease in the serum or urine, with serum immunoglobulin free light chain $\geq$ 10 mg/dL and abnormal serum immunoglobulin kappa lambda free light chain ratio; and/or Eastern Cooperative Oncology Group (ECOG) performance

status ≤ 1. In a more specific embodiment, said subject at the time of apheresis additionally: has received at least three of said lines of prior treatment, including prior treatment with a proteasome inhibitor, an immunomodulatory agent (lenalidomide or pomalidomide) and an anti-CD38 antibody; has undergone at least 2 consecutive cycles of treatment for each of said at least three lines of prior treatment, unless progressive disease was the best response to a line of treatment; has evidence of progressive disease on or within 60 days of the most recent line of prior treatment; and/or has achieved a response (minimal response or better) to at least one of said prior lines of treatment. In a specific embodiment of any of the above embodiments, said subject exhibits at the time of said administration: M-protein (serum protein electrophoresis [sPEP] or urine protein electrophoresis [uPEP]): sPEP ≥ 0.5 g/dL or uPEP ≥ 200 mg/24 hours; light chain multiple myeloma without measurable disease in the serum or urine, with serum immunoglobulin free light chain ≥ 10 mg/dL and abnormal serum immunoglobulin kappa lambda free light chain ratio; and/or Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1. In another more specific embodiment, said subject additionally: has received only one prior anti-myeloma treatment regimen; has the following high risk factors: R-ISS stage III, and early relapse, defined as (i) if the subject has undergone induction plus a stem cell transplant, progressive disease (PD) less than 12 months since date of first transplant; or (ii) if the subject has received only induction, PD < 12 months since date of last treatment regimen which must contain at minimum, a proteasome inhibitor, an immunomodulatory agent and dexamethasone.

[0121] In a specific embodiment of any of any of the above aspects or embodiments, said CAR comprises an antibody or antibody fragment that targets BCMA. In a more specific embodiment. said CAR comprises a single chain Fv antibody fragment (scFv). In a more specific embodiment, said CAR comprises a BCMA02 scFv. In a specific embodiment of any of the above aspects or embodiments, said immune cells are idecabtagene vicleucel cells. In one embodiment, the chimeric antigen receptor comprises a murine single chain Fv antibody fragment that targets BCMA, *e.g.*, BCMA. In one embodiment, the chimeric antigen receptor comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide a hinge domain comprising a CD8α polypeptide, a CD8α transmembrane domain, a CD137 (4-1BB) intracellular co-stimulatory signaling domain, and a CD3ζ primary signaling domain. In one embodiment, the chimeric antigen receptor comprises a murine scFv that targets BCMA, *e.g.*, BCMA, wherein the scFV is that of anti-BCMA02 CAR of SEQ ID NO: 9. In one embodiment,

the chimeric antigen receptor is or comprises SEQ ID NO: 9. In a more specific embodiment of any embodiment herein, said immune cells are idecabtagene vicleucel (ide-cel) cells. In one embodiment, the immune cells comprise a chimeric antigen receptor which comprises a murine single chain Fv antibody fragment that targets BCMA, *e.g.*, BCMA. In one embodiment, the immune cells comprise a chimeric antigen receptor which comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, BCMA, a hinge domain comprising a CD8α polypeptide, a CD8α transmembrane domain, a CD137 (4-1BB) intracellular co-stimulatory signaling domain, and a CD3ζ primary signaling domain. In one embodiment, the immune cells comprise a chimeric antigen receptor which is or comprises SEQ ID NO: 9.

[0122] In other embodiments, the genetically modified immune effector cells contemplated herein, are administered to a patient with a B cell related condition, *e.g.*, an autoimmune disease associated with B cells or a B cell malignancy.

[0123] The amount of soluble (*i.e.*, non-membrane-bound) BCMA (sBCMA) after administration of a CAR T cell therapy, *e.g.*, an anti-BCMA CAR T cell therapy, can be used to determine whether a subject can be expected to respond to the CAR T cell therapy appropriately, or whether the subject should be administered a different anticancer therapy. A greater drop in sBCMA levels in a tissue sample (*e.g.*, serum, plasma, lymph, or blood) after administration of a CAR T cell therapy is correlated with a more clinically beneficial outcome (*e.g.*, very good partial response, complete response or stringent complete response). In one aspect, for example, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and then determining a second level of soluble BCMA in a tissue sample from the subject; wherein, if said second level of sBCMA is greater than about 30% of said first level of sBCMA, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease; and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene

vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0124] In one aspect, for example, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; administering to the subject a first BCMA-based treatment modality comprising idecabtagene vicleucel cells, and then determining a second level of soluble BCMA in a tissue sample from the subject; wherein, if said second level of sBCMA is greater than about 30% of said first level of sBCMA, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease; and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0125] In one aspect, for example, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and then determining a second level of soluble BCMA in a tissue sample from the subject; wherein, if said second level of sBCMA is greater than about 20%, 25%, or 30% of said first level of sBCMA, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease; and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0126] In one aspect, for example, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject;

administering to the subject a first BCMA-based treatment modality comprising idecabtagene vicleucel cells, and then determining a second level of soluble BCMA in a tissue sample from the subject; wherein, if said second level of sBCMA is greater than about 20%, 25%, or 30% of said first level of sBCMA, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease; and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0127] In one aspect, for example, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and then determining a second level of soluble BCMA in a tissue sample from the subject; wherein, if said second level of sBCMA is greater than about 30%, 35%, 40%, 45%, or 50% of said first level of sBCMA, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease; and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities.

[0128] In one aspect, for example, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and then determining a second level of soluble BCMA in a tissue sample from the subject; wherein, if said second level of sBCMA is greater than about 30%, 35%, 40%, 45%, or 50% of said first level of sBCMA, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease; and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells.

In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0129] In one aspect, for example, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; administering to the subject a first BCMA-based treatment modality comprising idecabtagene vicleucel cells, and then determining a second level of soluble BCMA in a tissue sample from the subject; wherein, if said second level of sBCMA is greater than about 30%, 35%, 40%, 45%, or 50% of said first level of sBCMA, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease; and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0130] Also provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells); and (c) determining that a second level of soluble BCMA in a tissue sample from the subject is greater than about 30% of said first level, and on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject; wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0131] Also provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising idecabtagene vicleucel cells; and (c)

determining that a second level of soluble BCMA in a tissue sample from the subject is greater than about 30% of said first level, and on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject; wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0132] Also provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells); and (c) determining that a second level of soluble BCMA in a tissue sample from the subject is greater than about 20%, 25%, or 30% of said first level, and on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject; wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0133] Also provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising idecabtagene vicleucel cells; and (c) determining that a second level of soluble BCMA in a tissue sample from the subject is greater than about 20%, 25%, or 30% of said first level, and on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject; wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0134] Also provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells); and (c) determining that a second level of soluble BCMA in a tissue sample from the subject is greater than about 30%, 35%, 40%, 45%, or 50% of said first level, and on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject; wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0135] Also provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising idecabtagene vicleucel cells; and (c) determining that a second level of soluble BCMA in a tissue sample from the subject is greater than about 30%, 35%, 40%, 45%, or 50% of said first level, and on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject; wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0136] In a specific embodiment of either of the above embodiments, if said second level of sBCMA is greater than 40% of said first level, the subject is provided a second BCMA-based treatment modality to treat said disease.

[0137] In a embodiment of either of the above embodiments, said second level of sBCMA is determined at 25-35 days after said administering. In another specific embodiment, said second level of sBCMA is determined at 23-35, 24-35, 25-36, 25-37, 23-35, or 25-37 days after said administering. In another specific embodiment, said second level of sBCMA is determined at

23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, or 37 days after said administering. In another specific embodiment, said second level of sBCMA is determined at 28-31 days after said administering. In another specific embodiment, said second level of sBCMA is determined at 26-31, 27-31, 28-32, 28-33, 26-31, or 27-33 days after said administering. In another specific embodiment, said second level of sBCMA is determined at 26, 27, 28, 29, 30, 31, 32, or 33 days after said administering. In more specific embodiments, the subject is provided a second BCMA-based treatment modality within three months, two months, or one month after said determining a second level of sBCMA.

[0138] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a second BCMA-based treatment modality, wherein the patient has previously been administered a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, and wherein a tissue sample from the patient subsequent to said administration contained a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0139] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a second BCMA-based treatment modality, wherein the patient has previously been administered a first BCMA-based treatment modality comprising idecabtagene vicleucel cells, wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, and wherein a tissue sample from the patient subsequent to said administration contained a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration. In certain embodiments, the second BCMA-based treatment modality is not

idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0140] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a second BCMA-based treatment modality after treatment with a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, comprising determining a level of soluble BCMA (sBCMA) in a tissue sample from the patient, wherein the patient has previously been administered the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the second BCMA-based treatment modality. In a specific embodiment, the method further comprises administering the second BCMA-based treatment modality to the candidate for the second BCMA-based treatment modality. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0141] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a second BCMA-based treatment modality after treatment with a first BCMA-based treatment modality comprising idecabtagene vicleucel cells, wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, comprising determining a level of soluble BCMA (sBCMA) in a tissue sample from the patient, wherein the patient has previously been administered the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the second

BCMA-based treatment modality. In a specific embodiment, the method further comprises administering the second BCMA-based treatment modality to the candidate for the second BCMA-based treatment modality. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0142] The absolute level of sBCMA in a tissue sample (*e.g.*, plasma, serum, lymph or blood) may also be used to determine whether a person administered a CAR T cell therapy, *e.g.*, a BCMA CAR T cell therapy will appropriately benefit from that therapy, or should be administered a different anticancer therapy. Thus, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and determining a level of soluble BCMA (sBCMA) in a tissue sample from the subject; wherein, if said level of sBCMA is greater than 4000 ng/L, the subject is subsequently provided a second BCMA-based treatment modalityto treat said disease, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0143] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: administering to the subject a first BCMA-based treatment modality comprising idecabtagene vicleucel cells, and determining a level of soluble BCMA (sBCMA) in a tissue sample from the subject; wherein, if said level of sBCMA is greater than 4000 ng/L, the subject is subsequently provided a second BCMA-based treatment modalityto treat said disease, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0144] In a specific embodiment of either of the above embodiments, if said level of sBCMA is greater than about 3000 ng/L, 3500 ng/L, 4000 ng/L, 4500 ng/L, or 5000 ng/L the subject is subsequently provided a BCMA-based treatment modalityto treat said disease. In a specific embodiment, said first level of sBCMA is determined at 50-70 days after said administering. In a specific embodiment, said first level of sBCMA is determined at 45-70, 46-70, 47-70, 48-70, 49-70, 50-70, 50-71, 50-72, 50-73, or 50-75 days after said administering. In a specific embodiment, said first level of sBCMA is determined at 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 days after said administering. In another specific embodiment, said first level of sBCMA is determined at 55-65 days after said administering. In another specific embodiment, said first level of sBCMA is determined at 50-65, 51-65, 52-65, 53-65, 54-65, 55-64, 55-63, 55-62, or 55-61 days after said administering. In another specific embodiment, said first level of sBCMA is determined at 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, or 65 days after said administering. In another specific embodiment, said level of sBCMA is determined at 58-62 days after said administering. In another specific embodiment, said level of sBCMA is determined at 53-62, 54-62, 55-62, 56-62, 57-62, 58-68, 58-67, 58-66, 58-65, 58-64, or 58-63 days after said administering. In another specific embodiment, said level of sBCMA is determined at 58, 59, 60, 61, or 62 days after said administering. In a specific embodiment of the preceding embodiments, the subject is provided said second BCMA-based treatment modality within three months, two months, or one month after said determining a first level of sBCMA.

[0145] The levels of certain cytokines, *e.g.*, interleukin-6 (IL-6) and/or tumor necrosis factor alpha (TNFα) can also be used to determine whether a person administered a CAR T cell therapy, *e.g.*, a BCMA CAR T cell therapy will appropriately benefit from that therapy, or should be administered a different anticancer therapy. Thus, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα) or both in a tissue sample from the subject; administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and subsequently determining a second level of IL-6, TNFα or both in a tissue sample from the subject; wherein, if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both,

respectively, then the subject is subsequently provided a second BCMA-based treatment modality to treat said disease, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0146] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: determining a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα) or both in a tissue sample from the subject; administering to the subject a first BCMA-based treatment modality comprising idecabtagene vicleucel cells, and subsequently determining a second level of IL-6, TNFα or both in a tissue sample from the subject; wherein, if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, respectively, then the subject is subsequently provided a second BCMA-based treatment modalityto treat said disease, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0147]

In certain embodiments, said first level is determined on the day of said administering to the subject the first BCMA-based treatment modality comprising immune cells expressing a CAR directed to BCMA, and said second level is determined 1-4 days after said administering. In another specific embodiment, said second level is determined one day after said administering. In another specific embodiment, said second level is determined two days after said administering. In another specific embodiment, said second level is determined three days after said administering. In another specific embodiment, said second level is determined four days after said administering.

[0148] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA)-expressing cells in a subject in need thereof, comprising: administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed

to BCMA (BCMA CAR T cells), and determining a level of ferritin in a tissue sample from the subject; wherein, if said level of ferritin is greater than 1500 picomoles per liter, the subject is subsequently provided a therapy to treat cytokine release syndrome (CRS). In certain embodiments, said determining is performed within 0-4 days prior to said administering. In a specific embodiment, said determining is performed on the same day as said administering. In another specific embodiment, said therapy to treat CRS is first provided to said subject 0-5 days after said administering.

[0149] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and (c) determining a second level of sBCMA and/or a second level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA and/or if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0150] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising idecabtagene vicleucel cells, and (c) determining a second level of sBCMA and/or a second level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA

and/or if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0151] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), (c) determining that a second level of sBCMA in a tissue sample from the subject is greater than 30% of said first level of sBCMA and/or a second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, and (d) on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject, wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0152] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising: (a) determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject; (b) administering to the subject a first BCMA-based treatment modality comprising idecabtagene vicleucel cells, (c) determining that a second level of sBCMA in a tissue sample from the subject is greater than 30% of said first level of sBCMA and/or a second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, and (d) on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject,

wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0153] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a second BCMA-based treatment modality, wherein the patient has previously been administered a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, and wherein a tissue sample from the patient subsequent to said administration contained (i) a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) a level of IL-6, TNFα or both not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0154] In another aspect, provided herein is a method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a second BCMA-based treatment modality, wherein the patient has previously been administered a first BCMA-based treatment modality comprising idecabtagene vicleucel cells, wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, and wherein a tissue sample from the patient subsequent to said administration contained (i) a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) a level of IL-6, TNFα or both not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0155] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a second BCMA-based treatment modality after treatment with a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) and/or a level of IL-6, TNFα or both in a tissue sample from the patient, wherein the patient has previously been administered the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein if (i) the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) the level of IL-6, TNFα or both is not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the second BCMA-based treatment modality, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a specific embodiment, the method further comprises administering the second BCMA-based treatment modality to the candidate for the second BCMA-based treatment modality. In a particular embodiment, the immune cells are idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0156] In another aspect, provided herein is a method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a second BCMA-based treatment modality after treatment with a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) and/or a level of IL-6, TNFα or both in a tissue sample from the patient, wherein the patient has previously been administered the first BCMA-based treatment modality comprising idecabtagene vicleucel cells, wherein if (i) the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) the level of IL-6, TNFα or both is not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration,

then the patient is a candidate for the second BCMA-based treatment modality, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities. In a specific embodiment, the method further comprises administering the second BCMA-based treatment modality to the candidate for the second BCMA-based treatment modality. In certain embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells. In certain embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

[0157] In specific embodiments of any of the above aspects or embodiments, said CAR T cell therapy (e.g., immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), e.g., idecabtagene vicleucel (ide-cel) cells) comprises a population of cells that comprises about 10%, 5%, 3%, 2%, or 1% activated CAR T-cells, for example, activated CD8 CAR T-cells (CD3+/CD8+/CAR+/CD25+).

[0158] In specific embodiments of any of the above aspects or embodiments, said CAR T cell therapy (e.g., immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), e.g., idecabtagene vicleucel (ide-cel) cells) comprises a population of cells that comprises 10%, 5%, 3%, 2%, or 1% senescence population of CAR T-cells, for example, CD4 CAR T-cells (CD3+/CD4+/CAR+/CD57+). In a specific embodiments of any of the above aspects or embodiments, said tissue sample is blood, plasma or serum. In another specific embodiments of any of the above aspects or embodiments, said disease caused by BCMA-expressing cells is multiple myeloma, chronic lymphocytic leukemia, or a non-Hodgkins lymphoma (*e.g.*, Burkitt's lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma). In specific embodiments, the disease is multiple myeloma, *e.g.*, high-risk multiple myeloma or relapsed and refractory multiple myeloma. In other specific embodiments, the high risk multiple myeloma is R-ISS stage III disease and/or a disease characterized by early relapse (e.g., progressive disease within 12 months since the date of last treatment regimen, such as last treatment regimen with a proteasome inhibitor, an immunomodulatory agent and/or dexamethasone). In specific embodiments, said disease caused by BCMA-expressing cells is a non-Hodgkins lymphoma, and wherein the non-Hodgkins lymphoma is selected from the group consisting of: Burkitt's lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma

(CLL/SLL), diffuse large B cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma.

[0159] In one embodiment, before the administration of the T cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA), the subject having a tumor has been assessed for expression of BCMA by the tumor.

[0160] In specific embodiments of any of the above aspects or embodiments, the immune cells are T cells, *e.g.*, CD4+ T cells, CD8+ T cells or cytotoxic T lymphocytes (CTLs), T killer cells, or natural killer (NK) cells. In another specific embodiment specific embodiment, the immune cells are administered in a dosage of from 150 x 10^6 cells to 450 x 10^6 cells.

[0161] Generally, a BCMA-based treatment modality refers to a treatment modality that targets BCMA and/or cells expressing BCMA (e.g., cells expressing BCMA on the cell surface). For example the BCMA-based treatment modality (e.g., the first BCMA-based treatment modality or the second BCMA-based treatment modality) may be a BCMA-Antibody-Drug Conjugate (ADC), a bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA), a natural killer (NK) cell engager (NKCEs) that targets B-cell maturation antigen (BCMA), or immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality comprises a BCMA-Antibody-Drug Conjugate (ADC), a bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA), a natural killer (NK) cell engager (NKCEs) that targets B-cell maturation antigen (BCMA), or immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In certain embodiments, the second BCMA-based treatment modality comprises a BCMA-Antibody-Drug Conjugate (ADC), a bispecific T-cell engager (BiTE), natural killer (NK) cell engagers (NKCEs) that target B-cell maturation antigen (BCMA), or immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are not the same as the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In certain embodiments, the second BCMA-based treatment modality is a BCMA-Antibody-Drug Conjugate (ADC), a bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA), a natural killer (NK) cell engager (NKCEs) that targets B-cell maturation antigen (BCMA), or immune cells

expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In certain embodiments, the second BCMA-based treatment modality is selected from the group consisting of a BCMA-Antibody-Drug Conjugate (ADC), a bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA), a natural killer (NK) cell engager (NKCEs) that targets B-cell maturation antigen (BCMA), and immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In certain embodiments, the second BCMA-based treatment modality is a BCMA-Antibody-Drug Conjugate (ADC), a bispecific T-cell engager (BiTE), natural killer (NK) cell engagers (NKCEs) that target B-cell maturation antigen (BCMA), or immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are not the same as the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In certain embodiments, the second BCMA-based treatment modality is selected from the group consisting of a BCMA-Antibody-Drug Conjugate (ADC), a bispecific T-cell engager (BiTE), natural killer (NK) cell engagers (NKCEs) that target B-cell maturation antigen (BCMA), and immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are not the same as the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In a more specific embodiment, the patient has not received said second BCMA-based treatment modality prior to administration of said first BCMA-based treatment modality.

[0162] In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality comprises CC99712, GSK2857916 (belantamab mafodotin), CC-93269, AMG 420, JNJ-64007957, AMG 701, PF-06863135, REGN5458, REGN5459, TNB-383B, DF3001, AFM26, CTX-4419, CTX-8573, JCARH125, KITE-585, P-BCMA-101, LCAR-B38M, CT053, anti-CD19/BCMA CAR-T cells (Hrain Biotechnology), or CTX120. In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality is CC99712, GSK2857916 (belantamab mafodotin), CC-93269, AMG 420, JNJ-64007957, AMG 701, PF-06863135, REGN5458, REGN5459, TNB-383B, DF3001, AFM26, CTX-4419, CTX-8573, JCARH125, KITE-585, P-BCMA-101, LCAR-B38M, CT053, anti-CD19/BCMA CAR-T

cells (Hrain Biotechnology), or CTX120. In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality consists of CC99712, GSK2857916 (belantamab mafodotin), CC-93269, AMG 420, JNJ-64007957, AMG 701, PF-06863135, REGN5458, REGN5459, TNB-383B, DF3001, AFM26, CTX-4419, CTX-8573, JCARH125, KITE-585, P-BCMA-101, LCAR-B38M, CT053, anti-CD19/BCMA CAR-T cells (Hrain Biotechnology), or CTX120. In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality is selected from the group consisting of CC99712, GSK2857916 (belantamab mafodotin), CC-93269, AMG 420, JNJ-64007957, AMG 701, PF-06863135, REGN5458, REGN5459, TNB-383B, DF3001, AFM26, CTX-4419, CTX-8573, JCARH125, KITE-585, P-BCMA-101, LCAR-B38M, CT053, anti-CD19/BCMA CAR-T cells (Hrain Biotechnology), and CTX120.

[0163] In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality comprises a BCMA-Antibody-Drug Conjugate (ADC). In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality is a BCMA-Antibody-Drug Conjugate (ADC). In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality consists of a BCMA-Antibody-Drug Conjugate (ADC). In certain embodiments, the BCMA-Antibody-Drug Conjugate (ADC) comprises CC99712 or GSK2857916 (belantamab mafodotin). In certain embodiments, the BCMA-Antibody-Drug Conjugate (ADC) is CC99712 or GSK2857916 (belantamab mafodotin). In certain embodiments, the BCMA-Antibody-Drug Conjugate (ADC) consists of CC99712 or GSK2857916 (belantamab mafodotin). In certain embodiments, the BCMA-Antibody-Drug Conjugate (ADC) may be administered immediately after administration of the first BCMA-based treatment modality. In certain embodiments, the BCMA-Antibody-Drug Conjugate (ADC) may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the first BCMA-based treatment modality. In certain embodiments, the BCMA-Antibody-Drug Conjugate (ADC) may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the first BCMA-based treatment modality. In a certain embodiment, the BCMA-Antibody-Drug Conjugate (ADC) should be initiated upon adequate bone marrow recovery or from 90 days after administration of the first BCMA-based treatment modality, e.g., 90 days after administration of ide-cel, whichever is later.

[0164] In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality comprises a bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA). In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality is a bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA). In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality consists of a bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA). In certain embodiments, the bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA) comprises CC-93269, AMG 420, JNJ-64007957, AMG 701, PF-06863135, REGN5458, REGN5459, or TNB-383B. In certain embodiments, the bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA) is CC-93269, AMG 420, JNJ-64007957, AMG 701, PF-06863135, REGN5458, REGN5459, or TNB-383B. In certain embodiments, the bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA) consists of CC-93269, AMG 420, JNJ-64007957, AMG 701, PF-06863135, REGN5458, REGN5459, or TNB-383B. In certain embodiments, the bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA) is selected from the group consisting of CC-93269, AMG 420, JNJ-64007957, AMG 701, PF-06863135, REGN5458, REGN5459, and TNB-383B. In certain embodiments, the bispecific T-cell engager (BiTE) may be administered immediately after administration of the first BCMA-based treatment modality. In certain embodiments, the bispecific T-cell engager (BiTE) may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the first BCMA-based treatment modality. In certain embodiments, the bispecific T-cell engager (BiTE) may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the first BCMA-based treatment modality. In a certain embodiment, the bispecific T-cell engager (BiTE) should be initiated upon adequate bone marrow recovery or from 90 days after administration of the first BCMA-based treatment modality, e.g., 90 days after administration of ide-cel, whichever is later.

[0165] In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality comprises a natural killer (NK) cell engager (NKCE) that targets B-cell maturation antigen (BCMA). In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality is a natural killer (NK) cell engager (NKCE) that targets B-cell maturation antigen (BCMA). In a specific embodiment of any of the above

embodiments, the second BCMA-based treatment modality consists of a natural killer (NK) cell engager (NKCE) that targets B-cell maturation antigen (BCMA). In certain embodiments, the natural killer (NK) cell engager (NKCE) that targets B-cell maturation antigen (BCMA) comprises DF3001, AFM26, CTX-4419, or CTX-8573. In certain embodiments, the natural killer (NK) cell engager (NKCE) that targets B-cell maturation antigen (BCMA) is DF3001, AFM26, CTX-4419, or CTX-8573. In certain embodiments, the natural killer (NK) cell engager (NKCE) that targets B-cell maturation antigen (BCMA) consists of DF3001, AFM26, CTX-4419, or CTX-8573. In certain embodiments, the natural killer (NK) cell engager (NKCE) that targets B-cell maturation antigen (BCMA) is selected from the group consisting of DF3001, AFM26, CTX-4419, and CTX-8573. In certain embodiments, the natural killer (NK) cell engager (NKCE) that targets B-cell maturation antigen (BCMA) may be administered immediately after administration of the first BCMA-based treatment modality. In certain embodiments, the natural killer (NK) cell engager (NKCE) that targets B-cell maturation antigen (BCMA) may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the first BCMA-based treatment modality. In certain embodiments, the natural killer (NK) cell engager (NKCE) that targets B-cell maturation antigen (BCMA) may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the first BCMA-based treatment modality. In a certain embodiment, the natural killer (NK) cell engager (NKCE) that targets B-cell maturation antigen (BCMA) should be initiated upon adequate bone marrow recovery or 90 days after administration of the first BCMA-based treatment modality, e.g., 90 days after administration of ide-cel, whichever is later.

[0166] In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality comprises immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality comprises immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are not the same as the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality is

immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality is immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are not the same as the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality consists of immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In a specific embodiment of any of the above embodiments, the second BCMA-based treatment modality consists of immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are not the same as the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells). In certain embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) comprise JCARH125, KITE-585, P-BCMA-101, LCAR-B38M, CT053, anti-CD19/BCMA CAR-T cells (Hrain Biotechnology), and CTX120. In certain embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) may be administered immediately after administration of the first BCMA-based treatment modality. In certain embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the first BCMA-based treatment modality. In certain embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the first BCMA-based treatment modality. In a certain embodiment, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) should be initiated upon adequate bone marrow recovery or 90 days after administration of the first BCMA-based treatment modality, e.g., 90 days after administration of ide-cel, whichever is later.

[0167] In certain embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are not the same as the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), may be administered immediately after administration of the first BCMA-based treatment modality. In certain embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are not the same as the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the first BCMA-based treatment modality. In certain embodiments, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are not the same as the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the first BCMA-based treatment modality. In a certain embodiment, the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are not the same as the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), should be initiated upon adequate bone marrow recovery or 90 days after administration of the first BCMA-based treatment modality, e.g., 90 days after administration of ide-cel, whichever is later.

[0168] In specific embodiments of any of the above aspects or embodiments, the immune cells in the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells.

[0169] In specific embodiments of any of the above aspects or embodiments, the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells. In specific embodiments of any of the above aspects or embodiments, the second BCMA-based treatment modality is not idecabtagene vicleucel cells.

[0170] In specific embodiments of any of the above aspects or embodiments, the immune cells are T cells, *e.g.*, CD4+ T cells, CD8+ T cells or cytotoxic T lymphocytes (CTLs), T killer cells, or natural killer (NK) cells. In another specific embodiment specific embodiment, the immune cells are administered in a dosage of from 150 x 10^6 cells to 450 x 10^6 cells.

[0171] In a specific embodiment of any of the above embodiments, the non-CAR T cell therapy comprises a proteasome inhibitor, lenalidomide, pomalidomide, thalidomide, bortezomib, dexamethasone, cyclophosphamide, doxorubicin, carfilzomib, ixazomib, cisplatin, doxorubicin, etoposide, an anti-CD38 antibody panobinostat, or elotuzumab. In more specific embodiments, before said administering said subject has received one or more lines of prior therapy comprising: daratumumab, pomalidomide, and dexamethasone (DPd); daratumumab, bortezomib, and dexamethasone (DVd); ixazomib, lenalidomide, and dexamethasone (IRd); daratumumab, lenalidomide and dexamethasone; bortezomib, lenalidomide and dexamethasone (RVd); bortezomib, cyclophosphamide and dexamethasone (BCd); bortezomib, doxorubicin and dexamethasone; carfilzomib, lenalidomide and dexamethasone (CRd); bortezomib and dexamethasone; bortezomib, thalidomide and dexamethasone; lenalidomide and dexamethasone; dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide and bortezomib (VTD-PACE); lenalidomide and low-dose dexamethasone; bortezomib, cyclophosphamide and dexamethasone; carfilzomib and dexamethasone; lenalidomide alone; bortezomib alone; daratumumab alone; elotuzumab, lenalidomide, and dexamethasone; elotuzumab, lenalidomide and dexamethasone; bendamustine, bortezomib and dexamethasone; bendamustine, lenalidomide, and dexamethasone; pomalidomide and dexamethasone; pomalidomide, bortezomib and dexamethasone; pomalidomide, carfilzomib and dexamethasone; bortezomib and liposomal doxorubicin; cyclophosphamide, lenalidomide, and dexamethasone; elotuzumab, bortezomib and dexamethasone; ixazomib and dexamethasone; panobinostat, bortezomib and dexamethasone; panobinostat and carfilzomib; or pomalidomide, cyclophosphamide and dexamethasone; or any one of the other therapeutic agents listed in

Section 5.9, below. In a more specific embodiment, the patient has not received said non-CAR T cell therapy prior to administration of CAR T cells.

[0172] In a specific embodiment of any of the above embodiments, the non-CAR T cell therapy comprises lenalidomide. In certain embodiments, the lenalidomide is administered to a subject as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered at a dosage of about 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg, or 25 mg. In certain embodiments, the lenalidomide may be administered at a dosage of about 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg, or 25 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 25 mg once daily orally on Days 1-21 of repeated 28-day cycles. In certain embodiments, the lenalidomide may be administered at a dosage of about 25 mg once daily orally on Days 1-21 of repeated 28-day cycles to a subject for treating Multiple Myeloma (MM). In certain embodiments, the lenalidomide may be administered at a dosage of about 10 mg once daily continuously on Days 1-28 of repeated 28-day cycles. In certain embodiments, the lenalidomide may be administered at a dosage of about 2.5 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 5 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 10 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 15 mg every other day. In certain embodiments, the lenalidomide may be administered at a dosage of about 25 mg once daily orally on Days 1-21 of repeated 28-day cycles. In certain embodiments, the lenalidomide may be administered at a dosage of about 20 mg once daily orally on Days 1-21 of repeated 28-day cycles for up to 12 cycles. In a certain embodiment, lenalidomide maintenance therapy is recommended for all patients. In a certain embodiment, lenalidomide maintenance

therapy should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0173] In a specific embodiment of any of the above embodiments, the non-CAR T cell therapy comprises pomalidomide. In certain embodiments, the pomalidomide is administered to a subject as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered at a dosage of about 1 mg, 2 mg, 3 mg, or 4 mg. In certain embodiments, the pomalidomide may be administered at a dosage of about 1 mg, 2 mg, 3 mg, or 4 mg once daily. In certain embodiments, the pomalidomide may be administered at a dosage of about 4 mg per day taken orally on days 1-21 of repeated 28-day cycles until disease progression. In certain embodiments, the pomalidomide may be administered at a dosage of about 4 mg per day taken orally on days 1-21 of repeated 28-day cycles until disease progression to a subject for treating Multiple Myeloma (MM). In a certain embodiment, pomalidomide maintenance therapy is recommended for all patients. In a certain embodiment, pomalidomide maintenance therapy should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0174] In a specific embodiment of any of the above embodiments, the non-CAR T cell therapy comprises CC-220 (iberdomide; *see*, e.g., Bjorkland, C.C. *et al.*, 2019, *Leukemia*, doi: 10.1038/s41375-019-0620-8; U.S. Patent No. 9,828,361). In certain embodiments, the CC-220 is administered to a subject as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing

immune effector cells. In certain embodiments, the CC-220 may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg. In certain embodiments, the CC-220 may be administered orally. In certain embodiments, the CC-220 may be administered orally at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily for 21 days of a 28-day cycle, e.g., daily on days 1-21 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the CC-220 may be administered to a subject for treating Multiple Myeloma (MM). In a certain embodiment, CC-220 maintenance therapy is recommended for all patients. In a certain embodiment, the CC-220 maintenance therapy should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0175] In a specific embodiment of any of the above embodiments, the non-CAR T cell therapy comprises CC-220 (iberdomide) and dexamethasone. In certain embodiments, the CC-220 and dexamethasone are administered to a subject as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 and dexamethasone may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the dexamethasone may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 and dexamethasone may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the dexamethasone may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 and dexamethasone may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after

administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the dexamethasone may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg. In certain embodiments, the dexamethasone may be administered at a dosage of about 20 mg, 25 mg., 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg. In certain embodiments, the dexamethasone may be administered at a dosage of about 40 mg. In certain embodiments, the CC-220 may be administered orally. In certain embodiments, the CC-220 may be administered orally at a dosage of about 15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily for 21 days of a 28-day cycle, e.g., daily on days 1-21 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the dexamethasone may be administered orally. In certain embodiments, the dexamethasone may be administered at a dose of about 20-60 mgs. In certain embodiments, the dexamethasone may be administered orally at a dosage of about 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg on days 1, 8, 15, and 22 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the CC-220 may be administered orally at a dosage of about 15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily for 21 days of a 28-day cycle, e.g., daily on days 1-21 of a 28-day cycle, with the 28-day cycles repeated as needed, and the dexamethasone may be administered orally at a dosage of about 20 mg, 25 mg., 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg on days 1, 8, 15, and 22 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the CC-220 and dexamethasone may be administered to a subject for treating Multiple Myeloma (MM). In a certain embodiment, CC-220 and dexamethasone maintenance therapy is recommended for all patients. In a certain embodiment, the CC-220 and dexamethasone maintenance therapy should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0176] In another specific embodiment of any of the above aspects or embodiments, before said administering said subject has received three or more lines of prior therapy, or one or more lines of prior therapy. In more specific embodiments, said lines of prior therapy comprise a proteasome inhibitor, lenalidomide, pomalidomide, thalidomide, bortezomib, dexamethasone, cyclophosphamide, doxorubicin, carfilzomib, ixazomib, cisplatin, doxorubicin, etoposide, an anti-CD38 antibody panobinostat, or elotuzumab. In more specific embodiments, before said administering said subject has received one or more lines of prior therapy comprising: daratumumab, pomalidomide, and dexamethasone (DPd); daratumumab, bortezomib, and dexamethasone (DVd); ixazomib, lenalidomide, and dexamethasone (IRd); daratumumab, lenalidomide and dexamethasone; bortezomib, lenalidomide and dexamethasone (RVd); bortezomib, cyclophosphamide and dexamethasone (BCd); bortezomib, doxorubicin and dexamethasone; carfilzomib, lenalidomide and dexamethasone (CRd); bortezomib and dexamethasone; bortezomib, thalidomide and dexamethasone; lenalidomide and dexamethasone; dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide and bortezomib (VTD-PACE); lenalidomide and low-dose dexamethasone; bortezomib, cyclophosphamide and dexamethasone; carfilzomib and dexamethasone; lenalidomide alone; bortezomib alone; daratumumab alone; elotuzumab, lenalidomide, and dexamethasone; elotuzumab, lenalidomide and dexamethasone; bendamustine, bortezomib and dexamethasone; bendamustine, lenalidomide, and dexamethasone; pomalidomide and dexamethasone; pomalidomide, bortezomib and dexamethasone; pomalidomide, carfilzomib and dexamethasone; bortezomib and liposomal doxorubicin; cyclophosphamide, lenalidomide, and dexamethasone; elotuzumab, bortezomib and dexamethasone; ixazomib and dexamethasone; panobinostat, bortezomib and dexamethasone; panobinostat and carfilzomib; or pomalidomide, cyclophosphamide and dexamethasone. In various more specific embodiments, said subject has received two, three, four, five, six, seven or more of said lines of prior therapy; no more than three of said lines of prior therapy; no more than two of said lines of prior therapy; or no more than one of said lines of prior therapy.

[0177] In specific embodiments of any of the above aspects or embodiments, the immune cells are administered at a dose ranging from 150 x 10^6 cells to 450 x 10^6 cells, 300 x 10^6 cells to 600 x 10^6 cells, 350 x 10^6 cells to 600 x 10^6 cells, 350 x 10^6 cells to 550 x 10^6 cells, 400 x 10^6 cells to 600 x 10^6 cells, 150 x 10^6 cells to 300 x 10^6 cells, or 400 x 10^6 cells to 500 x 10^6 cells. In some

embodiments, the immune cells are administered at a dose of about 150 x 10^6 cells, about 200 x 10^6 cells, about 250 x 10^6 cells, about 300 x 10^6 cells, about 350 x 10^6 cells, about 400 x 10^6 cells, about 450 x 10^6 cells, about 500 x 10^6 cells, or about 550 x 10^6 cells. In one embodiment, the immune cells are administered at a dose of about 450 x 10^6 cells. In some embodiments, the subject is administered one infusion of the immune cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA). In some embodiments, the administration of the immune cells expressing a CAR directed to BCMA is repeated (e.g., a second dose of immune cells is administered to the subject).

[0178] In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 150 x 10^6 cells to about 300 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administererd in a dosage of from about 350 x 10^6 cells to about 550 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administererd in a dosage of from about 400 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 150 x 10^6 cells to about 250 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 350 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administererd in a dosage of from about 250 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 300 x 10^6 cells to about 600 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the

embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 350 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 400 x 10^6 cells to about 600 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 400 x 10^6 cells to about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 10^6 cells to about 400 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 10^6 cells to about 350 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 200 x 10^6 cells to about 300 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 450 x 10^6 cells to about 500 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 10^6 cells to about 400 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of from about 250 x 10^6 cells to about 350 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells expressing a CAR directed to BCMA are administered in a dosage of about 450 x 10^6 cells. In specific embodiments of any of the embodiments described herein, the immune cells are T cells (e.g., autologous T cells). In specific embodiments of any of the embodiments described herein, the subjects being treated undergo a leukapharesis procedure to collect autologous immune cells for the manufacture of the immune cells expressing a CAR directed to BCMA prior to their administration to the subject. In specific embodiments of any of the embodiments described herein, the immune cells (e.g., T cells) are administered by an intravenous infusion.

[0179] In specific embodiments of any of the aspects or embodiments disclosed herein, before administration of immune cells expressing a CAR directed to BCMA, the subject being treated is administered a lymphodepleting (LD) chemotherapy. In specific embodiments, LD

chemotherapy comprises fludarabine and/or cyclophosphamide. In specific embodiments, LD chemotherapy comprises fludarabine (e.g., about 30 mg/m^2 for intravenous administration) and cyclophosphamide (e.g., about 300 mg/m^2 for intravenous administration) for a duration of 1, 2, 3, 4, 5, 6, or 7 days (*e.g.*, 3 days). In other specific embodiments, LD chemotherapy comprises any of the chemotherapeutic agents described in Section 5.9. In specific embodiments, the subject is administered immune cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA) 1, 2, 3, 4, 5, 6, or 7 days after the administration of the LD chemotherapy (e.g., 2 or 3 days after the administration of the LD chemotherapy). In specific embodiments, the subject has not received any therapy prior to the initiation of the LD chemotherapy for at least or more than 1 week, at least or more than 2 weeks (at least or more than 14 days), at least or more than 3 weeks, at least or more than 4 weeks, at least or more than 5 weeks, or at least or more than 6 weeks. In specific embodiments of any of the embodiments disclosed herein, before administration of immune cells expressing a chimeric antigen receptor (CAR) directed to B Cell Maturation Antigen (BCMA), the subject being treated has received only a single prior treatment regimen.

[0180] For any of the above embodiments, the subject undergoes apheresis to collect and isolate said immune cells, *e.g.*, T cells. In a specific embodiment of any of the above embodiments, said subject exhibits at the time of said apheresis: M-protein (serum protein electrophoresis [sPEP] or urine protein electrophoresis [uPEP]): sPEP $\geq$ 0.5 g/dL or uPEP $\geq$ 200 mg/24 hours; light chain multiple myeloma without measurable disease in the serum or urine, with serum immunoglobulin free light chain $\geq$ 10 mg/dL and abnormal serum immunoglobulin kappa lambda free light chain ratio; and/or Eastern Cooperative Oncology Group (ECOG) performance status $\leq$ 1. In a more specific embodiment, said subject at the time of apheresis additionally: has received at least three of said lines of prior treatment, including prior treatment with a proteasome inhibitor, an immunomodulatory agent (lenalidomide or pomalidomide) and an anti-CD38 antibody; has undergone at least 2 consecutive cycles of treatment for each of said at least three lines of prior treatment, unless progressive disease was the best response to a line of treatment; has evidence of progressive disease on or within 60 days of the most recent line of prior treatment; and/or has achieved a response (minimal response or better) to at least one of said prior lines of treatment. In a specific embodiment of any of the above embodiments, said subject exhibits at the time of said administration: M-protein (serum protein electrophoresis

[sPEP] or urine protein electrophoresis [uPEP]): sPEP ≥ 0.5 g/dL or uPEP ≥ 200 mg/24 hours; light chain multiple myeloma without measurable disease in the serum or urine, with serum immunoglobulin free light chain ≥ 10 mg/dL and abnormal serum immunoglobulin kappa lambda free light chain ratio; and/or Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1. In another more specific embodiment, said subject additionally: has received only one prior anti-myeloma treatment regimen; has the following high risk factors: R-ISS stage III, and early relapse, defined as (i) if the subject has undergone induction plus a stem cell transplant, progressive disease (PD) less than 12 months since date of first transplant; or (ii) if the subject has received only induction, PD < 12 months since date of last treatment regimen which must contain at minimum, a proteasome inhibitor, an immunomodulatory agent and dexamethasone.

[0181] In a specific embodiment of any of any of the above aspects or embodiments, said CAR comprises an antibody or antibody fragment that targets BCMA. In a more specific embodiment. said CAR comprises a single chain Fv antibody fragment (scFv). In a more specific embodiment, said CAR comprises a BCMA02 scFv. In a specific embodiment of any of the above aspects or embodiments, said immune cells are idecabtagene vicleucel cells. In one embodiment, the chimeric antigen receptor comprises a murine single chain Fv antibody fragment that targets BCMA, *e.g.*, BCMA. In one embodiment, the chimeric antigen receptor comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide a hinge domain comprising a CD8α polypeptide, a CD8α transmembrane domain, a CD137 (4-1BB) intracellular co-stimulatory signaling domain, and a CD3ζ primary signaling domain. In one embodiment, the chimeric antigen receptor comprises a murine scFv that targets BCMA, *e.g.*, BCMA, wherein the scFV is that of anti-BCMA02 CAR of SEQ ID NO: 9. In one embodiment, the chimeric antigen receptor is or comprises SEQ ID NO: 9. In a more specific embodiment of any embodiment herein, said immune cells are idecabtagene vicleucel (ide-cel) cells. In one embodiment, the immune cells comprise a chimeric antigen receptor which comprises a murine single chain Fv antibody fragment that targets BCMA, *e.g.*, BCMA. In one embodiment, the immune cells comprise a chimeric antigen receptor which comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, BCMA, a hinge domain comprising a CD8α polypeptide, a CD8α transmembrane domain, a CD137 (4-1BB) intracellular co-stimulatory signaling domain, and a CD3ζ primary signaling domain. In one embodiment, the immune cells comprise a chimeric antigen receptor which is or comprises SEQ ID NO: 9.

[0182] In other embodiments, the genetically modified immune effector cells contemplated herein, are administered to a patient with a B cell related condition, *e.g.,* an autoimmune disease associated with B cells or a B cell malignancy.

[0183] The practice of the subject matter presented herein employs, 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); Harlow and Lane, *Antibodies*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1998) *Current Protocols in Immunology* Q. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, eds., 1991); *Annual Review of Immunology*; as well as monographs in journals such as *Advances in Immunology*.

5.2. DEFINITIONS

[0184] 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 disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, preferred embodiments of compositions, methods and materials are described herein. For the purposes of the present disclosure, the following terms are defined below.

[0185] The articles "a," "an," and "the" are used herein to refer to one or to more than one (*i.e.*, to at least one, or to one or more) of the grammatical object of the article. By way of example, "an element" means one element or one or more elements.

[0186] The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives.

[0187] The term "and/or" should be understood to mean either one, or both of the alternatives.

[0188] 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 15%, 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 one embodiment, the term "about" or "approximately" refers a range of quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length ± 15%, ± 10%, ± 9%, ± 8%, ± 7%, ± 6%, ± 5%, ± 4%, ± 3%, ± 2%, or ± 1% about a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[0189] 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 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 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 present that materially affect the activity or action of the listed elements.

[0190] Reference throughout this specification to "one embodiment," "an embodiment," "a particular embodiment," "a related embodiment," "a certain embodiment," "an additional embodiment," or "a further embodiment" or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the disclosure presented herein. 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. It is also understood that the

positive recitation of a feature in one embodiment, serves as a basis for excluding the feature in a particular embodiment.

5.3. Chimeric Antigen Receptors

[0191] In various embodiments, genetically engineered receptors that redirect cytotoxicity of immune effector cells toward B cells are provided. These genetically engineered receptors referred to herein as chimeric antigen receptors (CARs). CARs are molecules that combine antibody-based specificity for a desired antigen (*e.g.*, BCMA) with a T cell receptor-activating intracellular domain to generate a chimeric protein that exhibits a specific anti-BCMA cellular immune activity. As used herein, the term, "chimeric," describes being composed of parts of different proteins or DNAs from different origins.

[0192] CARs contemplated herein, comprise an extracellular domain (also referred to as a binding domain or antigen-specific binding domain) that binds to BCMA, a transmembrane domain, and an intracellular signaling domain. Engagement of the anti-BCMA antigen binding domain of the CAR with BCMA on the surface of a target cell results in clustering of the CAR and delivers an activation stimulus to the CAR-containing cell. The main characteristic of CARs are their ability to redirect immune effector cell specificity, thereby triggering proliferation, cytokine production, phagocytosis or production of molecules that can mediate cell death of the target antigen expressing cell in a major histocompatibility (MHC) independent manner, exploiting the cell specific targeting abilities of monoclonal antibodies, soluble ligands or cell specific co-receptors.

[0193] In various embodiments, a CAR comprises an extracellular binding domain that comprises a murine anti-BCMA (*e.g.*, human BCMA)-specific binding domain; a transmembrane domain; one or more intracellular co-stimulatory signaling domains; and a primary signaling domain.

[0194] In particular embodiments, a CAR comprises an extracellular binding domain that comprises a murine anti-BCMA (*e.g.*, human BCMA) antibody or antigen binding fragment thereof; one or more hinge domains or spacer domains; a transmembrane domain including; one or more intracellular co-stimulatory signaling domains; and a primary signaling domain.

5.3.1. BINDING DOMAIN

[0195] In particular embodiments, CARs contemplated herein comprise an extracellular binding domain that comprises a murine anti-BCMA antibody or antigen binding fragment thereof that specifically binds to a human BCMA polypeptide expressed on a B cell. As used herein, the terms, "binding domain," "extracellular domain," "extracellular binding domain," "antigen-specific binding domain," and "extracellular antigen specific binding domain," are used interchangeably and provide a CAR with the ability to specifically bind to the target antigen of interest, *e.g.*, BCMA. The binding domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source.

[0196] The terms "specific binding affinity" or "specifically binds" or "specifically bound" or "specific binding" or "specifically targets" as used herein, describe binding of an anti-BCMA antibody or antigen binding fragment thereof (or a CAR comprising the same) to BCMA at greater binding affinity than background binding. A binding domain (or a CAR comprising a binding domain or a fusion protein containing a binding domain) "specifically binds" to a BCMA if it binds to or associates with BCMA with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/M) of, for example, greater than or equal to about 10^5 M^{-1}. In certain embodiments, a binding domain (or a fusion protein thereof) binds to a target with a K_a greater than or equal to about 10^6 M^{-1}, 10^7 M^{-1}, 10^8 M^{-1}, 10^9 M^{-1}, 10^{10} M^{-1}, 10^{11} M^{-1}, 10^{12} M^{-1}, or 10^{13} M^{-1}. "High affinity" binding domains (or single chain fusion proteins thereof) refers to those binding domains with a K_a of at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, at least 10^{10} M^{-1}, at least 10^{11} M^{-1}, at least 10^{12} M^{-1}, at least 10^{13} M^{-1}, or greater.

[0197] Alternatively, affinity may be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (*e.g.*, 10^{-5} M to 10^{-13} M, or less). Affinities of binding domain polypeptides and CAR proteins according to the present disclosure can be readily determined using conventional techniques, *e.g.*, by competitive ELISA (enzyme-linked immunosorbent assay), or by binding association, or displacement assays using labeled ligands, or using a surface-plasmon resonance device such as the Biacore T100, which is available from Biacore, Inc., Piscataway, NJ, or optical biosensor technology such as the EPIC system or EnSpire that are available from Corning and Perkin Elmer respectively (*see also*, *e.g.*, Scatchard

et al. (1949) Ann. N.Y. Acad. Sci. 51:660; and U.S. Patent Nos. 5,283,173; 5,468,614, or the equivalent) .

[0198] In one embodiment, the affinity of specific binding 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.

[0199] In particular embodiments, the extracellular binding domain of a CAR comprises an antibody or antigen binding fragment thereof. An "antibody" refers to a binding agent that is a polypeptide comprising at least a light chain or heavy chain immunoglobulin variable region which specifically recognizes and binds an epitope of an antigen, such as a peptide, lipid, polysaccharide, or nucleic acid containing an antigenic determinant, such as those recognized by an immune cell.

[0200] An "antigen (Ag)" refers to a compound, composition, or substance that can stimulate the production of antibodies or a T cell response in an animal, including compositions (such as one that includes a cancer-specific protein) that are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous antigens, such as the disclosed antigens. In particular embodiments, the target antigen is an epitope of a BCMA polypeptide.

[0201] An "epitope" or "antigenic determinant" refers to the region of an antigen to which a binding agent binds. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5, about 9, or about 8-10 amino acids in a unique spatial conformation.

[0202] Antibodies include antigen binding fragments thereof, such as Camel Ig, Ig NAR, Fab fragments, Fab' fragments, $F(ab)'_2$ fragments, $F(ab)'_3$ fragments, Fv, single chain Fv proteins ("scFv"), bis-scFv, $(scFv)_2$, minibodies, diabodies, triabodies, tetrabodies, disulfide stabilized Fv proteins ("dsFv"), and single-domain antibody (sdAb, Nanobody) and portions of full length antibodies responsible for antigen binding. The term also includes genetically engineered forms

such as chimeric antibodies (for example, humanized murine antibodies), heteroconjugate antibodies (such as, bispecific antibodies) and antigen binding fragments thereof. See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, IL); Kuby, J., Immunology, 3rd Ed., W. H. Freeman & Co., New York, 1997.

[0203] As would be understood by the skilled person and as described elsewhere herein, a complete antibody comprises two heavy chains and two light chains. Each heavy chain consists of a variable region and a first, second, and third constant region, while each light chain consists of a variable region and a constant region. Mammalian heavy chains are classified as α, δ, ε, γ, and μ. Mammalian light chains are classified as λ or κ. Immunoglobulins comprising the α, δ, ε, γ, and μ heavy chains are classified as immunoglobulin (Ig)A, IgD, IgE, IgG, and IgM. The complete antibody forms a "Y" shape. The stem of the Y consists of the second and third constant regions (and for IgE and IgM, the fourth constant region) of two heavy chains bound together and disulfide bonds (inter-chain) are formed in the hinge. Heavy chains γ, α and δ have a constant region composed of three tandem (in a line) Ig domains, and a hinge region for added flexibility; heavy chains μ and ε have a constant region composed of four immunoglobulin domains. The second and third constant regions are referred to as "CH2 domain" and "CH3 domain", respectively. Each arm of the Y includes the variable region and first constant region of a single heavy chain bound to the variable and constant regions of a single light chain. The variable regions of the light and heavy chains are responsible for antigen binding.

[0204] Light and heavy chain variable regions contain a "framework" region interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs". The CDRs can be defined or identified by conventional methods, such as by sequence according to Kabat *et al* (Wu, TT and Kabat, E. A., *J Exp Med.* 132(2):211-50, (1970); Borden, P. and Kabat E. A., *PNAS*, 84: 2440-2443 (1987); (see, Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* U.S. Department of Health and Human Services, 1991, which is hereby incorporated by reference), or by structure according to Chothia *et al* (Chothia, C. and Lesk, A.M., *J Mol. Biol.*, 196(4): 901-917 (1987), Chothia, C. *et al*, *Nature*, 342: 877 - 883 (1989)).

[0205] The sequences of the framework regions of different light or heavy chains are relatively conserved within a species, such as humans. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space. The CDRs are primarily responsible for binding to

an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, the CDRs located in the variable domain of the heavy chain of the antibody are referred to as CDRH1, CDRH2, and CDRH3, whereas the CDRs located in the variable domain of the light chain of the antibody are referred to as CDRL1, CDRL2, and CDRL3. Antibodies with different specificities (*i.e.,* different combining sites for different antigens) have different CDRs. Although it is the CDRs that vary from antibody to antibody, only a limited number of amino acid positions within the CDRs are directly involved in antigen binding. These positions within the CDRs are called specificity determining residues (SDRs). Illustrative examples of light chain CDRs that are suitable for constructing humanized BCMA CARs contemplated herein include, but are not limited to the CDR sequences set forth in SEQ ID NOs: 1-3. Illustrative examples of heavy chain CDRs that are suitable for constructing humanized BCMA CARs contemplated herein include, but are not limited to the CDR sequences set forth in SEQ ID NOs: 4-6.

[0206] References to "V_H" or "VH" refer to the variable region of an immunoglobulin heavy chain, including that of an antibody, Fv, scFv, dsFv, Fab, or other antibody fragment as disclosed herein. References to "V_L" or "VL" refer to the variable region of an immunoglobulin light chain, including that of an antibody, Fv, scFv, dsFv, Fab, or other antibody fragment as disclosed herein.

[0207] A "monoclonal antibody" is an antibody produced by a single clone of B lymphocytes or by a cell into which the light and heavy chain genes of a single antibody have been transfected. Monoclonal antibodies are produced by methods known to those of skill in the art, for instance by making hybrid antibody-forming cells from a fusion of myeloma cells with immune spleen cells. Monoclonal antibodies include humanized monoclonal antibodies.

[0208] A "chimeric antibody" has framework residues from one species, such as human, and CDRs (which generally confer antigen binding) from another species, such as a mouse. In particular embodiments, a CAR contemplated herein comprises antigen-specific binding domain that is a chimeric antibody or antigen binding fragment thereof.

[0209] A "humanized" antibody is an immunoglobulin including a human framework region and one or more CDRs from a non-human (for example a mouse, rat, or synthetic) immunoglobulin.

The non-human immunoglobulin providing the CDRs is termed a "donor," and the human immunoglobulin providing the framework is termed an "acceptor."

[0210] In particular embodiments, a murine anti-BCMA (*e.g.*, human BCMA) antibody or antigen binding fragment thereof, includes but is not limited to a Camel Ig (a camelid antibody (VHH)), Ig NAR, Fab fragments, Fab' fragments, $F(ab)'_2$ fragments, $F(ab)'_3$ fragments, Fv, single chain Fv antibody ("scFv"), bis-scFv, $(scFv)_2$, minibody, diabody, triabody, tetrabody, disulfide stabilized Fv protein ("dsFv"), and single-domain antibody (sdAb, Nanobody).

[0211] "Camel Ig" or "camelid VHH" as used herein refers to the smallest known antigen-binding unit of a heavy chain antibody (Koch-Nolte, *et al*, FASEB J., 21: 3490-3498 (2007)). A "heavy chain antibody" or a "camelid antibody" refers to an antibody that contains two VH domains and no light chains (Riechmann L. *et al*, J. Immunol. Methods 231:25–38 (1999); WO94/04678; WO94/25591; U.S. Patent No. 6,005,079).

[0212] "IgNAR" of "immunoglobulin new antigen receptor" refers to class of antibodies from the shark immune repertoire that consist of homodimers of one variable new antigen receptor (VNAR) domain and five constant new antigen receptor (CNAR) domains. IgNARs represent some of the smallest known immunoglobulin-based protein scaffolds and are highly stable and possess efficient binding characteristics. The inherent stability can be attributed to both (i) the underlying Ig scaffold, which presents a considerable number of charged and hydrophilic surface exposed residues compared to the conventional antibody VH and VL domains found in murine antibodies; and (ii) stabilizing structural features in the complementary determining region (CDR) loops including inter-loop disulphide bridges, and patterns of intra-loop hydrogen bonds.

[0213] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab′)2 fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0214] "Fv" is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three hypervariable regions (HVRs) of

each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0215] The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab′ fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab′-SH is the designation herein for Fab′ in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab′)2 antibody fragments originally were produced as pairs of Fab′ fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0216] The term “diabodies” refers to antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO 1993/01161; Hudson *et al.*, Nat. Med. 9:129-134 (2003); and Hollinger *et al.*, PNAS USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson *et al.*, Nat. Med. 9:129-134 (2003).

[0217] “Single domain antibody” or “sdAb” or “nanobody” refers to an antibody fragment that consists of the variable region of an antibody heavy chain (VH domain) or the variable region of an antibody light chain (VL domain) (Holt, L., *et al*, 2003, Trends in Biotechnology, 21(11): 484-490).

[0218] “Single-chain Fv” or “scFv” antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain and in either orientation (*e.g.*, VL-VH or VH-VL). Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see, *e.g.*, Pluckthün, in The Pharmacology of

Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315.

[0219] In certain embodiments, a CAR contemplated herein comprises antigen-specific binding domain that is a murine scFv. Single chain antibodies may be cloned form the V region genes of a hybridoma specific for a desired target. The production of such hybridomas has become routine. A technique which can be used for cloning the variable region heavy chain (V_H) and variable region light chain (V_L) has been described, for example, in Orlandi *et al., PNAS,* 1989; 86: 3833-3837.

[0220] In particular embodiments, the antigen-specific binding domain that is a murine scFv that binds a human BCMA polypeptide. Illustrative examples of variable heavy chains that are suitable for constructing BCMA CARs contemplated herein include, but are not limited to the amino acid sequences set forth in SEQ ID NO: 8. Illustrative examples of variable light chains that are suitable for constructing BCMA CARs contemplated herein include, but are not limited to the amino acid sequences set forth in SEQ ID NO: 7.

[0221] BCMA-specific binding domains provided herein also comprise one, two, three, four, five, or six CDRs. Such CDRs may be nonhuman CDRs or altered nonhuman CDRs selected from CDRL1, CDRL2 and CDRL3 of the light chain and CDRH1, CDRH2 and CDRH3 of the heavy chain. In certain embodiments, a BCMA-specific binding domain comprises (a) a light chain variable region that comprises a light chain CDRL1, a light chain CDRL2, and a light chain CDRL3, and (b) a heavy chain variable region that comprises a heavy chain CDRH1, a heavy chain CDRH2, and a heavy chain CDRH3.

5.3.2. Linkers

[0222] In certain embodiments, the CARs contemplated herein may comprise linker residues between the various domains, *e.g.,* added for appropriate spacing and conformation of the molecule. In particular embodiments the linker is a variable region linking sequence. A "variable region linking sequence" is an amino acid sequence that connects the V_H and V_L domains and provides a spacer function compatible with interaction of the two sub-binding domains so that the resulting polypeptide retains a specific binding affinity to the same target molecule as an antibody that comprises the same light and heavy chain variable regions. CARs contemplated herein, may comprise one, two, three, four, or five or more linkers. In particular embodiments, the length of a linker is about 1 to about 25 amino acids, about 5 to about 20

amino acids, or about 10 to about 20 amino acids, or any intervening length of amino acids. In some embodiments, the linker is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more amino acids long.

[0223] Illustrative examples of linkers include glycine polymers $(G)_n$; glycine-serine polymers $(G_{1-5}S_{1-5})_n$, where n is an integer of at least one, two, three, four, or five; glycine-alanine polymers; alanine-serine polymers; and other flexible linkers known in the art. Glycine and glycine-serine polymers are relatively unstructured, and therefore may be able to serve as a neutral tether between domains of fusion proteins such as the CARs described herein. Glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (*see* Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). The ordinarily skilled artisan will recognize that design of a CAR in particular embodiments can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure to provide for a desired CAR structure.

[0224] Other exemplary linkers include, but are not limited to the following amino acid sequences: GGG; DGGGS (SEQ ID NO: 12); TGEKP (SEQ ID NO: 13) (see, *e.g.*, Liu *et al.*, PNAS 5525-5530 (1997)); GGRR (SEQ ID NO: 14) (Pomerantz *et al.* 1995, supra); $(GGGGS)_n$ wherein n = 1, 2, 3, 4 or 5, and where GGGGS is identified as SEQ ID NO: 15 (Kim *et al.*, PNAS 93, 1156-1160 (1996.); EGKSSGSGSESKVD (SEQ ID NO: 16) (Chaudhary *et al.*, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:1066-1070); KESGSVSSEQLAQFRSLD (SEQ ID NO: 17) (Bird *et al.*, 1988, Science 242:423-426), GGRRGGGS (SEQ ID NO: 18); LRQRDGERP (SEQ ID NO: 19); LRQKDGGGSERP (SEQ ID NO: 20); LRQKd$(GGGS)_2$ ERP (SEQ ID NO: 21). Alternatively, flexible linkers can be rationally designed using a computer program capable of modeling both DNA-binding sites and the peptides themselves (Desjarlais & Berg, *PNAS* 90:2256-2260 (1993), *PNAS* 91:11099-11103 (1994) or by phage display methods. In one embodiment, the linker comprises the following amino acid sequence: GSTSGSGKPGSGEGSTKG (SEQ ID NO: 22) (Cooper *et al.*, *Blood*, 101(4): 1637-1644 (2003)).

5.3.3. Spacer Domain

[0225] In particular embodiments, the binding domain of the CAR is followed by one or more "spacer domains," which refers to the region that moves the antigen binding domain away from

the effector cell surface to enable proper cell/cell contact, antigen binding and activation (Patel *et al.*, *Gene Therapy*, 1999; 6: 412-419). The spacer domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. In certain embodiments, a spacer domain is a portion of an immunoglobulin, including, but not limited to, one or more heavy chain constant regions, *e.g.*, CH2 and CH3. The spacer domain can include the amino acid sequence of a naturally occurring immunoglobulin hinge region or an altered immunoglobulin hinge region.

[0226] In one embodiment, the spacer domain comprises the CH2 and CH3 domains of IgG1 or IgG4.

5.3.4. Hinge Domain

[0227] The binding domain of the CAR is generally followed by one or more "hinge domains," which play a role in positioning the antigen binding domain away from the effector cell surface to enable proper cell/cell contact, antigen binding and activation. A CAR generally comprises one or more hinge domains between the binding domain and the transmembrane domain (TM). The hinge domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. The hinge domain can include the amino acid sequence of a naturally occurring immunoglobulin hinge region or an altered immunoglobulin hinge region.

[0228] An "altered hinge region" refers to (a) a naturally occurring hinge region with up to 30% amino acid changes (*e.g.*, up to 25%, 20%, 15%, 10%, or 5% amino acid substitutions or deletions), (b) a portion of a naturally occurring hinge region that is at least 10 amino acids (*e.g.*, at least 12, 13, 14 or 15 amino acids) in length with up to 30% amino acid changes (*e.g.*, up to 25%, 20%, 15%, 10%, or 5% amino acid substitutions or deletions), or (c) a portion of a naturally occurring hinge region that comprises the core hinge region (which may be 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15, or at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids in length). In certain embodiments, one or more cysteine residues in a naturally occurring immunoglobulin hinge region may be substituted by one or more other amino acid residues (*e.g.*, one or more serine residues). An altered immunoglobulin hinge region may alternatively or additionally have a proline residue of a wild type immunoglobulin hinge region substituted by another amino acid residue (*e.g.*, a serine residue).

[0229] Other illustrative hinge domains suitable for use in the CARs described herein include the hinge region derived from the extracellular regions of type 1 membrane proteins such as CD8α, CD4, CD28 and CD7, which may be wild-type hinge regions from these molecules or may be altered. In another embodiment, the hinge domain comprises a CD8α hinge region.

5.3.5. Transmembrane Domain

[0230] The transmembrane (TM) domain is the portion of the CAR that fuses the extracellular binding portion and intracellular signaling domain and anchors the CAR to the plasma membrane of the immune effector cell. The TM domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. The TM domain may be derived from (*i.e.*, comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD3ε, CD3ζ, CD4, CD5, CD8α, CD9, CD16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD152, CD154, and PD-1. In a particular embodiment, the TM domain is synthetic and predominantly comprises hydrophobic residues such as leucine and valine.

[0231] In one embodiment, the CARs contemplated herein comprise a TM domain derived from CD8α. In another embodiment, a CAR contemplated herein comprises a TM domain derived from CD8α and a short oligo- or polypeptide linker, preferably between 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length that links the TM domain and the intracellular signaling domain of the CAR. A glycine-serine based linker provides a particularly suitable linker.

5.3.6. Intracellular Signaling Domain

[0232] In particular embodiments, CARs contemplated herein comprise an intracellular signaling domain. An "intracellular signaling domain" refers to the part of a CAR that participates in transducing the message of effective BCMA CAR binding to a human BCMA polypeptide into the interior of the immune effector cell to elicit effector cell function, *e.g.,* activation, cytokine production, proliferation and cytotoxic activity, including the release of cytotoxic factors to the CAR-bound target cell, or other cellular responses elicited with antigen binding to the extracellular CAR domain.

[0233] The term "effector function" refers to a specialized function of an immune effector cell. Effector function of the T cell, for example, may be cytolytic activity or helper activity including the secretion of a cytokine. Thus, the term "intracellular signaling domain" refers to the portion of a protein which transduces the effector function signal and that directs the cell to perform a

specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire domain. To the extent that a truncated portion of an intracellular signaling domain is used, such truncated portion may be used in place of the entire domain as long as it transduces the effector function signal. The term intracellular signaling domain is meant to include any truncated portion of the intracellular signaling domain sufficient to transducing effector function signal.

[0234] It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary or co-stimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of intracellular signaling domains: primary signaling domains that initiate antigen-dependent primary activation through the TCR (*e.g.*, a TCR/CD3 complex) and co-stimulatory signaling domains that act in an antigen-independent manner to provide a secondary or co-stimulatory signal. In certain embodiments, a CAR contemplated herein comprises an intracellular signaling domain that comprises one or more "co-stimulatory signaling domain" and a "primary signaling domain."

[0235] Primary signaling domains regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

[0236] Illustrative examples of ITAM containing primary signaling domains that are of particular use in the subject matter presented herein include those derived from TCRζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d. In particular embodiments, a CAR comprises a CD3ζ primary signaling domain and one or more co-stimulatory signaling domains. The intracellular primary signaling and co-stimulatory signaling domains may be linked in any order in tandem to the carboxyl terminus of the transmembrane domain.

[0237] CARs contemplated herein comprise one or more co-stimulatory signaling domains to enhance the efficacy and expansion of T cells expressing CAR receptors. As used herein, the term, "co-stimulatory signaling domain," or "co-stimulatory domain", refers to an intracellular signaling domain of a co-stimulatory molecule. Co-stimulatory molecules are cell surface molecules other than antigen receptors or Fc receptors that provide a second signal required for efficient activation and function of T lymphocytes upon binding to antigen. Illustrative examples

of such co-stimulatory molecules include CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and ZAP70. In one embodiment, a CAR comprises one or more co-stimulatory signaling domains selected from the group consisting of CD28, CD137, and CD134, and a CD3ζ primary signaling domain.

[0238] In another embodiment, a CAR comprises CD28 and CD137 co-stimulatory signaling domains and a CD3ζ primary signaling domain.

[0239] In yet another embodiment, a CAR comprises CD28 and CD134 co-stimulatory signaling domains and a CD3ζ primary signaling domain.

[0240] In one embodiment, a CAR comprises CD137 and CD134 co-stimulatory signaling domains and a CD3ζ primary signaling domain.

[0241] In particular embodiments, CARs contemplated herein comprise a murine anti-BCMA antibody or antigen binding fragment thereof that specifically binds to a BCMA polypeptide expressed on B cells, *e.g.*, a human BCMA expressed on human B cells.

[0242] In one embodiment, a CAR comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide; a transmembrane domain derived from a polypeptide selected from the group consisting of: alpha, beta or zeta chain of the T-cell receptor, CD3ε, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD 154, and PD1; and one or more intracellular co-stimulatory signaling domains from a co-stimulatory molecule selected from the group consisting of: CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and ZAP70; and a primary signaling domain from TCRζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

[0243] In one embodiment, a CAR comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide; a transmembrane domain derived from a polypeptide selected from the group consisting of: alpha, beta or zeta chain of the T-cell receptor, CD3ε, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD 154, and PD1; and one or more

intracellular co-stimulatory signaling domains from a co-stimulatory molecule selected from the group consisting of: CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and ZAP70; and one or more primary signaling domains from a polypeptide selected from the group consisting of: TCRζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

[0244] In one embodiment, a CAR comprises a murine anti-BCMA scFv that binds a BCMA polypeptide;, *e.g.*, a human BCMA polypeptide, a hinge domain selected from the group consisting of: IgG1 hinge/CH2/CH3, IgG4 hinge/CH2/CH3, and a CD8α hinge; a transmembrane domain derived from a polypeptide selected from the group consisting of: alpha, beta or zeta chain of the T-cell receptor, CD3ε, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD 154, and PD1; and one or more intracellular co-stimulatory signaling domains from a co-stimulatory molecule selected from the group consisting of: CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and ZAP70; and a primary signaling domain from TCRζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

[0245] In one embodiment, a CAR comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide; a hinge domain selected from the group consisting of: IgG1 hinge/CH2/CH3, IgG4 hinge/CH2/CH3, and a CD8α hinge; a transmembrane domain derived from a polypeptide selected from the group consisting of: alpha, beta or zeta chain of the T-cell receptor, CD3ε, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD 154, and PD1; and one or more intracellular co-stimulatory signaling domains from a co-stimulatory molecule selected from the group consisting of: CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and ZAP70; and one or more primary signaling domains

from a polypeptide selected from the group consisting of: TCRζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

[0246] In one embodiment, a CAR comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide; a hinge domain selected from the group consisting of: IgG1 hinge/CH2/CH3, IgG4 hinge/CH2/CH3, and a CD8α hinge; a transmembrane domain derived from a polypeptide selected from the group consisting of: alpha, beta or zeta chain of the T-cell receptor, CD3ε, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD 154, and PD1; a short oligo- or polypeptide linker, preferably between 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length that links the TM domain to the intracellular signaling domain of the CAR; and one or more intracellular co-stimulatory signaling domains from a co-stimulatory molecule selected from the group consisting of: CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and ZAP70; and a primary signaling domain from TCRζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

[0247] In one embodiment, a CAR comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide; a hinge domain selected from the group consisting of: IgG1 hinge/CH2/CH3, IgG4 hinge/CH2/CH3, and a CD8α hinge; a transmembrane domain derived from a polypeptide selected from the group consisting of: alpha, beta or zeta chain of the T-cell receptor, CD3ε, CD3ζ, CD4, CD5, CD8α, CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD 154, and PD1; a short oligo- or polypeptide linker, preferably between 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length that links the TM domain to the intracellular signaling domain of the CAR; and one or more intracellular co-stimulatory signaling domains from a co-stimulatory molecule selected from the group consisting of: CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and ZAP70; and one or more primary signaling domains from a polypeptide selected from the group consisting of: TCRζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD3ζ, CD22, CD79a, CD79b, and CD66d.

[0248] In a particular embodiment, a CAR comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide; a hinge domain comprising an IgG1 hinge/CH2/CH3 polypeptide and a CD8α polypeptide; a CD8α transmembrane domain comprising a polypeptide linker of about 3 to about 10 amino acids; a CD137 intracellular co-stimulatory signaling domain; and a CD3ζ primary signaling domain.

[0249] In a particular embodiment, a CAR comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide; a hinge domain comprising a CD8α polypeptide; a CD8α transmembrane domain comprising a polypeptide linker of about 3 to about 10 amino acids; a CD134 intracellular co-stimulatory signaling domain; and a CD3ζ primary signaling domain.

[0250] In a particular embodiment, a CAR comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide; a hinge domain comprising a CD8α polypeptide; a CD8α transmembrane domain comprising a polypeptide linker of about 3 to about 10 amino acids; a CD28 intracellular co-stimulatory signaling domain; and a CD3ζ primary signaling domain.

[0251] In a particular embodiment, a CAR comprises a murine anti-BCMA scFv that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide; a hinge domain comprising a CD8α polypeptide; a CD8α transmembrane domain; a CD137 (4-1BB) intracellular co-stimulatory signaling domain; and a CD3ζ primary signaling domain.

[0252] Moreover, the design of the CARs contemplated herein enable improved expansion, long-term persistence, and tolerable cytotoxic properties in T cells expressing the CARs compared to non-modified T cells or T cells modified to express other CARs.

5.4. Polypeptides

[0253] The present disclosure contemplates, in part, CAR polypeptides and fragments thereof, cells and compositions comprising the same, and vectors that express polypeptides. In particular embodiments, a polypeptide comprising one or more CARs as set forth in SEQ ID NO:9 is provided.

[0254] "Polypeptide," "polypeptide fragment," "peptide" and "protein" are used interchangeably, unless specified to the contrary, and according to conventional meaning, *i.e.*, as a sequence of amino acids. Polypeptides are not limited to a specific length, *e.g.*, they may comprise a full length protein sequence or a fragment of a full length protein, and may include

post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. In various embodiments, the CAR polypeptides contemplated herein comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. Illustrative examples of suitable signal sequences useful in CARs disclosed herein include, but are not limited to, the IgG1 heavy chain signal sequence and the CD8α signal sequence. Polypeptides can be prepared using any of a variety of well-known recombinant and/or synthetic techniques. Polypeptides contemplated herein specifically encompass the CARs of the present disclosure, or sequences that have deletions from, additions to, and/or substitutions of one or more amino acid of a CAR as disclosed herein.

[0255] An "isolated peptide" or an "isolated polypeptide" and the like, as used herein, refer to *in vitro* isolation and/or purification of a peptide or polypeptide molecule from a cellular environment, and from association with other components of the cell, *i.e.*, it is not significantly associated with *in vivo* substances. Similarly, an "isolated cell" refers to a cell that has been obtained from an *in vivo* tissue or organ and is substantially free of extracellular matrix.

[0256] Polypeptides include "polypeptide variants." Polypeptide variants may differ from a naturally occurring polypeptide in one or more substitutions, deletions, additions and/or insertions. Such variants may be naturally occurring or may be synthetically generated, for example, by modifying one or more of the above polypeptide sequences. For example, in particular embodiments, it may be desirable to improve the binding affinity and/or other biological properties of the CARs by introducing one or more substitutions, deletions, additions and/or insertions into a binding domain, hinge, TM domain, co-stimulatory signaling domain or primary signaling domain of a CAR polypeptide. In certain embodiments, such polypeptides include polypeptides having at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% amino acid identity thereto.

[0257] Polypeptides include "polypeptide fragments." Polypeptide fragments refer to a polypeptide, which can be monomeric or multimeric, that has an amino-terminal deletion, a carboxyl-terminal deletion, and/or an internal deletion or substitution of a naturally-occurring or recombinantly-produced polypeptide. In certain embodiments, a polypeptide fragment can comprise an amino acid chain at least 5 to about 500 amino acids long. It will be appreciated

that in certain embodiments, fragments are at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 150, 200, 250, 300, 350, 400, or 450 amino acids long. Particularly useful polypeptide fragments include functional domains, including antigen-binding domains or fragments of antibodies. In the case of a murine anti-BCMA (*e.g.*, human BCMA) antibody, useful fragments include, but are not limited to: a CDR region, a CDR3 region of the heavy or light chain; a variable region of a heavy or light chain; a portion of an antibody chain or variable region including two CDRs; and the like.

[0258] The polypeptide may also be fused in-frame or conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support.

[0259] As noted above, polypeptides of the present disclosure may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of a reference polypeptide can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (1985, *Proc. Natl. Acad. Sci. USA*. 82: 488-492), Kunkel *et al.*, (1987, *Methods in Enzymol*, 154: 367-382), U.S. Pat. No. 4,873,192, Watson, J. D. *et al.*, (*Molecular Biology of the Gene*, Fourth Edition, Benjamin/Cummings, Menlo Park, Calif., 1987) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.*, (1978) *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.).

[0260] In certain embodiments, a variant will contain conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Modifications may be made in the structure of the polynucleotides and polypeptides of the present disclosure and still obtain a functional molecule that encodes a variant or derivative polypeptide with desirable characteristics. When it is desired to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, variant polypeptide, one skilled in

the art, for example, can change one or more of the codons of the encoding DNA sequence, *e.g.*, according to Table 2.

Table 2- Amino Acid Codons

Amino Acids	One letter code	Three letter code	Codons					
Alanine	A	Ala	GCA	GCC	GCG	GCU		
Cysteine	C	Cys	UGC	UGU				
Aspartic acid	D	Asp	GAC	GAU				
Glutamic acid	E	Glu	GAA	GAG				
Phenylalanine	F	Phe	UUC	UUU				
Glycine	G	Gly	GGA	GGC	GGG	GGU		
Histidine	H	His	CAC	CAU				
Isoleucine	I	Ile	AUA	AUC	AUU			
Lysine	K	Lys	AAA	AAG				
Leucine	L	Leu	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	M	Met	AUG					
Asparagine	N	Asn	AAC	AAU				
Proline	P	Pro	CCA	CCC	CCG	CCU		
Glutamine	Q	Gln	CAA	CAG				
Arginine	R	Arg	AGA	AGG	CGA	CGC	CGG	CGU
Serine	S	Ser	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	T	Thr	ACA	ACC	ACG	ACU		
Valine	V	Val	GUA	GUC	GUG	GUU		
Tryptophan	W	Trp	UGG					
Tyrosine	Y	Tyr	UAC	UAU				

[0261] Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological activity can be found using computer programs well known in the art, such as DNASTAR™ software. Preferably, amino acid changes in the protein variants disclosed herein are conservative amino acid changes, *i.e.,* substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a

family of amino acids which are related in their side chains. Naturally occurring amino acids are generally divided into four families: acidic (aspartate, glutamate), basic (lysine, arginine, histidine), non-polar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. In a peptide or protein, suitable conservative substitutions of amino acids are known to those of skill in this art and generally can be made without altering a biological activity of a resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, *e.g.*, Watson *et al. Molecular Biology of the Gene*, 4th Edition, 1987, The Benjamin/Cummings Pub. Co., p.224). Exemplary conservative substitutions are described in U.S. Provisional Patent Application No. 61/241,647 , the disclosure of which is herein incorporated by reference.

[0262] In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

[0263] It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.*, still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity.

[0264] As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate

(+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (–0.4); proline (–0.5 ± 1); alanine (–0.5); histidine (–0.5); cysteine (–1.0); methionine (–1.3); valine (–1.5); leucine (–1.8); isoleucine (–1.8); tyrosine (–2.3); phenylalanine (–2.5); tryptophan (–3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

[0265] As outlined above, amino acid substitutions may be based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like.

[0266] Polypeptide variants further include glycosylated forms, aggregative conjugates with other molecules, and covalent conjugates with unrelated chemical moieties (*e.g.*, pegylated molecules). Covalent variants can be prepared by linking functionalities to groups which are found in the amino acid chain or at the N- or C-terminal residue, as is known in the art. Variants also include allelic variants, species variants, and muteins. Truncations or deletions of regions which do not affect functional activity of the proteins are also variants.

[0267] In one embodiment, where expression of two or more polypeptides is desired, the polynucleotide sequences encoding them can be separated by and IRES sequence as discussed elsewhere herein. In another embodiment, two or more polypeptides can be expressed as a fusion protein that comprises one or more self-cleaving polypeptide sequences.

[0268] Polypeptides disclosed herein include fusion polypeptides. In certain embodiments, fusion polypeptides and polynucleotides encoding fusion polypeptides are provided, *e.g.*, CARs. Fusion polypeptides and fusion proteins refer to a polypeptide having at least two, three, four, five, six, seven, eight, nine, or ten or more polypeptide segments. Fusion polypeptides are typically linked C-terminus to N-terminus, although they can also be linked C-terminus to C-terminus, N-terminus to N-terminus, or N-terminus to C-terminus. The polypeptides of the fusion protein can be in any order or a specified order. Fusion polypeptides or fusion proteins can also include conservatively modified variants, polymorphic variants, alleles, mutants, subsequences, and interspecies homologs, so long as the desired transcriptional activity of the fusion polypeptide is preserved. Fusion polypeptides may be produced by chemical synthetic

methods or by chemical linkage between the two moieties or may generally be prepared using other standard techniques. Ligated DNA sequences comprising the fusion polypeptide are operably linked to suitable transcriptional or translational control elements as discussed elsewhere herein.

[0269] In one embodiment, a fusion partner comprises a sequence that assists in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments or to facilitate transport of the fusion protein through the cell membrane.

[0270] Fusion polypeptides may further comprise a polypeptide cleavage signal between each of the polypeptide domains described herein. In addition, a polypeptide site can be put into any linker peptide sequence. Exemplary polypeptide cleavage signals include polypeptide cleavage recognition sites such as protease cleavage sites, nuclease cleavage sites (*e.g.*, rare restriction enzyme recognition sites, self-cleaving ribozyme recognition sites), and self-cleaving viral oligopeptides (see deFelipe and Ryan, 2004. *Traffic*, 5(8); 616-26).

[0271] Suitable protease cleavages sites and self-cleaving peptides are known to the skilled person (*see, e.g.*, in Ryan *et al.*, 1997. *J. Gener. Virol.* 78, 699-722; Scymczak *et al.* (2004) Nature Biotech. 5, 589-594). Exemplary protease cleavage sites include, but are not limited to, the cleavage sites of potyvirus NIa proteases (*e.g.*, tobacco etch virus protease), potyvirus HC proteases, potyvirus P1 (P35) proteases, byovirus NIa proteases, byovirus RNA-2-encoded proteases, aphthovirus L proteases, enterovirus 2A proteases, rhinovirus 2A proteases, picorna 3C proteases, comovirus 24K proteases, nepovirus 24K proteases, RTSV (rice tungro spherical virus) 3C-like protease, PYVF (parsnip yellow fleck virus) 3C-like protease, heparin, thrombin, factor Xa and enterokinase. Due to its high cleavage stringency, TEV (tobacco etch virus) protease cleavage sites are preferred in one embodiment, *e.g.*, EXXYXQ(G/S) (SEQ ID NO: 23), for example, ENLYFQG (SEQ ID NO: 24) and ENLYFQS (SEQ ID NO: 25), wherein X represents any amino acid (cleavage by TEV occurs between Q and G or Q and S).

[0272] In a particular embodiment, self-cleaving peptides include those polypeptide sequences obtained from potyvirus and cardiovirus 2A peptides, FMDV (foot-and-mouth disease virus), equine rhinitis A virus, Thosea asigna virus and porcine teschovirus.

[0273] In certain embodiments, the self-cleaving polypeptide site comprises a 2A or 2A-like site, sequence or domain (Donnelly *et al.*, 2001. *J. Gen. Virol.* 82:1027-1041).

Table 3: Exemplary 2A sites include the following sequences:

SEQ ID NO: 26	LLNFDLLKLAGDVESNPGP
SEQ ID NO: 27	TLNFDLLKLAGDVESNPGP
SEQ ID NO: 28	LLKLAGDVESNPGP
SEQ ID NO: 29	NFDLLKLAGDVESNPGP
SEQ ID NO: 30	QLLNFDLLKLAGDVESNPGP
SEQ ID NO: 31	APVKQTLNFDLLKLAGDVESNPGP
SEQ ID NO: 32	VTELLYRMKRAETYCPRPLLAIHPTEARHKQKIVAPVKQT
SEQ ID NO: 33	LNFDLLKLAGDVESNPGP
SEQ ID NO: 34	LLAIHPTEARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP
SEQ ID NO: 35	EARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP

[0274] In certain embodiments, a polypeptide contemplated herein comprises a CAR polypeptide.

5.5. Polynucleotides

[0275] In certain embodiments, a polynucleotide encoding one or more CAR polypeptides is provided, *e.g.,* SEQ ID NO: 10. As used herein, the terms "polynucleotide" or "nucleic acid" refers to messenger RNA (mRNA), RNA, genomic RNA (gRNA), plus strand RNA (RNA(+)), minus strand RNA (RNA(-)), genomic DNA (gDNA), complementary DNA (cDNA) or recombinant DNA. Polynucleotides include single and double stranded polynucleotides. Preferably, polynucleotides disclosed herein include polynucleotides or variants having at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any of the reference sequences described herein (*see, e.g.,* Sequence Listing), typically where the variant maintains at least one biological activity of the reference sequence. In various illustrative embodiments, the present dislosure contemplates, in part, polynucleotides comprising expression vectors, viral vectors, and transfer plasmids, and compositions, and cells comprising the same.

[0276] In particular embodiments, polynucleotides are provided by this disclosure that encode at least about 5, 10, 25, 50, 100, 150, 200, 250, 300, 350, 400, 500, 1000, 1250, 1500, 1750, or

2000 or more contiguous amino acid residues of a polypeptide, as well as all intermediate lengths. It will be readily understood that "intermediate lengths," in this context, means any length between the quoted values, such as 6, 7, 8, 9, *etc.*, 101, 102, 103, *etc.*; 151, 152, 153, *etc.*; 201, 202, 203, *etc.*

[0277] As used herein, the terms "polynucleotide variant" and "variant" and the like refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridize with a reference sequence under stringent conditions that are defined hereinafter. These terms include polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides compared to a reference polynucleotide. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide.

[0278] The recitations "sequence identity" or, for example, comprising a "sequence 50% identical to," as used herein, refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.,* A, T, C, G, I) or the identical amino acid residue (*e.g.,* Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.,* the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Included are nucleotides and polypeptides having at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any of the reference sequences described herein, typically where the polypeptide variant maintains at least one biological activity of the reference polypeptide.

[0279] Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence," "comparison window," "sequence identity," "percentage of sequence identity," and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and

amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (i.e., only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (i.e., gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (*i.e.*, resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.,* 1997, *Nucl. Acids Res.* 25:3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.,* Current Protocols in Molecular Biology, John Wiley & Sons Inc, 1994-1998, Chapter 15.

[0280] As used herein, "isolated polynucleotide" refers to a polynucleotide that has been purified from the sequences which flank it in a naturally-occurring state, *e.g.,* a DNA fragment that has been removed from the sequences that are normally adjacent to the fragment. An "isolated polynucleotide" also refers to a complementary DNA (cDNA), a recombinant DNA, or other polynucleotide that does not exist in nature and that has been made by the hand of man.

[0281] Terms that describe the orientation of polynucleotides include: 5' (normally the end of the polynucleotide having a free phosphate group) and 3' (normally the end of the polynucleotide having a free hydroxyl (OH) group). Polynucleotide sequences can be annotated in the 5' to 3' orientation or the 3' to 5' orientation. For DNA and mRNA, the 5' to 3' strand is designated the "sense," "plus," or "coding" strand because its sequence is identical to the sequence of the premessenger (premRNA) [except for uracil (U) in RNA, instead of thymine (T) in DNA]. For

DNA and mRNA, the complementary 3' to 5' strand which is the strand transcribed by the RNA polymerase is designated as "template," "antisense," "minus," or "non-coding" strand. As used herein, the term "reverse orientation" refers to a 5' to 3' sequence written in the 3' to 5' orientation or a 3' to 5' sequence written in the 5' to 3' orientation.

[0282] The terms "complementary" and "complementarity" refer to polynucleotides *(i.e.,* a sequence of nucleotides) related by the base-pairing rules. For example, the complementary strand of the DNA sequence 5' A G T C A T G 3' is 3' T C A G T A C 5'. The latter sequence is often written as the reverse complement with the 5' end on the left and the 3' end on the right, 5' C A T G A C T 3'. A sequence that is equal to its reverse complement is said to be a palindromic sequence. Complementarity can be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there can be "complete" or "total" complementarity between the nucleic acids.

[0283] Moreover, it will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide, or fragment of variant thereof, as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present disclosure, for example polynucleotides that are optimized for human and/or primate codon selection. Further, alleles of the genes comprising the polynucleotide sequences provided herein may also be used. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides.

[0284] The term "nucleic acid cassette" as used herein refers to genetic sequences within a vector which can express a RNA, and subsequently a protein. The nucleic acid cassette contains the gene of interest, *e.g.,* a CAR. The nucleic acid cassette is positionally and sequentially oriented within the vector such that the nucleic acid in the cassette can be transcribed into RNA, and when necessary, translated into a protein or a polypeptide, undergo appropriate post-translational modifications required for activity in the transformed cell, and be translocated to the appropriate compartment for biological activity by targeting to appropriate intracellular compartments or secretion into extracellular compartments. Preferably, the cassette has its 3' and 5' ends adapted for ready insertion into a vector, *e.g.,* it has restriction endonuclease sites at each end. In one embodiment, the nucleic acid cassette contains the sequence of a chimeric antigen

receptor used to treat a B cell malignancy. The cassette can be removed and inserted into a plasmid or viral vector as a single unit.

[0285] In particular embodiments, polynucleotides include at least one polynucleotide-of-interest. As used herein, the term "polynucleotide-of-interest" refers to a polynucleotide encoding a polypeptide (*i.e.,* a polypeptide-of-interest), inserted into an expression vector that is desired to be expressed. A vector may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 polynucleotides-of-interest. In certain embodiments, the polynucleotide-of-interest encodes a polypeptide that provides a therapeutic effect in the treatment or prevention of a disease or disorder. Polynucleotides-of-interest, and polypeptides encoded therefrom, include both polynucleotides that encode wild-type polypeptides, as well as functional variants and fragments thereof. In particular embodiments, a functional variant has at least 80%, at least 90%, at least 95%, or at least 99% identity to a corresponding wild-type reference polynucleotide or polypeptide sequence. In certain embodiments, a functional variant or fragment has at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% of a biological activity of a corresponding wild-type polypeptide.

[0286] In one embodiment, the polynucleotide-of-interest does not encode a polypeptide but serves as a template to transcribe miRNA, siRNA, or shRNA, ribozyme, or other inhibitory RNA. In various other embodiments, a polynucleotide comprises a polynucleotide-of-interest encoding a CAR and one or more additional polynucleotides-of-interest including but not limited to an inhibitory nucleic acid sequence including, but not limited to: an siRNA, an miRNA, an shRNA, and a ribozyme.

[0287] As used herein, the terms "siRNA" or "short interfering RNA" refer to a short polynucleotide sequence that mediates a process of sequence-specific post-transcriptional gene silencing, translational inhibition, transcriptional inhibition, or epigenetic RNAi in animals (Zamore *et al.*, 2000, *Cell*, 101, 25-33; Fire *et al.*, 1998, *Nature*, 391, 806; Hamilton *et al.*, 1999, *Science*, 286, 950-951; Lin *et al.*, 1999, *Nature*, 402, 128-129; Sharp, 1999, *Genes & Dev.*, 13, 139-141; and Strauss, 1999, *Science*, 286, 886). In certain embodiments, an siRNA comprises a first strand and a second strand that have the same number of nucleosides; however, the first and second strands are offset such that the two terminal nucleosides on the first and second strands are not paired with a residue on the complimentary strand. In certain instances, the two nucleosides that are not paired are thymidine resides. The siRNA should include a region of

sufficient homology to the target gene, and be of sufficient length in terms of nucleotides, such that the siRNA, or a fragment thereof, can mediate down regulation of the target gene. Thus, an siRNA includes a region which is at least partially complementary to the target RNA. It is not necessary that there be perfect complementarity between the siRNA and the target, but the correspondence must be sufficient to enable the siRNA, or a cleavage product thereof, to direct sequence specific silencing, such as by RNAi cleavage of the target RNA. Complementarity, or degree of homology with the target strand, is most critical in the antisense strand. While perfect complementarity, particularly in the antisense strand, is often desired, some embodiments include one or more, but preferably 10, 8, 6, 5, 4, 3, 2, or fewer mismatches with respect to the target RNA. The mismatches are most tolerated in the terminal regions, and if present are preferably in a terminal region or regions, *e.g.*, within 6, 5, 4, or 3 nucleotides of the 5' and/or 3' terminus. The sense strand need only be sufficiently complementary with the antisense strand to maintain the overall double-strand character of the molecule.

[0288] In addition, an siRNA may be modified or include nucleoside analogs. Single stranded regions of an siRNA may be modified or include nucleoside analogs, *e.g.*, the unpaired region or regions of a hairpin structure, *e.g.*, a region which links two complementary regions, can have modifications or nucleoside analogs. Modification to stabilize one or more 3'- or 5'-terminus of an siRNA, *e.g.*, against exonucleases, or to favor the antisense siRNA agent to enter into RISC are also useful. Modifications can include C3 (or C6, C7, C12) amino linkers, thiol linkers, carboxyl linkers, non-nucleotidic spacers (C3, C6, C9, C12, abasic, triethylene glycol, hexaethylene glycol), special biotin or fluorescein reagents that come as phosphoramidites and that have another DMT-protected hydroxyl group, allowing multiple couplings during RNA synthesis. Each strand of an siRNA can be equal to or less than 30, 25, 24, 23, 22, 21, or 20 nucleotides in length. The strand is preferably at least 19 nucleotides in length. For example, each strand can be between 21 and 25 nucleotides in length. Preferred siRNAs have a duplex region of 17, 18, 19, 29, 21, 22, 23, 24, or 25 nucleotide pairs, and one or more overhangs of 2-3 nucleotides, preferably one or two 3' overhangs, of 2-3 nucleotides.

[0289] As used herein, the terms “miRNA” or “microRNA” refer to small non-coding RNAs of 20–22 nucleotides, typically excised from ~70 nucleotide fold-back RNA precursor structures known as pre-miRNAs. miRNAs negatively regulate their targets in one of two ways depending on the degree of complementarity between the miRNA and the target. First, miRNAs that bind

with perfect or nearly perfect complementarity to protein-coding mRNA sequences induce the RNA-mediated interference (RNAi) pathway. miRNAs that exert their regulatory effects by binding to imperfect complementary sites within the 3' untranslated regions (UTRs) of their mRNA targets, repress target-gene expression post-transcriptionally, apparently at the level of translation, through a RISC complex that is similar to, or possibly identical with, the one that is used for the RNAi pathway. Consistent with translational control, miRNAs that use this mechanism reduce the protein levels of their target genes, but the mRNA levels of these genes are only minimally affected. miRNAs encompass both naturally occurring miRNAs as well as artificially designed miRNAs that can specifically target any mRNA sequence. For example, in one embodiment, the skilled artisan can design short hairpin RNA constructs expressed as human miRNA (*e.g.*, miR-30 or miR-21) primary transcripts. This design adds a Drosha processing site to the hairpin construct and has been shown to greatly increase knockdown efficiency (Pusch *et al.*, 2004). The hairpin stem consists of 22-nt of dsRNA (*e.g.*, antisense has perfect complementarity to desired target) and a 15-19-nt loop from a human miR. Adding the miR loop and miR30 flanking sequences on either or both sides of the hairpin results in greater than 10-fold increase in Drosha and Dicer processing of the expressed hairpins when compared with conventional shRNA designs without microRNA. Increased Drosha and Dicer processing translates into greater siRNA/miRNA production and greater potency for expressed hairpins.

[0290] As used herein, the terms “shRNA” or “short hairpin RNA” refer to double-stranded structure that is formed by a single self-complementary RNA strand. shRNA constructs containing a nucleotide sequence identical to a portion, of either coding or non-coding sequence, of the target gene are preferred for inhibition. RNA sequences with insertions, deletions, and single point mutations relative to the target sequence have also been found to be effective for inhibition. Greater than 90% sequence identity, or even 100% sequence identity, between the inhibitory RNA and the portion of the target gene is preferred. In certain preferred embodiments, the length of the duplex-forming portion of an shRNA is at least 20, 21 or 22 nucleotides in length, *e.g.*, corresponding in size to RNA products produced by Dicer-dependent cleavage. In certain embodiments, the shRNA construct is at least 25, 50, 100, 200, 300 or 400 bases in length. In certain embodiments, the shRNA construct is 400-800 bases in length. shRNA constructs are highly tolerant of variation in loop sequence and loop size.

[0291] As used herein, the term "ribozyme" refers to a catalytically active RNA molecule capable of site-specific cleavage of target mRNA. Several subtypes have been described, *e.g.*, hammerhead and hairpin ribozymes. Ribozyme catalytic activity and stability can be improved by substituting deoxyribonucleotides for ribonucleotides at noncatalytic bases. While ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy particular mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA has the following sequence of two bases: 5′-UG-3′. The construction and production of hammerhead ribozymes is well known in the art.

[0292] In certain embodiments, a method of delivery of a polynucleotide-of-interest that comprises an siRNA, an miRNA, an shRNA, or a ribozyme comprises one or more regulatory sequences, such as, for example, a strong constitutive pol III, *e.g.*, human U6 snRNA promoter, the mouse U6 snRNA promoter, the human and mouse H1 RNA promoter and the human tRNA-val promoter, or a strong constitutive pol II promoter, as described elsewhere herein.

[0293] The polynucleotides disclosed herein, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters and/or enhancers, untranslated regions (UTRs), signal sequences, Kozak sequences, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, internal ribosomal entry sites (IRES), recombinase recognition sites (*e.g.*, LoxP, FRT, and Att sites), termination codons, transcriptional termination signals, and polynucleotides encoding self-cleaving polypeptides, epitope tags, as disclosed elsewhere herein or as known in the art, such that their overall length may vary considerably. It is therefore contemplated that a polynucleotide fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol.

[0294] Polynucleotides can be prepared, manipulated and/or expressed using any of a variety of well-established techniques known and available in the art. In order to express a desired polypeptide, a nucleotide sequence encoding the polypeptide, can be inserted into appropriate vector. Examples of vectors are plasmid, autonomously replicating sequences, and transposable elements. Additional exemplary vectors include, without limitation, plasmids, phagemids, cosmids, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial

chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. Examples of categories of animal viruses useful as vectors include, without limitation, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, and papovavirus (*e.g.*, SV40). Examples of expression vectors are pClneo vectors (Promega) for expression in mammalian cells; pLenti4/V5-DEST™, pLenti6/V5-DEST™, and pLenti6.2/V5-GW/lacZ (Invitrogen) for lentivirus-mediated gene transfer and expression in mammalian cells. In particular embodiments, he coding sequences of the chimeric proteins disclosed herein can be ligated into such expression vectors for the expression of the chimeric protein in mammalian cells.

[0295] In one embodiment, a vector encoding a CAR contemplated herein comprises the polynucleotide sequence set forth in SEQ ID NO: 36.

[0296] In particular embodiments, the vector is an episomal vector or a vector that is maintained extrachromosomally. As used herein, the term "episomal" refers to a vector that is able to replicate without integration into host's chromosomal DNA and without gradual loss from a dividing host cell also meaning that said vector replicates extrachromosomally or episomally. The vector is engineered to harbor the sequence coding for the origin of DNA replication or "ori" from a lymphotrophic herpes virus or a gamma herpesvirus, an adenovirus, SV40, a bovine papilloma virus, or a yeast, specifically a replication origin of a lymphotrophic herpes virus or a gamma herpesvirus corresponding to oriP of EBV. In a particular aspect, the lymphotrophic herpes virus may be Epstein Barr virus (EBV), Kaposi's sarcoma herpes virus (KSHV), Herpes virus saimiri (HS), or Marek's disease virus (MDV). Epstein Barr virus (EBV) and Kaposi's sarcoma herpes virus (KSHV) are also examples of a gamma herpesvirus. Typically, the host cell comprises the viral replication transactivator protein that activates the replication.

[0297] The "control elements" or "regulatory sequences" present in an expression vector are those non-translated regions of the vector—origin of replication, selection cassettes, promoters, enhancers, translation initiation signals (Shine Dalgarno sequence or Kozak sequence) introns, a polyadenylation sequence, 5' and 3' untranslated regions—which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable

transcription and translation elements, including ubiquitous promoters and inducible promoters may be used.

[0298] In particular embodiments, a vector for utilization herein include, but are not limited to expression vectors and viral vectors, will include exogenous, endogenous, or heterologous control sequences such as promoters and/or enhancers. An "endogenous" control sequence is one which is naturally linked with a given gene in the genome. An "exogenous" control sequence is one which is placed in juxtaposition to a gene by means of genetic manipulation (*i.e.*, molecular biological techniques) such that transcription of that gene is directed by the linked enhancer/promoter. A "heterologous" control sequence is an exogenous sequence that is from a different species than the cell being genetically manipulated.

[0299] The term "promoter" as used herein refers to a recognition site of a polynucleotide (DNA or RNA) to which an RNA polymerase binds. An RNA polymerase initiates and transcribes polynucleotides operably linked to the promoter. In particular embodiments, promoters operative in mammalian cells comprise an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated and/or another sequence found 70 to 80 bases upstream from the start of transcription, a CNCAAT region where N may be any nucleotide.

[0300] The term "enhancer" refers to a segment of DNA which contains sequences capable of providing enhanced transcription and in some instances can function independent of their orientation relative to another control sequence. An enhancer can function cooperatively or additively with promoters and/or other enhancer elements. The term "promoter/enhancer" refers to a segment of DNA which contains sequences capable of providing both promoter and enhancer functions.

[0301] The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. In one embodiment, the term refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, and/or enhancer) and a second polynucleotide sequence, *e.g.*, a polynucleotide-of-interest, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

[0302] As used herein, the term "constitutive expression control sequence" refers to a promoter, enhancer, or promoter/enhancer that continually or continuously allows for transcription of an

operably linked sequence. A constitutive expression control sequence may be a "ubiquitous" promoter, enhancer, or promoter/enhancer that allows expression in a wide variety of cell and tissue types or a "cell specific," "cell type specific," "cell lineage specific," or "tissue specific" promoter, enhancer, or promoter/enhancer that allows expression in a restricted variety of cell and tissue types, respectively.

[0303] Illustrative ubiquitous expression control sequences suitable for use in particular embodiments presented hereininclude, but are not limited to, a cytomegalovirus (CMV) immediate early promoter, a viral simian virus 40 (SV40) (*e.g.*, early or late), a Moloney murine leukemia virus (MoMLV) LTR promoter, a Rous sarcoma virus (RSV) LTR, a herpes simplex virus (HSV) (thymidine kinase) promoter, H5, P7.5, and P11 promoters from vaccinia virus, an elongation factor 1-alpha (EF1a) promoter, early growth response 1 (EGR1), ferritin H (FerH), ferritin L (FerL), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), eukaryotic translation initiation factor 4A1 (EIF4A1), heat shock 70kDa protein 5 (HSPA5), heat shock protein 90kDa beta, member 1 (HSP90B1), heat shock protein 70kDa (HSP70), β-kinesin (β-KIN), the human ROSA 26 locus (Irions *et al.*, *Nature Biotechnology* 25, 1477 - 1482 (2007)), a Ubiquitin C promoter (UBC), a phosphoglycerate kinase-1 (PGK) promoter, a cytomegalovirus enhancer/chicken β-actin (CAG) promoter, a β-actin promoter and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) promoter (Challita *et al.*, *J Virol.* 69(2):748-55 (1995)).

[0304] In one embodiment, a vector of the present disclosure comprises a MND promoter.

[0305] In one embodiment, a vector of the present disclosure comprises an EF1a promoter comprising the first intron of the human EF1a gene.

[0306] In one embodiment, a vector of the present disclosure comprises an EF1a promoter that lacks the first intron of the human EF1a gene.

[0307] In a particular embodiment, it may be desirable to express a polynucleotide comprising a CAR from a T cell specific promoter.

[0308] As used herein, "conditional expression" may refer to any type of conditional expression including, but not limited to, inducible expression; repressible expression; expression in cells or tissues having a particular physiological, biological, or disease state, *etc.* This definition is not intended to exclude cell type or tissue specific expression. Certain embodiments provide conditional expression of a polynucleotide-of-interest, *e.g.*, expression is controlled by subjecting

a cell, tissue, organism, *etc.*, to a treatment or condition that causes the polynucleotide to be expressed or that causes an increase or decrease in expression of the polynucleotide encoded by the polynucleotide-of-interest.

[0309] Illustrative examples of inducible promoters/systems include, but are not limited to, steroid-inducible promoters such as promoters for genes encoding glucocorticoid or estrogen receptors (inducible by treatment with the corresponding hormone), metallothionine promoter (inducible by treatment with various heavy metals), MX-1 promoter (inducible by interferon), the "GeneSwitch" mifepristone-regulatable system (Sirin *et al.*, 2003, *Gene*, 323:67), the cumate inducible gene switch (WO 2002/088346), tetracycline-dependent regulatory systems, *etc.*

[0310] Conditional expression can also be achieved by using a site specific DNA recombinase. According to certain embodiments, the vector comprises at least one (typically two) site(s) for recombination mediated by a site specific recombinase. As used herein, the terms "recombinase" or "site specific recombinase" include excisive or integrative proteins, enzymes, co-factors or associated proteins that are involved in recombination reactions involving one or more recombination sites (*e.g.*, two, three, four, five, seven, ten, twelve, fifteen, twenty, thirty, fifty, *etc.*), which may be wild-type proteins (*see* Landy, Current Opinion in Biotechnology 3:699-707 (1993)), or mutants, derivatives (*e.g.*, fusion proteins containing the recombination protein sequences or fragments thereof), fragments, and variants thereof. Illustrative examples of recombinases suitable for use herein include, but are not limited to: Cre, Int, IHF, Xis, Flp, Fis, Hin, Gin, ΦC31, Cin, Tn3 resolvase, TndX, XerC, XerD, TnpX, Hjc, Gin, SpCCE1, and ParA.

[0311] The vectors may comprise one or more recombination sites for any of a wide variety of site specific recombinases. It is to be understood that the target site for a site specific recombinase is in addition to any site(s) required for integration of a vector, *e.g.*, a retroviral vector or lentiviral vector. As used herein, the terms "recombination sequence," "recombination site," or "site specific recombination site" refer to a particular nucleic acid sequence to which a recombinase recognizes and binds.

[0312] For example, one recombination site for Cre recombinase is loxP which is a 34 base pair sequence comprising two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence (*see* FIG. 1 of Sauer, B., *Current Opinion in Biotechnology* 5:521-527 (1994)). Other exemplary loxP sites include, but are not limited to: lox511 (Hoess *et*

al., 1996; Bethke and Sauer, 1997), lox5171 (Lee and Saito, 1998), lox2272 (Lee and Saito, 1998), m2 (Langer *et al.*, 2002), lox71 (Albert *et al.*, 1995), and lox66 (Albert *et al.*, 1995).

[0313] Suitable recognition sites for the FLP recombinase include, but are not limited to: FRT (McLeod, *et al.*, 1996), F_1, F_2, F_3 (Schlake and Bode, 1994), F_4, F_5 (Schlake and Bode, 1994), FRT(LE) (Senecoff *et al.*, 1988), FRT(RE) (Senecoff *et al.*, 1988).

[0314] Other examples of recognition sequences are the attB, attP, attL, and attR sequences, which are recognized by the recombinase enzyme λ Integrase, *e.g.,* phi-c31. The φC31 SSR mediates recombination only between the heterotypic sites attB (34 bp in length) and attP (39 bp in length) (Groth *et al.*, 2000). attB and attP, named for the attachment sites for the phage integrase on the bacterial and phage genomes, respectively, both contain imperfect inverted repeats that are likely bound by φC31 homodimers (Groth *et al.*, 2000). The product sites, attL and attR, are effectively inert to further φC31-mediated recombination (Belteki *et al.*, 2003), making the reaction irreversible. For catalyzing insertions, it has been found that attB-bearing DNA inserts into a genomic attP site more readily than an attP site into a genomic attB site (Thyagarajan *et al.*, 2001; Belteki *et al.*, 2003). Thus, typical strategies position by homologous recombination an attP-bearing "docking site" into a defined locus, which is then partnered with an attB-bearing incoming sequence for insertion.

[0315] As used herein, an "internal ribosome entry site" or "IRES" refers to an element that promotes direct internal ribosome entry to the initiation codon, such as ATG, of a cistron (a protein encoding region), thereby leading to the cap-independent translation of the gene. *See, e.g.,* Jackson *et al.*, 1990. *Trends Biochem Sci* 15(12):477-83) and Jackson and Kaminski. 1995. *RNA* 1(10):985-1000. In particular embodiments, the vectors contemplated herein include one or more polynucleotides-of-interest that encode one or more polypeptides. In particular embodiments, to achieve efficient translation of each of the plurality of polypeptides, the polynucleotide sequences can be separated by one or more IRES sequences or polynucleotide sequences encoding self-cleaving polypeptides.

[0316] As used herein, the term "Kozak sequence" refers to a short nucleotide sequence that greatly facilitates the initial binding of mRNA to the small subunit of the ribosome and increases translation. The consensus Kozak sequence is (GCC)RCCATGG, where R is a purine (A or G) (Kozak, 1986. *Cell.* 44(2):283-92, and Kozak, 1987. *Nucleic Acids Res.* 15(20):8125-48). In

particular embodiments, the vectors contemplated herein comprise polynucleotides that have a consensus Kozak sequence and that encode a desired polypeptide, *e.g.*, a CAR.

[0317] In some embodiments, a polynucleotide or cell harboring the polynucleotide utilizes a suicide gene, including an inducible suicide gene to reduce the risk of direct toxicity and/or uncontrolled proliferation. In specific aspects, the suicide gene is not immunogenic to the host harboring the polynucleotide or cell. A certain example of a suicide gene that may be used is caspase-9 or caspase-8 or cytosine deaminase. Caspase-9 can be activated using a specific chemical inducer of dimerization (CID).

[0318] In certain embodiments, vectors comprise gene segments that cause the immune effector cells of the present disclosure, *e.g.*, T cells, to be susceptible to negative selection *in vivo*. By "negative selection" is meant that the infused cell can be eliminated as a result of a change in the *in vivo* condition of the individual. The negative selectable phenotype may result from the insertion of a gene that confers sensitivity to an administered agent, for example, a compound. Negative selectable genes are known in the art, and include, inter alia the following: the Herpes simplex virus type I thymidine kinase (HSV-I TK) gene (Wigler et al., Cell 11:223, 1977) which confers ganciclovir sensitivity; the cellular hypoxanthine phosphoribosyltransferase (HPRT) gene, the cellular adenine phosphoribosyltransferase (APRT) gene, and bacterial cytosine deaminase, (Mullen et al., Proc. Natl. Acad. Sci. USA. 89:33 (1992)).

[0319] In some embodiments, genetically modified immune effector cells, such as T cells, comprise a polynucleotide further comprising a positive marker that enables the selection of cells of the negative selectable phenotype *in vitro*. The positive selectable marker may be a gene which, upon being introduced into the host cell expresses a dominant phenotype permitting positive selection of cells carrying the gene. Genes of this type are known in the art, and include, inter alia, hygromycin-B phosphotransferase gene (hph) which confers resistance to hygromycin B, the amino glycoside phosphotransferase gene (neo or aph) from Tn5 which codes for resistance to the antibiotic G418, the dihydrofolate reductase (DHFR) gene, the adenosine deaminase gene (ADA), and the multi-drug resistance (MDR) gene.

[0320] Preferably, the positive selectable marker and the negative selectable element are linked such that loss of the negative selectable element necessarily also is accompanied by loss of the positive selectable marker. Even more preferably, the positive and negative selectable markers are fused so that loss of one obligatorily leads to loss of the other. An example of a fused

polynucleotide that yields as an expression product a polypeptide that confers both the desired positive and negative selection features described above is a hygromycin phosphotransferase thymidine kinase fusion gene (HyTK). Expression of this gene yields a polypeptide that confers hygromycin B resistance for positive selection in vitro, and ganciclovir sensitivity for negative selection *in vivo*. *See* Lupton S. D., et al, Mol. and Cell. Biology 1 1:3374- 3378, 1991. In addition, in certain embodiments, polynucleotides encoding the chimeric receptors are in retroviral vectors containing the fused gene, particularly those that confer hygromycin B resistance for positive selection *in vitro*, and ganciclovir sensitivity for negative selection *in vivo*, for example the HyTK retroviral vector described in Lupton, S. D. et al. (1991), supra. See also the publications of PCT US91/08442 and PCT/US94/05601, by S. D. Lupton, describing the use of bifunctional selectable fusion genes derived from fusing a dominant positive selectable markers with negative selectable markers.

[0321] Positive selectable markers can, for example, be derived from genes selected from the group consisting of hph, nco, and gpt, and negative selectable markers can, for example, bederived from genes selected from the group consisting of cytosine deaminase, HSV-I TK, VZV TK, HPRT, APRT and gpt. In specific embodiments, markers are bifunctional selectable fusion genes wherein the positive selectable marker is derived from hph or neo, and the negative selectable marker is derived from cytosine deaminase or a TK gene or selectable marker.

[0322] Viral Vectors

[0323] In particular embodiments, a cell (*e.g.*, an immune effector cell) is transduced with a retroviral vector, *e.g.*, a lentiviral vector, encoding a CAR. For example, an immune effector cell is transduced with a vector encoding a CAR that comprises a murine anti-BCMA antibody or antigen binding fragment thereof that binds a BCMA polypeptide,, *e.g.*, a human BCMA polypeptide, with an intracellular signaling domain of CD3ζ, CD28, 4-1BB, Ox40, or any combinations thereof. Thus, these transduced cells can elicit a CAR-mediated cytotoxic response.

[0324] Retroviruses are a common tool for gene delivery (Miller, 2000, *Nature*. 357: 455-460). In particular embodiments, a retrovirus is used to deliver a polynucleotide encoding a chimeric antigen receptor (CAR) to a cell. As used herein, the term “retrovirus” refers to an RNA virus that reverse transcribes its genomic RNA into a linear double-stranded DNA copy and subsequently covalently integrates its genomic DNA into a host genome. Once the virus is

integrated into the host genome, it is referred to as a "provirus." The provirus serves as a template for RNA polymerase II and directs the expression of RNA molecules which encode the structural proteins and enzymes needed to produce new viral particles.

[0325] Illustrative retroviruses suitable for use in particular embodiments, include, but are not limited to: Moloney murine leukemia virus (MMuLV), Moloney murine sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV)) and lentivirus.

[0326] As used herein, the term "lentivirus" refers to a group (or genus) of complex retroviruses. Illustrative lentiviruses include, but are not limited to: HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi virus (VMV) virus; the caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). In one embodiment, HIV based vector backbones (*i.e.*, HIV cis-acting sequence elements) are utilized. In particular embodiments, a lentivirus is used to deliver a polynucleotide comprising a CAR to a cell.

[0327] Retroviral vectors and more particularly lentiviral vectors may be used in practicing particular embodiments disclosed herein. Accordingly, the term "retrovirus" or "retroviral vector", as used herein is meant to include "lentivirus" and "lentiviral vectors" respectively.

[0328] The term "vector" is used herein to refer to a nucleic acid molecule capable transferring or transporting another nucleic acid molecule. The transferred nucleic acid is generally linked to, *e.g.*, inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell, or may include sequences sufficient to allow integration into host cell DNA. Useful vectors include, for example, plasmids (*e.g.*, DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. Useful viral vectors include, *e.g.*, replication defective retroviruses and lentiviruses.

[0329] As will be evident to one of skill in the art, the term "viral vector" is widely used to refer either to a nucleic acid molecule (*e.g.*, a transfer plasmid) that includes virus-derived nucleic acid elements that typically facilitate transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. Viral particles will

typically include various viral components and sometimes also host cell components in addition to nucleic acid(s).

[0330] The term viral vector may refer either to a virus or viral particle capable of transferring a nucleic acid into a cell or to the transferred nucleic acid itself. Viral vectors and transfer plasmids contain structural and/or functional genetic elements that are primarily derived from a virus. The term "retroviral vector" refers to a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, that are primarily derived from a retrovirus. The term "lentiviral vector" refers to a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, including LTRs that are primarily derived from a lentivirus. The term "hybrid vector" refers to a vector, LTR or other nucleic acid containing both retroviral, *e.g.,* lentiviral, sequences and non-lentiviral viral sequences. In one embodiment, a hybrid vector refers to a vector or transfer plasmid comprising retroviral *e.g.,* lentiviral, sequences for reverse transcription, replication, integration and/or packaging.

[0331] In particular embodiments, the terms "lentiviral vector" and "lentiviral expression vector" may be used to refer to lentiviral transfer plasmids and/or infectious lentiviral particles. Where reference is made herein to elements such as cloning sites, promoters, regulatory elements, heterologous nucleic acids, *etc.*, it is to be understood that the sequences of these elements are present in RNA form in the lentiviral particles of the present disclosure and are present in DNA form in the DNA plasmids of the present disclosure.

[0332] At each end of the provirus are structures called "long terminal repeats" or "LTRs." The term "long terminal repeat (LTR)" refers to domains of base pairs located at the ends of retroviral DNAs which, in their natural sequence context, are direct repeats and contain U3, R and U5 regions. LTRs generally provide functions fundamental to the expression of retroviral genes (*e.g.,* promotion, initiation and polyadenylation of gene transcripts) and to viral replication. The LTR contains numerous regulatory signals including transcriptional control elements, polyadenylation signals and sequences needed for replication and integration of the viral genome. The viral LTR is divided into three regions called U3, R and U5. The U3 region contains the enhancer and promoter elements. The U5 region is the sequence between the primer binding site and the R region and contains the polyadenylation sequence. The R (repeat) region is flanked by the U3 and U5 regions. The LTR composed of U3, R and U5 regions and appears at both the 5' and 3' ends of the viral genome. Adjacent to the 5' LTR are sequences necessary

for reverse transcription of the genome (the tRNA primer binding site) and for efficient packaging of viral RNA into particles (the Psi site).

[0333] As used herein, the term "packaging signal" or "packaging sequence" refers to sequences located within the retroviral genome which are required for insertion of the viral RNA into the viral capsid or particle, see *e.g.*, Clever *et al.*, 1995. *J. of Virology*, Vol. 69, No. 4; pp. 2101–2109. Several retroviral vectors use the minimal packaging signal (also referred to as the psi [Ψ] sequence) needed for encapsidation of the viral genome. Thus, as used herein, the terms "packaging sequence," "packaging signal," "psi" and the symbol "Ψ," are used in reference to the non-coding sequence required for encapsidation of retroviral RNA strands during viral particle formation.

[0334] In various embodiments, vectors comprise modified 5' LTR and/or 3' LTRs. Either or both of the LTR may comprise one or more modifications including, but not limited to, one or more deletions, insertions, or substitutions. Modifications of the 3' LTR are often made to improve the safety of lentiviral or retroviral systems by rendering viruses replication-defective. As used herein, the term "replication-defective" refers to virus that is not capable of complete, effective replication such that infective virions are not produced (*e.g.*, replication-defective lentiviral progeny). The term "replication-competent" refers to wild-type virus or mutant virus that is capable of replication, such that viral replication of the virus is capable of producing infective virions (*e.g.*, replication-competent lentiviral progeny).

[0335] "Self-inactivating" (SIN) vectors refers to replication-defective vectors, *e.g.*, retroviral or lentiviral vectors, in which the right (3') LTR enhancer-promoter region, known as the U3 region, has been modified (*e.g.*, by deletion or substitution) to prevent viral transcription beyond the first round of viral replication. This is because the right (3') LTR U3 region is used as a template for the left (5') LTR U3 region during viral replication and, thus, the viral transcript cannot be made without the U3 enhancer-promoter. In a further embodiment, the 3' LTR is modified such that the U5 region is replaced, for example, with an ideal poly(A) sequence. It should be noted that modifications to the LTRs such as modifications to the 3' LTR, the 5' LTR, or both 3' and 5' LTRs, are also included herein.

[0336] An additional safety enhancement is provided by replacing the U3 region of the 5' LTR with a heterologous promoter to drive transcription of the viral genome during production of viral particles. Examples of heterologous promoters which can be used include, for example,

viral simian virus 40 (SV40) (*e.g.*, early or late), cytomegalovirus (CMV) (*e.g.*, immediate early), Moloney murine leukemia virus (MoMLV), Rous sarcoma virus (RSV), and herpes simplex virus (HSV) (thymidine kinase) promoters. Typical promoters are able to drive high levels of transcription in a Tat-independent manner. This replacement reduces the possibility of recombination to generate replication-competent virus because there is no complete U3 sequence in the virus production system. In certain embodiments, the heterologous promoter has additional advantages in controlling the manner in which the viral genome is transcribed. For example, the heterologous promoter can be inducible, such that transcription of all or part of the viral genome will occur only when the induction factors are present. Induction factors include, but are not limited to, one or more chemical compounds or the physiological conditions such as temperature or pH, in which the host cells are cultured.

[0337] In some embodiments, viral vectors comprise a TAR element. The term "TAR" refers to the "trans-activation response" genetic element located in the R region of lentiviral (*e.g.*, HIV) LTRs. This element interacts with the lentiviral trans-activator (tat) genetic element to enhance viral replication. However, this element is not required in embodiments wherein the U3 region of the 5' LTR is replaced by a heterologous promoter.

[0338] The "R region" refers to the region within retroviral LTRs beginning at the start of the capping group (*i.e.*, the start of transcription) and ending immediately prior to the start of the poly A tract. The R region is also defined as being flanked by the U3 and U5 regions. The R region plays a role during reverse transcription in permitting the transfer of nascent DNA from one end of the genome to the other.

[0339] As used herein, the term "FLAP element" refers to a nucleic acid whose sequence includes the central polypurine tract and central termination sequences (cPPT and CTS) of a retrovirus, *e.g.*, HIV-1 or HIV-2. Suitable FLAP elements are described in U.S. Pat. No. 6,682,907 and in Zennou, *et al.*, 2000, *Cell*, 101:173. During HIV-1 reverse transcription, central initiation of the plus-strand DNA at the central polypurine tract (cPPT) and central termination at the central termination sequence (CTS) lead to the formation of a three-stranded DNA structure: the HIV-1 central DNA flap. While not wishing to be bound by any theory, the DNA flap may act as a cis-active determinant of lentiviral genome nuclear import and/or may increase the titer of the virus. In particular embodiments, the retroviral or lentiviral vector backbones comprise one or more FLAP elements upstream or downstream of the heterologous

genes of interest in the vectors. For example, in particular embodiments a transfer plasmid includes a FLAP element. In one embodiment, a vector comprises a FLAP element isolated from HIV-1.

[0340] In one embodiment, retroviral or lentiviral transfer vectors comprise one or more export elements. The term "export element" refers to a cis-acting post-transcriptional regulatory element which regulates the transport of an RNA transcript from the nucleus to the cytoplasm of a cell. Examples of RNA export elements include, but are not limited to, the human immunodeficiency virus (HIV) rev response element (RRE) (*see e.g.,* Cullen *et al.*, 1991. *J. Virol.* 65: 1053; and Cullen *et al.*, 1991. *Cell* 58: 423), and the hepatitis B virus post-transcriptional regulatory element (HPRE). Generally, the RNA export element is placed within the 3' UTR of a gene, and can be inserted as one or multiple copies.

[0341] In particular embodiments, expression of heterologous sequences in viral vectors is increased by incorporating posttranscriptional regulatory elements, efficient polyadenylation sites, and optionally, transcription termination signals into the vectors. A variety of posttranscriptional regulatory elements can increase expression of a heterologous nucleic acid at the protein, *e.g.,* woodchuck hepatitis virus posttranscriptional regulatory element (WPRE; Zufferey *et al.*, 1999, *J. Virol.*, 73:2886); the posttranscriptional regulatory element present in hepatitis B virus (HPRE) (Huang *et al., Mol. Cell. Biol.*, 5:3864); and the like (Liu *et al.,* 1995, *Genes Dev.*, 9:1766). In particular embodiments, a vector can comprise a posttranscriptional regulatory element such as a WPRE or HPRE

[0342] In particular embodiments, vectors lack or do not comprise a posttranscriptional regulatory element (PTE) such as a WPRE or HPRE because in some instances these elements increase the risk of cellular transformation and/or do not substantially or significantly increase the amount of mRNA transcript or increase mRNA stability. Therefore, in some embodiments, vectors lack or do not comprise a PTE. In other embodiments, vectors lack or do not comprise a WPRE or HPRE as an added safety measure.

[0343] Elements directing the efficient termination and polyadenylation of the heterologous nucleic acid transcripts increases heterologous gene expression. Transcription termination signals are generally found downstream of the polyadenylation signal. In particular embodiments, vectors comprise a polyadenylation sequence 3′ of a polynucleotide encoding a polypeptide to be expressed. The term "polyA site" or "polyA sequence" as used herein denotes

a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript by RNA polymerase II. Polyadenylation sequences can promote mRNA stability by addition of a polyA tail to the 3′ end of the coding sequence and thus, contribute to increased translational efficiency. Efficient polyadenylation of the recombinant transcript is desirable as transcripts lacking a poly A tail are unstable and are rapidly degraded. Illustrative examples of polyA signals that can be used in a vector herein, include an ideal polyA sequence (*e.g.*, AATAAA, ATTAAA, AGTAAA), a bovine growth hormone polyA sequence (BGHpA), a rabbit β-globin polyA sequence (rβgpA), or another suitable heterologous or endogenous polyA sequence known in the art.

[0344] In certain embodiments, a retroviral or lentiviral vector further comprises one or more insulator elements. Insulators elements may contribute to protecting lentivirus-expressed sequences, *e.g.*, therapeutic polypeptides, from integration site effects, which may be mediated by cis-acting elements present in genomic DNA and lead to deregulated expression of transferred sequences (*i.e.*, position effect; *see, e.g.*, Burgess-Beusse *et al.*, 2002, *Proc. Natl. Acad. Sci., USA*, 99:16433; and Zhan *et al.*, 2001, *Hum. Genet.*, 109:471). In some embodiments, transfer vectors comprise one or more insulator element the 3′ LTR and upon integration of the provirus into the host genome, the provirus comprises the one or more insulators at both the 5′ LTR or 3′ LTR, by virtue of duplicating the 3′ LTR. Suitable insulators for use herein include, but are not limited to, the chicken β-globin insulator (see Chung et al., 1993. Cell 74:505; Chung et al., 1997. PNAS 94:575; and Bell et al., 1999. Cell 98:387, incorporated by reference herein). Examples of insulator elements include, but are not limited to, an insulator from an β-globin locus, such as chicken HS4.

[0345] According to certain specific embodiments, most or all of the viral vector backbone sequences are derived from a lentivirus, e.g., HIV-1. However, it is to be understood that many different sources of retroviral and/or lentiviral sequences can be used, or combined and numerous substitutions and alterations in certain of the lentiviral sequences may be accommodated without impairing the ability of a transfer vector to perform the functions described herein. Moreover, a variety of lentiviral vectors are known in the art, *see* Naldini *et al.*, (1996a, 1996b, and 1998); Zufferey *et al.*, (1997); Dull *et al.*, 1998, U.S. Pat. Nos. 6,013,516; and 5,994,136, many of which may be adapted to produce a viral vector or transfer plasmid of the present disclosure.

[0346] In various embodiments, a vector described herein can comprise a promoter operably linked to a polynucleotide encoding a CAR polypeptide. The vectors may have one or more LTRs, wherein either LTR comprises one or more modifications, such as one or more nucleotide substitutions, additions, or deletions. The vectors may further comprise one of more accessory elements to increase transduction efficiency (*e.g.*, a cPPT/FLAP), viral packaging (*e.g.*, a Psi (Ψ) packaging signal, RRE), and/or other elements that increase therapeutic gene expression (*e.g.*, poly (A) sequences), and may optionally comprise a WPRE or HPRE.

[0347] In a particular embodiment, the transfer vector comprises a left (5') retroviral LTR; a central polypurine tract/DNA flap (cPPT/FLAP); a retroviral export element; a promoter active in a T cell, operably linked to a polynucleotide encoding CAR polypeptide contemplated herein; and a right (3') retroviral LTR; and optionally a WPRE or HPRE.

[0348] In a particular embodiment, the transfer vector comprises a left (5') retroviral LTR; a retroviral export element; a promoter active in a T cell, operably linked to a polynucleotide encoding CAR polypeptide contemplated herein; a right (3') retroviral LTR; and a poly (A) sequence; and optionally a WPRE or HPRE. In another particular embodiment, provided herein i a lentiviral vector comprising: a left (5') LTR; a cPPT/FLAP; an RRE; a promoter active in a T cell, operably linked to a polynucleotide encoding CAR polypeptide contemplated herein; a right (3') LTR; and a polyadenylation sequence; and optionally a WPRE or HPRE.

[0349] In a certain embodiment, provide herein is a lentiviral vector comprising: a left (5') HIV-1 LTR; a Psi (Ψ) packaging signal; a cPPT/FLAP; an RRE; a promoter active in a T cell, operably linked to a polynucleotide encoding CAR polypeptide contemplated herein; a right (3') self-inactivating (SIN) HIV-1 LTR; and a rabbit β-globin polyadenylation sequence; and optionally a WPRE or HPRE.

[0350] In another embodiment, provided herein is a vector comprising: at least one LTR; a central polypurine tract/DNA flap (cPPT/FLAP); a retroviral export element; and a promoter active in a T cell, operably linked to a polynucleotide encoding CAR polypeptide contemplated herein; and optionally a WPRE or HPRE.

[0351] In particular embodiment, provided herein is a vector comprising at least one LTR; a cPPT/FLAP; an RRE; a promoter active in a T cell, operably linked to a polynucleotide encoding CAR polypeptide contemplated herein; and a polyadenylation sequence; and optionally a WPRE or HPRE.

[0352] In a certain embodiment, provided herein is at least one SIN HIV-1 LTR; a Psi (Ψ) packaging signal; a cPPT/FLAP; an RRE; a promoter active in a T cell, operably linked to a polynucleotide encoding CAR polypeptide contemplated herein; and a rabbit β-globin polyadenylation sequence; and optionally a WPRE or HPRE.

[0353] In various embodiments, the vector is an integrating viral vector.

[0354] In various other embodiments, the vector is an episomal or non-integrating viral vector.

[0355] In various embodiments, vectors contemplated herein, comprise non-integrating or integration defective retrovirus. In one embodiment, an "integration defective" retrovirus or lentivirus refers to retrovirus or lentivirus having an integrase that lacks the capacity to integrate the viral genome into the genome of the host cells. In various embodiments, the integrase protein is mutated to specifically decrease its integrase activity. Integration-incompetent lentiviral vectors are obtained by modifying the pol gene encoding the integrase protein, resulting in a mutated pol gene encoding an integrative deficient integrase. Such integration-incompetent viral vectors have been described in patent application WO 2006/010834, which is herein incorporated by reference in its entirety.

[0356] Illustrative mutations in the HIV-1 pol gene suitable to reduce integrase activity include, but are not limited to: H12N, H12C, H16C, H16V, S81 R, D41A, K42A, H51A, Q53C, D55V, D64E, D64V, E69A, K71A, E85A, E87A, D116N, D1161, D116A, N120G, N1201, N120E, E152G, E152A, D35E, K156E, K156A, E157A, K159E, K159A, K160A, R166A, D167A, E170A, H171A, K173A, K186Q, K186T, K188T, E198A, R199c, R199T, R199A, D202A, K211A, Q214L, Q216L, Q221 L, W235F, W235E, K236S, K236A, K246A, G247W, D253A, R262A, R263A and K264H.

[0357] Illustrative mutations in the HIV-1 pol gene suitable to reduce integrase activity include, but are not limited to: D64E, D64V, E92K, D116N, D1161, D116A, N120G, N1201, N120E, E152G, E152A, D35E, K156E, K156A, E157A, K159E, K159A, W235F, and W235E.

[0358] In a particular embodiment, an integrase comprises a mutation in one or more of amino acids, D64, D116 or E152. In one embodiment, an integrase comprises a mutation in the amino acids, D64, D116 and E152. In a particular embodiment, a defective HIV-1 integrase comprises a D64V mutation.

[0359] A "host cell" includes cells electroporated, transfected, infected, or transduced *in vivo, ex vivo,* or *in vitro* with a recombinant vector or a polynucleotide disclosed herein. Host cells may

include packaging cells, producer cells, and cells infected with viral vectors. In particular embodiments, host cells infected with a viral vector disclosed herein are administered to a subject in need of therapy. In certain embodiments, the term "target cell" is used interchangeably with host cell and refers to transfected, infected, or transduced cells of a desired cell type. In particular embodiments, the target cell is a T cell.

[0360] Large scale viral particle production is often necessary to achieve a reasonable viral titer. Viral particles are produced by transfecting a transfer vector into a packaging cell line that comprises viral structural and/or accessory genes, *e.g.*, gag, pol, env, tat, rev, vif, vpr, vpu, vpx, or nef genes or other retroviral genes.

[0361] As used herein, the term "packaging vector" refers to an expression vector or viral vector that lacks a packaging signal and comprises a polynucleotide encoding one, two, three, four or more viral structural and/or accessory genes. Typically, the packaging vectors are included in a packaging cell, and are introduced into the cell via transfection, transduction or infection. Methods for transfection, transduction or infection are well known by those of skill in the art. A retroviral/lentiviral transfer vector disclosed herein can be introduced into a packaging cell line, via transfection, transduction or infection, to generate a producer cell or cell line. The packaging vectors disclosed herein can be introduced into human cells or cell lines by standard methods including, *e.g.*, calcium phosphate transfection, lipofection or electroporation. In some embodiments, the packaging vectors are introduced into the cells together with a dominant selectable marker, such as neomycin, hygromycin, puromycin, blastocidin, zeocin, thymidine kinase, DHFR, Gln synthetase or ADA, followed by selection in the presence of the appropriate drug and isolation of clones. A selectable marker gene can be linked physically to genes encoding by the packaging vector, *e.g.*, by IRES or self-cleaving viral peptides.

[0362] Viral envelope proteins (env) determine the range of host cells which can ultimately be infected and transformed by recombinant retroviruses generated from the cell lines. In the case of lentiviruses, such as HIV-1, HIV-2, SIV, FIV and EIV, the env proteins include gp41 and gp120. Preferably, the viral env proteins expressed by packaging cells disclosed herein are encoded on a separate vector from the viral gag and pol genes, as has been previously described.

[0363] Illustrative examples of retroviral-derived env genes which can be employed herein include, but are not limited to: MLV envelopes, 10A1 envelope, BAEV, FeLV-B, RD114, SSAV, Ebola, Sendai, FPV (Fowl plague virus), and influenza virus envelopes. Similarly, genes

encoding envelopes from RNA viruses (*e.g.,* RNA virus families of Picornaviridae, Calciviridae, Astroviridae, Togaviridae, Flaviviridae, Coronaviridae, Paramyxoviridae, Rhabdoviridae, Filoviridae, Orthomyxoviridae, Bunyaviridae, Arenaviridae, Reoviridae, Birnaviridae, Retroviridae) as well as from the DNA viruses (families of Hepadnaviridae, Circoviridae, Parvoviridae, Papovaviridae, Adenoviridae, Herpesviridae, Poxyiridae, and Iridoviridae) may be utilized. Representative examples include FeLV, VEE, HFVW, WDSV, SFV, Rabies, ALV, BIV, BLV, EBV, CAEV, SNV, ChTLV, STLV, MPMV, SMRV, RAV, FuSV, MH2, AEV, AMV, CT10, and EIAV.

[0364] In other embodiments, envelope proteins for pseudotyping a virus in connection with the present disclosure include, but are not limited to, any from the following viruses: Influenza A such as H1N1, H1N2, H3N2 and H5N1 (bird flu), Influenza B, Influenza C virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rotavirus, any virus of the Norwalk virus group, enteric adenoviruses, parvovirus, Dengue fever virus, Monkey pox, Mononegavirales, Lyssavirus such as rabies virus, Lagos bat virus, Mokola virus, Duvenhage virus, European bat virus 1 & 2 and Australian bat virus, Ephemerovirus, Vesiculovirus, Vesicular Stomatitis Virus (VSV), Herpesviruses such as Herpes simplex virus types 1 and 2, varicella zoster, cytomegalovirus, Epstein-Bar virus (EBV), human herpesviruses (HHV), human herpesvirus type 6 and 8, Human immunodeficiency virus (HIV), papilloma virus, murine gammaherpesvirus, Arenaviruses such as Argentine hemorrhagic fever virus, Bolivian hemorrhagic fever virus, Sabia-associated hemorrhagic fever virus, Venezuelan hemorrhagic fever virus, Lassa fever virus, Machupo virus, Lymphocytic choriomeningitis virus (LCMV), Bunyaviridiae such as Crimean-Congo hemorrhagic fever virus, Hantavirus, hemorrhagic fever with renal syndrome causing virus, Rift Valley fever virus, Filoviridae (filovirus) including Ebola hemorrhagic fever and Marburg hemorrhagic fever, Flaviviridae including Kaysanur Forest disease virus, Omsk hemorrhagic fever virus, Tick-borne encephalitis causing virus and Paramyxoviridae such as Hendra virus and Nipah virus, variola major and variola minor (smallpox), alphaviruses such as Venezuelan equine encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, SARS-associated coronavirus (SARS-CoV), West Nile virus, and any encephalitis causing virus.

[0365] In one embodiment, provided herein are packaging cells which produce recombinant retrovirus, *e.g.,* lentivirus, pseudotyped with the VSV-G glycoprotein.

[0366] The terms "pseudotype" or "pseudotyping" as used herein, refer to a virus whose viral envelope proteins have been substituted with those of another virus possessing preferable characteristics. For example, HIV can be pseudotyped with vesicular stomatitis virus G-protein (VSV-G) envelope proteins, which allows HIV to infect a wider range of cells because HIV envelope proteins (encoded by the env gene) normally target the virus to CD4+ presenting cells. In one embodiment, lentiviral envelope proteins are pseudotyped with VSV-G. In one embodiment, provided herein are packaging cells which produce recombinant retrovirus, *e.g.*, lentivirus, pseudotyped with the VSV-G envelope glycoprotein.

[0367] As used herein, the term "packaging cell lines" is used in reference to cell lines that do not contain a packaging signal, but do stably or transiently express viral structural proteins and replication enzymes (*e.g.*, gag, pol and env) which are necessary for the correct packaging of viral particles. Any suitable cell line can be employed to prepare packaging cells in connection with the present disclosure. Generally, the cells are mammalian cells. In a particular embodiment, the cells used to produce the packaging cell line are human cells. Suitable cell lines which can be used include, for example, CHO cells, BHK cells, MDCK cells, C3H 10T1/2 cells, FLY cells, Psi-2 cells, BOSC 23 cells, PA317 cells, WEHI cells, COS cells, BSC 1 cells, BSC 40 cells, BMT 10 cells, VERO cells, W138 cells, MRC5 cells, A549 cells, HT1080 cells, 293 cells, 293T cells, B-50 cells, 3T3 cells, NIH3T3 cells, HepG2 cells, Saos-2 cells, Huh7 cells, HeLa cells, W163 cells, 211 cells, and 211A cells. In specific embodiments, the packaging cells are 293 cells, 293T cells, or A549 cells. In another specific embodiment, the cells are A549 cells.

[0368] As used herein, the term "producer cell line" refers to a cell line which is capable of producing recombinant retroviral particles, comprising a packaging cell line and a transfer vector construct comprising a packaging signal. The production of infectious viral particles and viral stock solutions may be carried out using conventional techniques. Methods of preparing viral stock solutions are known in the art and are illustrated by, *e.g.*, Y. Soneoka *et al.* (1995) *Nucl. Acids Res.* 23:628-633, and N. R. Landau *et al.* (1992) *J. Virol.* 66:5110-5113. Infectious virus particles may be collected from the packaging cells using conventional techniques. For example, the infectious particles can be collected by cell lysis, or collection of the supernatant of the cell culture, as is known in the art. Optionally, the collected virus particles may be purified if desired. Suitable purification techniques are well known to those skilled in the art.

[0369] The delivery of a gene(s) or other polynucleotide sequence using a retroviral or lentiviral vector by means of viral infection rather than by transfection is referred to as "transduction." In one embodiment, retroviral vectors are transduced into a cell through infection and provirus integration. In certain embodiments, a target cell, *e.g.,* a T cell, is "transduced" if it comprises a gene or other polynucleotide sequence delivered to the cell by infection using a viral or retroviral vector. In particular embodiments, a transduced cell comprises one or more genes or other polynucleotide sequences delivered by a retroviral or lentiviral vector in its cellular genome.

[0370] In particular embodiments, host cells transduced with a viral vector as disclosed herein that expresses one or more polypeptides are administered to a subject to treat and/or prevent a B cell malignancy. Other methods relating to the use of viral vectors in gene therapy, which may be utilized according to certain embodiments herein, can be found in, *e.g.,* Kay, M. A. (1997) *Chest* 111(6 Supp.):138S-142S; Ferry, N. and Heard, J. M. (1998) *Hum. Gene Ther*. 9:1975-81; Shiratory, Y. *et al.* (1999) Liver 19:265-74; Oka, K. *et al.* (2000) *Curr. Opin. Lipidol*. 11:179-86; Thule, P. M. and Liu, J. M. (2000) *Gene Ther*. 7:1744-52; Yang, N. S. (1992) *Crit. Rev. Biotechnol*. 12:335-56; Alt, M. (1995) *J. Hepatol*. 23:746-58; Brody, S. L. and Crystal, R. G. (1994) *Ann. N.Y. Acad. Sci*. 716:90-101; Strayer, D. S. (1999) *Expert Opin. Investig. Drugs* 8:2159-2172; Smith-Arica, J. R. and Bartlett, J. S. (2001) *Curr. Cardiol. Rep*. 3:43-49; and Lee, H. C. *et al.* (2000) *Nature* 408:483-8.

5.6. Genetically Modified Cells

[0371] In particular embodiments, disclosed herein are cells genetically modified to express the CARs contemplated herein, for use in the treatment of B cell related conditions. As used herein, the term "genetically engineered" or "genetically modified" refers to the addition of extra genetic material in the form of DNA or RNA into the total genetic material in a cell. The terms, "genetically modified cells," "modified cells," and, "redirected cells," are used interchangeably. As used herein, the term "gene therapy" refers to the introduction of extra genetic material in the form of DNA or RNA into the total genetic material in a cell that restores, corrects, or modifies expression of a gene, or for the purpose of expressing a therapeutic polypeptide, *e.g.,* a CAR.

[0372] In particular embodiments, the CARs contemplated herein are introduced and expressed in immune effector cells so as to redirect their specificity to a target antigen of interest, *e.g.,* a BCMA polypeptide. An "immune effector cell," is any cell of the immune system that has one

or more effector functions (*e.g.,* cytotoxic cell killing activity, secretion of cytokines, induction of ADCC and/or CDC).

[0373] Immune effector cells of the present disclosure can be autologous/autogeneic ("self") or non-autologous ("non-self," *e.g.,* allogeneic, syngeneic or xenogeneic).

[0374] "Autologous," as used herein, refers to cells from the same subject.

[0375] "Allogeneic," as used herein, refers to cells of the same species that differ genetically to the cell in comparison.

[0376] "Syngeneic," as used herein, refers to cells of a different subject that are genetically identical to the cell in comparison.

[0377] "Xenogeneic," as used herein, refers to cells of a different species to the cell in comparison. In certain embodiments, the cells of the present disclosure are allogeneic.

[0378] Illustrative immune effector cells used with the CARs contemplated herein include T lymphocytes. The terms "T cell" or "T lymphocyte" are art-recognized and are intended to include thymocytes, immature T lymphocytes, mature T lymphocytes, resting T lymphocytes, or activated T lymphocytes. A T cell can be a T helper (Th) cell, for example a T helper 1 (Th1) or a T helper 2 (Th2) cell. The T cell can be a helper T cell (HTL; $CD4^+$ T cell) $CD4^+$ T cell, a cytotoxic T cell (CTL; $CD8^+$ T cell), $CD4^+CD8^+$ T cell, $CD4^-CD8^-$ T cell, or any other subset of T cells. Other illustrative populations of T cells suitable for use in particular embodiments include naïve T cells and memory T cells.

[0379] As would be understood by the skilled person, other cells may also be used as immune effector cells with the CARs as described herein. In particular, immune effector cells also include NK cells, NKT cells, neutrophils, and macrophages. Immune effector cells also include progenitors of effector cells wherein such progenitor cells can be induced to differentiate into an immune effector cells *in vivo* or *in vitro*. Thus, in particular embodiments, immune effector cell includes progenitors of immune effectors cells such as hematopoietic stem cells (HSCs) contained within the $CD34_+$ population of cells derived from cord blood, bone marrow or mobilized peripheral blood which upon administration in a subject differentiate into mature immune effector cells, or which can be induced *in vitro* to differentiate into mature immune effector cells.

[0380] As used herein, immune effector cells genetically engineered to contain BCMA-specific CAR may be referred to as, "BCMA-specific redirected immune effector cells."

[0381] The term, "$CD34^+$ cell" as used herein refers to a cell expressing the CD34 protein on its cell surface. "CD34" as used herein refers to a cell surface glycoprotein (*e.g.,* sialomucin protein) that often acts as a cell-cell adhesion factor and is involved in T cell entrance into lymph nodes. The $CD34^+$ cell population contains hematopoietic stem cells (HSC), which upon administration to a patient differentiate and contribute to all hematopoietic lineages, including T cells, NK cells, NKT cells, neutrophils and cells of the monocyte/macrophage lineage.

[0382] In certain embodiments, provided herein are methods for making the immune effector cells which express the CAR contemplated herein. In one embodiment, the method comprises transfecting or transducing immune effector cells isolated from an individual such that the immune effector cells express one or more CAR as described herein. In certain embodiments, the immune effector cells are isolated from an individual and genetically modified without further manipulation *in vitro*. Such cells can then be directly re-administered into the individual. In further embodiments, the immune effector cells are first activated and stimulated to proliferate *in vitro* prior to being genetically modified to express a CAR. In this regard, the immune effector cells may be cultured before and/or after being genetically modified (i.e., transduced or transfected to express a CAR contemplated herein).

[0383] In particular embodiments, prior to *in vitro* manipulation or genetic modification of the immune effector cells described herein, the source of cells is obtained from a subject. In particular embodiments, the CAR-modified immune effector cells comprise T cells. T cells can be obtained from a number of sources including, but not limited to, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled person, such as sedimentation, *e.g.,* FICOLL™ separation. In one embodiment, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocyte, B cells, other nucleated white blood cells, red blood cells, and platelets. In one embodiment, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing. The cells can be washed with PBS or with another suitable solution that lacks calcium, magnesium, and most, if not all other, divalent cations. As would be appreciated by those of ordinary skill in the art, a

washing step may be accomplished by methods known to those in the art, such as by using a semiautomated flowthrough centrifuge. For example, the Cobe 2991 cell processor, the Baxter CytoMate, or the like. After washing, the cells may be resuspended in a variety of biocompatible buffers or other saline solution with or without buffer. In certain embodiments, the undesirable components of the apheresis sample may be removed in the cell directly resuspended culture media.

[0384] In certain embodiments, T cells are isolated from peripheral blood mononuclear cells (PBMCs) by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient. A specific subpopulation of T cells, expressing one or more of the following markers: CD3, CD28, CD4, CD8, CD45RA, and CD45RO, can be further isolated by positive or negative selection techniques. In one embodiment, a specific subpopulation of T cells, expressing CD3, CD28, CD4, CD8, CD45RA, and CD45RO is further isolated by positive or negative selection techniques. For example, enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method for use herein is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for $CD4^+$ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. Flow cytometry and cell sorting may also be used to isolate cell populations of interest for use accordance with the present disclosure.

[0385] PBMC may be directly genetically modified to express CARs using methods contemplated herein. In certain embodiments, after isolation of PBMC, T lymphocytes are further isolated and in certain embodiments, both cytotoxic and helper T lymphocytes can be sorted into naïve, memory, and effector T cell subpopulations either before or after genetic modification and/or expansion.

[0386] $CD8^+$ cells can be obtained by using standard methods. In some embodiments, $CD8^+$ cells are further sorted into naive, central memory, and effector cells by identifying cell surface antigens that are associated with each of those types of $CD8^+$ cells.

[0387] In certain embodiments, naive $CD8^+$ T lymphocytes are characterized by the expression of phenotypic markers of naive T cells including CD62L, CCR7, CD28, CD3, CD 127, and CD45RA.

[0388] In particular embodiments, memory T cells are present in both $CD62L^+$ and $CD62L^-$ subsets of $CD8^+$ peripheral blood lymphocytes. PBMC are sorted into $CD62L^-CD8^+$ and $CD62L^+CD8^+$ fractions after staining with anti-CD8 and anti-CD62L antibodies. I n some embodiments, the expression of phenotypic markers of central memory T cells include CD45RO, CD62L, CCR7, CD28, CD3, and CD127 and are negative for granzyme B. In some embodiments, central memory T cells are $CD45RO^+$, $CD62L^+$, $CD8^+$ T cells.

[0389] In some embodiments, effector T cells are negative for CD62L, CCR7, CD28, and CD127, and positive for granzyme B and perforin.

[0390] In certain embodiments, $CD4^+$ T cells are further sorted into subpopulations. For example, $CD4^+$ T helper cells can be sorted into naive, central memory, and effector cells by identifying cell populations that have cell surface antigens. $CD4^+$ lymphocytes can be obtained by standard methods. In some embodiments, naïve $CD4^+$ T lymphocytes are $CD45RO^-$, $CD45RA^+$, $CD62L^+$ $CD4^+$ T cell. In some embodiments, central memory $CD4^+$ cells are CD62L positive and CD45RO positive. In some embodiments, effector $CD4^+$ cells are CD62L and CD45RO negative.

[0391] The immune effector cells, such as T cells, can be genetically modified following isolation using known methods, or the immune effector cells can be activated and expanded (or differentiated in the case of progenitors) *in vitro* prior to being genetically modified. In a particular embodiment, the immune effector cells, such as T cells, are genetically modified with the chimeric antigen receptors contemplated herein (*e.g.*, transduced with a viral vector comprising a nucleic acid encoding a CAR) and then are activated and expanded *in vitro*. In various embodiments, T cells can be activated and expanded before or after genetic modification to express a CAR, using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681 ; 7, 144,575; 7,067,318; 7, 172,869; 7,232,566; 7, 175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

[0392] Generally, the T cells are expanded by contact with a surface having attached thereto an agent that stimulates a CD3 TCR complex associated signal and a ligand that stimulates a co-

stimulatory molecule on the surface of the T cells. T cell populations may be stimulated by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (*e.g.*, bryostatin) in conjunction with a calcium ionophore. Co-stimulation of accessory molecules on the surface of T cells, is also contemplated.

[0393] In particular embodiments, PBMCs or isolated T cells are contacted with a stimulatory agent and costimulatory agent, such as anti-CD3 and anti-CD28 antibodies, generally attached to a bead or other surface, in a culture medium with appropriate cytokines, such as IL-2, IL-7, and/or IL-15. To stimulate proliferation of either $CD4^+$ T cells or $CD8^+$ T cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diacione, Besancon, France) can be used as can other methods commonly known in the art (Berg *et al., Transplant Proc*. 30(8):3975-3977, 1998; Haanen *et al., J. Exp. Med.* 190(9): 13191328, 1999; Garland *et al., J. Immunol Meth*. 227(1 -2):53-63, 1999). Anti-CD3 and anti-CD28 antibodies attached to the same bead serve as a "surrogate" antigen presenting cell (APC). In other embodiments, the T cells may be activated and stimulated to proliferate with feeder cells and appropriate antibodies and cytokines using methods such as those described in US6040177; US5827642; and WO2012129514.

[0394] In other embodiments, artificial APC (aAPC) made by engineering K562, U937, 721.221, T2, and C1R cells to direct the stable expression and secretion, of a variety of co-stimulatory molecules and cytokines. In a particular embodiment K32 or U32 aAPCs are used to direct the display of one or more antibody-based stimulatory molecules on the AAPC cell surface. Expression of various combinations of genes on the aAPC enables the precise determination of human T-cell activation requirements, such that aAPCs can be tailored for the optimal propagation of T-cell subsets with specific growth requirements and distinct functions. The aAPCs support ex vivo growth and long-term expansion of functional human CD8 T cells without requiring the addition of exogenous cytokines, in contrast to the use of natural APCs. Populations of T cells can be expanded by aAPCs expressing a variety of costimulatory molecules including, but not limited to, CD137L (4-1BBL), CD134L (OX40L), and/or CD80 or CD86. Finally, the aAPCs provide an efficient platform to expand genetically modified T cells and to maintain CD28 expression on CD8 T cells. aAPCs provided in WO 03/057171 and US2003/0147869 are hereby incorporated by reference in their entirety.

[0395] In one embodiment, $CD34^+$ cells are transduced with a nucleic acid construct in accordance with the present disclosure. In certain embodiments, the transduced $CD34^+$ cells differentiate into mature immune effector cells *in vivo* following administration into a subject, generally the subject from whom the cells were originally isolated. In another embodiment, $CD34^+$ cells may be stimulated *in vitro* prior to exposure to or after being genetically modified with a CAR as described herein, with one or more of the following cytokines: Flt-3 ligand (FLT3), stem cell factor (SCF), megakaryocyte growth and differentiation factor (TPO), IL-3 and IL-6 according to the methods described previously (Asheuer *et al.*, 2004, PNAS 101(10):3557-3562; Imren, *et al.*, 2004).

[0396] In certain embodiments, provided herein is a population of modified immune effector cells for the treatment of cancer, the modified immune effector cells comprising a CAR as disclosed herein. For example, a population of modified immune effector cells are prepared from peripheral blood mononuclear cells (PBMCs) obtained from a patient diagnosed with B cell malignancy described herein (autologous donors). The PBMCs form a heterogeneous population of T lymphocytes that can be $CD4^+$, $CD8^+$, or $CD4^+$ and $CD8^+$.

[0397] The PBMCs also can include other cytotoxic lymphocytes such as NK cells or NKT cells. An expression vector carrying the coding sequence of a CAR contemplated herein can be introduced into a population of human donor T cells, NK cells or NKT cells. Successfully transduced T cells that carry the expression vector can be sorted using flow cytometry to isolate CD3 positive T cells and then further propagated to increase the number of these CAR protein expressing T cells in addition to cell activation using anti-CD3 antibodies and or anti-CD28 antibodies and IL-2 or any other methods known in the art as described elsewhere herein. Standard procedures are used for cryopreservation of T cells expressing the CAR protein T cells for storage and/or preparation for use in a human subject. In one embodiment, the *in vitro* transduction, culture and/or expansion of T cells are performed in the absence of non-human animal derived products such as fetal calf serum and fetal bovine serum. Since a heterogeneous population of PBMCs is genetically modified, the resultant transduced cells are a heterogeneous population of modified cells comprising a BCMA targeting CAR as contemplated herein.

[0398] In a further embodiment, a mixture of, *e.g.*, one, two, three, four, five or more, different expression vectors can be used in genetically modifying a donor population of immune effector cells wherein each vector encodes a different chimeric antigen receptor protein as contemplated

herein. The resulting modified immune effector cells forms a mixed population of modified cells, with a proportion of the modified cells expressing more than one different CAR proteins.

[0399] In one embodiment, provided herein is a method of storing genetically modified murine, human or humanized CAR protein expressing immune effector cells which target a BCMA protein, comprising cryopreserving the immune effector cells such that the cells remain viable upon thawing. A fraction of the immune effector cells expressing the CAR proteins can be cryopreserved by methods known in the art to provide a permanent source of such cells for the future treatment of patients afflicted with the B cell related condition. When needed, the cryopreserved transformed immune effector cells can be thawed, grown and expanded for more such cells.

[0400] As used herein, "cryopreserving," refers to the preservation of cells by cooling to sub-zero temperatures, such as (typically) 77 K or −196° C. (the boiling point of liquid nitrogen). Cryoprotective agents are often used at sub-zero temperatures to prevent the cells being preserved from damage due to freezing at low temperatures or warming to room temperature. Cryopreservative agents and optimal cooling rates can protect against cell injury. Cryoprotective agents which can be used include but are not limited to dimethyl sulfoxide (DMSO) (Lovelock and Bishop, Nature, 1959; 183: 1394-1395; Ashwood-Smith, Nature, 1961; 190: 1204-1205), glycerol, polyvinylpyrrolidone (Rinfret, Ann. N.Y. Acad. Sci., 1960; 85: 576), and polyethylene glycol (Sloviter and Ravdin, Nature, 1962; 196: 48). The preferred cooling rate is 1° to 3° C/minute. After at least two hours, the T cells have reached a temperature of −80° C. and can be placed directly into liquid nitrogen (−196° C.) for permanent storage such as in a long-term cryogenic storage vessel.

5.7. T Cell Manufacturing Process

[0401] The T cells manufactured by the methods contemplated herein provide improved adoptive immunotherapy compositions. Without wishing to be bound to any particular theory, it is believed that the T cell compositions manufactured by the methods contemplated herein are imbued with superior properties, including increased survival, expansion in the relative absence of differentiation, and persistence *in vivo*. In one embodiment, a method of manufacturing T cells comprises contacting the cells with one or more agents that modulate a PI3K cell signaling pathway. In one embodiment, a method of manufacturing T cells comprises contacting the cells with one or more agents that modulate a PI3K/Akt/mTOR cell signaling pathway. In various

embodiments, the T cells may be obtained from any source and contacted with the agent during the activation and/or expansion phases of the manufacturing process. The resulting T cell compositions are enriched in developmentally potent T cells that have the ability to proliferate and express one or more of the following biomarkers: CD62L, CCR7, CD28, CD27, CD122, CD127, CD197, and CD38. In one embodiment, populations of cell comprising T cells, that have been treated with one or more PI3K inhibitors is enriched for a population of CD8+ T cells co-expressing one or more or, or all of, the following biomarkers: CD62L, CD127, CD197, and CD38.

[0402] In one embodiment, modified T cells comprising maintained levels of proliferation and decreased differentiation are manufactured. In a particular embodiment, T cells are manufactured by stimulating T cells to become activated and to proliferate in the presence of one or more stimulatory signals and an agent that is an inhibitor of a PI3K cell signaling pathway.

[0403] The T cells can then be modified to express anti-BCMA CARs. In one embodiment, the T cells are modified by transducing the T cells with a viral vector comprising an anti-BCMA CAR contemplated herein. In a certain embodiment, the T cells are modified prior to stimulation and activation in the presence of an inhibitor of a PI3K cell signaling pathway. In another embodiment, T cells are modified after stimulation and activation in the presence of an inhibitor of a PI3K cell signaling pathway. In a particular embodiment, T cells are modified within 12 hours, 24 hours, 36 hours, or 48 hours of stimulation and activation in the presence of an inhibitor of a PI3K cell signaling pathway.

[0404] After T cells are activated, the cells are cultured to proliferate. T cells may be cultured for at least 1, 2, 3, 4, 5, 6, or 7 days, at least 2 weeks, at least 1, 2, 3, 4, 5, or 6 months or more with 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more rounds of expansion.

[0405] In various embodiments, T cell compositions are manufactured in the presence of one or more inhibitors of the PI3K pathway. The inhibitors may target one or more activities in the pathway or a single activity. Without wishing to be bound to any particular theory, it is contemplated that treatment or contacting T cells with one or more inhibitors of the PI3K pathway during the stimulation, activation, and/or expansion phases of the manufacturing process preferentially increases young T cells, thereby producing superior therapeutic T cell compositions.

[0406] In a particular embodiment, a method for increasing the proliferation of T cells expressing an engineered T cell receptor is provided. Such methods may comprise, for example, harvesting a source of T cells from a subject, stimulating and activating the T cells in the presence of one or more inhibitors of the PI3K pathway, modification of the T cells to express an anti-BCMA CAR, *e.g.*, anti-BCMA02 CAR, and expanding the T cells in culture.

[0407] In a certain embodiment, a method for producing populations of T cells enriched for expression of one or more of the following biomarkers: CD62L, CCR7, CD28, CD27, CD122, CD127, CD197, and CD38. In one embodiment, young T cells comprise one or more of, or all of the following biological markers: CD62L, CD127, CD197, and CD38. In one embodiment, the young T cells lack expression of CD57, CD244, CD160, PD-1, CTLA4, TIM3, and LAG3 are provided. As discussed elsewhere herein, the expression levels young T cell biomarkers is relative to the expression levels of such markers in more differentiated T cells or immune effector cell populations.

[0408] In one embodiment, peripheral blood mononuclear cells (PBMCs) are used as the source of T cells in the T cell manufacturing methods contemplated herein. PBMCs form a heterogeneous population of T lymphocytes that can be $CD4^+$, $CD8^+$, or $CD4^+$ and $CD8^+$ and can include other mononuclear cells such as monocytes, B cells, NK cells and NKT cells. An expression vector comprising a polynucleotide encoding an engineered TCR or CAR contemplated herein can be introduced into a population of human donor T cells, NK cells or NKT cells. Successfully transduced T cells that carry the expression vector can be sorted using flow cytometry to isolate CD3 positive T cells and then further propagated to increase the number of the modified T cells in addition to cell activation using anti-CD3 antibodies and or anti-CD28 antibodies and IL-2, IL-7, and/or IL-15 or any other methods known in the art as described elsewhere herein.

[0409] Manufacturing methods contemplated herein may further comprise cryopreservation of modified T cells for storage and/or preparation for use in a human subject. T cells are cryopreserved such that the cells remain viable upon thawing. When needed, the cryopreserved transformed immune effector cells can be thawed, grown and expanded for more such cells. As used herein, “cryopreserving,” refers to the preservation of cells by cooling to sub-zero temperatures, such as (typically) 77 K or −196° C. (the boiling point of liquid nitrogen). Cryoprotective agents are often used at sub-zero temperatures to prevent the cells being

preserved from damage due to freezing at low temperatures or warming to room temperature. Cryopreservative agents and optimal cooling rates can protect against cell injury. Cryoprotective agents which can be used include but are not limited to dimethyl sulfoxide (DMSO) (Lovelock and Bishop, *Nature*, 1959; 183: 1394-1395; Ashwood-Smith, *Nature*, 1961; 190: 1204-1205), glycerol, polyvinylpyrrolidone (Rinfret, *Ann. N.Y. Acad. Sci.*, 1960; 85: 576), and polyethylene glycol (Sloviter and Ravdin, *Nature*, 1962; 196: 48). The preferred cooling rate is 1° to 3° C/minute. After at least two hours, the T cells have reached a temperature of −80° C. and can be placed directly into liquid nitrogen (−196° C.) for permanent storage such as in a long-term cryogenic storage vessel.

5.8. T Cells

[0410] The present disclosure contemplates the manufacture of improved CAR T cell compositions. T cells used for CAR T cell production may be autologous/autogeneic ("self") or non-autologous ("non-self," *e.g.*, allogeneic, syngeneic or xenogeneic). In certain embodiments, the T cells are obtained from a mammalian subject. In a more specific embodiment, the T cells are obtained from a primate subject. In a preferred embodiment, the T cells are obtained from a human subject.

[0411] T cells can be obtained from a number of sources including, but not limited to, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled person, such as sedimentation, *e.g.*, FICOLL™ separation. In one embodiment, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one embodiment, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing. The cells can be washed with PBS or with another suitable solution that lacks calcium, magnesium, and most, if not all other, divalent cations. As would be appreciated by those of ordinary skill in the art, a washing step may be accomplished by methods known to those in the art, such as by using a semiautomated flowthrough centrifuge. For example, the Cobe 2991 cell processor, the Baxter CytoMate, or the like. After washing, the cells may be resuspended in a variety of biocompatible

buffers or other saline solution with or without buffer. In certain embodiments, the undesirable components of the apheresis sample may be removed in the cell directly resuspended culture media.

[0412] In particular embodiments, a population of cells comprising T cells, *e.g.*, PBMCs, is used in the manufacturing methods contemplated herein. In other embodiments, an isolated or purified population of T cells is used in the manufacturing methods contemplated herein. Cells can be isolated from peripheral blood mononuclear cells (PBMCs) by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient. In some embodiments, after isolation of PBMC, both cytotoxic and helper T lymphocytes can be sorted into naïve, memory, and effector T cell subpopulations either before or after activation, expansion, and/or genetic modification.

[0413] A specific subpopulation of T cells, expressing one or more of the following markers: CD3, CD4, CD8, CD28, CD45RA, CD45RO, CD62, CD127, and HLA-DR can be further isolated by positive or negative selection techniques. In one embodiment, a specific subpopulation of T cells, expressing one or more of the markers selected from the group consisting of (i) CD62L, CCR7, CD28, CD27, CD122, CD127, CD197; or (ii) CD38 or CD62L, CD127, CD197, and CD38, is further isolated by positive or negative selection techniques. In various embodiments, the manufactured T cell compositions do not express or do not substantially express one or more of the following markers: CD57, CD244, CD160, PD-1, CTLA4, TIM3, and LAG3.

[0414] In one embodiment, expression of one or more of the markers selected from the group consisting of CD62L, CD127, CD197, and CD38 is increased at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 25 fold, or more compared to a population of T cells activated and expanded without a PI3K inhibitor.

[0415] In one embodiment, expression of one or more of the markers selected from the group consisting of CD57, CD244, CD160, PD-1, CTLA4, TIM3, and LAG3 is decreased at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 25 fold, or more compared to a population of T cells activated and expanded with a PI3K inhibitor.

[0416] In one embodiment, the manufacturing methods contemplated herein increase the number CAR T cells comprising one or more markers of naïve or developmentally potent T cells. Without wishing to be bound to any particular theory, the present inventors believe that treating a population of cells comprising T cells with one or more PI3K inhibitors results in an increase an expansion of developmentally potent T cells and provides a more robust and efficacious adoptive CAR T cell immunotherapy compared to existing CAR T cell therapies.

[0417] Illustrative examples of markers of naïve or developmentally potent T cells increased in T cells manufactured using the methods contemplated herein include, but are not limited to CD62L, CD127, CD197, and CD38. In particular embodiments, naïve T cells do not express do not express or do not substantially express one or more of the following markers: CD57, CD244, CD160, PD-1, BTLA, CD45RA, CTLA4, TIM3, and LAG3.

[0418] With respect to T cells, the T cell populations resulting from the various expansion methodologies contemplated herein may have a variety of specific phenotypic properties, depending on the conditions employed. In various embodiments, expanded T cell populations comprise one or more of the following phenotypic markers: CD62L, CD127, CD197, CD38, and HLA-DR.

[0419] In one embodiment, such phenotypic markers include enhanced expression of one or more of, or all of CD62L, CD127, CD197, and CD38. In particular embodiments, $CD8^+$ T lymphocytes characterized by the expression of phenotypic markers of naive T cells including CD62L, CD127, CD197, and CD38 are expanded.

[0420] In particular embodiments, T cells characterized by the expression of phenotypic markers of central memory T cells including CD45RO, CD62L, CD127, CD197, and CD38 and negative for granzyme B are expanded. In some embodiments, the central memory T cells are $CD45RO^+$, $CD62L^+$, $CD8^+$ T cells.

[0421] In certain embodiments, $CD4^+$ T lymphocytes characterized by the expression of phenotypic markers of naïve $CD4^+$ cells including CD62L and negative for expression of CD45RA and/or CD45RO are expanded. In some embodiments, $CD4^+$ cells characterized by the expression of phenotypic markers of central memory $CD4^+$ cells including CD62L and CD45RO positive. In some embodiments, effector $CD4^+$ cells are CD62L positive and CD45RO negative.

[0422] In certain embodiments, the T cells are isolated from an individual and activated and stimulated to proliferate *in vitro* prior to being genetically modified to express an anti-BCMA

CAR. In this regard, the T cells may be cultured before and/or after being genetically modified (*i.e.*, transduced or transfected to express an anti-BCMA CAR contemplated herein).

5.8.1. Activation and Expansion

[0423] In order to achieve sufficient therapeutic doses of T cell compositions, T cells are often subject to one or more rounds of stimulation, activation and/or expansion. T cells can be activated and expanded generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; and 6,867,041, each of which is incorporated herein by reference in its entirety. T cells modified to express an anti-BCMA CAR can be activated and expanded before and/or after the T cells are modified. In addition, T cells may be contacted with one or more agents that modulate the PI3K cell signaling pathway before, during, and/or after activation and/or expansion. In one embodiment, T cells manufactured by the methods contemplated herein undergo one, two, three, four, or five or more rounds of activation and expansion, each of which may include one or more agents that modulate the PI3K cell signaling pathway.

[0424] In one embodiment, a costimulatory ligand is presented on an antigen presenting cell (*e.g.*, an aAPC, dendritic cell, B cell, and the like) that specifically binds a cognate costimulatory molecule on a T cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex, mediates a desired T cell response. Suitable costimulatory ligands include, but are not limited to, CD7, B7-1 (CD80), B7-2 (CD86), PD-L 1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, ILT3, ILT4, an agonist or antibody that binds Toll ligand receptor, and a ligand that specifically binds with B7-H3.

[0425] In a particular embodiment, a costimulatory ligand comprises an antibody or antigen binding fragment thereof that specifically binds to a costimulatory molecule present on a T cell, including but not limited to, CD27, CD28, 4- IBB, OX40, CD30, CD40, PD-1, 1COS, lymphocyte function-associated antigen 1 (LFA-1), CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83.

[0426] Suitable costimulatory ligands further include target antigens, which may be provided in soluble form or expressed on APCs or aAPCs that bind engineered TCRs or CARs expressed on modified T cells.

[0427] In various embodiments, a method for manufacturing T cells contemplated herein comprises activating a population of cells comprising T cells and expanding the population of T cells. T cell activation can be accomplished by providing a primary stimulation signal through the T cell TCR/CD3 complex or via stimulation of the CD2 surface protein and by providing a secondary costimulation signal through an accessory molecule, *e.g,* CD28.

[0428] The TCR/CD3 complex may be stimulated by contacting the T cell with a suitable CD3 binding agent, *e.g.,* a CD3 ligand or an anti-CD3 monoclonal antibody. Illustrative examples of CD3 antibodies include, but are not limited to, OKT3, G19-4, BC3, and 64.1.

[0429] In another embodiment, a CD2 binding agent may be used to provide a primary stimulation signal to the T cells. Illustrative examples of CD2 binding agents include, but are not limited to, CD2 ligands and anti-CD2 antibodies, *e.g.,* the T11.3 antibody in combination with the T11.1 or T11.2 antibody (Meuer, S. C. *et al.* (1984) *Cell* 36:897-906) and the 9.6 antibody (which recognizes the same epitope as TI 1.1) in combination with the 9-1 antibody (Yang, S. Y. *et al.* (1986) *J. Immunol.* 137:1097-1100). Other antibodies which bind to the same epitopes as any of the above described antibodies can also be used. Additional antibodies, or combinations of antibodies, can be prepared and identified by standard techniques as disclosed elsewhere herein.

[0430] In addition to the primary stimulation signal provided through the TCR/CD3 complex, or via CD2, induction of T cell responses requires a second, costimulatory signal. In particular embodiments, a CD28 binding agent can be used to provide a costimulatory signal. Illustrative examples of CD28 binding agents include but are not limited to: natural CD 28 ligands, *e.g.,* a natural ligand for CD28 (*e.g.,* a member of the B7 family of proteins, such as B7-1(CD80) and B7-2 (CD86); and anti-CD28 monoclonal antibody or fragment thereof capable of crosslinking the CD28 molecule, *e.g.,* monoclonal antibodies 9.3, B-T3, XR-CD28, KOLT-2, 15E8, 248.23.2, and EX5.3D10.

[0431] In one embodiment, the molecule providing the primary stimulation signal, for example a molecule which provides stimulation through the TCR/CD3 complex or CD2, and the costimulatory molecule are coupled to the same surface.

[0432] In certain embodiments, binding agents that provide stimulatory and costimulatory signals are localized on the surface of a cell. This can be accomplished by transfecting or transducing a cell with a nucleic acid encoding the binding agent in a form suitable for its expression on the cell surface or alternatively by coupling a binding agent to the cell surface.

[0433] In another embodiment, the molecule providing the primary stimulation signal, for example a molecule which provides stimulation through the TCR/CD3 complex or CD2, and the costimulatory molecule are displayed on antigen presenting cells.

[0434] In one embodiment, the molecule providing the primary stimulation signal, for example a molecule which provides stimulation through the TCR/CD3 complex or CD2, and the costimulatory molecule are provided on separate surfaces.

[0435] In a certain embodiment, one of the binding agents that provide stimulatory and costimulatory signals is soluble (provided in solution) and the other agent(s) is provided on one or more surfaces.

[0436] In a particular embodiment, the binding agents that provide stimulatory and costimulatory signals are both provided in a soluble form (provided in solution).

[0437] In various embodiments, the methods for manufacturing T cells contemplated herein comprise activating T cells with anti-CD3 and anti-CD28 antibodies.

[0438] T cell compositions manufactured by the methods contemplated herein comprise T cells activated and/or expanded in the presence of one or more agents that inhibit a PI3K cell signaling pathway. T cells modified to express an anti-BCMA CAR can be activated and expanded before and/or after the T cells are modified. In particular embodiments, a population of T cells is activated, modified to express an anti-BCMA CAR, and then cultured for expansion.

[0439] In one embodiment, T cells manufactured by the methods contemplated herein comprise an increased number of T cells expressing markers indicative of high proliferative potential and the ability to self-renew but that do not express or express substantially undetectable markers of T cell differentiation. These T cells may be repeatedly activated and expanded in a robust fashion and thereby provide an improved therapeutic T cell composition.

[0440] In one embodiment, a population of T cells activated and expanded in the presence of one or more agents that inhibit a PI3K cell signaling pathway is expanded at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 250 fold, at

least 500 fold, at least 1000 fold, or more compared to a population of T cells activated and expanded without a PI3K inhibitor.

[0441] In one embodiment, a population of T cells characterized by the expression of markers young T cells are activated and expanded in the presence of one or more agents that inhibit a PI3K cell signaling pathway is expanded at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 25 fold, at least 50 fold, at least 100 fold, at least 250 fold, at least 500 fold, at least 1000 fold, or more compared the population of T cells activated and expanded without a PI3K inhibitor.

[0442] In one embodiment, expanding T cells activated by the methods contemplated herein further comprises culturing a population of cells comprising T cells for several hours (about 3 hours) to about 7 days to about 28 days or any hourly integer value in between. In another embodiment, the T cell composition may be cultured for 14 days. In a particular embodiment, T cells are cultured for about 21 days. In another embodiment, the T cell compositions are cultured for about 2-3 days. Several cycles of stimulation/activation/expansion may also be desired such that culture time of T cells can be 60 days or more.

[0443] In particular embodiments, conditions appropriate for T cell culture include an appropriate media (*e.g.*, Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) and one or more factors necessary for proliferation and viability including, but not limited to serum (*e.g.*, fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-γ, IL-4, IL-7, IL-21, GM-CSF, IL- 10, IL- 12, IL-15, TGFβ, and TNF-α or any other additives suitable for the growth of cells known to the skilled artisan.

[0444] Further illustrative examples of cell culture media include, but are not limited to RPMI 1640, Clicks, AIM-V, DMEM, MEM, a-MEM, F-12, X-Vivo 1 5, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells.

[0445] Illustrative examples of other additives for T cell expansion include, but are not limited to, surfactant, piasmanate, pH buffers such as HEPES, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol

[0446] Antibiotics, *e.g.*, penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (*e.g.*, 37° C) and atmosphere (*e.g.*, air plus 5% C02).

[0447] In particular embodiments, PBMCs or isolated T cells are contacted with a stimulatory agent and costimulatory agent, such as anti-CD3 and anti-CD28 antibodies, generally attached to a bead or other surface, in a culture medium with appropriate cytokines, such as IL-2, IL-7, and/or IL-15.

[0448] In other embodiments, artificial APC (aAPC) may be made by engineering K562, U937, 721.221, T2, and C1R cells to direct the stable expression and secretion, of a variety of costimulatory molecules and cytokines. In a particular embodiment K32 or U32 aAPCs are used to direct the display of one or more antibody-based stimulatory molecules on the AAPC cell surface. Populations of T cells can be expanded by aAPCs expressing a variety of costimulatory molecules including, but not limited to, CD137L (4-1BBL), CD134L (OX40L), and/or CD80 or CD86. Finally, the aAPCs provide an efficient platform to expand genetically modified T cells and to maintain CD28 expression on CD8 T cells. aAPCs provided in WO 03/057171 and US2003/0147869 are hereby incorporated by reference in their entirety.

5.8.2. Agents

[0449] In various embodiments, a method for manufacturing T cells is provided that expands undifferentiated or developmentally potent T cells comprising contacting T cells with an agent that modulates a PI3K pathway in the cells. In various embodiments, a method for manufacturing T cells is provided that expands undifferentiated or developmentally potent T cells comprising contacting T cells with an agent that modulates a PI3K/AKT/mTOR pathway in the cells. The cells may be contacted prior to, during, and/or after activation and expansion. The T cell compositions retain sufficient T cell potency such that they may undergo multiple rounds of expansion without a substantial increase in differentiation.

[0450] As used herein, the terms “modulate,” “modulator,” or “modulatory agent” or comparable term refer to an agent’s ability to elicit a change in a cell signaling pathway. A modulator may increase or decrease an amount, activity of a pathway component or increase or decrease a desired effect or output of a cell signaling pathway. In one embodiment, the modulator is an inhibitor. In another embodiment, the modulator is an activator.

[0451] An "agent" refers to a compound, small molecule, *e.g.*, small organic molecule, nucleic acid, polypeptide, or a fragment, isoform, variant, analog, or derivative thereof used in the modulation of a PI3K/AKT/mTOR pathway.

[0452] A "small molecule" refers to a composition that has a molecular weight of less than about 5 kD, less than about 4 kD, less than about 3 kD, less than about 2 kD, less than about 1 kD, or less than about .5kD. Small molecules may comprise nucleic acids, peptides, polypeptides, peptidomimetics, peptoids, carbohydrates, lipids, components thereof or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the present disclosure. Methods for the synthesis of molecular libraries are known in the art (see, e.g., Carell *et al.*, 1994a; Carell *et al.*, 1994b; Cho *et al.*, 1993; DeWitt *et al.*, 1993; Gallop *et al.*, 1994; Zuckermann *et al.*, 1994).

[0453] An "analog" refers to a small organic compound, a nucleotide, a protein, or a polypeptide that possesses similar or identical activity or function(s) as the compound, nucleotide, protein or polypeptide or compound having the desired activity of the present disclosure, but need not necessarily comprise a sequence or structure that is similar or identical to the sequence or structure of a preferred embodiment.

[0454] A "derivative" refers to either a compound, a protein or polypeptide that comprises an amino acid sequence of a parent protein or polypeptide that has been altered by the introduction of amino acid residue substitutions, deletions or additions, or a nucleic acid or nucleotide that has been modified by either introduction of nucleotide substitutions or deletions, additions or mutations. The derivative nucleic acid, nucleotide, protein or polypeptide possesses a similar or identical function as the parent polypeptide.

[0455] In various embodiments, the agent that modulates a PI3K pathway activates a component of the pathway. An "activator," or "agonist" refers to an agent that promotes, increases, or induces one or more activities of a molecule in a PI3K/AKT/mTOR pathway including, without limitation, a molecule that inhibits one or more activities of a PI3K.

[0456] In various embodiments, the agent that modulates a PI3K pathway inhibits a component of the pathway. An "inhibitor" or "antagonist" refers to an agent that inhibits, decreases, or reduces one or more activities of a molecule in a PI3K pathway including, without limitation, a PI3K. In one embodiment, the inhibitor is a dual molecule inhibitor. In particular embodiment,

the inhibitor may inhibit a class of molecules have the same or substantially similar activities (a pan-inhibitor) or may specifically inhibit a molecule's activity (a selective or specific inhibitor). Inhibition may also be irreversible or reversible.

[0457] In one embodiment, the inhibitor has an IC50 of at least 1nM, at least 2nM, at least 5nM, at least 10nM, at least 50nM, at least 100nM, at least 200nM, at least 500nM, at least 1μM, at least 10μM, at least 50μM, or at least 100μM. IC50 determinations can be accomplished using any conventional techniques known in the art. For example, an IC50 can be determined by measuring the activity of a given enzyme in the presence of a range of concentrations of the inhibitor under study. The experimentally obtained values of enzyme activity then are plotted against the inhibitor concentrations used. The concentration of the inhibitor that shows 50% enzyme activity (as compared to the activity in the absence of any inhibitor) is taken as the "IC50" value. Analogously, other inhibitory concentrations can be defined through appropriate determinations of activity.

[0458] In various embodiments, T cells are contacted or treated or cultured with one or more modulators of a PI3K pathway at a concentration of at least1nM, at least 2nM, at least 5nM, at least 10nM, at least 50nM, at least 100nM, at least 200nM, at least 500nM, at least 1μM, at least 10μM, at least 50μM, at least 100μM, or at least 1 M.

[0459] In particular embodiments, T cells may be contacted or treated or cultured with one or more modulators of a PI3K pathway for at least 12 hours, 18 hours, at least 1, 2, 3, 4, 5, 6, or 7 days, at least 2 weeks, at least 1, 2, 3, 4, 5, or 6 months or more with 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more rounds of expansion.

5.8.3. PI3K/Akt/mTOR Pathway

[0460] The phosphatidyl-inositol-3 kinase/Akt/mammalian target of rapamycin pathway serves as a conduit to integrate growth factor signaling with cellular proliferation, differentiation, metabolism, and survival. PI3Ks are a family of highly conserved intracellular lipid kinases. Class IA PI3Ks are activated by growth factor receptor tyrosine kinases (RTKs), either directly or through interaction with the insulin receptor substrate family of adaptor molecules. This activity results in the production of phosphatidyl-inositol-3,4,5-trisphospate (PIP3) a regulator of the serine/threonine kinase Akt. mTOR acts through the canonical PI3K pathway via 2 distinct complexes, each characterized by different binding partners that confer distinct activities. mTORC1 (mTOR in complex with PRAS40, raptor, and mLST8/GbL) acts as a downstream

effector of PI3K/Akt signaling, linking growth factor signals with protein translation, cell growth, proliferation, and survival. mTORC2 (mTOR in complex with rictor, mSIN1, protor, and mLST8) acts as an upstream activator of Akt.

[0461] Upon growth factor receptor-mediated activation of PI3K, Akt is recruited to the membrane through the interaction of its pleckstrin homology domain with PIP3, thus exposing its activation loop and enabling phosphorylation at threonine 308 (Thr308) by the constitutively active phosphoinositide-dependent protein kinase 1 (PDK1). For maximal activation, Akt is also phosphorylated by mTORC2, at serine 473 (Ser473) of its C-terminal hydrophobic motif. DNA-PK and HSP have also been shown to be important in the regulation of Akt activity. Akt activates mTORC1 through inhibitory phosphorylation of TSC2, which along with TSC1, negatively regulates mTORC1 by inhibiting the Rheb GTPase, a positive regulator of mTORC1. mTORC1 has 2 well-defined substrates, p70S6K (referred to hereafter as S6K1) and 4E-BP1, both of which critically regulate protein synthesis. Thus, mTORC1 is an important downstream effector of PI3K, linking growth factor signaling with protein translation and cellular proliferation.

5.8.4. PI3K Inhibitors

[0462] As used herein, the term "PI3K inhibitor" refers to a nucleic acid, peptide, compound, or small organic molecule that binds to and inhibits at least one activity of PI3K. The PI3K proteins can be divided into three classes, class 1 PI3Ks, class 2 PI3Ks, and class 3 PI3Ks. Class 1 PI3Ks exist as heterodimers consisting of one of four p110 catalytic subunits (p110α, p110β, p110δ, and p110γ) and one of two families of regulatory subunits. In a particular embodiment, a PI3K inhibitor of the present disclosure targets the class 1 PI3K inhibitors. In one embodiment, a PI3K inhibitor will display selectivity for one or more isoforms of the class 1 PI3K inhibitors (i.e., selectivity for p110α, p110β, p110δ, and p110γ or one or more of p110α, p110β, p110δ, and p110γ). In another aspect, a PI3K inhibitor will not display isoform selectivity and be considered a "pan-PI3K inhibitor." In one embodiment, a PI3K inhibitor will compete for binding with ATP to the PI3K catalytic domain.

[0463] In certain embodiments, a PI3K inhibitor can, for example, target PI3K as well as additional proteins in the PI3K-AKT-mTOR pathway. In particular embodiments, a PI3K inhibitor that targets both mTOR and PI3K can be referred to as either an mTOR inhibitor or a PI3K inhibitor. A PI3K inhibitor that only targets PI3K can be referred to as a selective PI3K

inhibitor. In one embodiment, a selective PI3K inhibitor can be understood to refer to an agent that exhibits a 50% inhibitory concentration with respect to PI3K that is at least 10-fold, at least 20-fold, at least 30-fold, at least 50-fold, at least 100-fold, at least 1000-fold, or more, lower than the inhibitor's IC50 with respect to mTOR and/or other proteins in the pathway.

[0464] In a particular embodiment, exemplary PI3K inhibitors inhibit PI3K with an IC50 (concentration that inhibits 50% of the activity) of about 200 nM or less, preferably about 100 nm or less, even more preferably about 60 nM or less, about 25 nM, about 10 nM, about 5 nM, about 1 nM, 100 μM, 50 μM, 25 μM, 10 μM, 1 μM, or less. In one embodiment, a PI3K inhibitor inhibits PI3K with an IC50 from about 2 nM to about 100 nm, more preferably from about 2 nM to about 50 nM, even more preferably from about 2 nM to about 15 nM.

[0465] Illustrative examples of PI3K inhibitors suitable for use in the T cell manufacturing methods contemplated herein include, but are not limited to, BKM120 (class 1 PI3K inhibitor, Novartis), XL147 (class 1 PI3K inhibitor, Exelixis), (pan-PI3K inhibitor, GlaxoSmithKline), and PX-866 (class 1 PI3K inhibitor; p110α, p110β, and p110γ isoforms, Oncothyreon).

[0466] Other illustrative examples of selective PI3K inhibitors include, but are not limited to BYL719, GSK2636771, TGX-221, AS25242, CAL-101, ZSTK474, and IPI-145.

[0467] Further illustrative examples of pan-PI3K inhibitors include, but are not limited to BEZ235, LY294002, GSK1059615, TG100713, and GDC-0941.

5.8.5. AKT Inhibitors

[0468] As used herein, the term "AKT inhibitor" refers to a nucleic acid, peptide, compound, or small organic molecule that inhibits at least one activity of AKT. AKT inhibitors can be grouped into several classes, including lipid-based inhibitors (*e.g.*, inhibitors that target the pleckstrin homology domain of AKT which prevents AKT from localizing to plasma membranes), ATP-competitive inhibitors, and allosteric inhibitors. In one embodiment, AKT inhibitors act by binding to the AKT catalytic site. In a particular embodiment, Akt inhibitors act by inhibiting phosphorylation of downstream AKT targets such as mTOR. In another embodiment, AKT activity is inhibited by inhibiting the input signals to activate Akt by inhibiting, for example, DNA-PK activation of AKT, PDK-1 activation of AKT, and/or mTORC2 activation of Akt.

[0469] AKT inhibitors can target all three AKT isoforms, AKT1, AKT2, AKT3 or may be isoform selective and target only one or two of the AKT isoforms. In one embodiment, an AKT inhibitor can target AKT as well as additional proteins in the PI3K-AKT-mTOR pathway. An

AKT inhibitor that only targets AKT can be referred to as a selective AKT inhibitor. In one embodiment, a selective AKT inhibitor can be understood to refer to an agent that exhibits a 50% inhibitory concentration with respect to AKT that is at least 10-fold, at least 20-fold, at least 30-fold, at least 50-fold, at least 100-fold, at least 1000-fold, or more lower than the inhibitor's IC50 with respect to other proteins in the pathway.

[0470] In a particular embodiment, exemplary AKT inhibitors inhibit AKT with an IC50 (concentration that inhibits 50% of the activity) of about 200 nM or less, preferably about 100 nm or less, even more preferably about 60 nM or less, about 25 nM, about 10 nM, about 5 nM, about 1 nM, 100 μM, 50 μM, 25 μM, 10 μM, 1 μM, or less. In one embodiment, an AKT inhibits AKT with an IC50 from about 2 nM to about 100 nm, more preferably from about 2 nM to about 50 nM, even more preferably from about 2 nM to about 15 nM.

[0471] Illustrative examples of AKT inhibitors for use in combination with auristatin based antibody-drug conjugates include, for example, perifosine (Keryx), MK2206 (Merck), VQD-002 (VioQuest), XL418 (Exelixis), GSK690693, GDC-0068, and PX316 (PROLX Pharmaceuticals).

[0472] An illustrative, non-limiting example of a selective Akt1 inhibitor is A-674563.

[0473] An illustrative, non-limiting example of a selective Akt2 inhibitor is CCT128930.

[0474] In particular embodiments, the Akt inhibitor DNA-PK activation of Akt, PDK-1 activation of Akt, mTORC2 activation of Akt, or HSP activation of Akt.

[0475] Illustrative examples of DNA-PK inhibitors include, but are not limited to, NU7441, PI-103, NU7026, PIK-75, and PP-121.

5.8.6. mTOR Inhibitors

[0476] The terms “mTOR inhibitor” or “agent that inhibits mTOR” refers to a nucleic acid, peptide, compound, or small organic molecule that inhibits at least one activity of an mTOR protein, such as, for example, the serine/threonine protein kinase activity on at least one of its substrates (*e.g.*, p70S6 kinase 1, 4E-BP1, AKT/PKB and eEF2). mTOR inhibitors are able to bind directly to and inhibit mTORC1, mTORC2 or both mTORC1 and mTORC2.

[0477] Inhibition of mTORC1 and/or mTORC2 activity can be determined by a reduction in signal transduction of the PI3K/Akt/mTOR pathway. A wide variety of readouts can be utilized to establish a reduction of the output of such signaling pathway. Some non-limiting exemplary readouts include (1) a decrease in phosphorylation of Akt at residues, including but not limited to 5473 and T308; (2) a decrease in activation of Akt as evidenced, for example, by a reduction of

phosphorylation of Akt substrates including but not limited to Fox01/O3a T24/32, GSK3a/β; S21/9, and TSC2 T1462; (3) a decrease in phosphorylation of signaling molecules downstream of mTOR, including but not limited to ribosomal S6 S240/244, 70S6K T389, and 4EBP1 T37/46; and (4) inhibition of proliferation of cancerous cells.

[0478] In one embodiment, the mTOR inhibitors are active site inhibitors. These are mTOR inhibitors that bind to the ATP binding site (also referred to as ATP binding pocket) of mTOR and inhibit the catalytic activity of both mTORC1 and mTORC2. One class of active site inhibitors suitable for use in the T cell manufacturing methods contemplated herein are dual specificity inhibitors that target and directly inhibit both PI3K and mTOR. Dual specificity inhibitors bind to both the ATP binding site of mTOR and PI3K. Illustrative examples of such inhibitors include, but are not limited to: imidazoquinazolines, wortmannin, LY294002, PI-103 (Cayman Chemical), SF1126 (Semafore), BGT226 (Novartis), XL765 (Exelixis) and NVP-BEZ235 (Novartis).

[0479] Another class of mTOR active site inhibitors suitable for use in the methods contemplated herein selectively inhibit mTORC1 and mTORC2 activity relative to one or more type I phosphatidylinositol 3-kinases, *e.g.,* PI3 kinase α, β, γ, or δ. These active site inhibitors bind to the active site of mTOR but not PI3K. Illustrative examples of such inhibitors include, but are not limited to: pyrazolopyrimidines, Torin1 (Guertin and Sabatini), PP242 (2-(4-Amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-1H-indol-5-ol), PP30, Ku-0063794, WAY-600 (Wyeth), WAY-687 (Wyeth), WAY-354 (Wyeth), and AZD8055 (Liu *et al.*, Nature Review, 8, 627-644, 2009).

[0480] In one embodiment, a selective mTOR inhibitor refers to an agent that exhibits a 50% inhibitory concentration (IC50) with respect to mTORC1 and/or mTORC2, that is at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 1000-fold, or more, lower than the inhibitor's IC50 with respect to one, two, three, or more type I PI3-kinases or to all of the type I PI3-kinases.

[0481] Another class of mTOR inhibitors for use in the present disclosure isreferred to herein as "rapalogs." As used herein the term "rapalogs" refers to compounds that specifically bind to the mTOR FRB domain (FKBP rapamycin binding domain), are structurally related to rapamycin, and retain the mTOR inhibiting properties. The term rapalogs excludes rapamycin. Rapalogs include esters, ethers, oximes, hydrazones, and hydroxylamines of rapamycin, as well as

compounds in which functional groups on the rapamycin core structure have been modified, for example, by reduction or oxidation. Pharmaceutically acceptable salts of such compounds are also considered to be rapamycin derivatives. Illustrative examples of rapalogs suitable for use in the methods contemplated herein include, without limitation, temsirolimus (CC1779), everolimus (RAD001), deforolimus (AP23573), AZD8055 (AstraZeneca), and OSI-027 (OSI).

[0482] In one embodiment, the agent is the mTOR inhibitor rapamycin (sirolimus).

[0483] In a particular embodiment, exemplary mTOR inhibitors for use herein inhibit either mTORC1, mTORC2 or both mTORC1 and mTORC2 with an IC50 (concentration that inhibits 50% of the activity) of about 200 nM or less, preferably about 100 nm or less, even more preferably about 60 nM or less, about 25 nM, about 10 nM, about 5 nM, about 1 nM, 100 μM, 50 μM, 25 μM, 10 μM, 1 μM, or less. In one aspect, a mTOR inhibitor for use herein inhibits either mTORC1, mTORC2 or both mTORC1 and mTORC2 with an IC50 from about 2 nM to about 100 nm, more preferably from about 2 nM to about 50 nM, even more preferably from about 2 nM to about 15 nM.

[0484] In one embodiment, exemplary mTOR inhibitors inhibit either PI3K and mTORC1 or mTORC2 or both mTORC1 and mTORC2 and PI3K with an IC50 (concentration that inhibits 50% of the activity) of about 200 nM or less, preferably about 100 nm or less, even more preferably about 60 nM or less, about 25 nM, about 10 nM, about 5 nM, about 1 nM, 100 μM, 50 μM, 25 μM, 10 μM, 1 μM, or less. In one aspect, a mTOR inhibitor for use herein inhibits PI3K and mTORC1 or mTORC2 or both mTORC1 and mTORC2 and PI3K with an IC50 from about 2 nM to about 100 nm, more preferably from about 2 nM to about 50 nM, even more preferably from about 2 nM to about 15 nM.

[0485] Further illustrative examples of mTOR inhibitors suitable for use in particular embodiments contemplated herein include, but are not limited to AZD8055, INK128, rapamycin, PF-04691502, and everolimus.

[0486] mTOR has been shown to demonstrate a robust and specific catalytic activity toward the physiological substrate proteins, p70 S6 ribosomal protein kinase I (p70S6K1) and eIF4E binding protein 1 (4EBP1) as measured by phosphor-specific antibodies in Western blotting.

[0487] In one embodiment, the inhibitor of the PI3K/AKT/mTOR pathway is a s6 kinase inhibitor selected from the group consisting of: BI-D1870, H89, PF-4708671, FMK, and AT7867.

5.9. Compositions and Formulations

[0488] The compositions contemplated herein may comprise one or more polypeptides, polynucleotides, vectors comprising same, genetically modified immune effector cells, *etc.*, as contemplated herein. Compositions include, but are not limited to pharmaceutical compositions. A "pharmaceutical composition" refers to a composition formulated in pharmaceutically-acceptable or physiologically-acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy. It will also be understood that, if desired, the compositions of the present disclosure may be administered in combination with other agents as well, such as, *e.g.*, cytokines, growth factors, hormones, small molecules, chemotherapeutics, pro-drugs, drugs, antibodies, or other various pharmaceutically-active agents. There is virtually no limit to other components that may also be included in the compositions, provided that the additional agents do not adversely affect the ability of the composition to deliver the intended therapy.

[0489] The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0490] As used herein "pharmaceutically acceptable carrier, diluent or excipient" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, surfactant, or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans or domestic animals. Exemplary pharmaceutically acceptable carriers include, but are not limited to, to sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; tragacanth; malt; gelatin; talc; cocoa butter, waxes, animal and vegetable fats, paraffins, silicones, bentonites, silicic acid, zinc oxide; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and

aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and any other compatible substances employed in pharmaceutical formulations.

[0491] In particular embodiments, compositions presented hereincomprise an amount of CAR-expressing immune effector cells contemplated herein. As used herein, the term "amount" refers to "an amount effective" or "an effective amount" of a genetically modified therapeutic cell, *e.g.*, T cell, to achieve a beneficial or desired prophylactic or therapeutic result, including clinical results.

[0492] A "prophylactically effective amount" refers to an amount of a genetically modified therapeutic cell effective to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount is less than the therapeutically effective amount.

[0493] A "therapeutically effective amount" of a genetically modified therapeutic cell may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the stem and progenitor cells to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the virus or transduced therapeutic cells are outweighed by the therapeutically beneficial effects. The term "therapeutically effective amount" includes an amount that is effective to "treat" a subject (*e.g.*, a patient). When a therapeutic amount is indicated, the precise amount of a compositions of the present disclosure to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising the T cells described herein may be administered at a dosage of 10^2 to 10^{10} cells/kg body weight, preferably 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. The number of cells will depend upon the ultimate use for which the composition is intended as will the type of cells included therein. For uses provided herein, the cells are generally in a volume of a liter or less, can be 500 mL or less, even 250 mL or 100 mL or less. Hence the density of the desired cells is typically greater than 10^6 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, or 10^{12} cells. In some aspects, particularly since all the infused cells will be redirected

to a particular target antigen (*e.g.*, κ or λ light chain), lower numbers of cells, in the range of 10^6/kilogram (10^6-10^{11} per patient) may be administered. CAR expressing cell compositions may be administered multiple times at dosages within these ranges. The cells may be allogeneic, syngeneic, xenogeneic, or autologous to the patient undergoing therapy. If desired, the treatment may also include administration of mitogens (*e.g.*, PHA) or lymphokines, cytokines, and/or chemokines (*e.g.*, IFN-γ, IL-2, IL-12, TNF-alpha, IL-18, and TNF-beta, GM-CSF, IL-4, IL-13, Flt3-L, RANTES, MIP1α, etc.) as described herein to enhance induction of the immune response.

[0494] Generally, compositions comprising the cells activated and expanded as described herein may be utilized in the treatment and prevention of diseases that arise in individuals who are immunocompromised. In particular, compositions comprising the CAR-modified T cells contemplated herein are used in the treatment of B cell malignancies. The CAR-modified T cells of the present disclosure may be administered either alone, or as a pharmaceutical composition in combination with carriers, diluents, excipients, and/or with other components such as IL-2 or other cytokines or cell populations. In particular embodiments, pharmaceutical compositions contemplated herein comprise an amount of genetically modified T cells, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients.

[0495] Pharmaceutical compositions of the present disclosure comprising a CAR-expressing immune effector cell population, such as T cells, may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. In certain aspects, compositions of the present disclosure are formulated for parenteral administration, *e.g.*, intravascular (intravenous or intraarterial), intraperitoneal or intramuscular administration.

[0496] The liquid pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite;

chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. An injectable pharmaceutical composition is preferably sterile.

[0497] In a particular embodiment, compositions contemplated herein comprise an effective amount of CAR-expressing immune effector cells, alone or in combination with one or more therapeutic agents. Thus, the CAR-expressing immune effector cell compositions may be administered alone or in combination with other known cancer treatments, such as radiation therapy, chemotherapy, transplantation, immunotherapy, hormone therapy, photodynamic therapy, *etc*. The compositions may also be administered in combination with antibiotics. Such therapeutic agents may be accepted in the art as a standard treatment for a particular disease state as described herein, such as a particular cancer. Exemplary therapeutic agents contemplated include cytokines, growth factors, steroids, NSAIDs, DMARDs, anti-inflammatories, chemotherapeutics, radiotherapeutics, therapeutic antibodies, or other active and ancillary agents.

[0498] In certain embodiments, compositions comprising CAR-expressing immune effector cells disclosed herein may be administered to a subject in conjunction with any number of chemotherapeutic, *e.g.*, anti-cancer, agents. In certain embodiments, a chemotherapeutic, *e.g.*, anti-cancer, agent, is administered to a subject after the administration of a CAR T cell therapy, *e.g*, BCMA CAR T cell therapy, if certain conditions, described elsewhere herein, occur that indicate the CAR T cell therapy will not be therapeutically beneficial to the subject. Illustrative examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine resume; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-

norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2”-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (“Ara-C”); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxetaxel (TAXOTERE®, Rhone-Poulenc Rohrer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoic acid derivatives such as Targretin™ (bexarotene), Panretin™ (alitretinoin); ONTAK™ (denileukin diftitox); esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on cancers such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0499] In certain embodiments, compositions comprising CAR-expressing immune effector cells (e.g., immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), e.g., idecabtagene vicleucel (ide-cel) cells) disclosed herein may be administered to a subject in conjunction with lenalidomide as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the lenalidomide may be administered at a dosage of about 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg, or 25 mg. In certain embodiments, the lenalidomide may be administered at a dosage of about 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg, or 25 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 25 mg once daily orally on Days 1-21 of repeated 28-day cycles. In certain embodiments, the lenalidomide may be administered at a dosage of about 25 mg once daily orally on Days 1-21 of repeated 28-day cycles to a subject for treating Multiple Myeloma (MM). In certain embodiments, the lenalidomide may be administered at a dosage of about 10 mg once daily continuously on Days 1-28 of repeated 28-day cycles. In certain embodiments, the lenalidomide may be administered at a dosage of about 2.5 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 5 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 10 mg once daily. In certain embodiments, the lenalidomide may be administered at a dosage of about 15 mg every other day. In certain embodiments, the lenalidomide may be administered at a dosage of about 25 mg once daily orally on Days 1-21 of repeated 28-day cycles. In certain embodiments, the lenalidomide may be administered at a dosage of about 20 mg once daily orally on Days 1-21 of repeated 28-day cycles for up to 12 cycles. In a certain embodiment, lenalidomide maintenance therapy is recommended for all patients. In a certain embodiment, lenalidomide maintenance therapy should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0500] In certain embodiments, compositions comprising CAR-expressing immune effector cells (e.g., immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), e.g., idecabtagene vicleucel (ide-cel) cells) disclosed herein may be administered to a subject in conjunction with pomalidomide as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the pomalidomide may be administered at a dosage of about 1 mg, 2 mg, 3 mg, or 4 mg. In certain embodiments, the pomalidomide may be administered at a dosage of about 1 mg, 2 mg, 3 mg, or 4 mg once daily. In certain embodiments, the pomalidomide may be administered at a dosage of about 4 mg per day taken orally on days 1-21 of repeated 28-day cycles until disease progression. In certain embodiments, the pomalidomide may be administered at a dosage of about 4 mg per day taken orally on days 1-21 of repeated 28-day cycles until disease progression to a subject for treating Multiple Myeloma (MM). In a certain embodiment, pomalidomide maintenance therapy is recommended for all patients. In a certain embodiment, pomalidomide maintenance therapy should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0501] In certain embodiments, compositions comprising CAR-expressing immune effector cells (e.g., immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), e.g., idecabtagene vicleucel (ide-cel) cells) disclosed herein may be administered to a subject in conjunction with CC-220 (iberdomide) as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the

CC-220 may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg. In certain embodiments, the CC-220 may be administered orally. In certain embodiments, the CC-220 may be administered orally at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily for 21 days of a 28-day cycle, e.g., daily on days 1-21 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the CC-220 may be administered to a subject for treating Multiple Myeloma (MM). In a certain embodiment, CC-220 maintenance therapy is recommended for all patients. In a certain embodiment, the CC-220 maintenance therapy should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0502] In certain embodiments, compositions comprising CAR-expressing immune effector cells (e.g., immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), e.g., idecabtagene vicleucel (ide-cel) cells) disclosed herein may be administered to a subject in conjunction with CC-220 (iberdomide) and dexamethasone as a maintenance therapy after administration of compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 and dexamethasone may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the dexamethasone may be administered immediately after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 and dexamethasone may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the dexamethasone may be administered 1 week, 2 weeks, 3 weeks, or 4 weeks after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 and dexamethasone may be administered 1 month, 2 months, 3

months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the dexamethasone may be administered 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months after administration of the compositions comprising CAR-expressing immune effector cells. In certain embodiments, the CC-220 may be administered at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg. In certain embodiments, the dexamethasone may be administered at a dosage of about 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg. In certain embodiments, the dexamethasone may be administered at a dosage of about 40 mg. In certain embodiments, the CC-220 may be administered orally. In certain embodiments, the CC-220 may be administered orally at a dosage of about 15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily for 21 days of a 28-day cycle, e.g., daily on days 1-21 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the dexamethasone may be administered orally. In certain embodiments, the dexamethasone may be administered at a dose of about 20-60 mgs. In certain embodiments, the dexamethasone may be administered orally at a dosage of about 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg on days 1, 8, 15, and 22 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the CC-220 may be administered orally at a dosage of about 15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily for 21 days of a 28-day cycle, e.g., daily on days 1-21 of a 28-day cycle, with the 28-day cycles repeated as needed, and the dexamethasone may be administered orally at a dosage of about 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg on days 1, 8, 15, and 22 of a 28-day cycle, with the 28-day cycles repeated as needed. In certain embodiments, the CC-220 and dexamethasone may be administered to a subject for treating Multiple Myeloma (MM). In a certain embodiment, CC-220 and dexamethasone maintenance therapy is recommended for all patients. In a certain embodiment, the CC-220 and dexamethasone maintenance therapy should be initiated upon adequate bone marrow recovery or from 90-day post-ide-cel infusion, whichever is later.

[0503] A variety of other therapeutic agents may be used in conjunction with the compositions described herein. In one embodiment, the composition comprising CAR-expressing immune effector cells is administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs include, but are not limited to, steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDS) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate.

[0504] Other exemplary NSAIDs are chosen from the group consisting of ibuprofen, naproxen, naproxen sodium, Cox-2 inhibitors such as VIOXX® (rofecoxib) and CELEBREX® (celecoxib), and sialylates. Exemplary analgesics are chosen from the group consisting of acetaminophen, oxycodone, tramadol, and propoxyphene hydrochloride. Exemplary glucocorticoids are chosen from the group consisting of cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, and prednisone. Exemplary biological response modifiers include molecules directed against cell surface markers (*e.g.*, CD4, CD5, etc.), cytokine inhibitors, such as the TNF antagonists (*e.g.*, etanercept (ENBREL®), adalimumab (HUMIRA®) and infliximab (REMICADE®), chemokine inhibitors and adhesion molecule inhibitors. The biological response modifiers include monoclonal antibodies as well as recombinant forms of molecules. Exemplary DMARDs include azathioprine, cyclophosphamide, cyclosporine, methotrexate, penicillamine, leflunomide, sulfasalazine, hydroxychloroquine, Gold (oral (auranofin) and intramuscular) and minocycline.

[0505] Illustrative examples of therapeutic antibodies suitable for combination with the CAR modified T cells contemplated herein, include, but are not limited to, bavituximab, bevacizumab (avastin), bivatuzumab, blinatumomab, conatumumab, daratumumab, duligotumab, dacetuzumab, dalotuzumab, elotuzumab (HuLuc63), gemtuzumab, ibritumomab, indatuximab, inotuzumab, lorvotuzumab, lucatumumab, milatuzumab, moxetumomab, ocaratuzumab, ofatumumab, rituximab, siltuximab, teprotumumab, and ublituximab.

[0506] Antibodies against PD-1 or, PD-L1 and/or CTLA-4 may be used in combination with the CAR T cells disclosed herein, e.g., BCMA CAR T cells, e.g., CAR T cells expressing a chimeric antigen receptor comprising a BCMA-2 single chain Fv fragment, e.g., idecabtagene vicleucel cells. In particular embodiments, the PD-1 antibody is selected from the group consisting of:

nivolumab, pembrolizumab, and pidilizumab. In particular embodiments, the PD-L1 antibody is selected from the group consisting of: atezolizumab, avelumab, durvalumab, and BMS-986559. In particular embodiments, the CTLA-4 antibody is selected from the group consisting of: ipilimumab and tremelimumab.

[0507] In certain embodiments, the compositions described herein are administered in conjunction with a cytokine. By "cytokine" as used herein is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-alpha and -beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, IL-21, a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

[0508] In certain embodiments, the compositions described herein are administered in conjunction with a therapy to treat Cytokine Release Syndrome (CRS). CRS is a systemic inflammatory immune response that can occur after administration of certain biologic therapeutics, *e.g.*, chimeric antigen receptor-expressing T cells or NK cells (CAR T cells or CAR NK cells), *e.g.*, BCMA CAR T cells. CRS can be distinguished from cytokine storm, a condition with a similar clinical phenotype and biomarker signature, as follows. In CRS, T cells become activated upon recognition of a tumor antigen, while in cytokine storm, the immune system is

activated independently of tumor targeting; in CRS, IL-6 is a key mediator, and thus symptoms may be relieved using an anti-IL-6 or anti-IL-6 receptor (IL-6R) inhibitor, while in cytokine storm, Tumor Necrosis Factor alpha (TNFα) and interferon gamma (IFNγ) are the key mediators, and symptoms may be relieved using anti-inflammatory therapy, *e.g.*, corticosteroids. An anti-IL-6 receptor (IL-6R) antibody such as tocilizumab may be used to manage CRS, optionally with supportive care. An anti-IL-6 antibody such as siltuximab may additionally or alternatively be used to manage CRS, optionally with supportive care. IL-6 blockade (e.g., using an anti-IL-6R antibody or anti-IL-6 antibody) can be used if a patient infused with CAR T cells or CAR NK cells displays any of grade 1, grade 2, grade 3 or grade 4 CRS, but is typically reserved for more severe grades (*e.g.*, grade 3 or grade 4). Corticosteroids can be administered to manage neurotoxicities that accompany or are caused by CRS, or to patients treated with an IL-6 blockade, but are generally not used as a first-line treatment for CRS. Other modalities for the management of CRS are described in, *e.g.*, Shimabukuro-Vornhagen *et al.*, "Cytokine Release Syndrome," *J. Immunother. Cancer* 6:56 (2018).

[0509] Table 4: CRS may be graded using the Penn grading scale:

GRADE	SYMPTOMS	MANAGEMENT
1	Mild reaction (fever, nausea, fatigue, headache, myalgia, malaise)	Supportive care, e.g., antiemetics, antipyretics
2	Moderate reaction (some signs of organ dysfunction such as grade 2 creatinine or grade 3 liver function test (LFT))	Hospitalization for fever with neutropenia
3	Severe reaction (signs of worse organ dysfunction such as grade 4 LFT, grade 3 creatinine; coagulopathy; dyspnea or hypoxia)	Hospitalization for one or more of IV fluids, low-dose vasosuppressors, fresh frozen plasma or fibrinogen concentrate; provision of oxygen or CPAP
4	Life-threatening reaction (hypotension, hypoxia)	Hospitalization for vasosuppressors, mechanical ventilation

[0510] Table 5: CRS may also be graded by the CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) v4.0:

GRADE	SYMPTOMS	MANAGEMENT
1	Mild reaction (fever, nausea, fatigue, headache, myalgia, malaise)	Supportive care, e.g., antiemetics, antipyretics - infusion interruption not indicated
2	Moderate reaction; patient responds promptly to supportive care, e.g. antihistamines, NSAIDs, narcotics, IV fluids	Interruption of infusion
3	Prolonged reaction; patient does not respond promptly to supportive care, e.g. antihistamines, NSAIDs, narcotics, IV fluids; recurrence of symptoms following initial improvement; renal impairment and/or pulmonary infiltrates	Interruption of infusion; hospitalization for sequelae
4	Life-threatening reaction (hypotension, hypoxia)	Hospitalization for vasopressors, mechanical ventilation

[0511] Table 6: CRS may also be graded by the system of Lee *et al.* ("Current concepts in the diagnosis and management of cytokine release syndrome," Blood, 2014, 124:188–195):

GRADE	SYMPTOMS	MANAGEMENT
1	Non-life-threatening symptoms (fever, nausea, fatigue, headache, myalgia, malaise)	Supportive care, e.g., antiemetics, antipyretics
2	Moderate reaction; symptoms require, and patient responds to, intervention	O2 requirement <40%, fluids for hypotension, vasopressors

GRADE	SYMPTOMS	MANAGEMENT
3	More severe reaction (e.g., hypoxia and/or hypotension; grade 3 organ toxicity, grade 4 transaminitis); symptoms require and respond to aggressive intervention.	O2 requirement > 40%; high-dose vasopressors for hypotension
4	Life-threatening reaction (hypotension, hypoxia)	Hospitalization for vasopressors, mechanical ventilation

[0512] In particular embodiments, a composition comprises CAR T cells contemplated herein that are cultured in the presence of a PI3K inhibitor as disclosed herein and express one or more of the following markers: CD3, CD4, CD8, CD28, CD45RA, CD45RO, CD62, CD127, and HLA-DR can be further isolated by positive or negative selection techniques. In one embodiment, a composition comprises a specific subpopulation of T cells, expressing one or more of the markers selected from the group consisting of CD62L, CCR7, CD28, CD27, CD122, CD127, CD197; and CD38 or CD62L, CD127, CD197, and CD38, is further isolated by positive or negative selection techniques. In various embodiments, compositions do not express or do not substantially express one or more of the following markers: CD57, CD244, CD160, PD-1, CTLA4, TIM3, and LAG3.

[0513] In one embodiment, expression of one or more of the markers selected from the group consisting of CD62L, CD127, CD197, and CD38 is increased at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 25 fold, or more compared to a population of T cells activated and expanded without a PI3K inhibitor.

[0514] In one embodiment, expression of one or more of the markers selected from the group consisting of CD57, CD244, CD160, PD-1, CTLA4, TIM3, and LAG3 is decreased at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 25 fold, or more compared to a population of T cells activated and expanded with a PI3K inhibitor.

5.10. Therapeutic Methods

[0515] The genetically modified immune effector cells contemplated herein provide improved methods of adoptive immunotherapy for use in the treatment of B cell related conditions that include, but are not limited to immunoregulatory conditions and hematological malignancies.

5.10.1. General Embodiments

[0516] In particular embodiments, the specificity of a primary immune effector cell is redirected to B cells by genetically modifying the primary immune effector cell with a CAR contemplated herein. In various embodiments, a viral vector is used to genetically modify an immune effector cell with a particular polynucleotide encoding a CAR comprising a murine anti-BCMA antigen binding domain that binds a BCMA polypeptide, *e.g.*, a human BCMA polypeptide; a hinge domain; a transmembrane (TM) domain, a short oligo- or polypeptide linker, that links the TM domain to the intracellular signaling domain of the CAR; and one or more intracellular co-stimulatory signaling domains; and a primary signaling domain.

[0517] In one embodiment, a type of cellular therapy is included where T cells are genetically modified to express a CAR that targets BCMA expressing B cells. In another embodiment, anti-BCMA CAR T cells are cultured in the presence of IL-2 and a PI3K inhibitor to increase the therapeutic properties and persistence of the CAR T cells. The CAR T cell are then infused to a recipient in need thereof. The infused cell is able to kill disease causing B cells in the recipient. Unlike antibody therapies, CAR T cells are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained cancer therapy.

[0518] In one embodiment, the CAR T cells can undergo robust *in vivo* T cell expansion and can persist for an extended amount of time. In another embodiment, the CAR T cells evolve into specific memory T cells that can be reactivated to inhibit any additional tumor formation or growth.

[0519] In particular embodiments, compositions comprising immune effector cells comprising the CARs contemplated herein are used in the treatment of conditions associated with abnormal B cell activity.

[0520] Illustrative examples of conditions that can be treated, prevented or ameliorated using the immune effector cells comprising the CARs contemplated herein include, but are not limited to: systemic lupus erythematosus, rheumatoid arthritis, myasthenia gravis, autoimmune hemolytic anemia, idiopathic thrombocytopenia purpura, anti-phospholipid syndrome, Chagas' disease,

Grave's disease, Wegener's granulomatosis, poly-arteritis nodosa, Sjogren's syndrome, pemphigus vulgaris, scleroderma, multiple sclerosis, anti-phospholipid syndrome, ANCA associated vasculitis, Goodpasture's disease, Kawasaki disease, and rapidly progressive glomerulonephritis.

[0521] The modified immune effector cells may also have application in plasma cell disorders such as heavy-chain disease, primary or immunocyte-associated amyloidosis, and monoclonal gammopathy of undetermined significance (MGUS).

[0522] As use herein, "B cell malignancy" refers to a type of cancer that forms in B cells (a type of immune system cell) as discussed *infra*.

[0523] In particular embodiments, compositions comprising CAR-modified T cells contemplated herein are used in the treatment of hematologic malignancies, including but not limited to B cell malignancies such as, for example, multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL).

[0524] Multiple myeloma is a B cell malignancy of mature plasma cell morphology characterized by the neoplastic transformation of a single clone of these types of cells. These plasma cells proliferate in bone marrow (BM) and may invade adjacent bone and sometimes the blood. Variant forms of multiple myeloma include overt multiple myeloma, smoldering multiple myeloma, plasma cell leukemia, non-secretory myeloma, IgD myeloma, osteosclerotic myeloma, solitary plasmacytoma of bone, and extramedullary plasmacytoma (see, for example, Braunwald, *et al.* (eds), *Harrison's Principles of Internal Medicine*, 15th Edition (McGraw-Hill 2001)).

[0525] Multiple myeloma can be staged as follows:

Table 7: Durie-Salmon MM Staging Criteria

Stage	Durie-Salmon Criteria
I	*All of the following:* Hemoglobin value > 10 g/dL Serum calcium value normal or < 12 mg/dL Bone x-ray, normal bone structure (scale 0), or solitary bone plasmacytoma only Low M-component production rates IgG value < 5 g/dL; IgA value < 3 g/dL Urine light chain M-component on electrophoresis < 4 g/24h
II	Neither Stage I nor Stage III
III	*One or more of the following:* Hemoglobin value < 8.5 g/dL Serum calcium value normal or > 12 mg/dL Advanced lytic bone lesions (scale 3) High M-component production rates IgG value > 7 g/dL; IgA value > 5 g/dL Urine light chain M-component on electrophoresis > 12 g/24h
Subclassification Criteria A Normal renal function (serum creatinine value < 2.0 mg/dL) B Abnormal renal function (serum creatinine value ≥ 2.0 mg/dL)	

Table 8: International Staging System MM Staging Criteria

Stage	International Staging System (ISS) Criteria	Revised International Staging System (ISS) Criteria
I	Serum beta-2 microglobulin < 3.5 mg/L Serum albumin ≥ 3.5 g/dL	ISS stage I and standard-risk CA by iFISH and normal LDH
II	Neither Stage I nor Stage III	Neither Stage I nor Stage III
III	Serum beta-2 microglobulin ≥ 5.5 mg/L	ISS stage III and either high-risk CA by iFISH[c] or high LDH

[0526] Non-Hodgkin lymphoma encompasses a large group of cancers of lymphocytes (white blood cells). Non-Hodgkin lymphomas can occur at any age and are often marked by lymph nodes that are larger than normal, fever, and weight loss. There are many different types of non-Hodgkin lymphoma. For example, non-Hodgkin's lymphoma can be divided into aggressive (fast-growing) and indolent (slow-growing) types. Although non-Hodgkin lymphomas can be derived from B cells and T-cells, as used herein, the term "non-Hodgkin lymphoma" and "B cell non-Hodgkin lymphoma" are used interchangeably. B cell non-Hodgkin lymphomas (NHL) include Burkitt's lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma. Lymphomas that occur after bone marrow or stem cell transplantation are usually B cell non-Hodgkin lymphomas.

[0527] Chronic lymphocytic leukemia (CLL) is an indolent (slow-growing) cancer that causes a slow increase in immature white blood cells called B lymphocytes, or B cells. Cancer cells spread through the blood and bone marrow, and can also affect the lymph nodes or other organs such as the liver and spleen. CLL eventually causes the bone marrow to fail. Sometimes, in later stages of the disease, the disease is called small lymphocytic lymphoma.

[0528] In particular embodiments, methods comprising administering a therapeutically effective amount of CAR-expressing immune effector cells contemplated herein or a composition comprising the same, to a patient in need thereof, alone or in combination with one or more therapeutic agents, are provided. In certain embodiments, the cells of the prewent disclosure are used in the treatment of patients at risk for developing a condition associated with abnormal B cell activity or a B cell malignancy. Thus, in certain embodiments, presented herein are methods for the treatment or prevention of a condition associated with abnormal B cell activity or a B cell malignancy comprising administering to a subject in need thereof, a therapeutically effective amount of the CAR-modified cells contemplated herein.

[0529] As used herein, the terms "individual" and "subject" are often used interchangeably and refer to any animal that exhibits a symptom of a disease, disorder, or condition that can be treated with the gene therapy vectors, cell-based therapeutics, and methods disclosed elsewhere herein. In specific embodiments, a subject includes any animal that exhibits symptoms of a disease, disorder, or condition of the hematopoietic system, *e.g.,* a B cell malignancy, that can be

treated with the gene therapy vectors, cell-based therapeutics, and methods disclosed elsewhere herein. Suitable subjects (*e.g.*, patients) include laboratory animals (such as mouse, rat, rabbit, or guinea pig), farm animals, and domestic animals or pets (such as a cat or dog). Non-human primates and, preferably, human patients, are included. Typical subjects include human patients that have a B cell malignancy, have been diagnosed with a B cell malignancy, or are at risk or having a B cell malignancy.

[0530] As used herein, the term "patient" refers to a subject that has been diagnosed with a particular disease, disorder, or condition that can be treated with the gene therapy vectors, cell-based therapeutics, and methods disclosed elsewhere herein.

[0531] As used herein "treatment" or "treating," includes any beneficial or desirable effect on the symptoms or pathology of a disease or pathological condition, and may include even minimal reductions in one or more measurable markers of the disease or condition being treated. Treatment can involve optionally either the reduction or amelioration of symptoms of the disease or condition, or the delaying of the progression of the disease or condition. "Treatment" does not necessarily indicate complete eradication or cure of the disease or condition, or associated symptoms thereof.

[0532] As used herein, "prevent," and similar words such as "prevented," "preventing" *etc.*, indicate an approach for preventing, inhibiting, or reducing the likelihood of the occurrence or recurrence of, a disease or condition. It also refers to delaying the onset or recurrence of a disease or condition or delaying the occurrence or recurrence of the symptoms of a disease or condition. As used herein, "prevention" and similar words also includes reducing the intensity, effect, symptoms and/or burden of a disease or condition prior to onset or recurrence of the disease or condition.

[0533] By "enhance" or "promote," or "increase" or "expand" refers generally to the ability of a composition contemplated herein, *e.g.*, a genetically modified T cell or vector encoding a CAR, to produce, elicit, or cause a greater physiological response (*i.e.*, downstream effects) compared to the response caused by either vehicle or a control molecule/composition. A measurable physiological response may include an increase in T cell expansion, activation, persistence, and/or an increase in cancer cell killing ability, among others apparent from the understanding in the art and the description herein. An "increased" or "enhanced" amount is typically a "statistically significant" amount, and may include an increase that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7,

8, 9, 10, 15, 20, 30 or more times (*e.g.,* 500, 1000 times) (including all integers and decimal points in between and above 1, *e.g.,* 1.5, 1.6, 1.7. 1.8, *etc.*) the response produced by vehicle or a control composition.

[0534] By "decrease" or "lower," or "lessen," or "reduce," or "abate" refers generally to the ability of composition contemplated herein to produce, elicit, or cause a lesser physiological response (*i.e.*, downstream effects) compared to the response caused by either vehicle or a control molecule/composition. A "decrease" or "reduced" amount is typically a "statistically significant" amount, and may include an decrease that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (*e.g.,* 500, 1000 times) (including all integers and decimal points in between and above 1, *e.g.,* 1.5, 1.6, 1.7. 1.8, *etc.*) the response (reference response) produced by vehicle, a control composition, or the response in a particular cell lineage.

[0535] By "maintain," or "preserve," or "maintenance," or "no change," or "no substantial change," or "no substantial decrease" refers generally to the ability of a composition contemplated herein to produce, elicit, or cause a substantially similar physiological response (*i.e.*, downstream effects) in a cell, as compared to the response caused by either vehicle, a control molecule/composition, or the response in a particular cell lineage. A comparable response is one that is not significantly different or measurably different from the reference response.

[0536] In one embodiment, a method of treating a B cell related condition in a subject in need thereof comprises administering an effective amount, *e.g.,* a therapeutically effective amount of a composition comprising genetically modified immune effector cells contemplated herein. The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

[0537] In one embodiment, the amount of T cells in the composition administered to a subject is at least 0.1×10^5 cells, at least 0.5×10^5 cells, at least 1×10^5 cells, at least 5×10^5 cells, at least 1×10^6 cells, at least 0.5×10^7 cells, at least 1×10^7 cells, at least 0.5×10^8 cells, at least 1×10^8 cells, at least 0.5×10^9 cells, at least 1×10^9 cells, at least 2×10^9 cells, at least 3×10^9 cells, at least 4×10^9 cells, at least 5×10^9 cells, or at least 1×10^{10} cells. In particular embodiments, about 1×10^7 CAR T cells to about 1×10^9 CAR T cells, about 2×10^7 CAR T cells to about 0.9×10^9 CAR T cells, about 3×10^7 CAR T cells to about 0.8×10^9 CAR T cells, about 4×10^7

CAR T cells to about 0.7 x 10^9 CAR T cells, about 5 x 10^7 CAR T cells to about 0.6 x 10^9 CAR T cells, or about 5 x 10^7 CAR T cells to about 0.5 x 10^9 CAR T cells are administered to a subject.

[0538] In one embodiment, the amount of T cells in the composition administered to a subject is at least 0.1 x 10^4 cells/kg of bodyweight, at least 0.5 x 10^4 cells/kg of bodyweight, at least 1 x 10^4 cells/kg of bodyweight, at least 5 x 10^4 cells/kg of bodyweight, at least 1 x 10^5 cells/kg of bodyweight, at least 0.5 x 10^6 cells/kg of bodyweight, at least 1 x 10^6 cells/kg of bodyweight, at least 0.5 x 10^7 cells/kg of bodyweight, at least 1 x 10^7 cells/kg of bodyweight, at least 0.5 x 10^8 cells/kg of bodyweight, at least 1 x 10^8 cells/kg of bodyweight, at least 2 x 10^8 cells/kg of bodyweight, at least 3 x 10^8 cells/kg of bodyweight, at least 4 x 10^8 cells/kg of bodyweight, at least 5 x 10^8 cells/kg of bodyweight, or at least 1 x 10^9 cells/kg of bodyweight. In particular embodiments, about 1 x 10^6 CAR T cells/kg of bodyweight to about 1 x 10^8 CAR T cells/kg of bodyweight, about 2 x 10^6 CAR T cells/kg of bodyweight to about 0.9 x 10^8 CAR T cells/kg of bodyweight, about 3 x 10^6 CAR T cells/kg of bodyweight to about 0.8 x 10^8 CAR T cells/kg of bodyweight, about 4 x 10^6 CAR T cells/kg of bodyweight to about 0.7 x 10^8 CAR T cells/kg of bodyweight, about 5 x 10^6 CAR T cells/kg of bodyweight to about 0.6 x 10^8 CAR T cells/kg of bodyweight, or about 5 x 10^6 CAR T cells/kg of bodyweight to about 0.5 x 10^8 CAR T cells/kg of bodyweight are administered to a subject.

[0539] One of ordinary skill in the art would recognize that multiple administrations of the compositions of the present disclosure may be required to effect the desired therapy. For example a composition may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more times over a span of 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, 2 years, 5, years, 10 years, or more.

[0540] In certain embodiments, it may be desirable to administer activated immune effector cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate immune effector cells therefrom according to the present disclosure, and reinfuse the patient with these activated and expanded immune effector cells. This process can be carried out multiple times every few weeks. In certain embodiments, immune effector cells can be activated from blood draws of from 10cc to 400cc. In certain embodiments, immune effector cells are activated from blood draws of 20cc, 30cc, 40cc, 50cc, 60cc, 70cc, 80cc, 90cc, 100cc, 150cc, 200cc, 250cc, 300cc, 350cc, or 400cc or more. Not to be bound by theory, using this multiple blood

draw/multiple reinfusion protocol may serve to select out certain populations of immune effector cells.

[0541] The administration of the compositions contemplated herein may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. In one embodiment, compositions are administered parenterally. The phrases "parenteral administration" and "administered parenterally" as used herein refers to modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravascular, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intratumoral, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion. In one embodiment, the compositions contemplated herein are administered to a subject by direct injection into a tumor, lymph node, or site of infection.

[0542] In one embodiment, a subject in need thereof is administered an effective amount of a composition to increase a cellular immune response to a B cell related condition in the subject. The immune response may include cellular immune responses mediated by cytotoxic T cells capable of killing infected cells, regulatory T cells, and helper T cell responses. Humoral immune responses, mediated primarily by helper T cells capable of activating B cells thus leading to antibody production, may also be induced. A variety of techniques may be used for analyzing the type of immune responses induced by the compositions of the present disclosure, which are well described in the art; *e.g.*, *Current Protocols in Immunology*, Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober (2001) John Wiley & Sons, NY, N.Y.

[0543] In the case of T cell-mediated killing, CAR-ligand binding initiates CAR signaling to the T cell, resulting in activation of a variety of T cell signaling pathways that induce the T cell to produce or release proteins capable of inducing target cell apoptosis by various mechanisms. These T cell-mediated mechanisms include (but are not limited to) the transfer of intracellular cytotoxic granules from the T cell into the target cell, T cell secretion of pro-inflammatory cytokines that can induce target cell killing directly (or indirectly via recruitment of other killer effector cells), and up regulation of death receptor ligands (*e.g.* FasL) on the T cell surface that induce target cell apoptosis following binding to their cognate death receptor (*e.g.* Fas) on the target cell.

[0544] In one embodiment, provided herein i a method of treating a subject diagnosed with a B cell related condition comprising removing immune effector cells from a subject diagnosed with a BCMA-expressing B cell related condition, genetically modifying said immune effector cells with a vector comprising a nucleic acid encoding a CAR as contemplated herein, thereby producing a population of modified immune effector cells, and administering the population of modified immune effector cells to the same subject. In a particular embodiment, the immune effector cells comprise T cells.

[0545] In certain embodiments, also provided herein are methods for stimulating an immune effector cell mediated immune modulator response to a target cell population in a subject comprising the steps of administering to the subject an immune effector cell population expressing a nucleic acid construct encoding a CAR molecule.

[0546] The methods for administering the cell compositions described herein includes any method which is effective to result in reintroduction of *ex vivo* genetically modified immune effector cells that either directly express a CAR of the present disclosure in the subject or on reintroduction of the genetically modified progenitors of immune effector cells that on introduction into a subject differentiate into mature immune effector cells that express the CAR. One method comprises transducing peripheral blood T cells *ex vivo* with a nucleic acid construct in accordance with the present disclosure and returning the transduced cells into the subject.

[0547] All publications, patent applications, and issued patents cited in this specification are hereby incorporated by reference herein in their entireties as if each individual publication, patent application, or issued patent were specifically and individually indicated to be incorporated by reference.

[0548] 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.

6. EXAMPLES

6.1. EXAMPLE 1: CONSTRUCTION OF BCMA CARS

[0549] CARs containing anti-BCMA scFv antibodies were designed to contain an MND promoter operably linked to anti-BMCA scFv, a hinge and transmembrane domain from CD8α and a CD137 co-stimulatory domain followed by the intracellular signaling domain of the CD3ζ chain. *See, e.g.,* Figure 1. *See*, also, International Publication No. WO 2016/094304, which is incorporated by reference herein in its entirety, and in particular incorporates the disclosure of BCMA CARs and their characterization. The BCMA CAR shown in Figure 1 comprises a CD8α signal peptide (SP) sequence for the surface expression on immune effector cells. The polynucleotide sequence of an exemplary BCMA CAR is set forth in SEQ ID NO: 10 (polynucleotide sequence of anti-BCMA02 CAR); an exemplary polypeptide sequence of a BCMA CAR is set forth in SEQ ID NO: 9 (polypeptide sequence of anti-BCMA02 CAR); and a vector map of an exemplary CAR construct is shown in Figure 1. Table 9 shows the identity, GenBank Reference (where applicable), Source Name and Citation for the various nucleotide segments of a BCMA CAR lentiviral vector that comprise a BCMA CAR construct as shown in Figure 1.

Table 9.

Nucleotides	Identity	GenBank Reference	Source Name	Citation
1-185	pUC19 plasmid backbone	Accession #L09137.2 nt 1 – 185	pUC19	New England Biolabs
185-222	Linker	Not applicable	Synthetic	Not applicable
223-800	CMV	Not Applicable	pHCMV	Yee, et al., (1994) PNAS 91: 9564-68
801-1136	R, U5, PBS, and packaging sequences	Accession #M19921.2 nt 454-789	pNL4-3	Maldarelli, et.al. (1991) J Virol: 65(11):5732-43
1137-1139	Gag start codon (ATG) changed to stop codon (TAG)	Not Applicable	Synthetic	Not applicable

Nucleotides	Identity	GenBank Reference	Source Name	Citation
1140-1240	HIV-1 gag sequence	Accession #M19921.2 nt 793-893	pNL4-3	Maldarelli, et.al. (1991) J Virol: 65(11):5732-43
1241-1243	HIV-1 gag sequence changed to a second stop codon	Not Applicable	Synthetic	Not applicable
1244-1595	HIV-1 gag sequence	Accession #M19921.2 nt 897-1248	pNL4-3	Maldarelli, et.al. (1991) J Virol: 65(11):5732-43
1596-1992	HIV-1 pol cPPT/CTS	Accession #M19921.2 nt 4745-5125	pNL4-3	Maldarelli, et.al. (1991) J Virol: 65(11):5732-43
1993-2517	HIV-1, isolate HXB3 env region (RRE)	Accession #M14100.1 nt 1875-2399	PgTAT-CMV	Malim, M. H. Nature (1988) 335:181-183
2518-2693	HIV-1 env sequences S/A	Accession #M19921.2 nt 8290-8470	pNL4-3	Maldarelli, et.al. (1991) J Virol: 65(11):5732-43
2694-2708	Linker	Not applicable	Synthetic	Not applicable
2709-3096	MND	Not applicable	pccl-c-MNDU3c-x2	Challita et al. (1995) J. Virol. 69: 748-755
3097-3124	Linker	Not applicable	Synthetic	Not applicable
3125-3187	Signal peptide	Accession # NM_001768	CD8a signal peptide	Not applicable
3188-3934	BCMA02 scFv	Not applicable	Synthetic	Not applicable

Nucleotides	Identity	GenBank Reference	Source Name	Citation
3935-4141	CD8a hinge and TM	Accession # NM_001768	CD8a hinge and TM	Milone et al (2009) Mol Ther 17(8):1453-64
4144-4269	CD137 (4-1BB) signaling domain	Accession # NM_001561	CD137 signaling domain	Milone et al (2009) Mol Ther 17(8):1453-64
4270-4606	CD3-ζ signaling domain	Accession # NM_000734	CD3-ζ signaling domain	Milone et al (2009) Mol Ther 17(8):1453-64
4607-4717	HIV-1 ppt and part of 3' U3	Accession #M19921.2 nt 9005-9110	pNL4-3	Maldarelli, et.al. (1991) J Virol: 65(11):5732-43
4718-4834	HIV-1 part of U3 (399bp deletion) and R	Accession #M19921.2 nt 9511-9627	pNL4-3	Maldarelli, et.al. (1991) J Virol: 65(11):5732-43
4835-4858	Synthetic polyA	Not applicable	Synthetic	Levitt, N. Genes & Dev (1989) 3:1019-1025
4859-4877	Linker	Not applicable	Synthetic	Not Applicable
4878-7350	pUC19 backbone	Accession #L09137.2 nt 2636-2686	pUC19	New England Biolabs

6.2. EXAMPLE 2: BIOMARKERS IN IDE-CEL TREATMENT

[0550] A phase 1 clinical trial was conducted of idecabtagene vicleucel (ide-cel), an autologous T lymphocyte-enriched population comprising cells transduced with an anti-B cell maturation antigen (BCMA) chimeric antigen receptor (CAR) (anti-BCMA02 CAR, described above) lentiviral vector encoding a CAR targeting human BCMA. This study was an open-label, multicenter Phase 1 dose escalation and expansion study to determine the safety and efficacy of ide-cel in subjects with relapsed and refractory multiple myeloma (RRMM), where each of the

subjects had ≥3 prior lines of therapy, including lenalidomide, PI (Proteasome Inhibitor) and/or anti-CD38 antibody. The study enrolled 33 subjects, without regard to the level of BCMA expression. Subjects were lymphodepleted with 30 mg/m^2 fludarabine and 300 mg/m^2 cytarabine on days -5, -4 and -3 prior to CAR T cell administration. Ide-cel was administered in dosage amounts of 50 x 10^5 cells, 150 x 10^5 cells, 450 x 10^5 cells, and 800 x 10^5 cells. Subjects were monitored for the development of cytokine response syndrome (CRS) over the first week post-administration of ide-cel. Efficacy was assessed at Month 1 following ide-cel administration, and monthly thereafter for at least 18 months.

[0551] Retrospective analysis was performed on data collected from the 33 patients in the trial of ide-cel in relapsed/refractory multiple myeloma and results were qualitatively validated in a larger cohort of 128 patients in the subsequent Phase 2 trial of ide-cel (NCT03361748). Amounts of soluble BCMA (sBCMA) in subject serum, and of IL-6, TNFα, and ferritin in subject plasma, were assessed on Day 0 (day of administration). Amounts of IL-6, TNFα and ferritin in subject plasma were assessed at Days 1, 2, 3, 4, 7, 9, 11, 14, 21 and Month 1 post-infusion, and sBCMA in subject serum was assessed as Days 2, 4, 7, 9, 11, 14, 21, and Months 1, 2, 3, 4, 6 and at subsequent three-month intervals post-infusion of ide-cel.

[0552] The fold-changes in IL-6 and TNF were computed relative to the day of infusion of ide-cel for all post-infusion sample collections. The fold-changes of IL-6 at days 1, 2, 7 and 9 were significantly lower ($p < .01$) in patients who failed to achieve a response of Partial Response (PR) or better (Figure 2A). The fold-changes of TNF at days 2, 7 and 9 were significantly lower ($p < 0.02$) in patients who failed to achieve a response of PR or better (Figure 2B).

[0553] The fold-changes in soluble BCMA were computed relative to the day of infusion for all post-infusion sample collections. Subjects who failed to achieve a response of PR or better showed significantly less reduction of soluble BCMA ($p < 0.03$ for the phase 1 study) at Days 7, 9, 11, 14, 21, and Months 1 and 2 than in patients who remained in stable or progressive disease. In particular, at Month 1 subjects who displayed progressive disease or stable disease showed significantly less reduction in sBCMA after ide-cel administration than subjects who achieved a response of PR or better (Figure 3; Wilcoxon AUC = .01, $p = .00013$ for the phase 1 trial, $p = 9.43 \times 10^{-8}$ for the phase 2 trial with a median % soluble BCMA reduction of non-responders in month 1 of 6.8% compared with a median % soluble BCMA reduction of PR or better responders of 96.9%).

[0554] Seven out of the thirty-three subjects maintained progression-free survival for at least 18 months. Soluble BCMA concentrations at month 2 were significantly lower in these seven subjects than in the rest of the cohort ($p = .0016$, Figure 4).

6.3. EXAMPLE 3: BIOMARKERS IN IDE-CEL TREATMENT

[0555] Ide-cel has demonstrated promising efficacy in a phase I clinical trial in relapsed/refractory multiple myeloma (MM) [Objective response rate, 85%; median Progression Free Survival (PFS) 11.8 months (95% CI 6.2, 17.8); median duration of response was 10.9 months (95% CI, 7.2 to not estimable)], but a subset of enrolled patients failed to respond to ide-cel and the duration of response varied among responders (Raje *et al.*, *N. Engl. J. Med.* 2019, 380:1726-1737). To gain insight into this observation, a retrospective analysis of 33 patients from the phase I study was performed.

[0556] The concentrations of ten immune-related factors in the blood (GMCSF, IFN-γ, IL-10, IL-1β, IL-2, IL-6, IL-8, MCP-1, TNF-α) and soluble BCMA were measured by ELISA before and after infusion with ide-cel along with 290 ide-cel CAR T-cell drug product attributes measured by flow cytometry and Luminex. The absolute concentrations and fold-changes from baseline were assessed for correlation with overall and long-term response using univariate and multivariate (random forests) approaches.

[0557] Results: Pre-infusion (i.e., baseline) levels of soluble BCMA significantly correlated with serum monoclonal protein (M-protein) levels in 20 of 33 patients for whom M-protein levels were measurable ($\rho = .49$; $P = 0.03$) and with concentrations of the involved free light chain (FLC) ($\rho = .59$; $P = 0.005$) in 23 of 33 patients with measurable levels. These results indicate that sBCMA aligns with existing tumor burden measures and is assessable in all patients (whereas M-protein and FLC were not assessable in all patients), and is an attractive potential pan-patient biomarker for monitoring response and relapse. The investigation of soluble BCMA levels in patients achieving a partial response (PR) or better confirmed significant decreases in soluble BCMA levels relative to non-responders (NR) as early as seven days post-infusion (median reduction of 50% for ≥ PR vs. median increase of 27% for NR, $P = 0.02$) (Figure 5A). Decreases in soluble BCMA levels were greatest at month 1 post-infusion (Figure 5B). The fold-change in soluble BCMA one month after infusion stratified patients who achieved a PR or better from those who did not ($P = 0.0001$) (Figure 5B). Patients who maintained a response to ide-cel for ≥ 18 months (i.e., durable responders/M18 R) experienced a greater depth of

clearance of soluble BCMA at month 2 (median concentration of 1835 ng/L for durable responders/M18 R vs. 6299 ng/L for nondurable responders/M18 NR, $P = 0.002$) (Figure 6). In particular, durable responders had significantly lower levels of sBCMA vs nondurable responders at month 2 (Figure 6). Thus, a lack of sBCMA clearance at 2 months post-infusion retrospectively identified patients at risk of early progression.

[0558] The induction of IL-6 and TNF-α in blood on days 1–9 post-infusion was also significantly higher in patients with a PR or better in response to ide-cel (e.g. IL-6 median fold change increase at Day 2 of 2.9 for ≥ PR vs. 0.7 for NR, $P = 0.00$, as shown in Figure 7A, and TNF-α median fold change increase at Day 2 of approximately 2-log-fold change for ≥ PR vs. NR, $P = 0.006$, as shown in Figure 7B), consistent with an active inflammatory response (i.e., a cytokine response induced by T-cell activation) and higher levels of CAR T expansion. *See*, also, Fig. 2A-2B.

[0559] Several CAR T-cell drug product covariates associated with longer progression-free survival (PFS) including: (1) higher IL-2 and TNF-α production (P = 0.03); (2) reduced composition of activated CD8 CAR T-cells (CD3+/CD8+/CAR+/CD25+; P = 0.015); and (3) reduced senescence population in CD4 CAR T-cells (CD3+/CD4+/CAR+/CD57+; P = 0.05). Longer PFS was associated with lower expression of CD57 (a marker of senescence) in CD4+ CAR T cells and lower expression of CD25 (a marker of activation) in CD8+ CAR T cells in the ide-cel drug product.

[0560] Survival and safety correlations were analyzed among 33 patients who received ≥ 150 × 10^6 CAR+ T cells. Partial dependence and local effect plots were generated from multivariate analyses based on the final ide-cel drug product. Longer PFS was associated with increased IL-2 production by CAR T cells and lower CD25 positivity in CD8+ CAR T cells (Figure 8). Longer PFS was also associated with a higher dose of ide-cel (data not shown).

[0561] A univariate analysis based on the final ide-cel drug product was performed. Univariate and multivariate (random forests) models were used to correlate patient response with absolute concentrations and fold-changes from baseline in immune-related factors and drug product attributes. Figure 9A shows that longer PFS trended with decreased expression of CD25 (a marker of activation) in CD8+CAR T cells. In particular, the hazard ratio (HR) for CD3+, CD8+, CAR+, CD25+ cells is 1.0396 ($P = 0.041$). CD3+, CD8+, CAR+, CD25+ cell subset represents activated CD8 CAR T cells. Figure 9B shows that higher doses of CAR T cells and

increased IL-2 production by CAR T cells correlated with a greater likelihood of cytokine release syndrome (CRS).

[0562] Conclusions: The results from the clinical trial indicate that soluble BCMA could provide a universal surrogate of tumor-burden and response assessment for multiple myeloma. The biomarker analyses from the clinical trial identified candidate drug product attributes and soluble factors that correlate with response to ide-cel. The data presented herein indicate that changes in soluble BCMA correlate with both early and durable responses to ide-cel. Strikingly, the depth of clearance of soluble BCMA, as measured 2 months after infusion, provided a strong correlation with long-term response, potentially allowing for the identification of patients at risk of progression before standard markers of myeloma progression have emerged. The rapid and significant reduction in sBCMA expression may be a robust biomarker of both early and durable responses to ide-cel. In addition, a lack of sBCMA clearance at 2 months post-infusion may be superior to standard markers of myeloma response (e.g., M-protein and FLC) to identify patients at risk of progression.

[0563] Measurement of drug product attributes and protein concentrations in the blood provided useful correlates of response to ide-cel, and random (survival) forests were used to identify candidate attributes of higher importance and to generate hypotheses for validation in a larger cohort (e.g., Efficacy and Safety Study of bb2121 in Subjects With Relapsed and Refractory Multiple Myeloma (KarMMa study), NCT03361748). Ide-cel drug product attributes associated with both increased functional cytokine expression and reduced activation and senescent T cell markers are also associated with greater PFS. A lack of post-infusion induction of IL-6 and TNF-α in non-responding patients was observed, consistent with the observation that these patients also experienced lower levels of CAR T expansion (Raje et al, *N Engl J Med.* 2019, 380:1726-1737).

6.4 EXAMPLE 4: TUMOR CELL EXPRESSION OF BCMA IN PATIENTS HAVING RELAPSED AND REFRACTORY MULTIPLE MYELOMA FOLLOWING TREATMENT WITH IDE-CEL

[0564] Treatment of patients having relapsed and refractory multiple myeloma (RRMM) with idecabtagene vicleucel (ide-cel; bb2121) has yielded frequent and deep responses in these patients, but non-response and progressive disease (PD) have also been observed after an initial response. Acquired resistance to CD19-directed CAR T cells mediated by CD19 antigen loss

has been reported with relatively high frequency in patients with B cell malignancies, especially acute lymphoblastic leukemia. B-cell maturation antigen (BCMA), a member of the tumor necrosis factor receptor superfamily, is nearly universally expressed on multiple myeloma cells, whereas normal expression is restricted to plasma cells and some mature B cells. While anecdotal cases of BCMA antigen loss have been reported in patients who have been administered BCMA-directed CAR T cells, the overall frequency of BCMA antigen loss as a mechanism of escape (i.e., acquired resistance) has not been reported. Idecabtagene vicleucel (ide-cel, bb2121) is a BCMA-directed chimeric antigen receptor (CAR) T cell therapy that demonstrated deep and durable responses in patients with relapsed and refractory multiple myeloma (RRMM) treated in the phase 2 KarMMa study. The overall response rate was 73% and the complete response rate was 33%. Nevertheless, some patients did not experience deep responses, or progressed after initial response to ide-cel. To gain insight into tumor BCMA expression at the time of progressive disease (PD), an analysis of direct and indirect data evaluating tumor BCMA expression was performed based on the primary analysis of patients treated with ide-cel in the phase 2 KarMMa study (NCT03361748). In particular, assessment of pretreatment BCMA expression on CD138+ bone marrow plasma cells and associations with response to ide-cel in the KarMMa trial was conducted. In addition, BCMA antigen expression on CD138+ bone marrow plasma cells and soluble BCMA (sBCMA) levels at progression in ide-cel–treated patients was conducted.

[0565] Methods: Tumor-associated BCMA expression was assessed in formalin-fixed paraffin-embedded decalcified bone marrow biopsies (BMBs) by immunohistochemistry (IHC) using a monoclonal antibody directed against an intracellular BCMA epitope. BCMA expression was assessed by IHC on bone marrow biopsies collected prior to ide-cel infusion. The fraction of CD138+ cells within the biopsy expressing BCMA and the average staining intensity of BCMA was evaluated by a trained pathologist. ≥3% CD138+ cells (indicating tumor) were required to score BCMA expression. BCMA+ cell percentage and average expression were manually scored in CD138+ cells within the bone marrow biopsy and the average BCMA intensity was determined on a 0, 1, 2, 3+ scale. Tumor cell surface BCMA expression was quantified in bone marrow (BM) aspirates by flow cytometry. BCMA-receptor density was detected in bone marrow mononuclear cells that were isolated from fresh bone marrow aspirates and analyzed by flow cytometry. BCMA-receptor density was quantified using Quantibrite beads™ (BD

Biosciences) and scored on malignant plasma cells within the bone marrow aspirate. Soluble BCMA (sBCMA), representing secreted protein from BCMA-expressing cells, was assessed in blood. sBCMA was evaluated by immunoassay (Luminex, catalog no. LXSAHM-01) in serum isolated from peripheral blood. The lower limit of quantification (LLOQ) was 4.4 ng/mL and was the threshold used for undetectable levels of sBCMA. Values below the LLOQ were imputed as 2.2 ng/mL. Minimal residual disease (MRD) was evaluated by next-generation sequencing (NGS)-based approaches (Adaptive clonoSEQ®). Tumor response was assessed using International Myeloma Working group criteria.

[0566] Results: Tumor BCMA expression was observed in all evaluable patients prior to infusion (see Figure 10). Before ide-cel infusion (N=128), all treated patients with evaluable BMBs (112/112) demonstrated BCMA expression on CD138+ tumor cells by IHC (two patients were not evaluable (NE) due to insufficient (≤ 1%) %CD138+ cells in the bone marrow biopsy to enable scoring of BCMA). 79% of patients (n = 88/112) expressed BCMA on all malignant plasma cells present in their pretreatment bone marrow biopsy (Figure 10). In 79% (88/112), 100% of CD138+ cells expressed BCMA with varying levels of staining intensity (1+ to 3+); and only 3 patients showed BCMA expression on <50% of CD138+ cells (indicating malignant plasma cells. A range of BCMA staining intensities was observed across the patient cohort.

[0567] A higher BCMA receptor density rather than a higher percentage of BCMA-positive cells, was associated with a deeper tumor response (see Figure 11A-D). In particular, a higher baseline BCMA receptor density was associated with a deeper tumor response, although significant overlap was observed across response groups. BCMA receptor surface density was evaluated at baseline for correlation with BOR and PFS. With these data, no clear association was observed between the percentage of BCMA-expressing myeloma cells (%BCMA+) and tumor response or depth of response (see Figures 11A and 11B, which show % BCMA+ by IHC in responders and nonresponders and %BCMA+ by IHC by best overall response (BOR), respectively). A statistically significant difference was observed in BCMA-receptor density for patients with a very good partial response (VGPR) or complete response/stringent complete response (CR/sCR) compared with nonresponders (Figure 11C). In Figure 11D, the hazard ratio (HR) for a PFS event is shown for patients with receptor densities higher than the median relative to those patients with receptor densities lower than the median. No statistically

significant association was observed between baseline BCMA-receptor density and progression-free survival (Figure 11D).

[0568] Most patients who relapsed had BCMA-expressing tumors (*See* Tables 10 and 11). BCMA was expressed in the majority (15 out of 16) of evaluable bone marrow biopsies at progression. One patient with a best overall response of partial response showed likely evidence of antigen loss at progression. There were no consistent trends in up- or down-regulation of BCMA expression or percent BCMA-positive cells 3 months post-infusion or at the time of relapse.

[0569] Table 10: BCMA expression in progressing patients with a BOR of partial response.

Baseline	Month 3	Progression
	%BCMA+	
100	NE	80
90	NE	NE
100	PD	NE
90	PD	80
90	PD	90
100	NE	NE
100	95	80
100	PD	70
100	50	90
100	100	100
100	NE	1
100	100	80

[0570] In the results summarized in Table 10, the frequency of BCMA-expressing cells was evaluated by IHC for available tumor biopsies from initially responding patients. The percentage of BCMA+ cells from individual patient data are reported. NE indicates that too few CD138+ ($\leq$ 1%) were present to interpret lack of BCMA staining. PD for month 3 visits indicate that the patient progressed at month 3. The month 3 sample was the progression sample and is captured in the progression column. Abbreviations in Table 10 are as follows: BCMA, B-cell maturation

antigen; BOR, best overall response; IHC, immunohistochemistry; NE, not evaluable; PD, progressive disease.

[0571] Table 11: BCMA expression in progressing patients with BOR of VGPR or better.

Baseline	Month 3	Progression
%BCMA+		
100	ND	100*
100	NE	50
100	NE	NE
100	ND	NE
100	NE	100
100	NE	NE
100	ND	50
100	NE	95
90	NE	80
100	NE	NE
100	NE	35

*Few CD138+ cells were present; however, all were BCMA-positive leading to interpretation of sample as BCMA+.

[0572] In the results summarized in Table 11, the frequency of BCMA-expressing cells was evaluated by IHC for available tumor biopsies from initially responding patients. The percentage of BCMA+ cells from individual patient data are reported. ND indicates that the biopsy sample was unavailable for testing. NE indicates that too few (≤ 1%) CD138+ cells were present to interpret lack of BCMA staining. Abbreviations in Table 11 are as follows: BCMA, B-cell maturation antigen; BOR, best overall response; IHC, immunohistochemistry; ND, not done; NE, not evaluable; VGPR, very good partial response.

[0573] There was no threshold for the BCMA-expressing cell percentage, or average intensity of BCMA staining, which stratified patients by overall response (responder vs nonresponder) or best overall response (BOR). In evaluable patients with longitudinal BMB samples available, no significant modulation of BCMA receptor levels was observed at 1 month postinfusion or at time of PD.

[0574] Table 12: Serial analysis of sBCMA showed that 96% of patients had rising sBCMA levels at the time of relapse consistent with persistent tumor BCMA expression.

Time Point	Nonresponders	Responders	Total
Screening, n	33	90	123
< LLOQ, n (%)	0	0	0
PD, n	27	44	71
< LLOQ, n (%)	0	2 (4.5)	2 (2.8)
> LLOQ, n (%)	27	42 (95.5)	69 (97.2)

[0575] The results summarized in Table 12 show that a majority of patients (96%) had increasing sBCMA levels at relapse (*See* Table 12). Efficacy measures reported with ide-cel in the study included an ORR of 73.4%, median response duration of 10.6 months, and median PFS of 8.6 months. sBCMA was measurable at levels exceeding those in healthy patients without MM in 100% (27/27) of evaluable non-responders (primary resistance) and 95.5% (42/44) of evaluable patients at the time of confirmed PD after an initial response (acquired resistance). These data provide an indirect indication that BCMA expression was still present on tumor cells at the time of PD in nearly all patients. Supporting this hypothesis, 94% (15/16) of patients with evaluable BMB at time of PD still demonstrated BCMA-expressing CD138+ cells by IHC (*See* Tables 10 and 11).

[0576] The Table 12 results also show that patients expressed detectable sBCMA at baseline and 2 patients displayed undetectable sBCMA at progression. Abbreviations in Table 12 are as follows: BCMA, B-cell maturation antigen; LLOQ, lower limit of quantification; PD, progressive disease; sBCMA, soluble B-cell maturation antigen.

[0577] sBCMA level, which is an easily accessible serum biomarker of tumor BCMA expression, was observed below or near the LLOQ at the time of clinical evidence of progression in 3 patients, consistent with BCMA antigen loss (*See* Table 13).

[0578] **Table 13. Summary of 3 relapsing patients showing evidence of antigen loss.**

Best Overall Response	CD138 IHC (%CD138+)	BCMA IHC (%BCMA+ of total CD138+)	sBCMA at PD (ng/mL)
PR	60	1	2.2*
SD	25	0	5.3
≥ VGPR	NA	NA	2.2*

*Values <LLOQ were imputed as 0.5 × LLOQ (LLOQ = 4.4 ng/mL).

[0579] Abbreviations in Table 13 are as follows: BCMA, B-cell maturation antigen; IHC, immunohistochemistry; LLOQ, lower limit of quantification; NA, not available; PD, progressive disease; PR, partial response; sBCMA, soluble B-cell maturation antigen; SD, stable disease; VGPR, very good partial response.

[0580] Evidence of BCMA loss at progression in the RRMM population treated with ide-cel was rarely observed, occurring in 4% of patients (n = 3/71). One or more indicators of BCMA antigen loss were observed in 4% (3/71) of patients at PD. One patient demonstrated undetectable sBCMA and negative BMB BCMA staining, 1 had undetectable sBCMA and no available BMB, and 1 had negative BMB BCMA staining and low levels of sBCMA (5 ng/mL). In one of these cases, genomic loss of tumor BCMA expression was subsequently identified. Figure 12 shows BCMA IHC staining (Figure 12A) and VDJ clone tracking (Figure 12B) in a patient with suspected antigen loss. BCMA IHC illustrated likely loss of tumor BCMA expression at disease progression in these patients, which was further supported by low levels of sBCMA at progression (Figure 12A). VDJ clone tracking illustrates return of initial and potential emergent clones at relapse in a Multiple myeloma patient with suspected antigen loss (Figure 12B). Exploratory analysis of NGS MRD results enabled tracking of the dominant baseline MRD clone, which was present over time based on variable, diversity, and joining sequences. The dominant baseline MRD clone was still present at progression. Additionally, expansion of a different clone, that was present at low frequency at baseline, was observed.

[0581] Conclusions: All evaluable patients infused with ide-cel in the study had tumors expressing BCMA before treatment. The majority of patients expressed BCMA on 100% of malignant plasma cells. BCMA-expression levels were positively correlated with depth of tumor response, although lower BCMA expression did not preclude patients from achieving a deep

clinical response. Evidence of BCMA antigen loss was rare (4% of patients) and does not appear to be a dominant mechanism of relapse in RRMM patients with 3 or more prior lines of therapy.

[0582] Together, these data indicate that loss of tumor BCMA expression may merely be an uncommon mechanism of escape in patients following ide-cel therapy and indicate that additional BCMA-targeted modalities may be administered sequentially to RRMM patients, particularly in patients that do not exhibit such a loss of tumor BCMA expression, as assessed herein.

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6.5 EXAMPLE 5: BASELINE AND PHARMACODYNAMIC BIOMARKERS ASSOCIATED WITH SAFETY AND EFFICACY FOLLOWING TREATMENT WITH IDE-CEL

[0591] To gain insight into baseline and pharmacodynamic biomarkers associated with safety and efficacy following ide-cel treatment, an analysis was performed based on the primary analysis of patients treated with ide-cel (N=128) in the phase 2 KarMMa study (NCT03361748). Despite a variety of approved therapies, multiple myeloma remains incurable and patients face persistent risk of relapse. Idecabtagene vicleucel (ide-cel, bb2121), a B-cell maturation antigen (BCMA)-directed CAR T cell therapy, demonstrated a favorable benefit-risk profile in triple-class exposed (to immunomodulatory agents, proteosome inhibitors, and anti-CD38 antibodies) RRMM patients in the phase 2, single-arm KarMMa trial (NCT03361748). Pharmacodynamic biomarkers of tumor responses and ide-cel activity include tumor-associated soluble factors, pro-inflammatory cytokines, and minimal residual disease (MRD). Pro-inflammatory cytokines related to CAR T cell activation can be potential indicators of ide-cel mechanism of action and safety events, such as cytokine release syndrome (CRS) and investigator-identified neurotoxicity (NT). BCMA cleaved from the surface of cells (ie, soluble BCMA; sBCMA) is a novel peripherally accessible biomarker in multiple myeloma that has shown utility for monitoring

tumor responses over time. MRD is a sensitive measure of tumor burden; the depth of tumor clearance by MRD assessment may be predictive of response duration.

[0592] Methods: Baseline and post-infusion levels of 25 immune-related soluble factors (Days 1−28) (GM-CSF, Granzyme A, Granzyme B, IFN-γ, IL-10, IL-13, IL-2, IL-4, IL-5, IL-6, MIP-1α, MIP-1β, perforin, sCD137, sFas, sFasL, TNFα, Ang-1, Ang-2, IL-15, IL-18, IL-2Rα, IL-7, IL-8, RANKL) in plasma and sBCMA (Day 1 through disease progression) in serum were evaluated by immunoassay in the peripheral blood using commercially available Luminex assays (Ampersand Biosciences, NY, USA). sBCMA was evaluated at screening, baseline (day 1), and until disease progression, and all other cytokines and soluble factors were evaluated at screening, baseline, days 1-6, and days 7, 9, 11, 14, and 21. All concentrations below the lower limit of quantitation (LLOQ) were imputed to LLOQ/2 (eg, sBCMA LLOQ is 4.4 ng/ mL, imputed as 2.2 ng/mL). Clinical markers of inflammation, C-reactive protein, and ferritin were measured locally at each clinical study center and included in this analysis.

[0593] Minimal residual disease (MRD) status was evaluated in bone marrow aspirate (BMA) by next-generation sequencing (NGS; ClonoSEQ®, Adaptive Biotechnologies) at baseline and postinfusion at fixed time points until progression (i.e., baseline, month 1 (M1), month 3 (M3), month 6 (M6), month 12 (M12), month 18 (M18), and month 24 (M24)) agnostic of response. Correlations between each biomarker and key safety and efficacy endpoints were assessed.

[0594] Correlations between each biomarker and key safety and efficacy endpoints were assessed. *P* values were 2-sided based on the Mann-Whitney-Wilcoxon test or Kruskal-Wallis test. A multiplicity adjusted P value using the Holm step-down Bonferroni method was provided across different immune-related soluble factors.

[0595] Results: The magnitude of postinfusion cytokine C_{max} was associated with CAR T cell activation and expansion, and tumor response (see Figure 13A-C). Figure 13A (entitled "Time course of ide-cel expansion and contraction") shows a time course of ide-cel expansion and contraction in patients following administration of ide-cel at doses of 150×10^6 cells (n=4), 300 $\times 10^6$ cells (n=69), and 450×10^6 cells (n=54). CAR T cell expansion was observed across all dose levels and was dose-dependent. In particular, median peak CAR+ T cell expansion was observed at day 11 (Figure 13A, entitled "Time course of ide-cel expansion and contraction"). Median expansion increased at higher target doses with overlapping profiles. Ide-cel expansion was assessed by clinical response (see Figure 13A, entitled "Ide-cel expansion by clinical

response"). Responders were defined as having had a partial response or better. Ide-cel expansion ($AUC_{0\text{-}28\ days}$, days x copies/μg) was higher in responders relative to non-responders (Figure 13A, entitled "Ide-cel expansion by clinical response"). Thus, CAR T cell (ide-cel) expansion was strongly correlated with response.

[0596] The magnitude of pro-inflammatory cytokine induction by dose level was assessed (see Figure 13B). The levels of pro-inflammatory cytokines IL-2, IL-6, and IFN-γ were measured following administration of ide-cel at doses of 150×10^6 cells (n=4), 300×10^6 cells (n=66), and 450×10^6 cells (n=54). Cytokine induction was observed across all dose levels and was dose-dependent (Figure 13B).

[0597] The magnitude of pro-inflammatory cytokine induction (C_{max}) by clinical response was assessed (see Figure 13C; responders were defined as having a partial response or better). The levels of C-reactive protein (CRP), IFN-γ, IL-10, and IL-6 were measured in responders and non-responders. No cytokines at baseline stratified patients by overall response, but significantly higher peak (C_{max}) CRP, IFNγ, IL-10, and IL-6 levels occurred in responders relative to non-responders (Figure 13C). Peak cytokine concentrations were generally reached within 7 days postinfusion; cytokine Cmax was generally higher at target dose level of 450×10^6 CAR+ T cells. Thus, pro-inflammatory cytokines were elevated to a greater degree in responders than nonresponders, which is consistent with CAR T cell activation and expansion.

[0598] The magnitude of postinfusion cytokine induction (cytokine C_{max}), but not baseline levels, was associated with higher grade CRS and investigator-identified neurotoxicity (NT) (see Figure 14). The magnitude of pro-inflammatory cytokine induction and grade of CRS was assessed (see Figure 14A). The levels of CRP, IFN-γ, IL-6, and IL-8 were measured and patients were assessed for cytokine resease syndrome (CRS) (grade 0, grades 1+2, and grade ≥3 are shown in Figure 14A; in each graph shown in Figure 14A, each rectangle with error bars is shown from left to right in the order of Grade 0, Grades 1+2, and Grade ≥ 3). No cytokines at baseline were statistically significantly correlated with higher-grade CRS (Figure 14A). Increased induction of CRP and a subset of cytokines (i.e., IFN-γ, IL-6, and IL-8) was observed with increasing grade of CRS ($P<0.05$) (Figure 14A).

[0599] The magnitude of pro-inflammatory cytokine induction and grade of investigator-identified NT was assessed (see Figure 14B). The levels of ferritin, IL-10, IFN-γ, IL-6, and IL-8 were measured. No cytokines at baseline were statistically significantly

correlated with higher-grade investigator-identified NT (for investigator-identified NT, grade 0, grades 1+2, and grade ≥3 are shown in Figure 14B; in each graph shown in Figure 14B, each rectangle with error bars is shown from left to right in the order of Grade 0, Grades 1+2, and Grade ≥ 3). Increased induction of ferritin and a subset of cytokines (i.e., IL-10, IFN-γ, IL-6, and IL-8) was observed with increasing grade of investigator-identified NT ($P<0.05$) (Figure 14B). In general, few higher-grade NT events were observed; however, a trend towards higher endothelial cell activation (increased angiopoietin ([Ang]-2) and lower endothelial cell stabilization (decreased Ang-1) compared with baseline was observed (Figure 14C; in each graph shown in Figure 14C, each rectangle with error bars is shown from left to right in the order of Grade 0, Grades 1+2, and Grade ≥ 3). All in all, none of the 27 factors measured at baseline were correlated with cytokine release syndrome (CRS), CRS requiring tocilizumab or corticosteroids, or investigator-identified NT endpoints, but several factors (eg, GM-CSF, IL-6, IFNγ, IL-8, IL-10) were induced to higher levels within 24 hours post-infusion in patients with CRS or investigator-identified NT, and peak levels increased with CRS or investigator-identified NT grade.

[0600] sBCMA clearance postinfusion was independent of baseline tumor burden (percentage of plasma cells in the bone marrow) or extramedullary plasmacytoma (EMP) involvement (see Figure 15). Baseline sBCMA levels were elevated in patients with higher baseline tumor burden (≥ 50% tumor burden, n=62, median baseline sBCMA was 386.0 ng/ml (First Quartile (Q1)=269.0 ng/ml, Third Quartile (Q3)=696.0 ng/ml), <LLOQ, n (%) = 0) compared to patients with lower baseline tumor burden (< 50% tumor burden, n=56, median baseline sBCMA was 177 ng/ml (Q1=57.5 ng/ml, Q3=327.0 ng/ml), <LLOQ, n (%) = 0) (Figure 15A, upper panel, *P* value < 0.0001). In patients with higher baseline tumor burden (≥ 50% tumor burden, n=62), median baseline sBCMA at nadir was 2.2 ng/ml (Q1=2.2 ng/ml, Q3=43.0 ng/ml, <LLOQ n=45 (59.2%)). In patients with lower baseline tumor burden (< 50% tumor burden, n=56), median baseline sBCMA at nadir was 2.2 ng/ml (Q1=2.2 ng/ml, Q3=11.0 ng/ml, <LLOQ n=30 (62.5%)). Baseline sBCMA levels were elevated in patients with EMP involvement (EMP, n=48, 345.5 ng/ml (Q1=195.0 ng/ml, Q3=670.0 ng/ml, <LLOQ, n (%) = 0) compared to patients with no EMP (No EMP, n=76, median baseline sBCMA was 238.0 ng/ml (Q1=76.0 ng/ml, Q3=424.5 ng/ml, <LLOQ, n (%) = 0) (Figure 15B, upper panel, *P* value = 0.0339). No differences in the

proportion of patients achieving sBCMA clearance were observed for high tumor burden or EMP involvement (see Figure 15A and 15B, lower panels, respectively).

[0601] A smaller proportion of patients with baseline sBCMA values in the upper quartile (>75th percentile sBCMA) achieved sBCMA clearance (Figure 15C, upper panel). Baseline tumor burden was determined by the percentage of plasma cells in the bone marrow (%CD138+). High tumor burden was defined as >50% bone marrow plasma cell involvement. In particular, patients with baseline sBCMA values in the upper quartile (>75th percentile sBCMA, n=31) had a median baseline sBCMA of 790.0 ng/ml (Q1=657.0 ng/ml, Q3=987.0 ng/ml, <LLOQ, n (%) = 0) and a median sBCMA at nadir of 25.0 ng/ml (Q1=2.2 ng/ml, Q3=507.0 ng/ml, <LLOQ, n (%)=11 (35.5%)). However, 35% of such patients achieved deep responses, as indicated by sBCMA clearance (Figure 15C, lower panel). Patients with baseline sBCMA values < 75% sBCMA (n=93) had a median baseline sBCMA of 191.0 ng/ml (Q1=73.0 ng/ml, Q3=314.0 ng/ml, <LLOQ, n (%) = 0) and a median sBCMA at nadir of 2.2 ng/ml (Q1=2.2 ng/ml, Q3=5.9 ng/ml, <LLOQ, n=64 (68.8%)).sBCMA clearance occurred rapidly in responding patients and a longer time to sBCMA rebound was associated with depth of tumor response (see Figure 16). Soluble BCMA (sBCMA) was a peripherally accessible biomarker with baseline serum levels tracking with clinical measures of MM tumor burden and post-infusion levels tracking with tumor response. Figure 16A shows median sBCMA stratified by best overall response post ide-cel infusion (the x-axis shown in Figure 16A was stretched from baseline to month 1 (M1) to allow better viewing of sBCMA dynamics during peak expansion; time to sBCMA rebound >LLOQ was defined as the last visit day prior to a sBCMA measure >LLOQ; patients without an sBCMA nadir <LLOQ were input as zeros; BL, baseline; CR, complete response; D, day; LLOQ, lower limit of quantitation; M, month; NPC, normal plasma cell; NR, no response; PD, progressive disease; PR, partial response; sBCMA, soluble B-cell maturation antigen; VGPR, very good partial response). Patients were excluded from analysis if baseline sBCMA value was unavailable (n = 4).

[0602] Median baseline sBCMA was 276.0 ng/mL in ide-cel–treated patients and decreased post-infusion in responders with patients achieving nadir within 3 months; median nadir was lower in responders (0-28d, measured by transgene level). Median sBCMA levels showed clearance <LLOQ of the assay in responding patients, indicating initial clearance of the tumor. The duration of median sBCMA levels <LLOQ was associated with increasing depth of tumor

response. sBCMA trajectories indicated that tumor clearance occurred within the first 1-2 months after ide-cel infusion. The proportion of patients who achieved sBCMA nadir <LLOQ by best overall response is shown in Figure 16B. The percentage of patients with sBCMA levels ≤LLOQ at nadir increased with best overall response (63% PR, 81% ≥VGPR, 95% ≥CR) (Figure 16B). Thus, the proportion of patients who achieved sBCMA nadir <LLOQ correlated with the depth of clinical response. Figure 16C shows the time to sBCMA rebound to detectable levels. The observed sBCMA level remained ≤LLOQ for a longer duration among patients who achieved ≥CR compared with patients who did not (Figure 16C). Thus, sBCMA levels were maintained <LLOQ for a longer duration of time in patients with increasing depth and duration of clinical response.

[0603] sBCMA response trajectories were consistent with sensitive minimal residual disease (MRD), as assessed by next-generation sequencing (NGS), and traditional serum markers of myeloma disease burden (i.e., M-protein and FLC) (see Figure 17A-B). MRD and sBCMA were assessed in patients that were administered ide-cel. In addition, standard markers of myeloma response (i.e, M-protein and FLC) were measured. Figure 17A shows MRD (as determined using next-generation sequencing, measured in cells/million) and levels of sBCMA, M-protein, and FLC in non-responders, responders (progressed), and responders (ongoing) (responses were characterized as nonresponders (<partial response), responders who relapsed at time of data cut (responders, progressed), and responders who were still in response at the data cut (responders, ongoing)). Patients were excluded from analysis if baseline sBCMA value was unavailable (n = 4). sBCMA and MRD status showed similar trajectories by responses and were consistent with traditional biomarkers of disease. sBCMA was evaluable for all patients (Figure 17B, entitled "Detectable Biomarkers"). The fraction of evaluable patients illustrates that sBCMA was a biomarker that was evaluable in a high percentage of patients, whereas the other biomarkers of tumor response (i.e., NGS MRD, multi-color flow cytometry (MFC) MRD, M-protein, and FLC) were evaluable in a subset of patients due to disease characteristics and/or technical limitations.

[0604] Conclusion: No preinfusion immune-related soluble factors evaluated were identified as predictive of high-grade CRS or NT. Pro-inflammatory cytokine induction occurred concurrently with CAR T cell activation and expansion, and was associated with higher grade of CRS and NT. sBCMA and MRD were robust biomarkers of tumor burden and were both rapidly cleared in responders within the first months after infusion. The depth and duration of sBCMA

clearance was associated with depth and duration of clinical response, respectively. sBCMA was peripherally accessible, could be frequently monitored, and was evaluable in a high percentage of ide-cel–treated patients as a universal surrogate measure for assessing tumor burden and tumor responses over time.

[0605] References:

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[0609] Pappa C, *et al.*, J. Cancer Res. Clin. Oncol., 2014, 140:1801-1805.

[0610] Terpos E, *et al.*, Int. J. Cancer, 2011, 130:735-742.

[0611] 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. All references cited herein, whether patent or non-patent, are incorporated by reference herein in their entireties.

WHAT IS CLAIMED IS:

1. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject;

b. administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

c. determining a second level of sBCMA in a tissue sample from the subject wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently provided a non-CAR T cell therapy to treat said disease.

2. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject;

b. administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells),

c. determining that a second level of sBCMA in a tissue sample from the subject is greater than 30% of said first level of sBCMA, and

d. on the basis of the determination in step c, subsequently providing a non-CAR T cell therapy to the subject.

3. The method of claim 1, wherein if said second level of sBCMA is greater than 40% of said first level of sBCMA, the subject is provided a non-CAR T cell therapy to treat said disease.

4. The method of any one of claims 1, 2 or 3, wherein said second level of sBCMA is determined at 25-35 days after said administering.

5. The method of any one of claims 1, 2, or 3, wherein said second level of sBCMA is determined at 28-31 days after said administering.

6. The method of any of claims 1-5, wherein the subject is provided a non-CAR T cell therapy within three months, two months, or one month after said determining the second level of sBCMA.

7. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a non-CAR T cell therapy, wherein the patient has previously been administered immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) and wherein a tissue sample from the patient subsequent to said administration contained a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration.

8. A method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a non-CAR T cell therapy after treatment with immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) in a tissue sample from the patient, wherein the patient has previously been administered the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the non-CAR T cell therapy.

9. The method of claim 8, further comprising administering the non-CAR T cell therapy to the candidate for the non-CAR T cell therapy.

10. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

b. determining a level of soluble BCMA (sBCMA) in a tissue sample from the subject

wherein, if said level of sBCMA is greater than 4000 ng/L, the subject is subsequently provided a non-CAR T cell therapy to treat said disease.

11. The method of claim 10, wherein said level of sBCMA is determined at 50-70 days after said administering.

12. The method of claim 10 or claim 11, wherein said level of sBCMA is determined at 55-65 days after said administering.

13. The method of any of claims 10-12, wherein said level of sBCMA is determined at 58-62 days after said administering.

14. The method of any of claims 11-13, wherein the subject is provided said non-CAR T cell therapy within three months, two months, or one month after said determining a level of sBCMA.

15. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα) or both in a tissue sample from the subject;

b. administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

c. subsequently determining a second level of IL-6, TNFα or both in a tissue sample from the subject;

wherein, if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, then the subject is subsequently provided a non-CAR T cell therapy to treat said disease.

16. The method of claim 15, wherein said first level is determined on the day of said administering to the subject immune cells expressing a CAR directed to BCMA, and said second level is determined 1-4 days after said administering.

17. The method of claim 16, wherein said second level is determined two days after said administering.

18. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

b. determining a level of ferritin in a tissue sample from the subject;

wherein, if said level of ferritin is greater than 1500 picomoles per liter, the subject is subsequently provided a therapy to treat cytokine release syndrome (CRS).

19. The method of claim 18, wherein said determining is performed within 0-4 days prior to said administering.

20. The method of claim 18, wherein said determining is performed on the same day as said administering.

21. The method of claim 18, wherein said therapy to treat CRS is first provided to said subject 0-5 days after said administering.

22. The method of any of claims 1-21, wherein said disease caused by BCMA-expressing cells is multiple myeloma, chronic lymphocytic leukemia, or a non-Hodgkins lymphoma.

23. The method of claim 22, wherein the disease caused by BCMA-expressing cells is multiple myeloma.

24. The method of claim 23, wherein said multiple myeloma is high-risk multiple myeloma or relapsed and refractory multiple myeloma.

25. The method of claim 22, wherein said disease caused by BCMA-expressing cellsis a non-Hodgkins lymphoma, and wherein the non-Hodgkins lymphoma is selected from the group consisting of: Burkitt's lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma.

26. The method of any of claims 1-25, wherein the immune cells are T cells.

27. The method of any of claims 1-26, wherein the immune cells are administered in a dosage of from 150 x 10^6 cells to 450 x 10^6 cells.

28. The method of any of claims 1-27, wherein before said administering said subject has received three or more lines of prior therapy.

29. The method of any of claims 1-27, wherein before said administering said subject has received one or more lines of prior therapy.

30. The method of claim 28 or 29, wherein said lines of prior therapy comprise a proteasome inhibitor, lenalidomide, pomalidomide, thalidomide, bortezomib, dexamethasone, cyclophosphamide, doxorubicin, carfilzomib, ixazomib, cisplatin, doxorubicin, etoposide, an anti-CD38 antibody panobinostat, or elotuzumab.

31. The method of claim 28 or claim 29, wherein before said administering said subject has received one or more lines of prior therapy comprising:

a. daratumumab, pomalidomide, and dexamethasone (DPd);

b. daratumumab, bortezomib, and dexamethasone (DVd);

c. ixazomib, lenalidomide, and dexamethasone (IRd);
d. daratumumab, lenalidomide and dexamethasone;
e. bortezomib, lenalidomide and dexamethasone (RVd);
f. bortezomib, cyclophosphamide and dexamethasone (BCd);
g. bortezomib, doxorubicin and dexamethasone;
h. carfilzomib, lenalidomide and dexamethasone (CRd);
i. bortezomib and dexamethasone;
j. bortezomib, thalidomide and dexamethasone;
k. lenalidomide and dexamethasone;
l. dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide and bortezomib (VTD-PACE);
m. lenalidomide and low-dose dexamethasone;
n. bortezomib, cyclophosphamide and dexamethasone;
o. carfilzomib and dexamethasone;
p. lenalidomide alone;
q. bortezomib alone;
r. daratumumab alone;
s. elotuzumab, lenalidomide, and dexamethasone;
t. elotuzumab, lenalidomide and dexamethasone;
u. bendamustine, bortezomib and dexamethasone;
v. bendamustine, lenalidomide, and dexamethasone;
w. pomalidomide and dexamethasone;
x. pomalidomide, bortezomib and dexamethasone;
y. pomalidomide, carfilzomib and dexamethasone;
z. bortezomib and liposomal doxorubicin;
aa. cyclophosphamide, lenalidomide, and dexamethasone;
bb. elotuzumab, bortezomib and dexamethasone;
cc. ixazomib and dexamethasone;
dd. panobinostat, bortezomib and dexamethasone;
ee. panobinostat and carfilzomib; or
ff. pomalidomide, cyclophosphamide and dexamethasone.

32. The method of claim 31, wherein said subject has received two, three, four, five, six, seven or more of said lines of prior therapy.

33. The method of claim 31, wherein said subject has received no more than three of said lines of prior therapy.

34. The method of claim 31, wherein said subject has received no more than two of said lines of prior therapy.

35. The method of claim 31, wherein said subject has received no more than one of said lines of prior therapy.

36. The method of any of claims 1-35, wherein said CAR comprises an antibody or antibody fragment that targets BCMA.

37. The method of any of claims 1-36, wherein said CAR comprises a single chain Fv antibody fragment (scFv).

38. The method of any of claims 1-36, wherein said CAR comprises a BCMA02 scFv.

39. The method of any of claims 1-36, wherein said immune cells are idecabtagene vicleucel cells.

40. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject;

b. administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

c. determining a second level of sBCMA and/or a second level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject

wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA and/or if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, the subject is subsequently provided a non-CAR T cell therapy to treat said disease.

41. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject;

b. administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells),

c. determining that a second level of sBCMA in a tissue sample from the subject is greater than 30% of said first level of sBCMA and/or a second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, and

d. on the basis of the determination in step c, subsequently providing a non-CAR T cell therapy to the subject.

42. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a non-CAR T cell therapy, wherein the patient has previously been administered immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) and wherein a tissue sample from the patient subsequent to said administration contained (i) a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) a level of IL-6, TNFα or both not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration.

43. A method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a non-CAR T cell therapy after treatment with immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) and/or a level of IL-6, TNFα or both in a tissue sample from the patient, wherein the patient has previously been administered the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if (i) the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) the level of IL-6, TNFα or both is not greater than

a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the non-CAR T cell therapy.

44. The method of claim 43, further comprising administering the non-CAR T cell therapy to the candidate for the non-CAR T cell therapy.

45. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject;

b. administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

c. determining a second level of sBCMA in a tissue sample from the subject

wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently administered lenalidomide to treat said disease.

46. The method of claim 45, wherein the lenalidomide is administered at a dosage of about 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg, or 25 mg.

47. The method of claim 45, wherein the lenalidomide is administered at a dosage of about 25 mg daily orally on days 1-21 of a 28-day cycle.

48. The method of any one of claims 45-47, wherein the disease is Multiple Myeloma (MM).

49. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject;

b. administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

c. determining a second level of sBCMA in a tissue sample from the subject

wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently administered pomalidomide to treat said disease.

50. The method of claim 49, wherein the pomalidomide is administered at a dosage of about 1 mg, 2 mg, 3 mg, or 4 mg once daily.

51. The method of claim 49, wherein the pomalidomide is administered at a dosage of about 4 mg per day taken orally on days 1-21 of repeated 28-day cycles until disease progression.

52. The method of any one of claims 49-51, wherein the wherein the disease is Multiple Myeloma (MM).

53. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject;

b. administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

c. determining a second level of sBCMA in a tissue sample from the subject

wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently administered CC-220 to treat said disease.

54. The method of claim 53, wherein the CC-220 is administered at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg.

55. The method of claim 53, wherein the CC-220 is administered orally at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily on days 1-21 of a 28-day cycle.

56. The method of any one of claims 53-55, wherein the disease is Multiple Myeloma (MM).

57. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject;

b. administering to the subject immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

c. determining a second level of sBCMA in a tissue sample from the subject

wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently administered CC-220 and dexamethasone to treat said disease.

58. The method of claim 57, wherein the CC-220 is administered at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg.

59. The method of claim 57 or claim 58, wherein the dexamethasone is administered at a dosage of about 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg.

60. The method of claim 57, wherein the CC-220 is administered orally at a dosage of about 0.15 mg, 0.3 mg, 0.45 mg, 0.6 mg, 0.75 mg, 0.9 mg, 1.0 mg, 1.1 mg, or 1.2 mg daily on days 1-21 of a 28-day cycle.

61. The method of any one of claims 57-60, wherein the dexamethasone is administered orally at a dosage of about 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, or 60 mg on days 1, 8, 15, and 22 of a 28-day cycle.

62. The method of any one of claims 57-61, wherein the disease is Multiple Myeloma (MM).

63. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject;

b. administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

c. determining a second level of sBCMA in a tissue sample from the subject

wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease; and

wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities.

64. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) in a tissue sample from the subject;

b. administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells),

c. determining that a second level of sBCMA in a tissue sample from the subject is greater than 30% of said first level of sBCMA, and

d. on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject;

wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities.

65. The method of claim 63, wherein if said second level of sBCMA is greater than 40% of said first level of sBCMA, the subject is provided a second BCMA-based treatment modality to treat said disease.

66. The method of any one of claims 63, 64, or 65, wherein said second level of sBCMA is determined at 25-35 days after said administering.

67. The method of any one of claims 63, 64, or 65, wherein said second level of sBCMA is determined at 28-31 days after said administering.

68. The method of any of claims 63-67, wherein the subject is provided a second BCMA-based treatment modality within three months, two months, or one month after said determining the second level of sBCMA.

69. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a second BCMA-based treatment modality, wherein the patient has previously been administered a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, and wherein a tissue sample from the patient subsequent to said administration contained a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration.

70. A method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a second BCMA-based treatment modality after treatment with a first BCMA-based treatment modality comprising

immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, comprising determining a level of soluble BCMA (sBCMA) in a tissue sample from the patient, wherein the patient has previously been administered the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and wherein if the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the second BCMA-based treatment modality.

71. The method of claim 70, further comprising administering the second BCMA-based treatment modality to the candidate for the second BCMA-based treatment modality.

72. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

b. determining a level of soluble BCMA (sBCMA) in a tissue sample from the subject

wherein, if said level of sBCMA is greater than 4000 ng/L, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease, and wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities.

73. The method of claim 72, wherein said level of sBCMA is determined at 50-70 days after said administering.

74. The method of claim 72 or claim 73, wherein said level of sBCMA is determined at 55-65 days after said administering.

75. The method of any of claims 72-74, wherein said level of sBCMA is determined at 58-62 days after said administering.

76. The method of any of claims 73-75, wherein the subject is provided said second BCMA-based treatment modality within three months, two months, or one month after said determining a level of sBCMA.

77. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα) or both in a tissue sample from the subject;

b. administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

c. subsequently determining a second level of IL-6, TNFα or both in a tissue sample from the subject;

wherein, if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, then the subject is subsequently provided a second BCMA-based treatment modality to treat said disease, and

wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities.

78. The method of claim 77, wherein said first level is determined on the day of said administering to the subject the first BCMA-based treatment modality comprising immune cells expressing a CAR directed to BCMA, and said second level is determined 1-4 days after said administering.

79. The method of claim 78, wherein said second level is determined two days after said administering.

80. The method of any of claims 1-79, wherein said disease caused by BCMA-expressing cells is multiple myeloma, chronic lymphocytic leukemia, or a non-Hodgkins lymphoma.

81. The method of claim 80, wherein the disease caused by BCMA-expressing cells is multiple myeloma.

82. The method of claim 81, wherein said multiple myeloma is high-risk multiple myeloma or relapsed and refractory multiple myeloma.

83. The method of claim 80, wherein said disease caused by BCMA-expressing cells is a non-Hodgkins lymphoma, and wherein the non-Hodgkins lymphoma is selected from the group consisting of: Burkitt's lymphoma, chronic lymphocytic leukemia/small lymphocytic

lymphoma (CLL/SLL), diffuse large B cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma.

84. The method of any of claims 1-83, wherein the immune cells are T cells.

85. The method of any of claims 1-84, wherein the immune cells are administered in a dosage of from 150 x 10^6 cells to 450 x 10^6 cells.

86. The method of any of claims 1-85, wherein before said administering said subject has received three or more lines of prior therapy.

87. The method of any of claims 1-85, wherein before said administering said subject has received one or more lines of prior therapy.

88. The method of claim 86 or 87, wherein said lines of prior therapy comprise a proteasome inhibitor, lenalidomide, pomalidomide, thalidomide, bortezomib, dexamethasone, cyclophosphamide, doxorubicin, carfilzomib, ixazomib, cisplatin, doxorubicin, etoposide, an anti-CD38 antibody panobinostat, or elotuzumab.

89. The method of claim 86 or claim 87, wherein before said administering said subject has received one or more lines of prior therapy comprising:

a. daratumumab, pomalidomide, and dexamethasone (DPd);
b. daratumumab, bortezomib, and dexamethasone (DVd);
c. ixazomib, lenalidomide, and dexamethasone (IRd);
d. daratumumab, lenalidomide and dexamethasone;
e. bortezomib, lenalidomide and dexamethasone (RVd);
f. bortezomib, cyclophosphamide and dexamethasone (BCd);
g. bortezomib, doxorubicin and dexamethasone;
h. carfilzomib, lenalidomide and dexamethasone (CRd);
i. bortezomib and dexamethasone;
j. bortezomib, thalidomide and dexamethasone;
k. lenalidomide and dexamethasone;
l. dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide and bortezomib (VTD-PACE);
m. lenalidomide and low-dose dexamethasone;
n. bortezomib, cyclophosphamide and dexamethasone;
o. carfilzomib and dexamethasone;

p. lenalidomide alone;

q. bortezomib alone;

r. daratumumab alone;

s. elotuzumab, lenalidomide, and dexamethasone;

t. elotuzumab, lenalidomide and dexamethasone;

u. bendamustine, bortezomib and dexamethasone;

v. bendamustine, lenalidomide, and dexamethasone;

w. pomalidomide and dexamethasone;

x. pomalidomide, bortezomib and dexamethasone;

y. pomalidomide, carfilzomib and dexamethasone;

z. bortezomib and liposomal doxorubicin;

aa. cyclophosphamide, lenalidomide, and dexamethasone;

bb. elotuzumab, bortezomib and dexamethasone;

cc. ixazomib and dexamethasone;

dd. panobinostat, bortezomib and dexamethasone;

ee. panobinostat and carfilzomib; or

ff. pomalidomide, cyclophosphamide and dexamethasone.

90. The method of claim 89, wherein said subject has received two, three, four, five, six, seven or more of said lines of prior therapy.

91. The method of claim 89, wherein said subject has received no more than three of said lines of prior therapy.

92. The method of claim 89, wherein said subject has received no more than two of said lines of prior therapy.

93. The method of claim 89, wherein said subject has received no more than one of said lines of prior therapy.

94. The method of any of claims 1-93, wherein said CAR comprises an antibody or antibody fragment that targets BCMA.

95. The method of any of claims 1-94, wherein said CAR comprises a single chain Fv antibody fragment (scFv).

96. The method of any of claims 1-94, wherein said CAR comprises a BCMA02 scFv.

97. The method of any of claims 1-94, wherein said immune cells are idecabtagene vicleucel cells.

98. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject;

b. administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), and

c. determining a second level of sBCMA and/or a second level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject;

wherein, if said second level of sBCMA is greater than 30% of said first level of sBCMA and/or if said second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, the subject is subsequently provided a second BCMA-based treatment modality to treat said disease, and

wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities.

99. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells in a subject in need thereof, comprising:

a. determining a first level of soluble BCMA (sBCMA) and/or a first level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or both in a tissue sample from the subject;

b. administering to the subject a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells),

c. determining that a second level of sBCMA in a tissue sample from the subject is greater than 30% of said first level of sBCMA and/or a second level of IL-6, TNFα or both is not greater than said first level of IL-6, TNFα or both, and

d. on the basis of the determination in step c, subsequently providing a second BCMA-based treatment modality to the subject,

wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities.

100. A method of treating a disease caused by B Cell Maturation Agent (BCMA) expressing cells, comprising administering to a patient diagnosed with said disease a second BCMA-based treatment modality, wherein the patient has previously been administered a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities, and wherein a tissue sample from the patient subsequent to said administration contained (i) a level of soluble BCMA (sBCMA) greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) a level of IL-6, TNFα or both not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration.

101. A method of determining whether a patient diagnosed with a disease caused by B Cell Maturation Agent (BCMA) expressing cells should be administered a second BCMA-based treatment modality after treatment with a first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), comprising determining a level of soluble BCMA (sBCMA) and/or a level of IL-6, TNFα or both in a tissue sample from the patient, wherein the patient has previously been administered the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells), wherein if (i) the level of sBCMA in the tissue sample is greater than 30% of a level of sBCMA found in a tissue sample obtained from the patient prior to said administration and/or (ii) the level of IL-6, TNFα or both is not greater than a level of IL-6, TNFα or both found in a tissue sample obtained from the patient prior to said administration, then the patient is a candidate for the second BCMA-based treatment modality, and

wherein the first BCMA-based treatment modality and the second BCMA-based treatment modality are different BCMA-based treatment modalities.

102. The method of claim 101, further comprising administering the second BCMA-based treatment modality to the candidate for the second BCMA-based treatment modality.

103. The method of any one of claims 63-102, wherein the second BCMA-based treatment modality comprises a BCMA-Antibody-Drug Conjugate (ADC), a bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA), a natural killer (NK) cell engager (NKCEs) that targets B-cell maturation antigen (BCMA), or immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells).

104. The method of claim 103, wherein the second BCMA-based treatment modality comprises a BCMA-Antibody-Drug Conjugate (ADC).

105. The method of claim 104, wherein the BCMA-Antibody-Drug Conjugate (ADC) comprises CC99712 or GSK2857916 (belantamab mafodotin).

106. The method of claim 103, wherein the second BCMA-based treatment modality comprises a bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA).

107. The method of claim 106, wherein the bispecific T-cell engager (BiTE) that targets B-cell maturation antigen (BCMA) comprises CC-93269, AMG 420, JNJ-64007957, AMG 701, PF-06863135, REGN5458, REGN5459, or TNB-383B.

108. The method of claim 103, wherein the second BCMA-based treatment modality comprises a natural killer (NK) cell engager (NKCEs) that targets B-cell maturation antigen (BCMA).

109. The method of claim 108, wherein the natural killer (NK) cell engager (NKCEs) that targets B-cell maturation antigen (BCMA) comprises DF3001, AFM26, CTX-4419, or CTX-8573.

110. The method of claim 103, wherein the second BCMA-based treatment modality comprises immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells).

111. The method of claim 103 or 110, wherein the immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) comprise JCARH125, KITE-585, P-BCMA-101, LCAR-B38M, CT053, anti-CD19/BCMA CAR-T cells, and CTX120.

112. The method of any one of claims 98-111, wherein the immune cells in the first BCMA-based treatment modality comprising immune cells expressing a chimeric antigen receptor (CAR) directed to BCMA (BCMA CAR T cells) are idecabtagene vicleucel cells.

113. The method of any one of claims 63-112, wherein the second BCMA-based treatment modality does not comprise idecabtagene vicleucel cells.

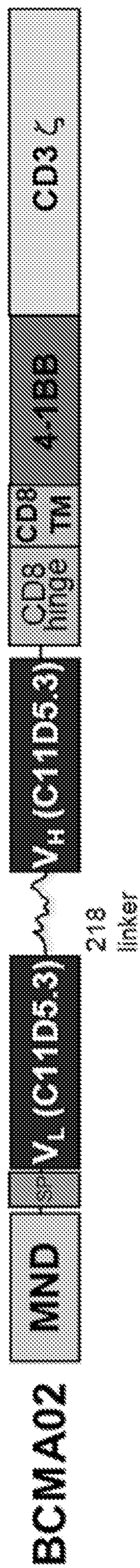

BCMA02
MND
VL (C11D5.3)
218
linker
VH (C11D5.3)
CD8
hinge
CD8
TM
4-1BB
CD3ζ

FIG. 1

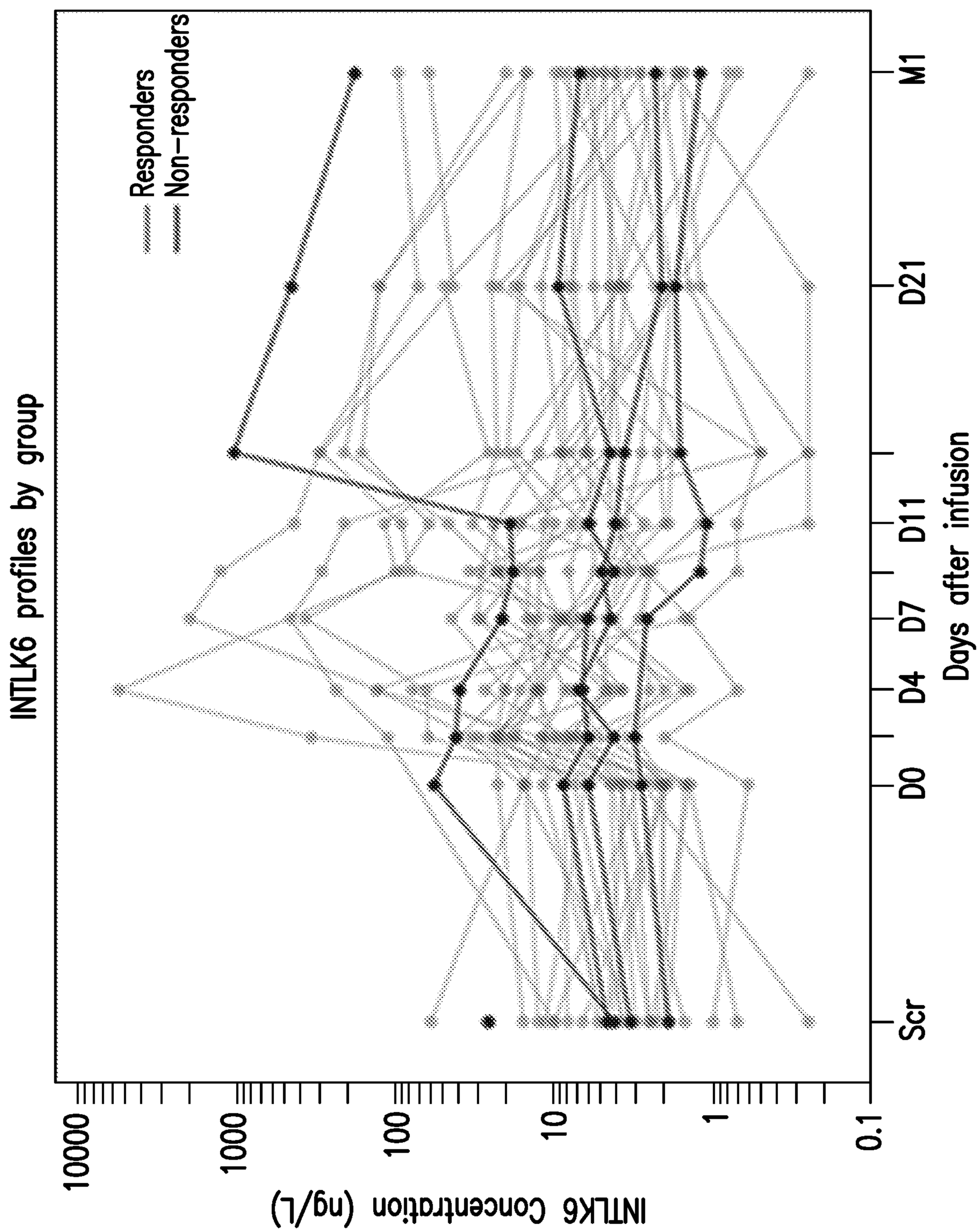

INTLK6 profiles by group
Responders
Non-responders
INTLK6 Concentration (ng/L)
10000
1000
100
10
1
0.1
Scr
D0
D4
D7
D11
D21
M1
Days after infusion

FIG. 2A

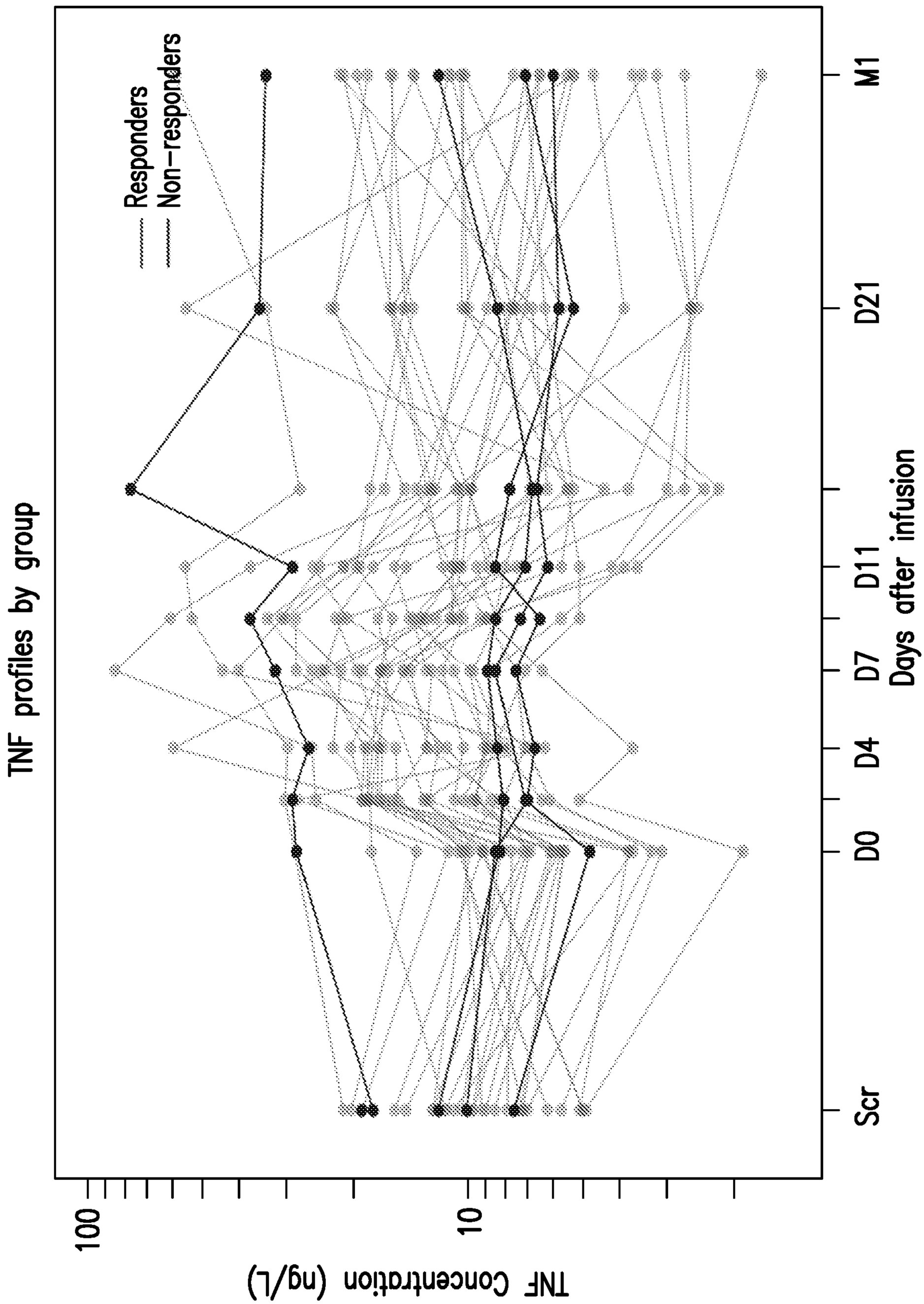

TNF profiles by group
Responders
Non−responders
TNF Concentration (ng/L)
100
10
Scr
D0
D4
D7
D11
D21
M1
Days after infusion

FIG. 2B

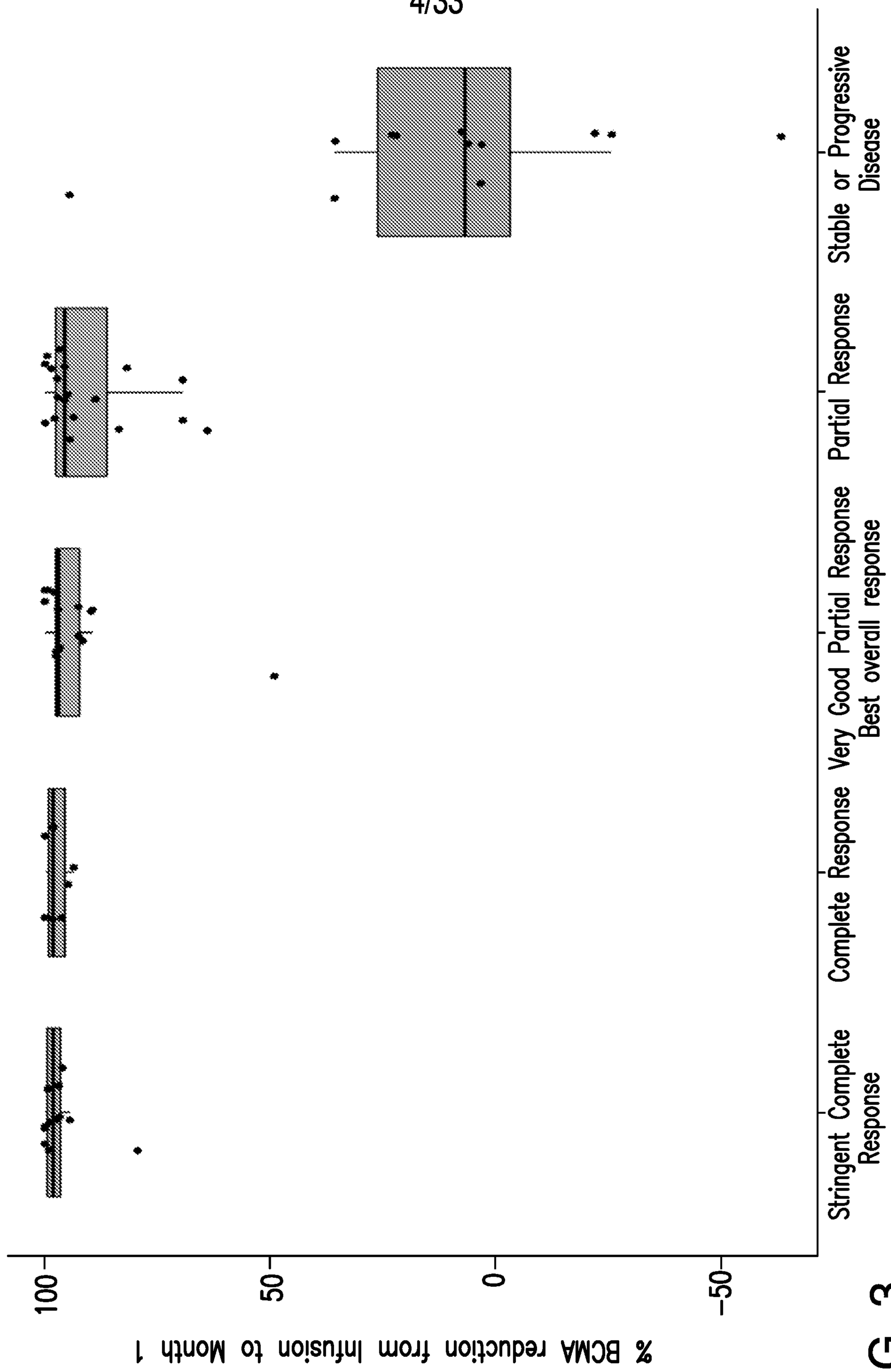

% BCMA reduction from Infusion to Month 1
100
50
0
−50
Stringent Complete Response
Complete Response
Very Good Partial Response
Best overall response
Partial Response
Stable or Progressive Disease

FIG. 3

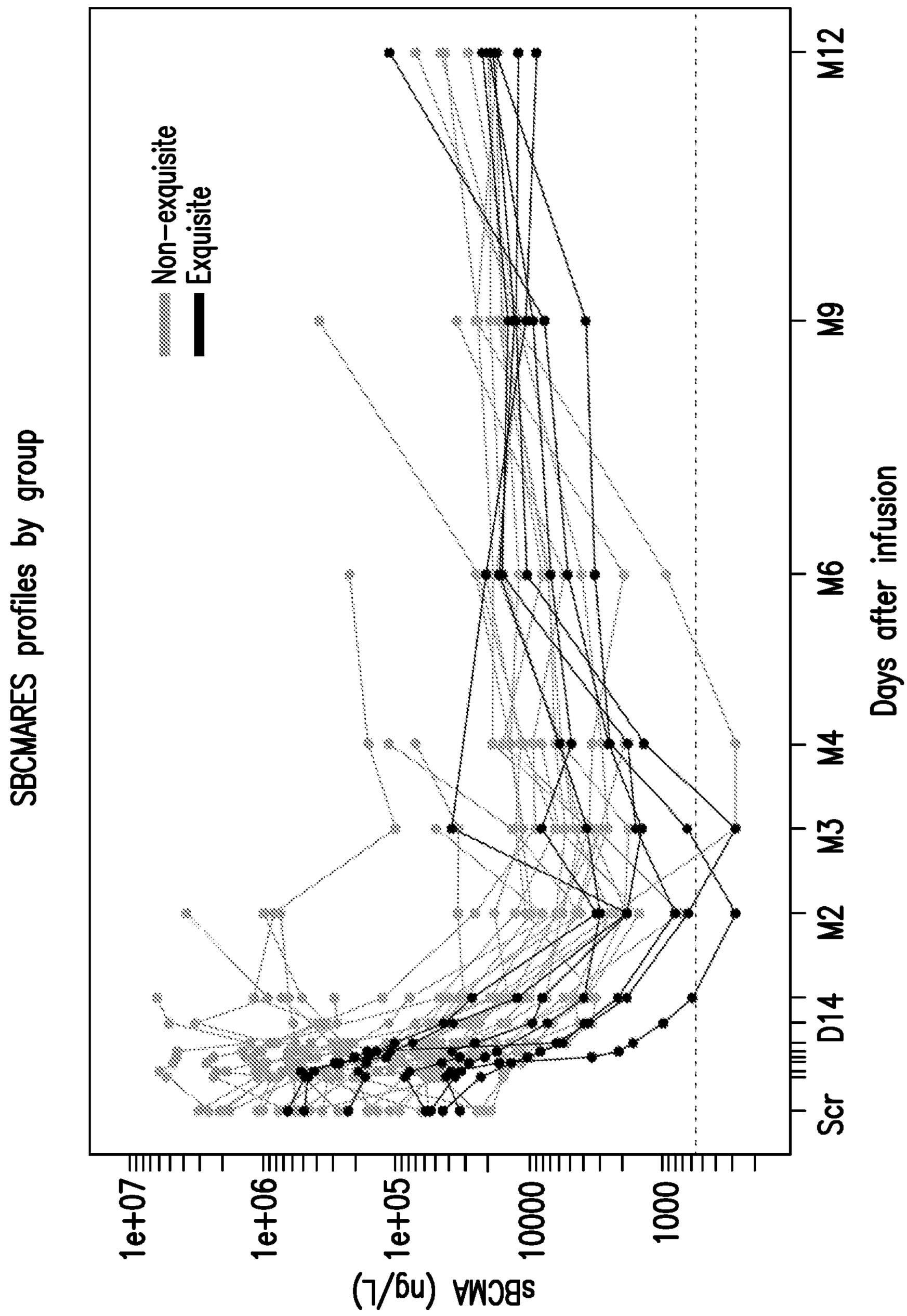

SBCMARES profiles by group
Non-exquisite
Exquisite
sBCMA (ng/L)
1e+07
1e+06
1e+05
10000
1000
Scr
D14
M2
M3
M4
M6
M9
M12
Days after infusion

FIG. 4

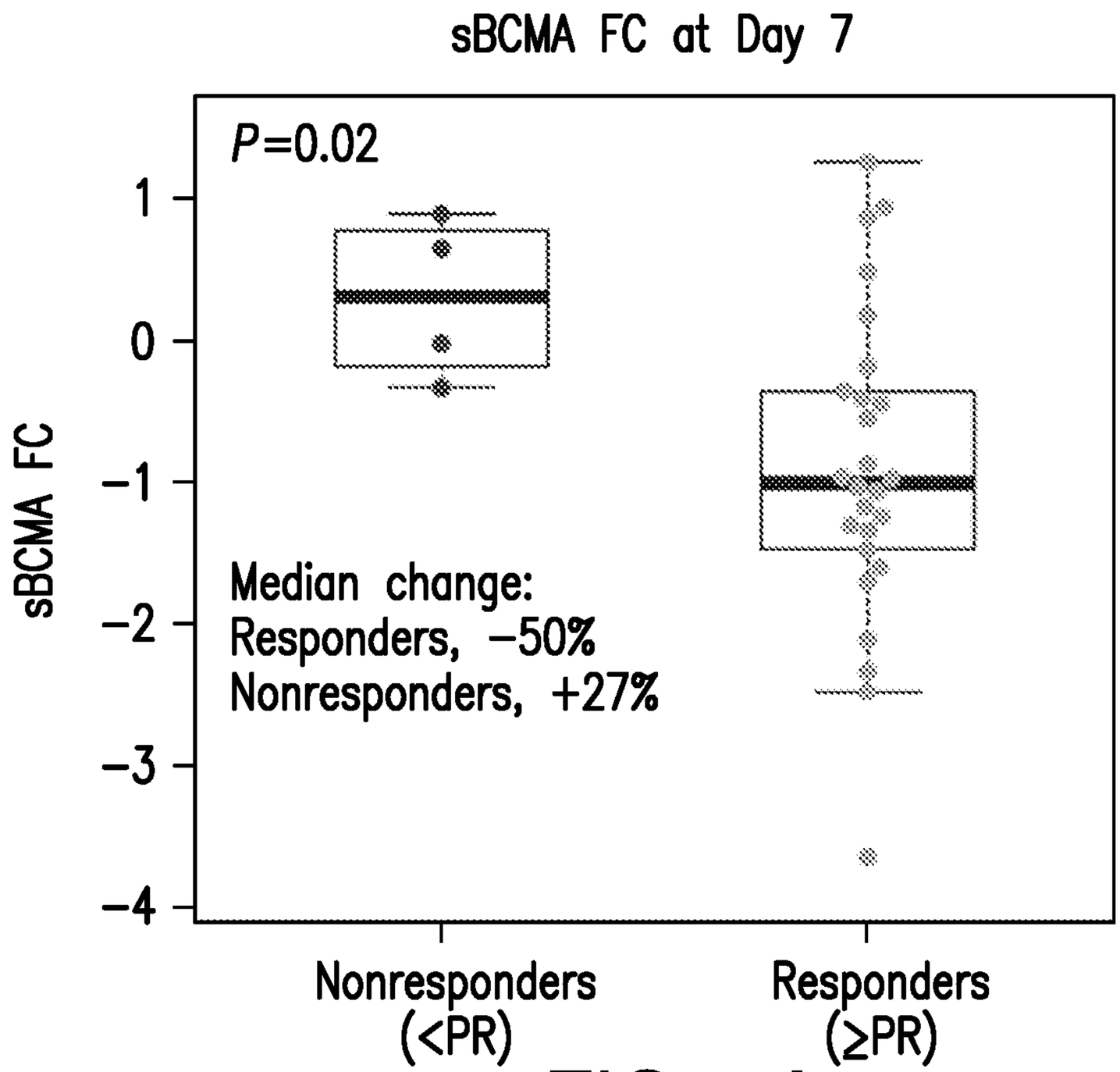

sBCMA FC at Day 7
P=0.02
1
0
−1
−2
−3
−4
sBCMA FC
Median change:
Responders, −50%
Nonresponders, +27%
Nonresponders
(<PR)
Responders
(≥PR)

FIG. 5A

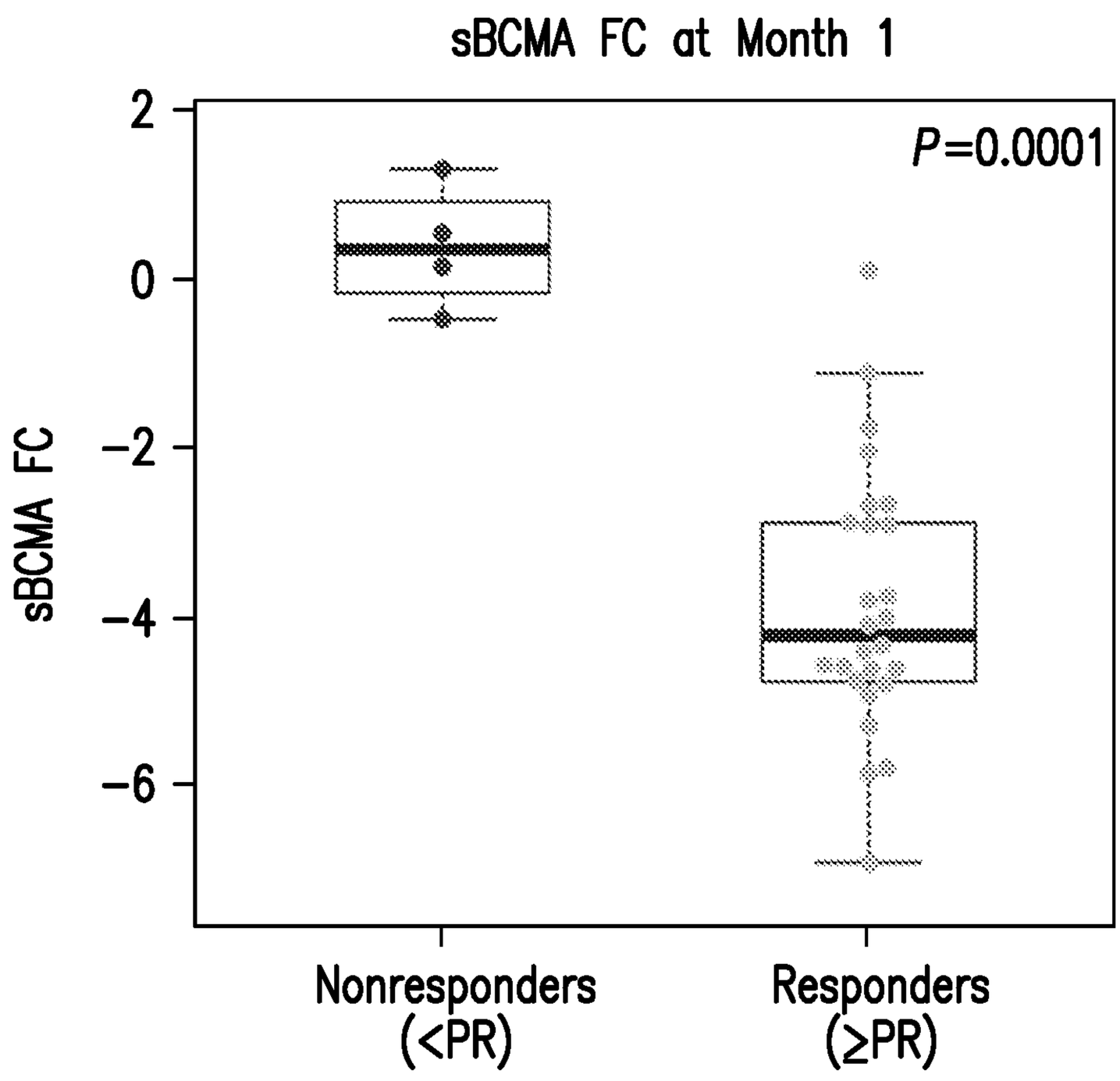

sBCMA FC at Month 1
P=0.0001
2
0
−2
−4
−6
sBCMA FC
Nonresponders
(<PR)
Responders
(≥PR)

FIG. 5B

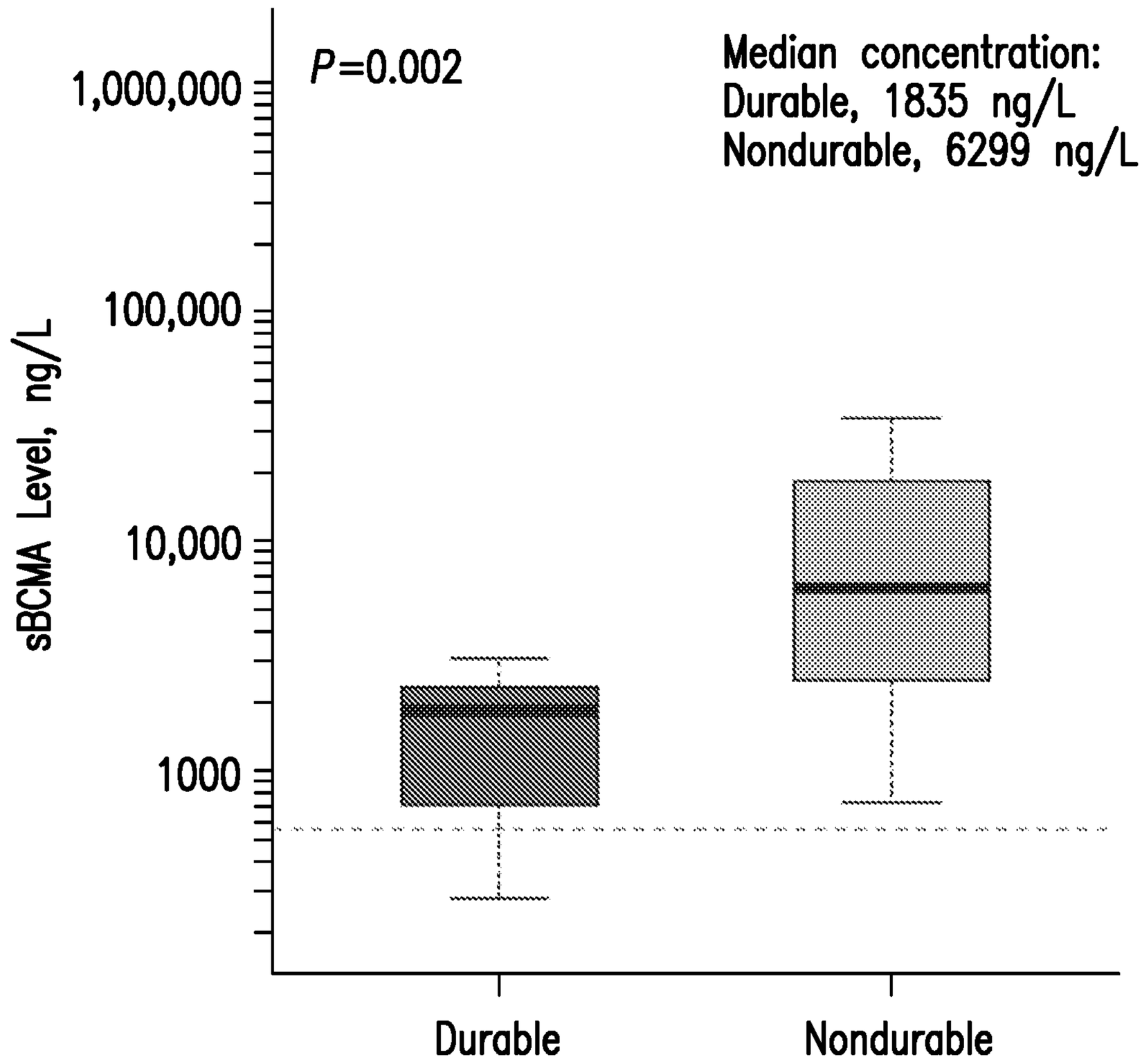

P=0.002
Median concentration:
Durable, 1835 ng/L
Nondurable, 6299 ng/L
sBCMA Level, ng/L
1,000,000
100,000
10,000
1000
Durable
Nondurable

FIG. 6

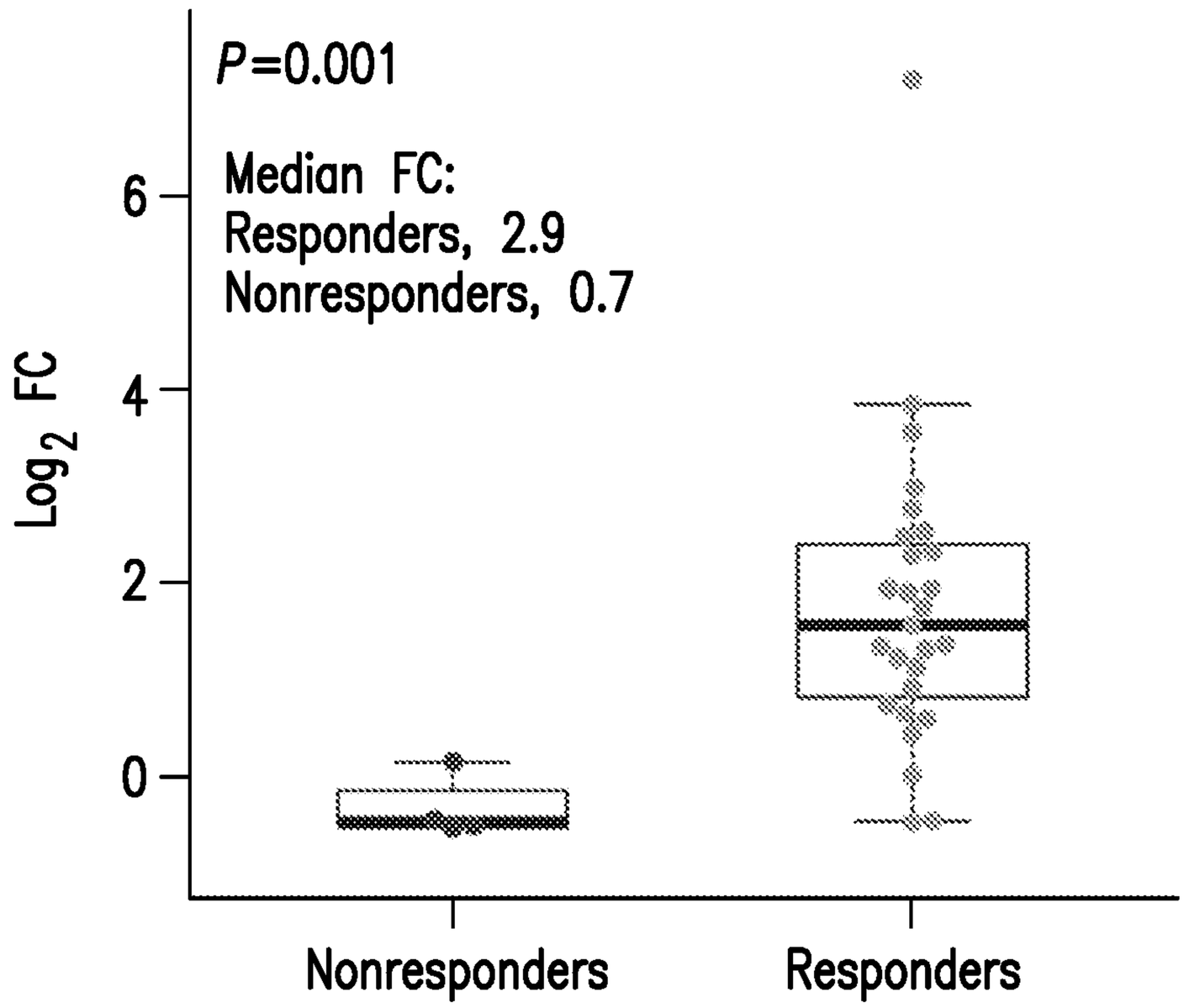

IL-6 FC at Day 2
P=0.001
Median FC:
Responders, 2.9
Nonresponders, 0.7
Log2 FC
6
4
2
0
Nonresponders
Responders

FIG. 7A

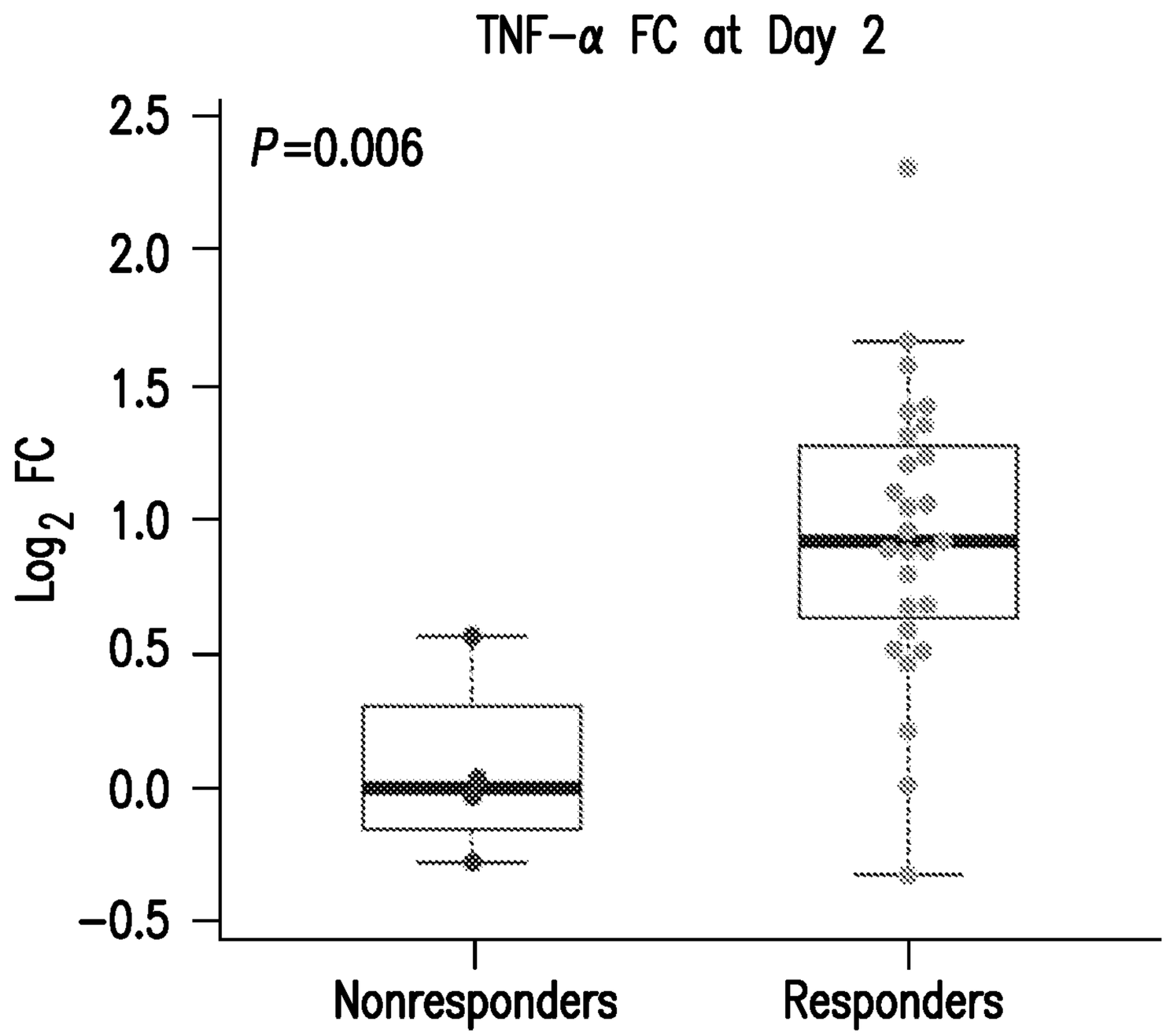

TNF-α FC at Day 2
P=0.006
Log2 FC
2.5
2.0
1.5
1.0
0.5
0.0
−0.5
Nonresponders
Responders

FIG. 7B

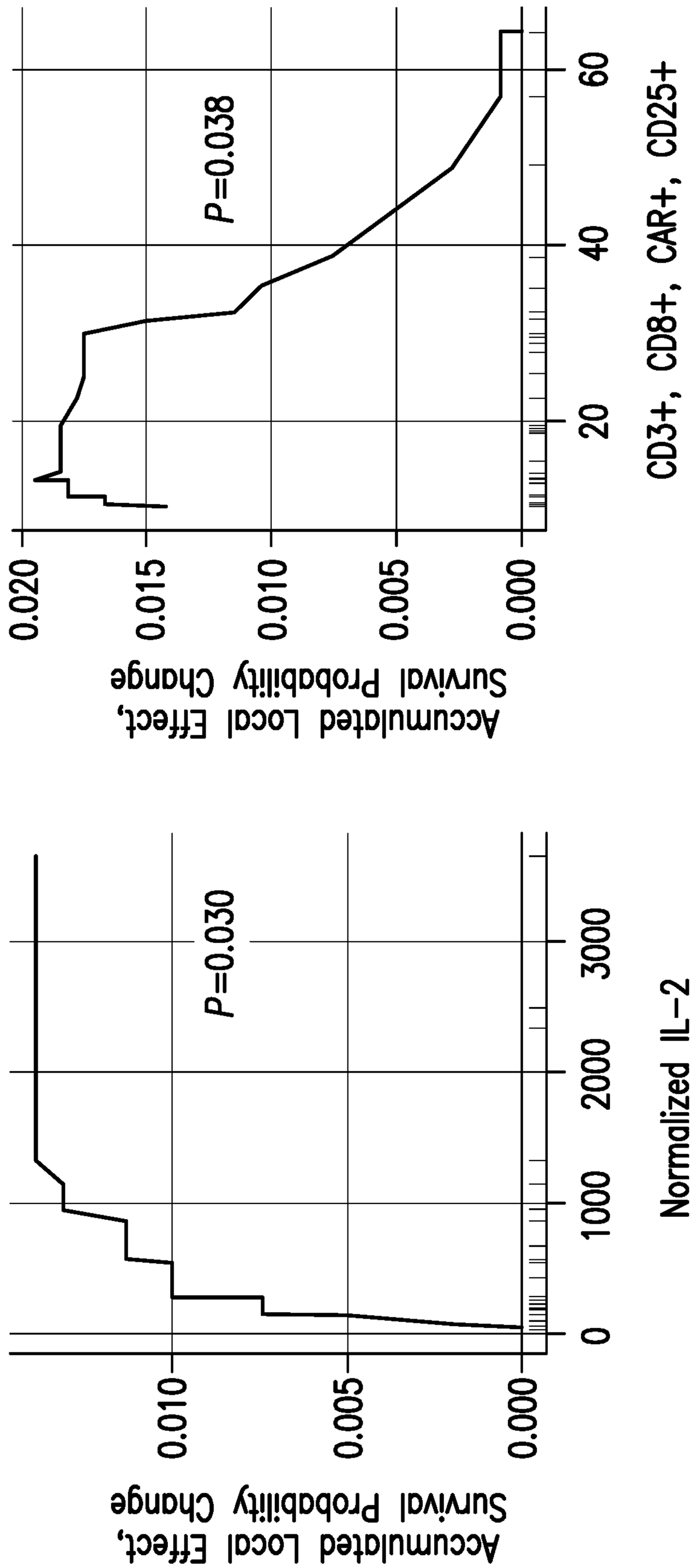

Accumulated Local Effect,
Survival Probability Change
0.020
0.015
0.010
0.005
0.000
P=0.038
20
40
60
CD3+, CD8+, CAR+, CD25+
Accumulated Local Effect,
Survival Probability Change
0.010
0.005
0.000
P=0.030
0
1000
2000
3000
Normalized IL-2

FIG. 8

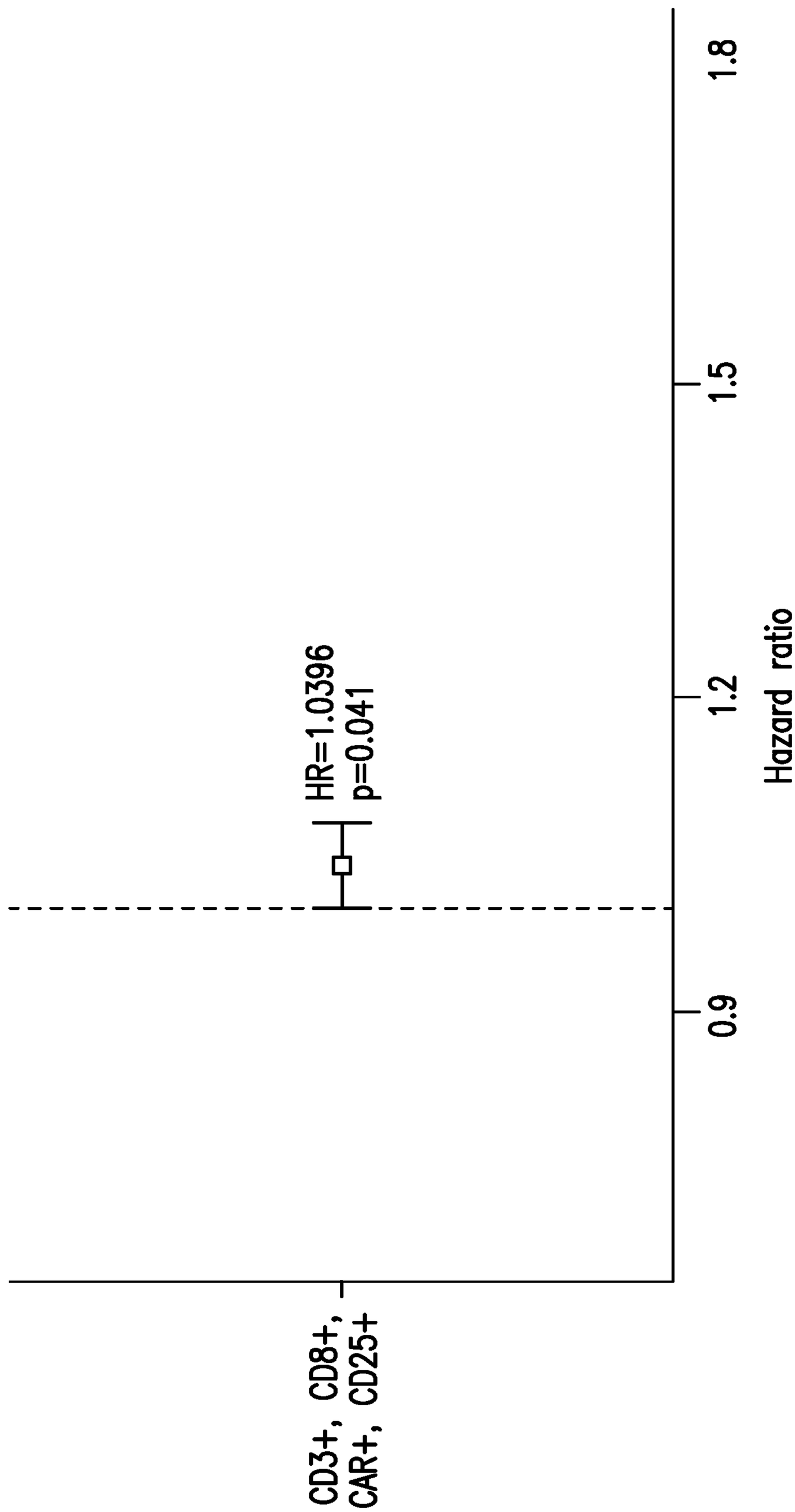


FIG. 9A

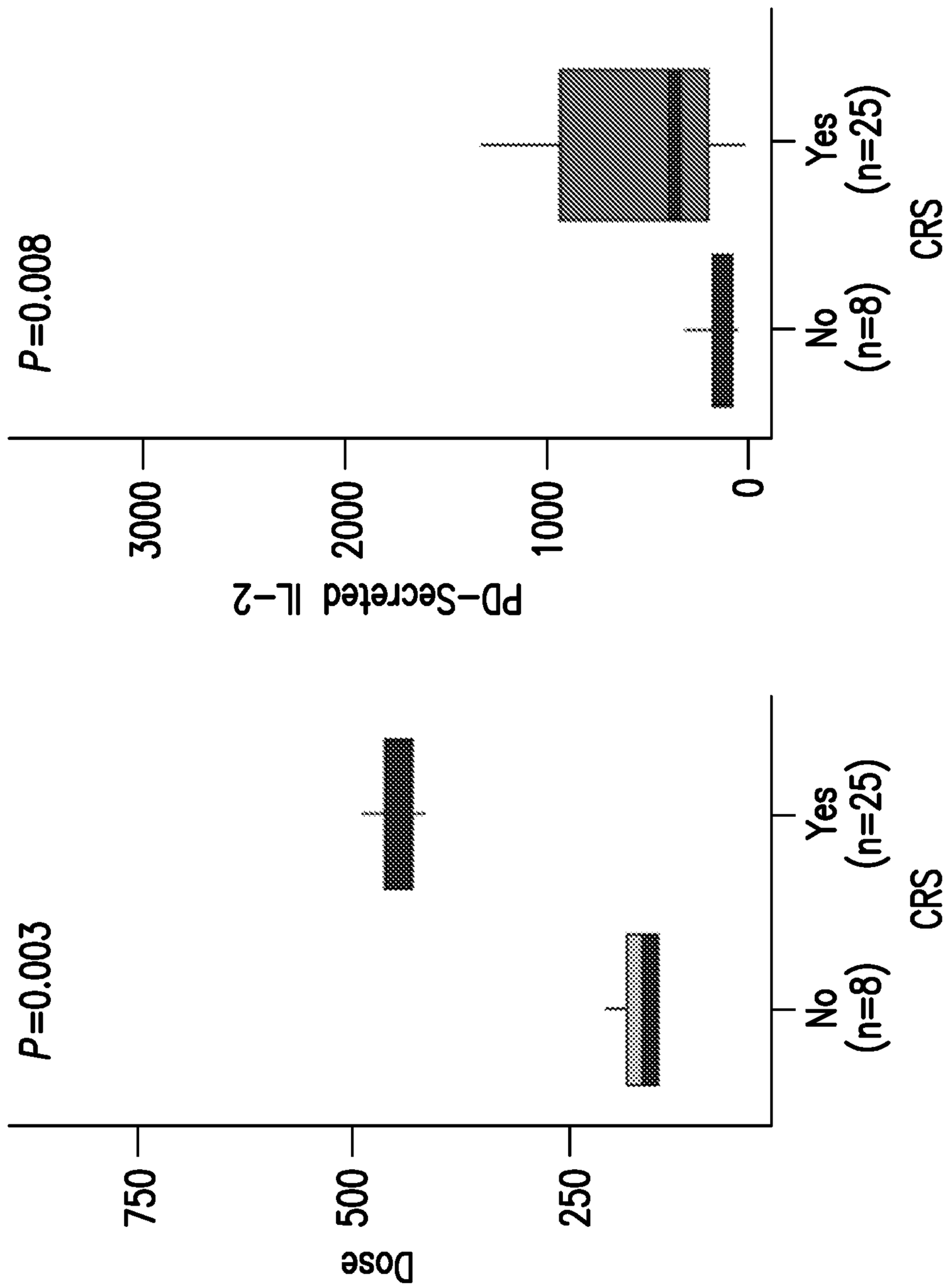

P=0.008
PD-Secreted IL-2
3000
2000
1000
0
No (n=8)
Yes (n=25)
CRS
P=0.003
Dose
750
500
250
No (n=8)
Yes (n=25)
CRS

FIG. 9B

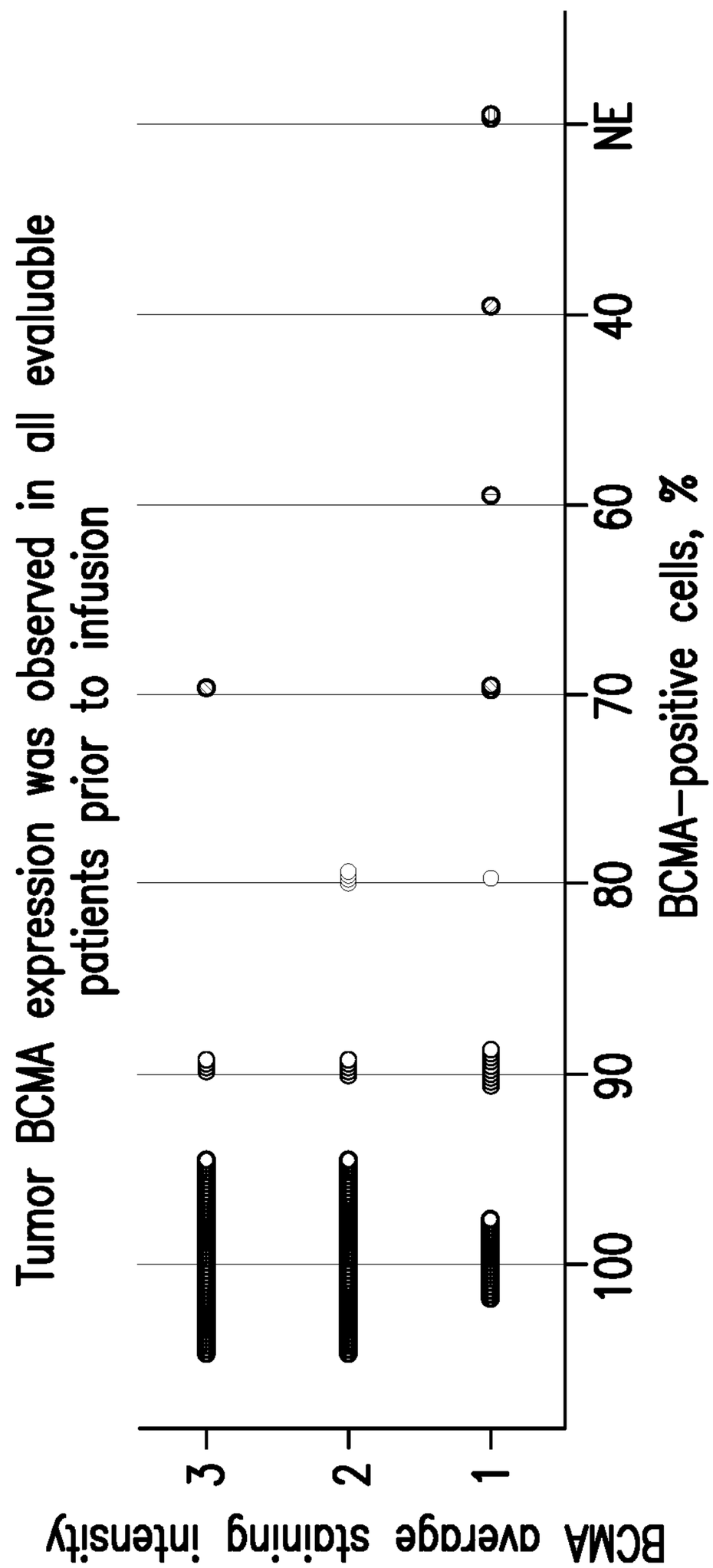

Tumor BCMA expression was observed in all evaluable patients prior to infusion
BCMA average staining intensity
3
2
1
100
90
80
70
60
40
NE
BCMA–positive cells, %

FIG. 10

Higher BCMA receptor density, rather than percentage of BCMA-positive cells, was associated with deeper tumor response

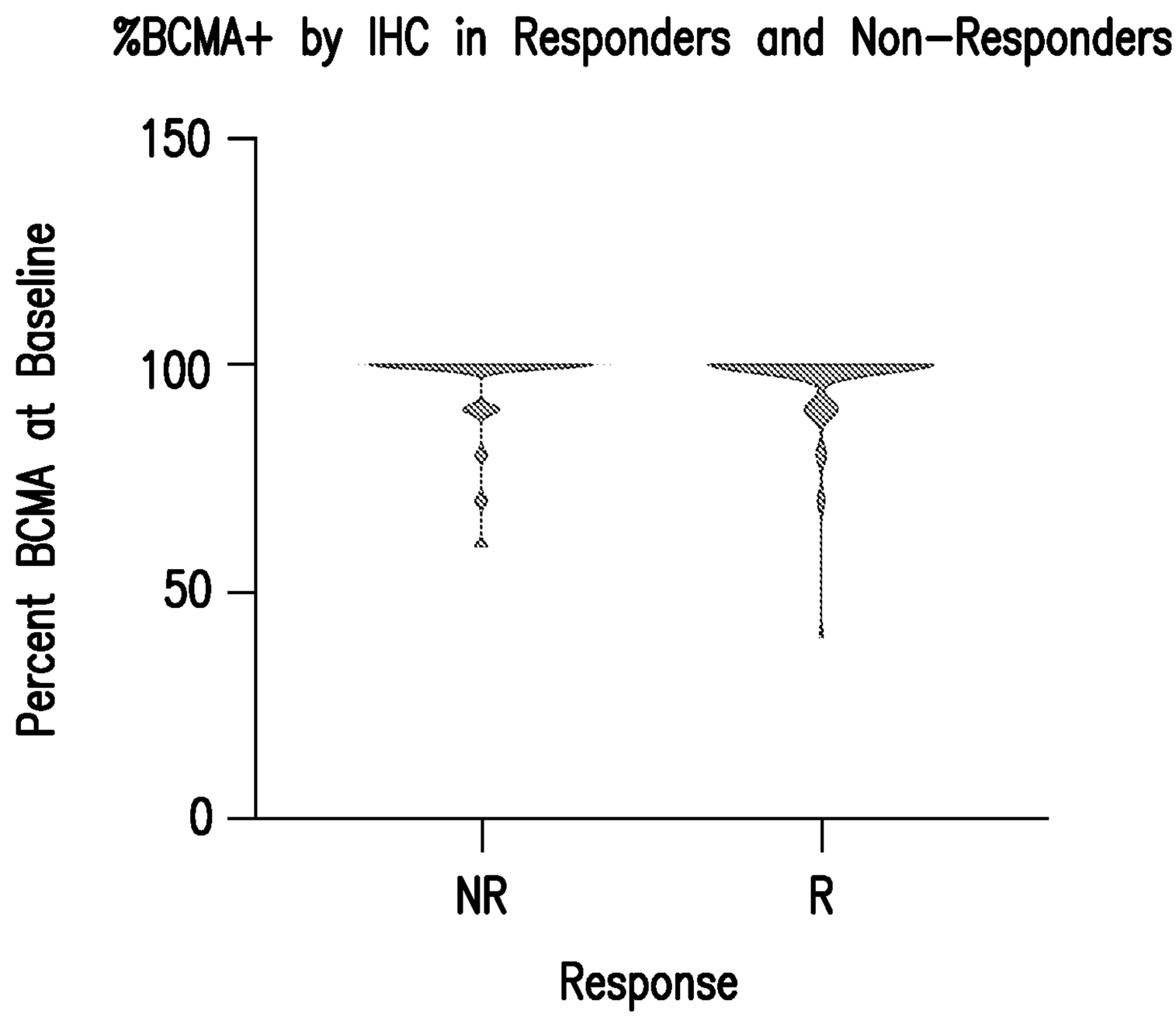


FIG. 11A

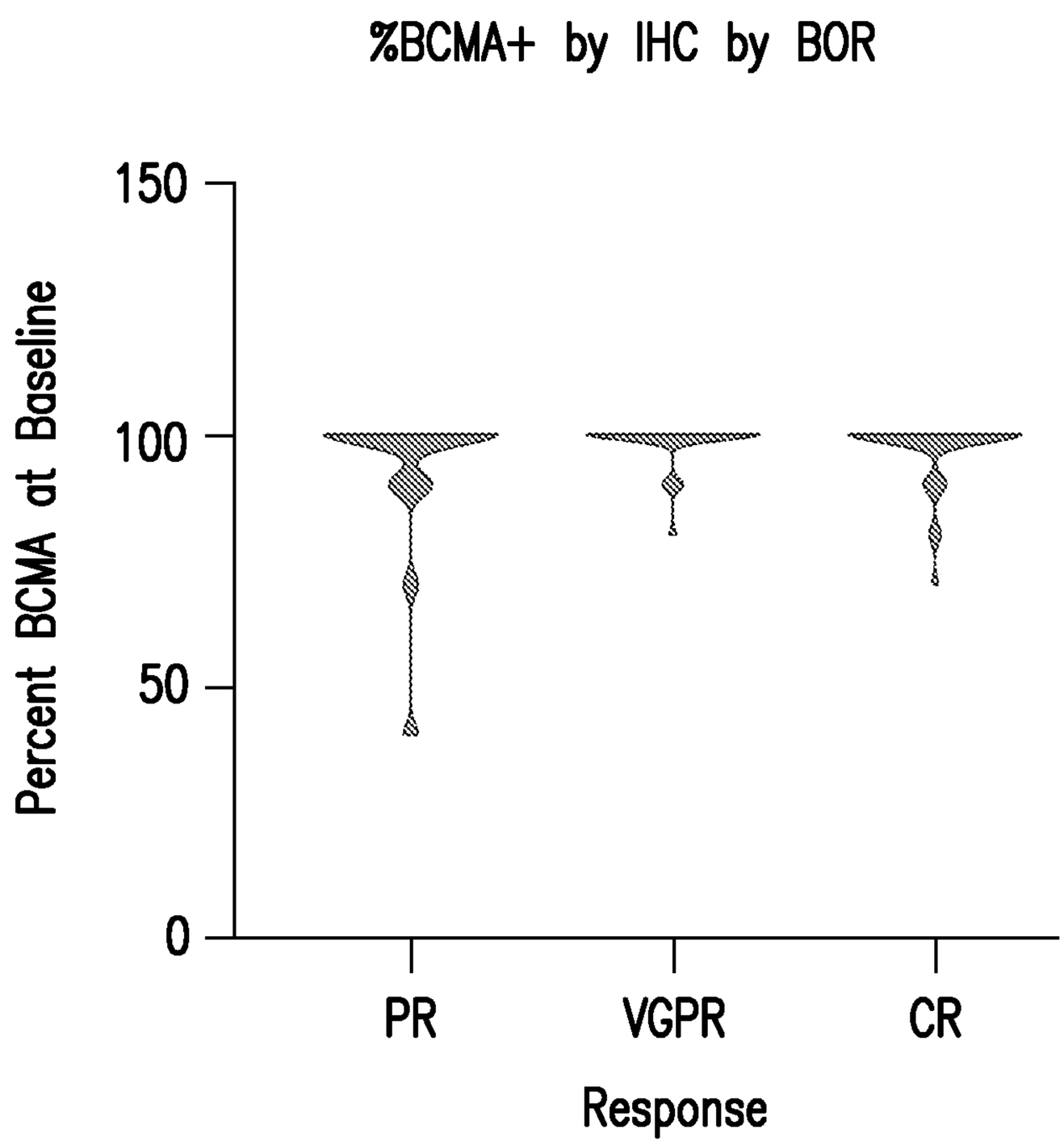

%BCMA+ by IHC by BOR
150
100
50
0
Percent BCMA at Baseline
PR
VGPR
CR
Response

FIG. 11B

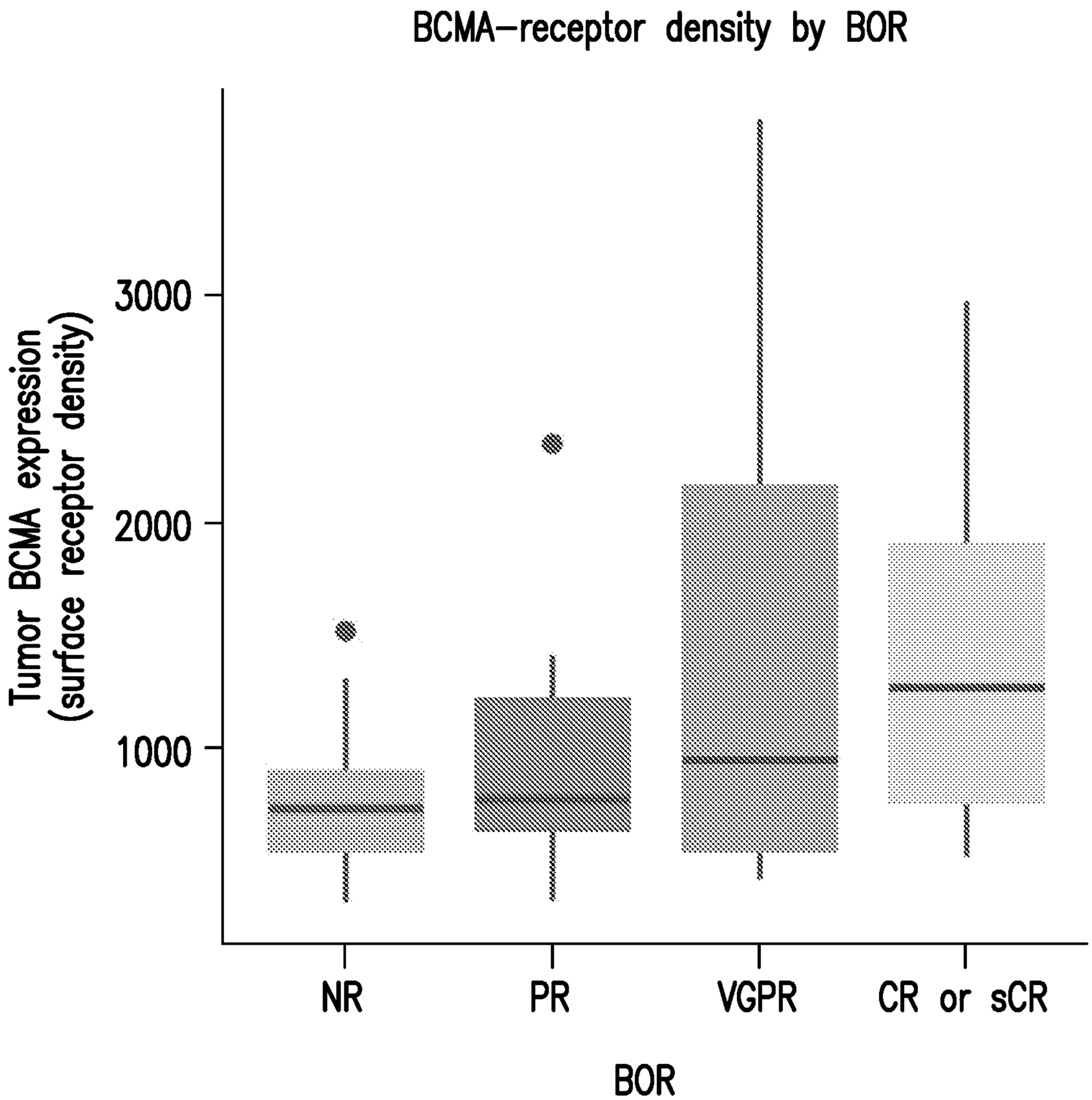

BCMA-receptor density by BOR
Tumor BCMA expression
(surface receptor density)
3000
2000
1000
NR
PR
VGPR
CR or sCR
BOR

FIG. 11C

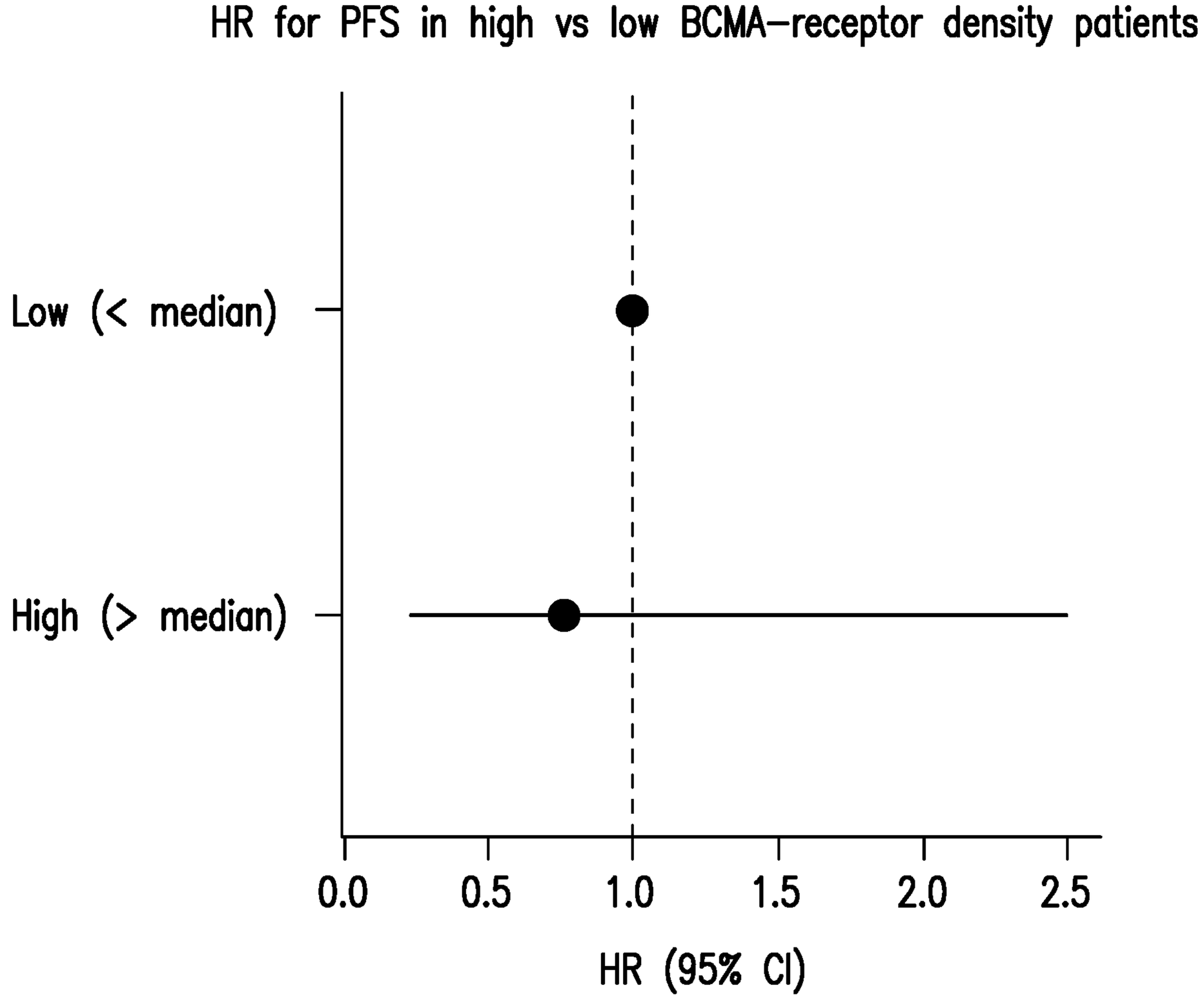

HR for PFS in high vs low BCMA-receptor density patients
Low (< median)
High (> median)
0.0
0.5
1.0
1.5
2.0
2.5
HR (95% CI)

FIG. 11D

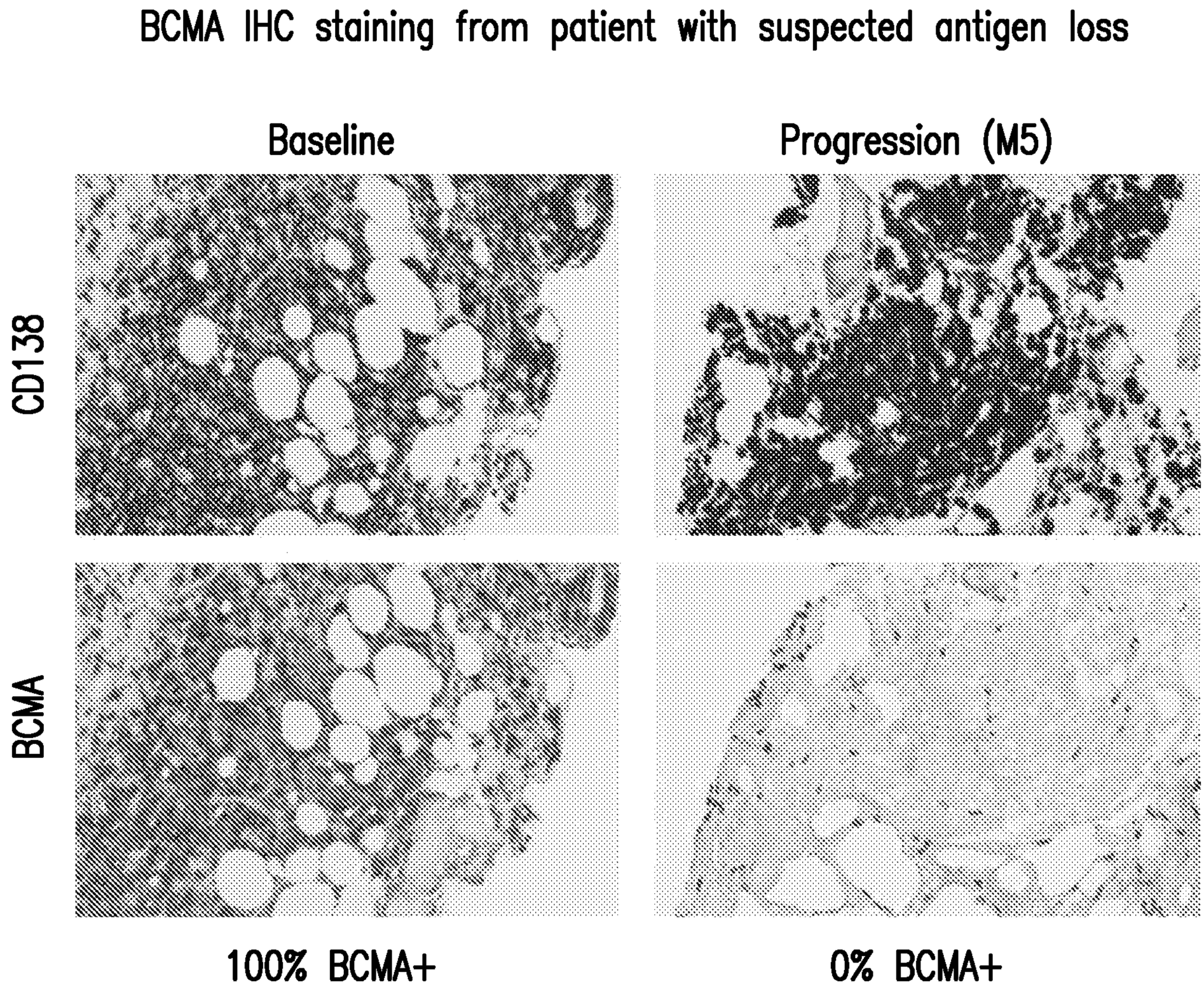
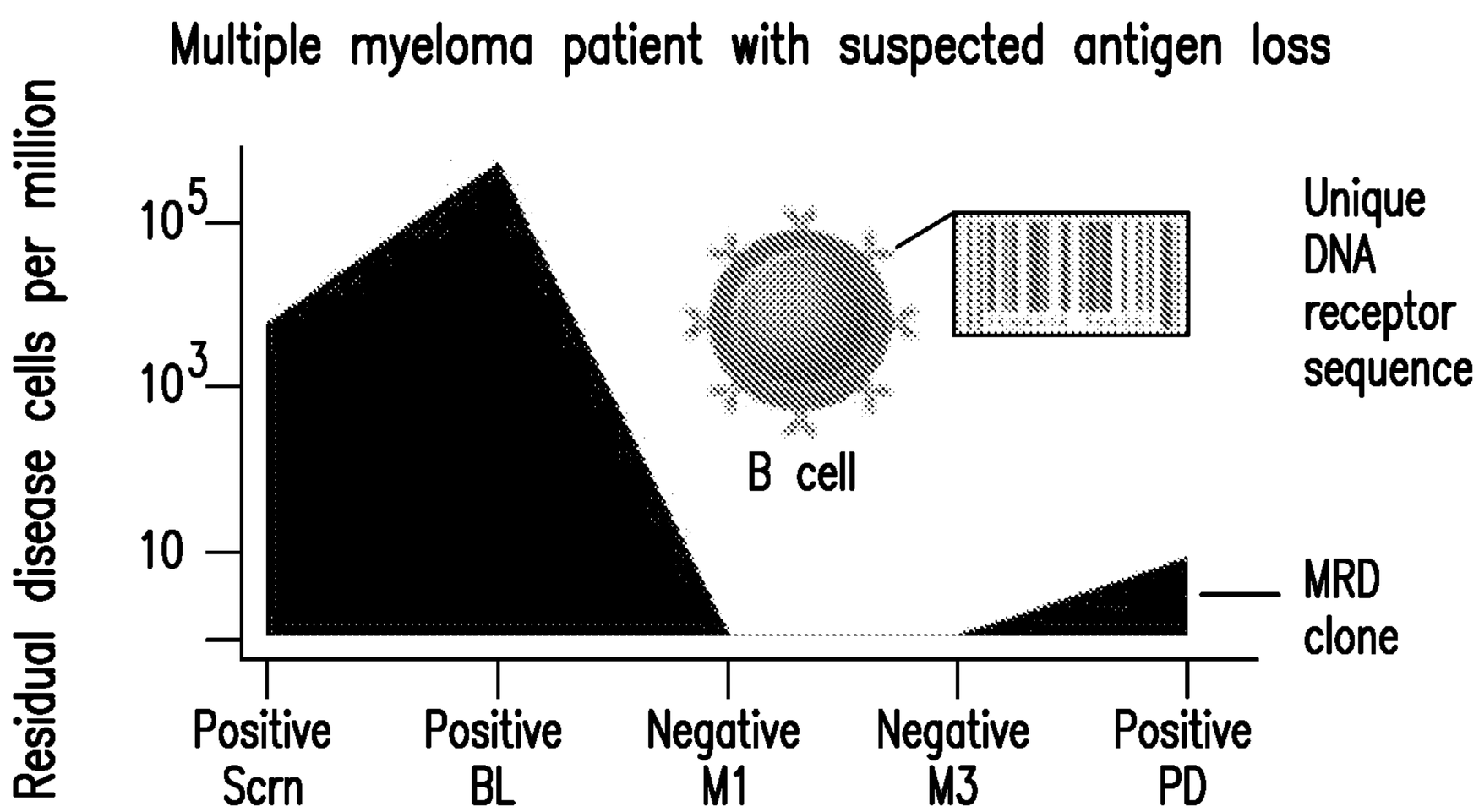

BCMA IHC staining from patient with suspected antigen loss
Baseline
Progression (M5)
CD138
BCMA
100% BCMA+
0% BCMA+

FIG. 12A

VDJ clone tracking illustrates return of initial and potential emergent clones at relapse



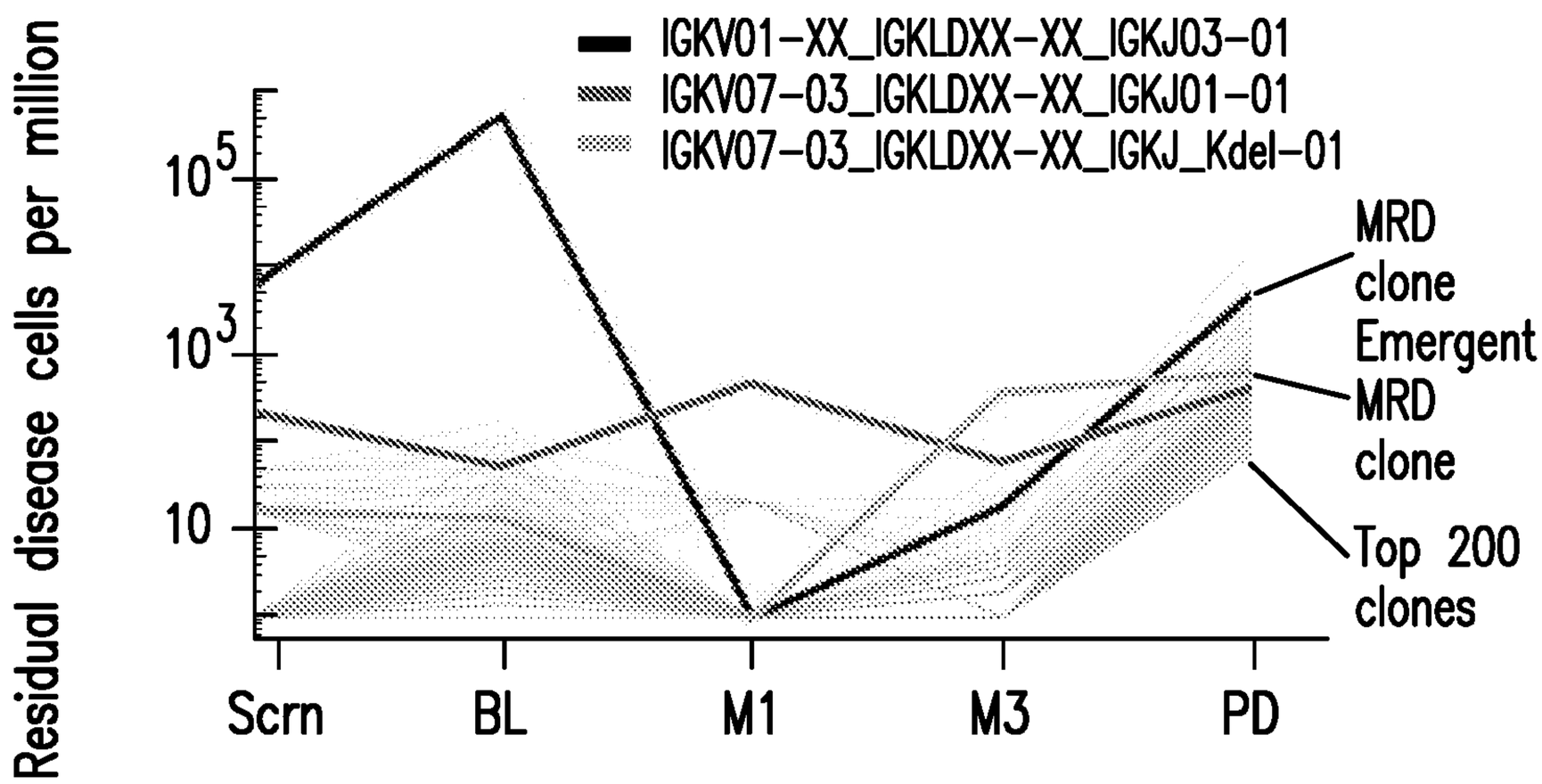


FIG. 12B

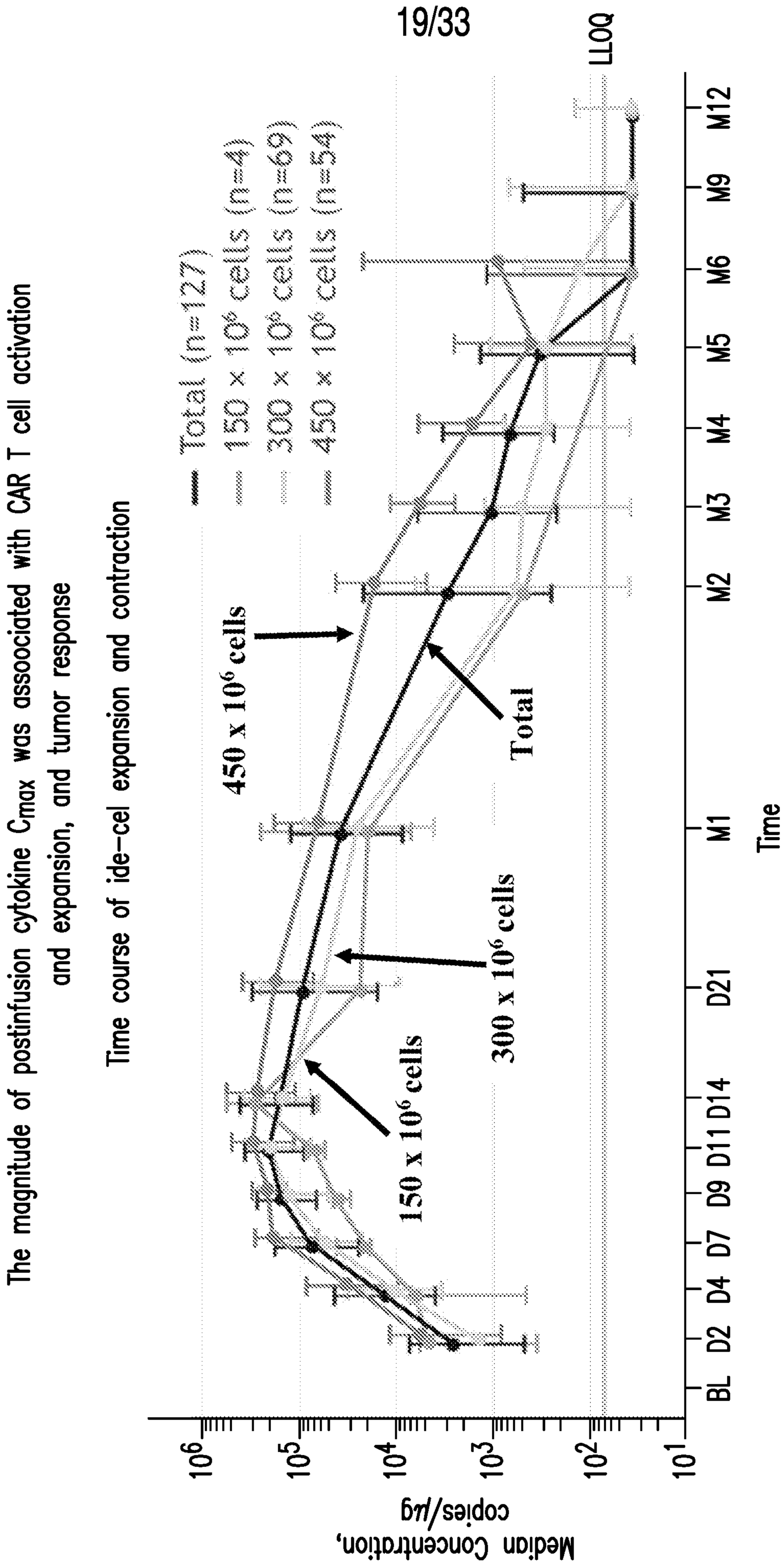

The magnitude of postinfusion cytokine C_{max} was associated with CAR T cell activation and expansion, and tumor response
Time course of ide-cel expansion and contraction
Total (n=127)
150 x 10^6 cells (n=4)
300 x 10^6 cells (n=69)
450 x 10^6 cells (n=54)
450 x 10^6 cells
Total
300 x 10^6 cells
150 x 10^6 cells
LLOQ
Median Concentration, copies/µg
10^6
10^5
10^4
10^3
10^2
10^1
BL D2 D4 D7 D9 D11 D14 D21 M1 M2 M3 M4 M5 M6 M9 M12
Time

FIG. 13A

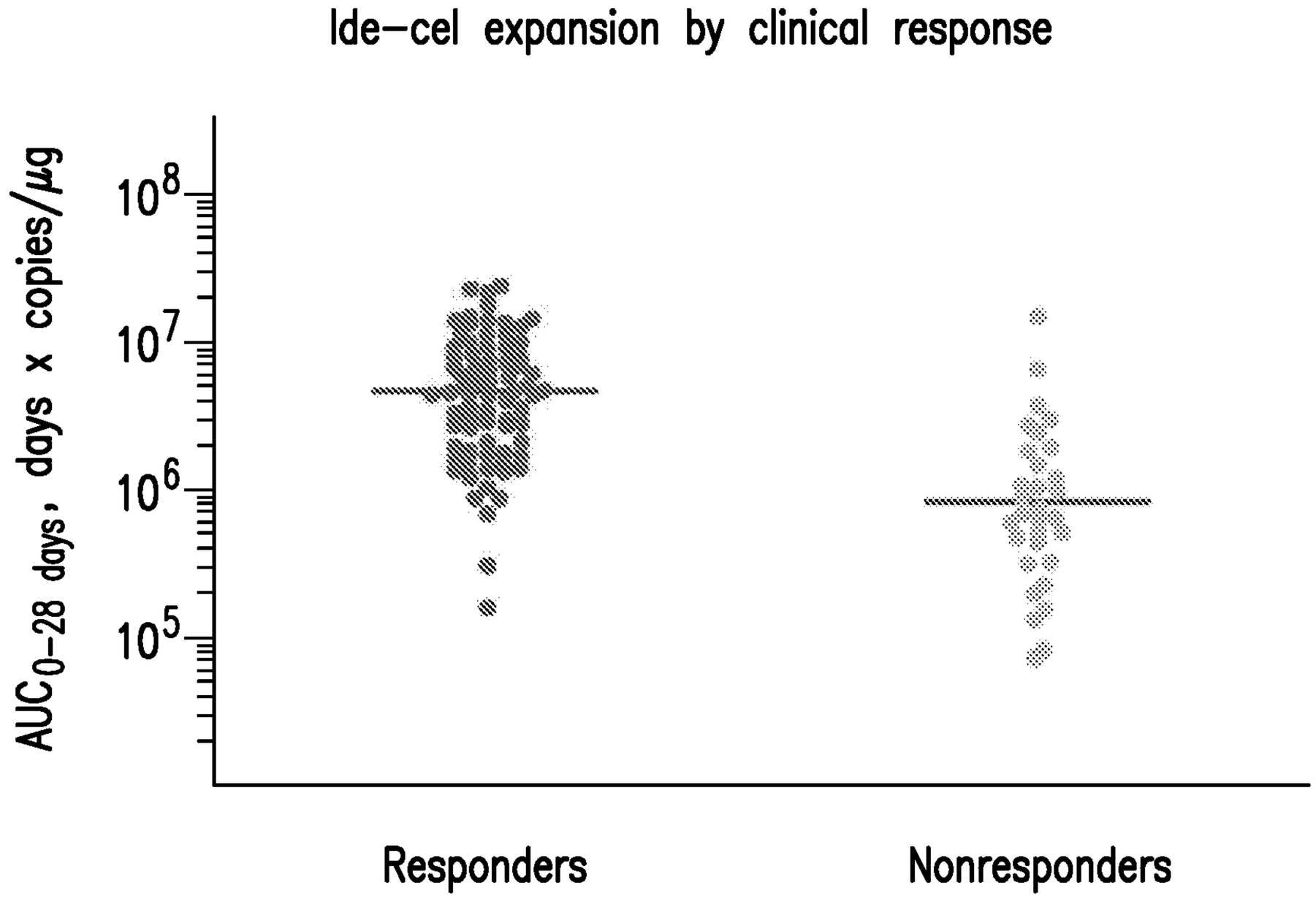


FIG. 13A (continued)

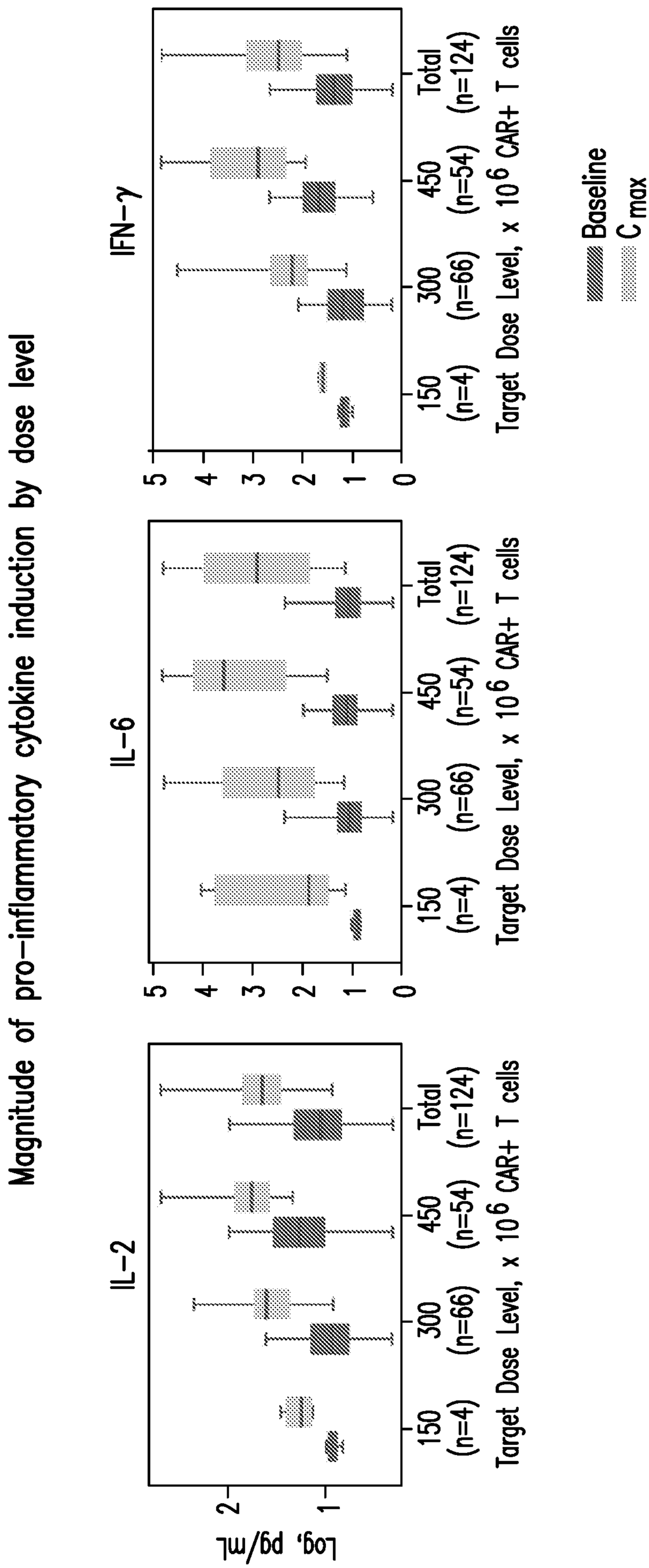

Magnitude of pro-inflammatory cytokine induction by dose level
IL-2
IL-6
IFN-γ
Log, pg/mL
5
4
3
2
1
0
150 (n=4)
300 (n=66)
450 (n=54)
Total (n=124)
Target Dose Level, x 10^6 CAR+ T cells
Baseline
C max

FIG. 13B

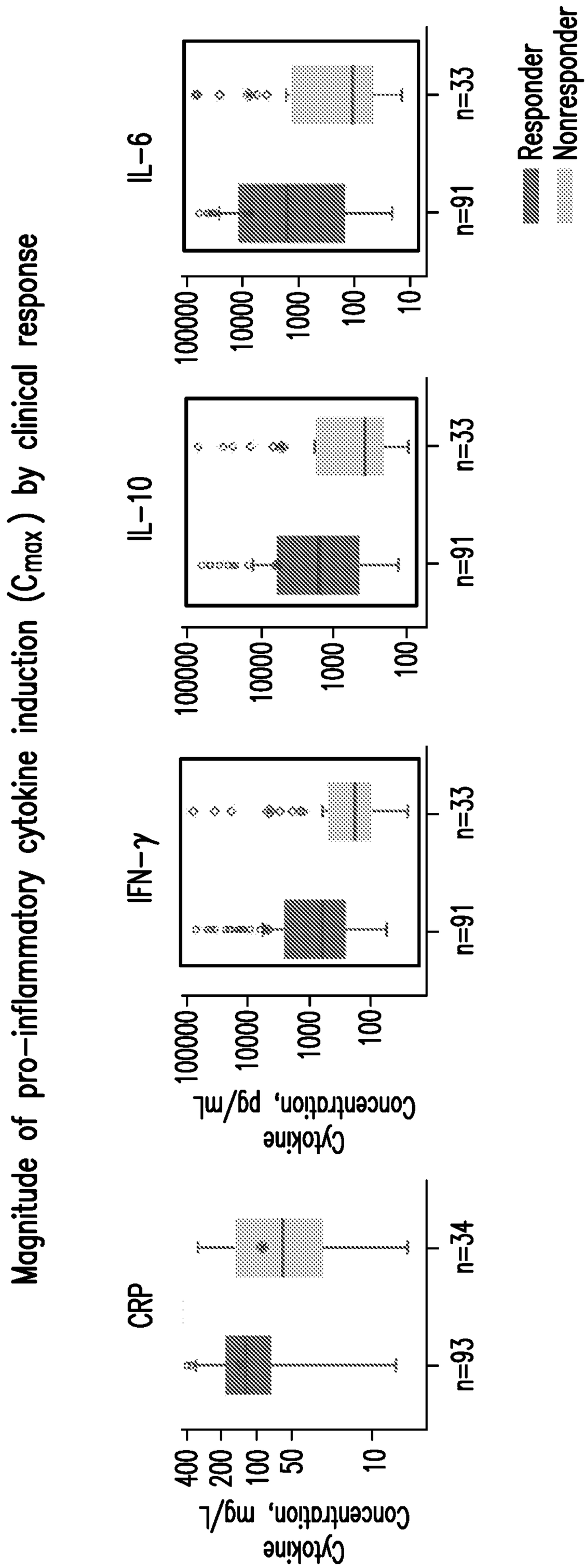

Magnitude of pro-inflammatory cytokine induction (C_{max}) by clinical response
CRP
IFN-γ
IL-10
IL-6
Cytokine Concentration, mg/L
Cytokine Concentration, pg/mL
400
200
100
50
10
100000
10000
1000
100
10
n=93
n=34
n=91
n=33
n=91
n=33
Responder
Nonresponder

FIG. 13C

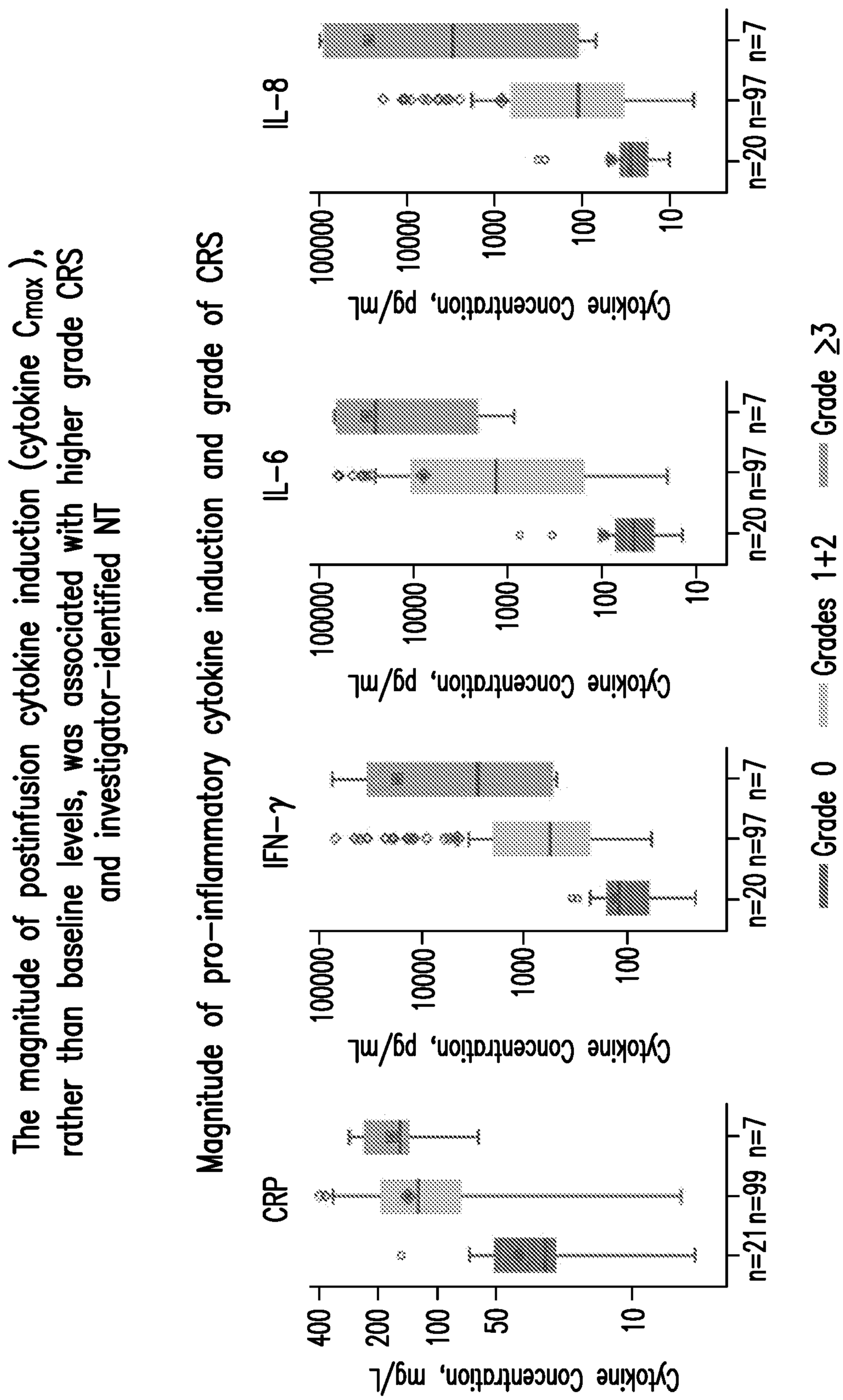

The magnitude of postinfusion cytokine induction (cytokine C_{max}), rather than baseline levels, was associated with higher grade CRS and investigator-identified NT
Magnitude of pro-inflammatory cytokine induction and grade of CRS
CRP
IFN-γ
IL-6
IL-8
Cytokine Concentration, mg/L
Cytokine Concentration, pg/mL
400
200
100
50
10
100000
10000
1000
100
10
n=21 n=99 n=7
n=20 n=97 n=7
Grade 0
Grades 1+2
Grade ≥3

FIG. 14A

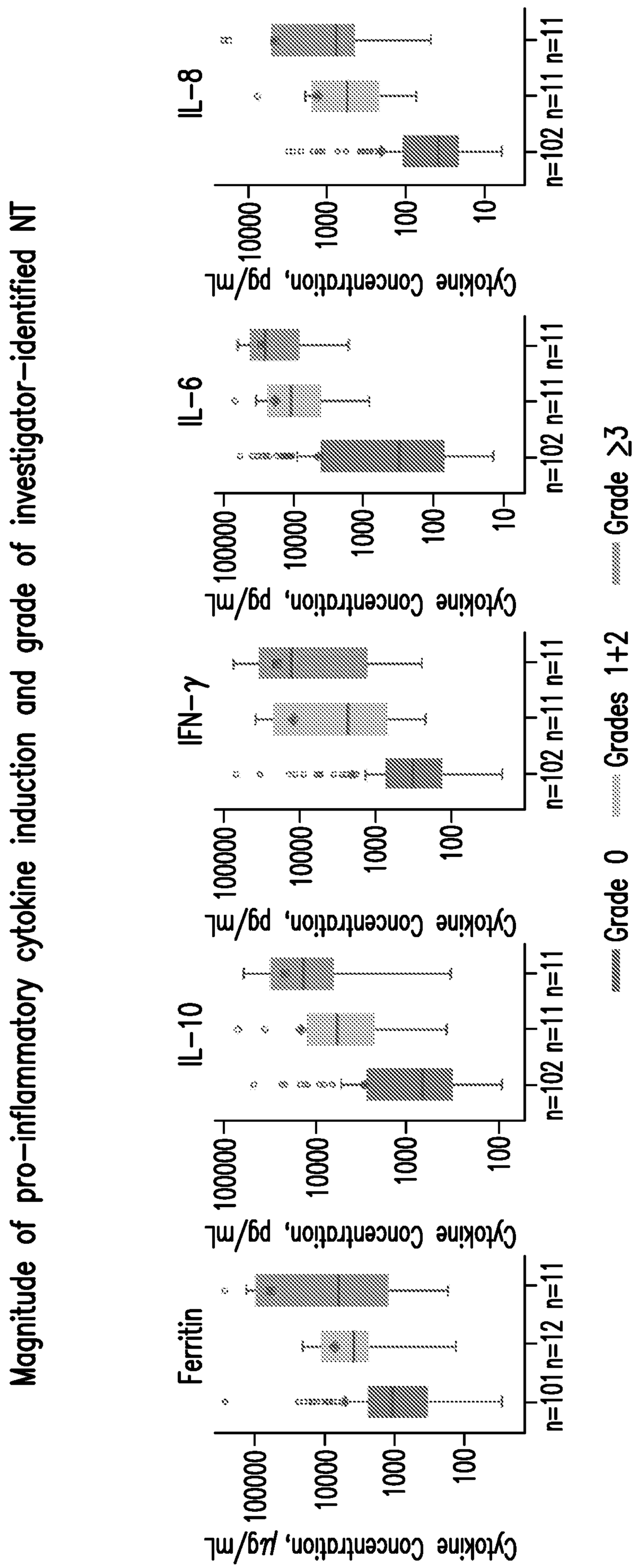

Magnitude of pro-inflammatory cytokine induction and grade of investigator-identified NT
Ferritin
IL-10
IFN-γ
IL-6
IL-8
Cytokine Concentration, μg/mL
Cytokine Concentration, pg/mL
100000
10000
1000
100
10
n=101 n=12 n=11
n=102 n=11 n=11
Grade 0
Grades 1+2
Grade ≥3

FIG. 14B

Baseline angiopoietin-1 and -2 levels and grade of investigator-identified NT

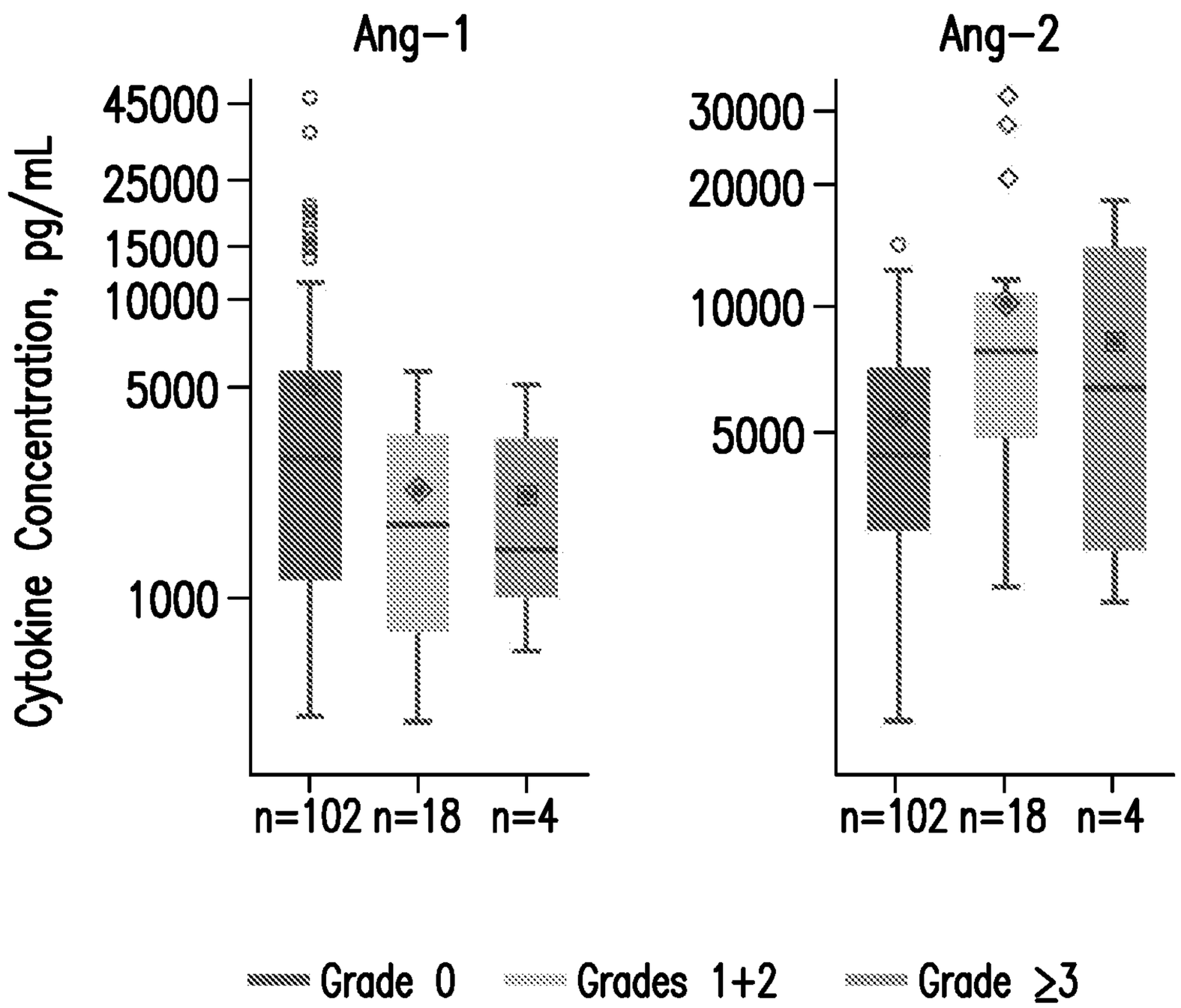


FIG. 14C

sBCMA clearance postinfusion was independent of baseline tumor burden or EMP involvement

Correlation between baseline sBCMA and tumor burden

Time point	< 50% Tumor Burden	≥ 50% Tumor Burden	*P* value
Baseline Median (Q1, Q3), ng/mL <LLOQ, n (%)	(n=56) 177 (57.5, 327.0) 0	(n=62) 386.0 (269.0, 696.0) 0	< 0.0001
Nadir Median (Q1, Q3), ng/mL <LLOQ, n (%)	(n=56) 2.2 (2.2, 11.0) 37 (66.1)	(n=62) 2.2 (2.2, 43.0) 34 (54.8)	0.1453

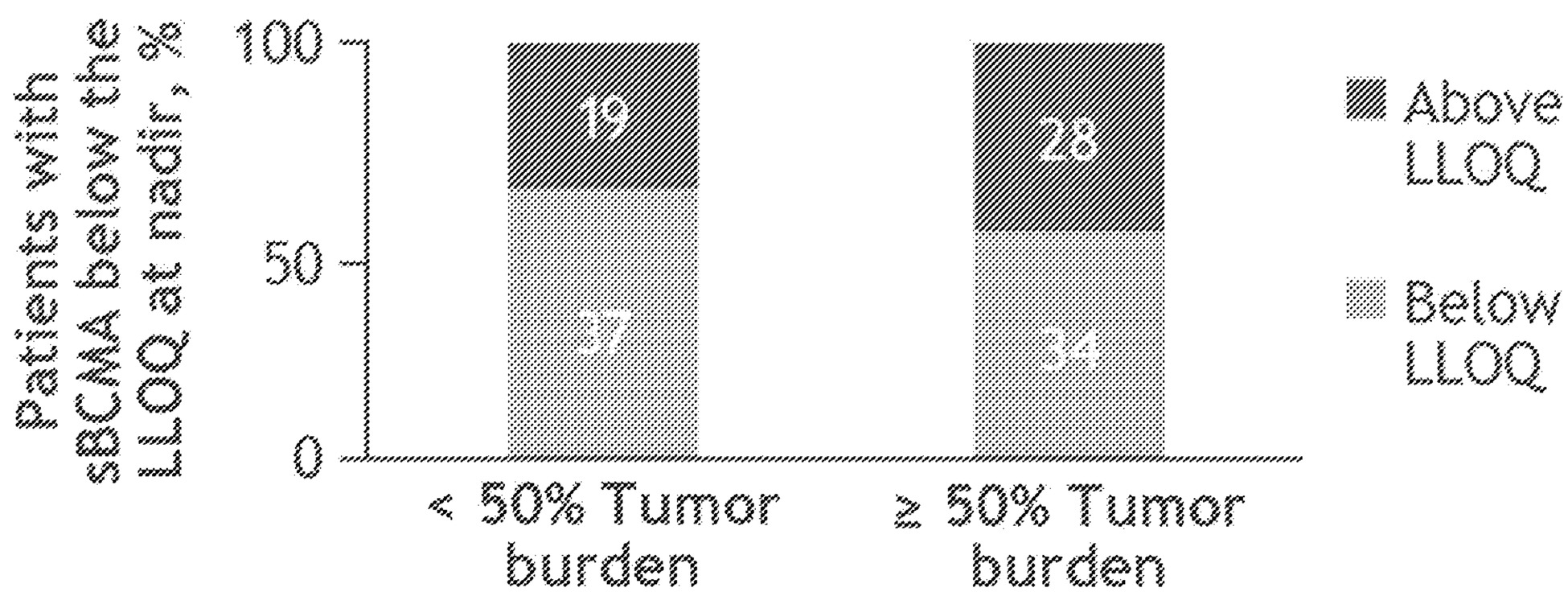


FIG. 15A

Correlation between baseline sBCMA and presence of EMP

Time point	EMP	No EMP	*P* value
Baseline Median (Q1, Q3), ng/mL <LLOQ, n (%)	(n=48) 345.5 (195.0, 670.0) 0 (0)	(n=76) 238.0 (76.0, 424.5) 0 (0)	0.0339
Nadir Median (Q1, Q3), ng/mL <LLOQ, n (%)	(n=48) 2.2 (2.2, 20.0) 30 (62.5)	(n=76) 2.2 (2.2, 19.0) 45 (59.2)	0.7869

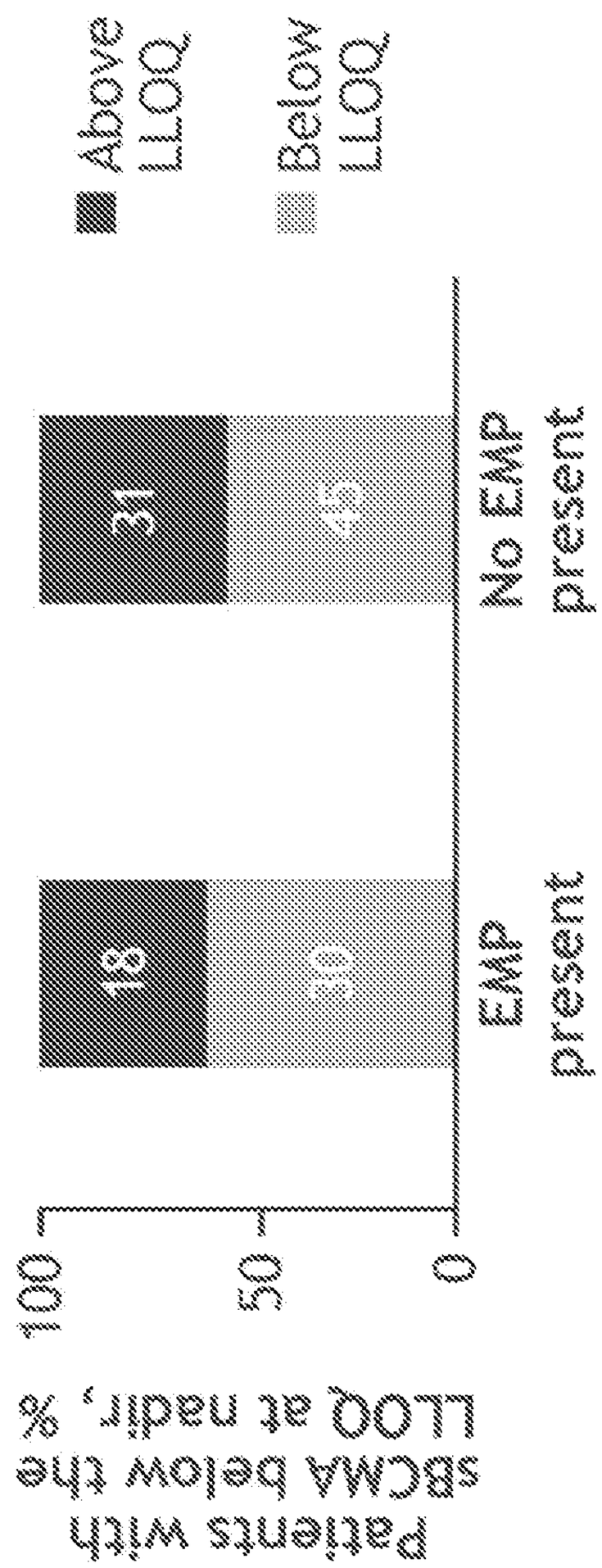


FIG. 15B

Correlation between baseline sBCMA and sBCMA clearance

Time point	< 75% sBCMA	≥ 75% sBCMA	*P* value
Baseline Median (Q1, Q3), ng/mL <LLOQ, n (%)	191.0 (n=93) (73.0, 314.0) 0 (0)	790.0 (n=31) (657.0, 987.0) 0 (0)	< 0.0001
Nadir Median (Q1, Q3), ng/mL <LLOQ, n (%)	2.2 (n=93) (2.2, 5.9) 64 (68.8)	25.0 (n=31) (2.2, 507.0) 11 (35.5)	< 0.0001

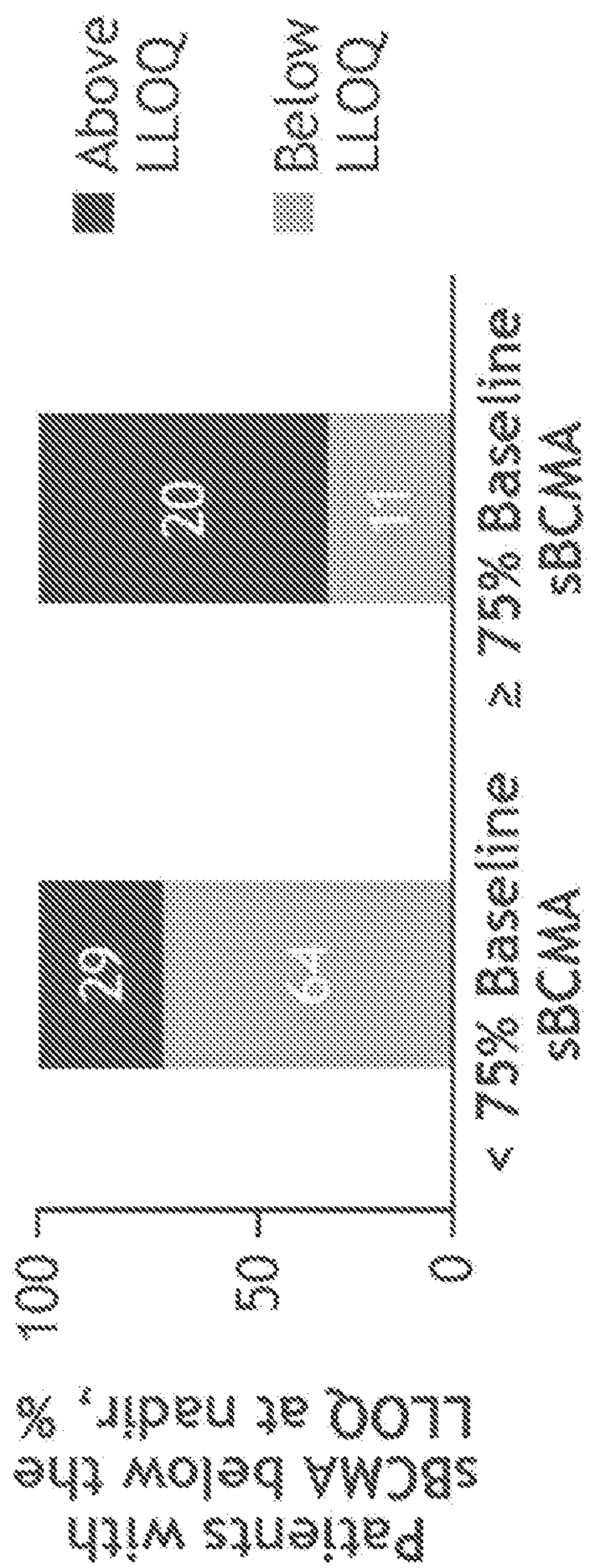


FIG. 15C

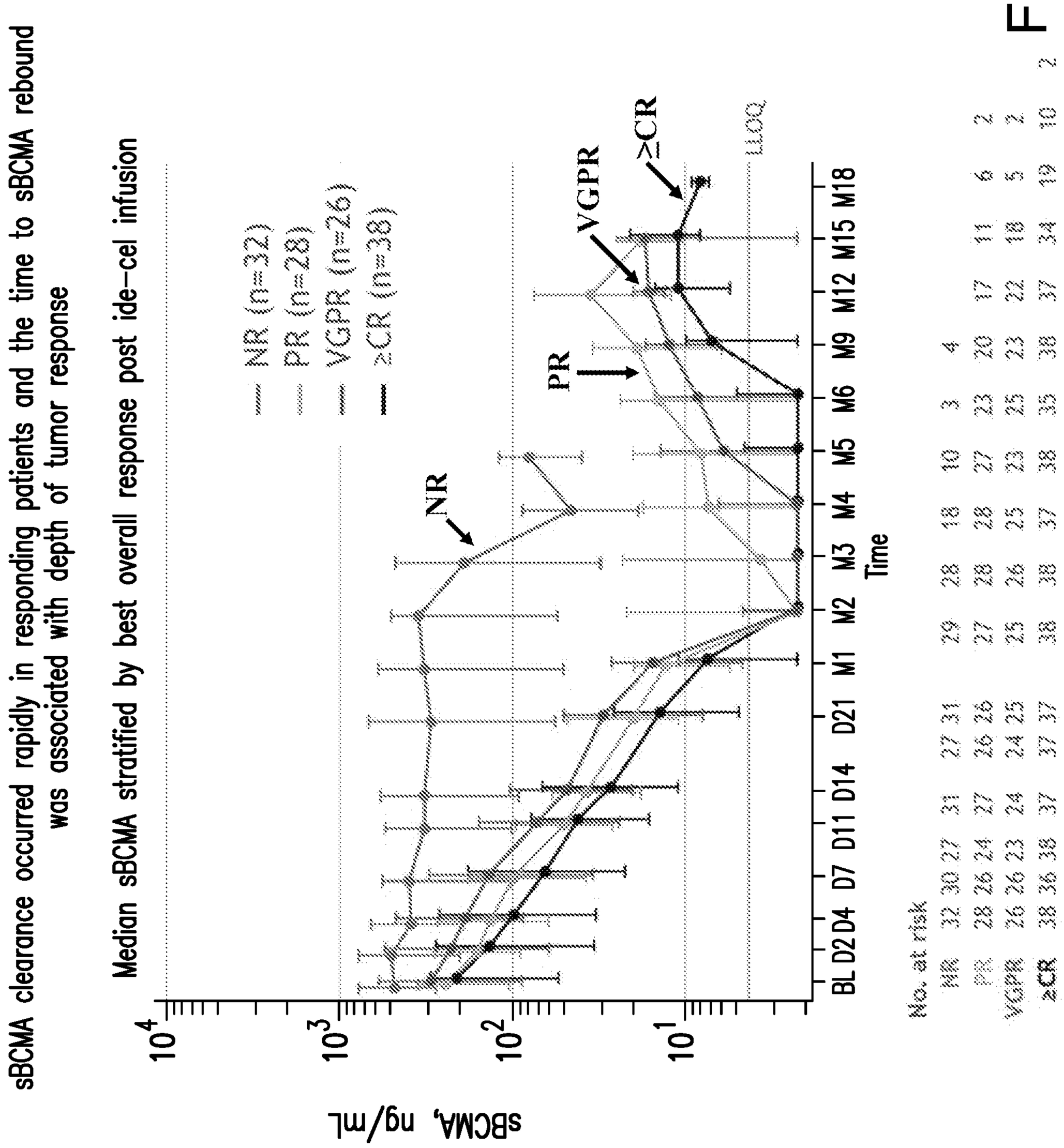

sBCMA clearance occurred rapidly in responding patients and the time to sBCMA rebound was associated with depth of tumor response
Median sBCMA stratified by best overall response post ide-cel infusion
NR (n=32)
PR (n=28)
VGPR (n=26)
≥CR (n=38)
NR
PR
VGPR
≥CR
LLOQ
sBCMA, ng/mL
10^4
10^3
10^2
10^1
BL D2 D4 D7 D11 D14 D21 M1 M2 M3 M4 M5 M6 M9 M12 M15 M18
Time
No. at risk
NR 32 30 27 31 27 31 29 28 28 18 10 3 4
PR 28 26 24 27 26 26 27 28 28 28 27 23 20 17 11 6 2
VGPR 26 26 23 24 24 25 25 26 26 25 23 25 23 22 18 5 2
≥CR 38 36 38 37 37 37 38 38 38 37 38 35 38 37 34 19 10 2

FIG. 16A

Proportion of patients achieving sBCMA nadir <LLOQ by best overall response

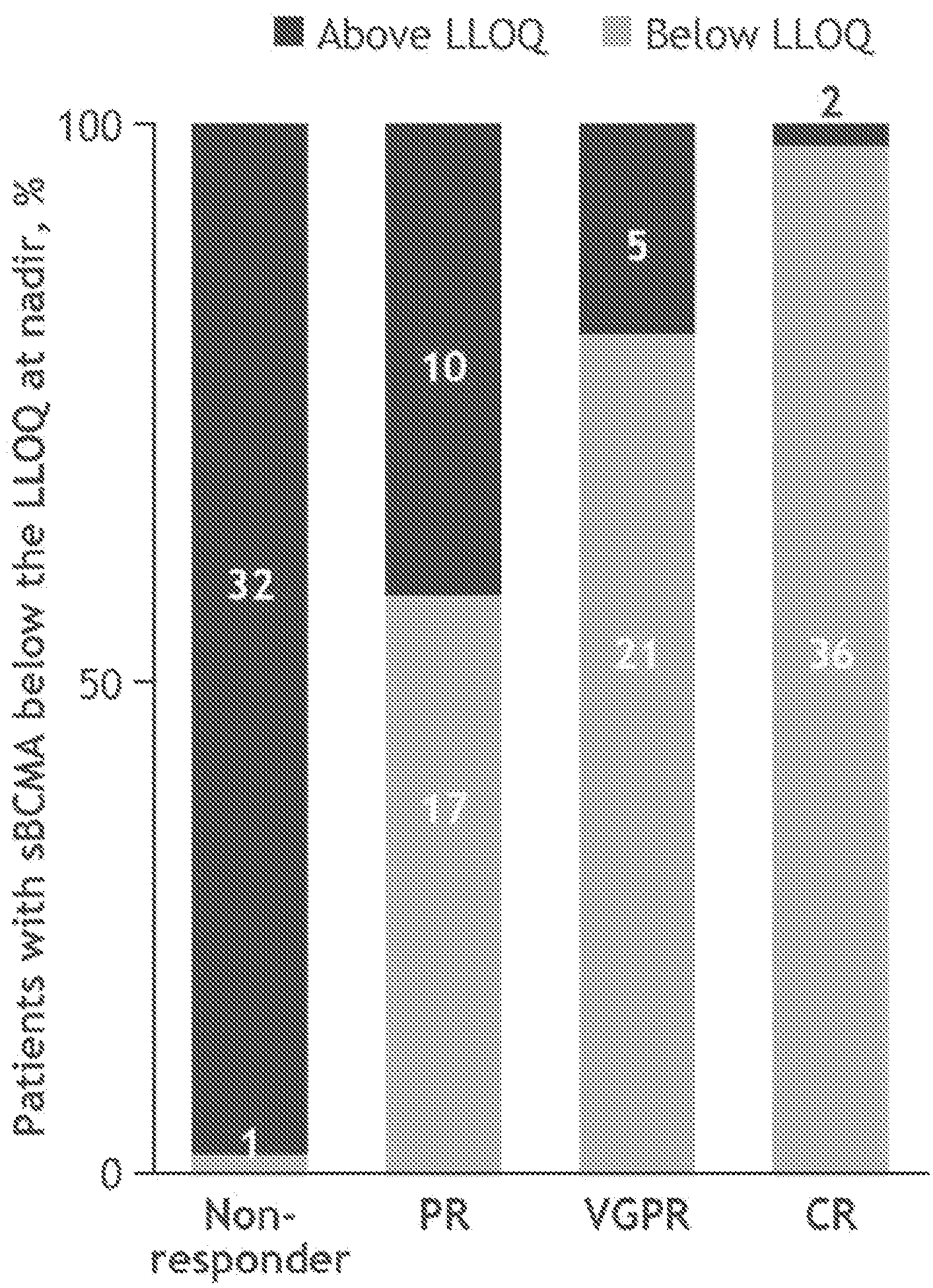


FIG. 16B

Time to rebound of sBCMA to detectable levels

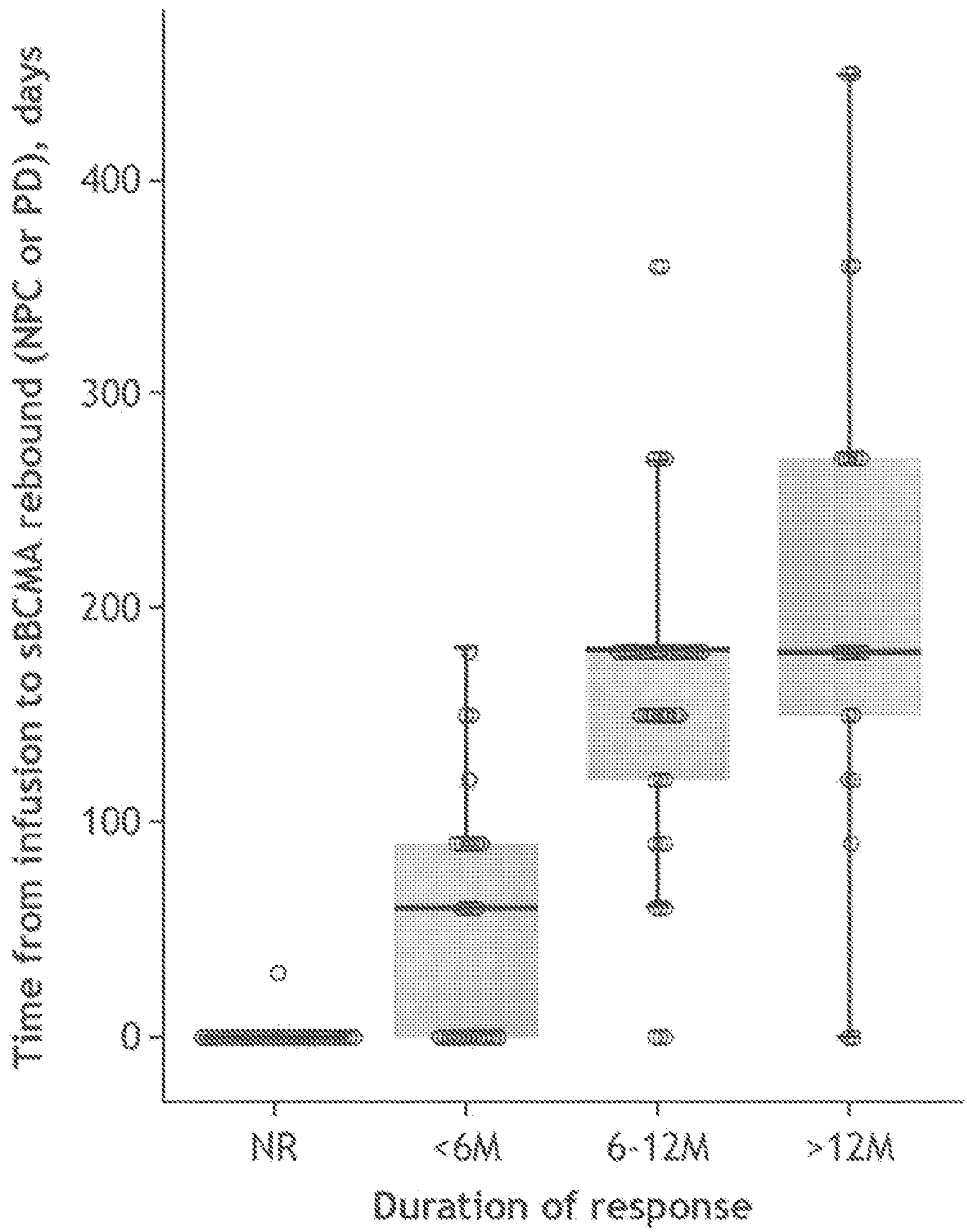


FIG. 16C

sBCMA response trajectories were consistent with sensitive MRD assessment by NGS and traditional serum markers of myeloma disease burden

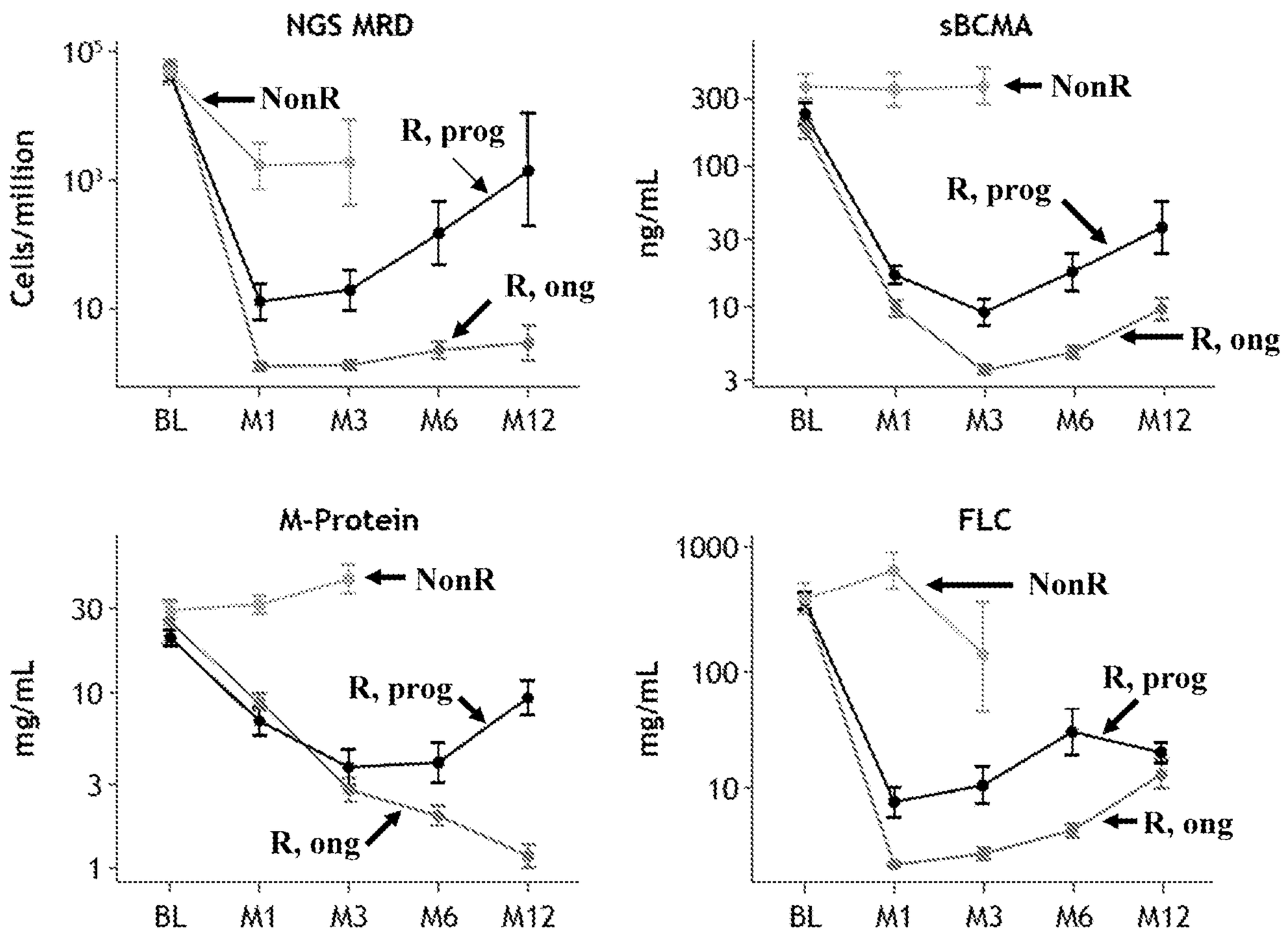


FIG. 17A

Detectable Biomarkers

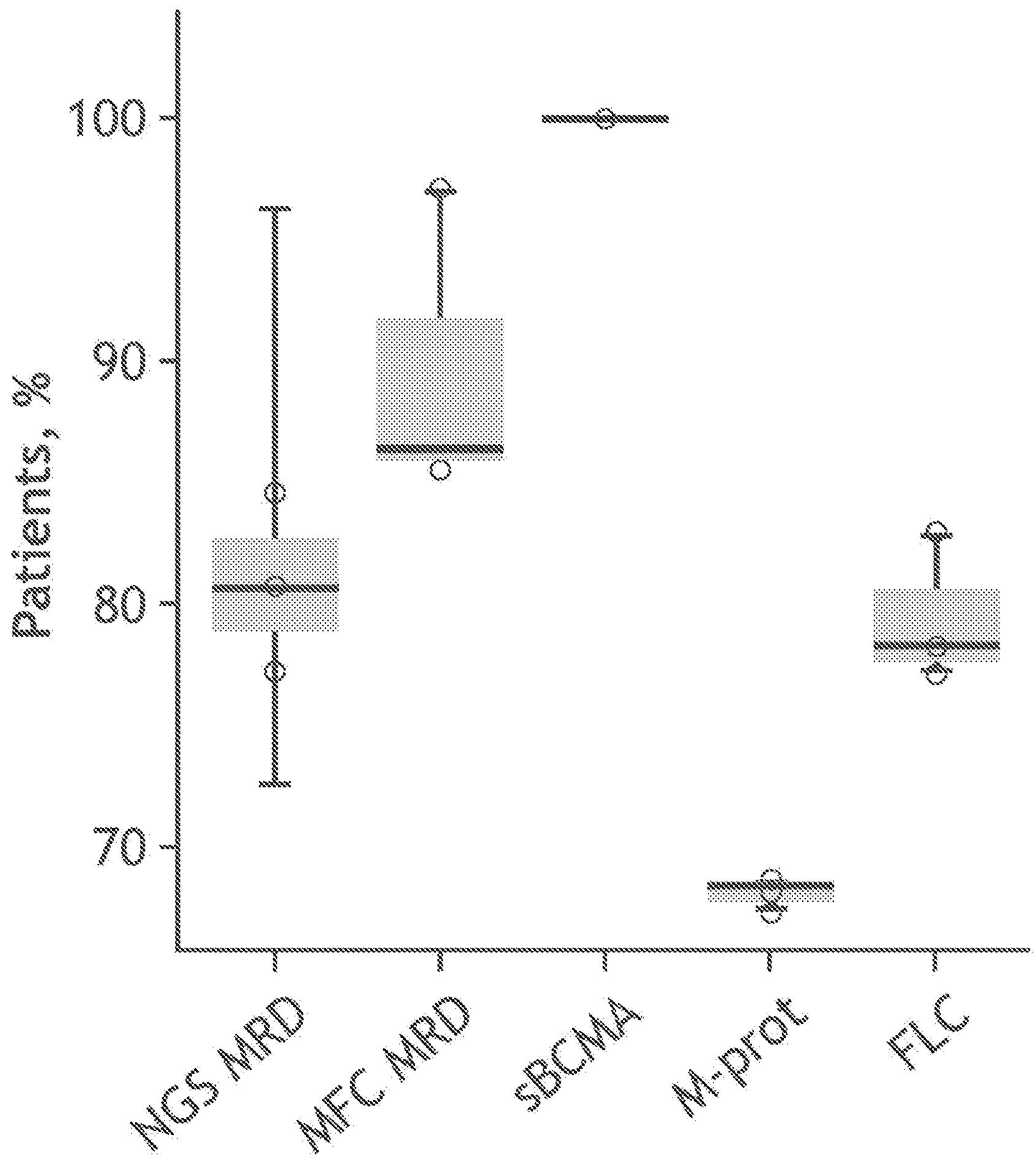


FIG. 17B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/058835

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/058835

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 G01N33/574 A61P35/00 C07K14/725
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N A61K A61P C07K

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2019/108900 A1 (NOVARTIS AG [CH]; UNIV PENNSYLVANIA [US]) 6 June 2019 (2019-06-06) the whole document -----	1-113
Y	LI CHUNRUI ET AL: "Clinical Responses and Pharmacoki- netics of fully human BCMA Target- ing CAR T Cell Therapy in Relapsed/ Refractory Multiple Myeloma", CLINICAL LYMPHOMA MYELOMA AND LEUKEMIA, vol. 19, no. 10_suppl, 18 October 2019 (2019-10-18), pages e23-e24, XP055787674, abstract -----	1-17, 22-113

-/--

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

[X] See patent family annex.

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"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
19 March 2021	07/04/2021

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

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/058835

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ADAM D. COHEN ET AL: "B cell maturation antigen-specific CAR T cells are clinically active in multiple myeloma", THE JOURNAL OF CLINICAL INVESTIGATION, vol. 129, no. 6, 29 April 2019 (2019-04-29), pages 2210-2221, XP055768828, GB ISSN: 0021-9738, DOI: 10.1172/JCI126397 the whole document -----	1-17, 22-113
Y	S. A. ALI ET AL: "T cells expressing an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of multiple myeloma", BLOOD, vol. 128, no. 13, 13 July 2016 (2016-07-13), pages 1688-1700, XP055334154, US ISSN: 0006-4971, DOI: 10.1182/blood-2016-04-711903 the whole document -----	1-17, 22-113
Y	SATTVA S. NEELAPU ET AL: "Chimeric antigen receptor T-cell therapy - assessment and management of toxicities", NATURE REVIEWS CLINICAL ONCOLOGY, vol. 15, no. 1, 19 September 2017 (2017-09-19), pages 47-62, XP055541211, NY, US ISSN: 1759-4774, DOI: 10.1038/nrclinonc.2017.148 the whole document -----	18-21
X,P	THOMPSON ETHAN G ET AL: "Markers of Initial and Long-Term Responses to Idecabtagene Vicleucel (Ide-Cel; bb2121) in the CRB-401 Study in Relapsed/Refractory Multiple Myeloma ¦ Blood ¦ American Society of Hematology", BLOOD, vol. 134, no. Suppl. 1, 13 November 2019 (2019-11-13), pages 4328-4328, XP055787860, DOI: 10.1182/blood-2019-124998 Retrieved from the Internet: URL:https://ashpublications.org/blood/article/134/Supplement_1/4328/424727/Markers-of-Initial-and-Long-Term-Responses-to> abstract -----	1-113

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/058835

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2019108900 A1	06-06-2019	AU 2018375738 A1	11-06-2020
		BR 112020010579 A2	10-11-2020
		CA 3083949 A1	06-06-2020
		CN 111727373 A	29-09-2020
		EP 3717907 A1	07-10-2020
		JP 2021509009 A	18-03-2021
		KR 20200096253 A	11-08-2020
		SG 11202005005Y A	29-06-2020
		TW 201925782 A	01-07-2019
		US 2020371091 A1	26-11-2020
		WO 2019108900 A1	06-06-2019