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(71) Applicant: AMPHIVENA THERAPEUTICS INC.

[US/US]; Two Tower Place, South San Francisco, California 94080 (US).

(72) Inventors: SMITH, Victoria; Two Tower Place, South

San Francisco, California 94080 (US). SARDE, Aurelien; 3868 Magnolia Drive, Palo Alto, California 94306 (US).

(74) Agent: MEIKLEJOHN, Anita L. et al.; Fish &

Richardson P.C., P.O. Box 1022, Minneapolis, Minnesota 55440-1022 (US).

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(54) Title: ASSESSMENT OF CANCERS FOR TREATMENT WITH A CD33/CD3 BISPECIFIC T CELL ENGAGER

(57) Abstract: Methods for assessing potential for treatment of certain cancers with a bispecific T cell engager having two CD33 binding domains and two CD3 binding domains are described. The methods entail assessing clustering of CD33 on tumor cells, e.g., leukemic blast.



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ASSESSMENT OF CANCERS FOR TREATMENT WITH A CD33/CD3 BISPECIFIC T CELL ENGAGER

BACKGROUND

T cell engagers, a particular class of bispecific antibody, mediate binding between a target cell and a T cell resulting in T cell directed lysis and T cell activation, differentiation and proliferation. While T cell engagers have demonstrated impressive potency and anti-tumor activity in some settings, a barrier to broader therapeutic success in many cases is undesirable activity against normal cells expressing the target of interest. This “on target, off tumor” toxicity can be significant, and has been reported widely for engineered T cell approaches.

Myeloid derived suppressor cells (MDSC) act locally and systemically to impair anti-tumor immunity, inhibiting effector T cell responses, promoting formation of immune suppressive regulatory T cells, inhibiting the maturation of dendritic cells and antigen presentation, and promoting formation of metastases. MDSC elicit a range of suppressive functions that inhibit normal T cell responses as well as causing unresponsiveness to immune checkpoint blockade. A dominant function of MDSC is the suppression of T cell activity in a variety of manners that are pathology and context dependent. The presence of MDSC is thought to be associated with poor outcomes and lack of response to certain therapies, e.g., therapies that activate T cells and therapies involving the use of a checkpoint inhibitor. Moreover, compensatory myelopoiesis in response to T cell activation can result in further generation of MDSC and recruitment to tumors.

Summary

Described herein is a method for treating a patient with a bispecific T cell engager having two CD33 binding domains and two CD3 binding domains, the method comprising: (a) providing a patient sample comprising CD33 expressing tumor cells; (b) measuring the binding of a CD33 antibody to the CD33 expressing cells; (c) measuring the binding of the bispecific T cell engager to the CD33 expressing cells; (d) selecting the patient for treatment with the bispecific T cell engager if the binding of the T cell engager to the CD33 expressing cells is equivalent to the binding of the CD33 antibody to the CD33 expressing cells; and (e) treating the CD33 expressing cells with the bispecific T cell engager.

Also described is a method for treating a patient suffering from acute myeloid leukemia (AML) with a bispecific T cell engager having two CD33 binding domains and two CD3 binding domains, the

method comprising: (a) determining the FAB subtype of the AML; and (b) treating the patient with a bispecific T cell engager having two CD33 binding domains and two CD3 binding domains if the FAB subtype is M2.

Also described is a method for stratifying patients for entry into a clinical trial for assessment of bispecific T cell engager having two CD33 binding domains and two CD3 binding domains for treatment of AML, the method comprising: (a) providing a patient sample comprising CD33 expressing tumor cells; (b) measuring the binding of a CD33 antibody to the CD33 expressing cells; (c) measuring the binding of the bispecific T cell engager to the CD33 expressing cells; (d) selecting the patient for inclusion in the clinical trial if the binding of the T cell engager to the CD33 expressing cells is equivalent to the binding of the CD33 antibody to the CD33 expressing cells otherwise excluding the patient from the clinical trial.

In various embodiments: the bispecific T cell engager having two CD33 binding domains and two CD3 binding domains is AMV564; the bispecific T cell engager having two CD33 binding domains and two CD3 binding domains is AMV564 and AMV564 is administered by subcutaneous injection; the dose of AMV564 injected is 5 – 150 mcg; AMV564 is administered on a least 7 days over a 14 day period; the AMV564 is administered on 10 days over a 14 day period; AMV564 is administered on 5 consecutive days on two occasions over a 14 day period; AMV564 is administered on 5 consecutive days, is not administered on the following two days and is administered on the following 5 consecutive days; the AMV564 is administered over a 21 day cycle in which AMV564 is administered on at least 7 days over a 14 day period and is not administered over the subsequent 7 day period; the 21 day cycle is repeated at least two times; AMV564 is administered on at least 10 days over a 14 day period with administration on 5 consecutive days followed by 2 days of no administration followed by administration on 5 consecutive days; the dose of AMV564 administered is 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 µg on each day when administered; the patient is being treated with a therapy that activates T cells (e.g., the therapy is a CAR T cell therapy, a CTL therapy); and patient is being treated with an antibody therapy.

Described herein are methods for using AMV564, a bispecific, bivalent molecule which binds to CD3 and CD33. AMV564 is homodimeric protein (i.e., a homodimer of a polypeptide having the amino acid sequence of SEQ ID NO: 1) having four single-chain variable fragment (scFv) binding sites, two that bind CD33 and two that bind CD3. A bivalent design can, in theory, restore selectivity to a T cell engager, directing preferential binding to regions of high local target density, such as found at sites of active signaling or associated with high receptor density or expression. Despite the fact that AMV564 binds CD33, which is broadly expressed across the myeloid lineage, it can be dosed in a

manner that provides a desirable therapeutic index, with selective binding of MDSC. AMV564 is very selective over a wide dose range, without being bound by any particular theory, this may be due to some combination of: bivalency, the affinity of the scFv and the geometry of the homodimer. For example, again without being bound by any particular theory, the structure of AMV564 may allow it to bind clusters of dimerized CD33.

AMV564 has dual activity: it induces T cell mediated killing of MDSC and drives T cell activation, promoting favorable polarization (e.g., Th1 CD4 T cells and effector CD8 T cells). AMV564 has an EC50 for MDSC that is less than about 3 pM. Dosed appropriately, AMV564 largely spares neutrophils, monocytes and many differentiated myeloid cells while directing killing of MDSC, thereby inhibiting MDSC-suppressive pathways.

Peripheral MDSC may play important roles in T cell suppression and T cell trafficking to tumor sites, a potentially rate-limiting factor for T cell activation-based therapies. For example, a 15 mcg dose of AMV564 can, in some circumstance, achieve depletion of MDSC populations. As MDSC are recruited from the bone marrow, peripheral depletion may enable sufficient control to benefit anti-tumor immunity over a dosing timeframe that exceeds the limited longevity of tissue-resident MDSC. However, distribution of AMV564 in the tumor microenvironment could target MDSC at tumor sites while promoting expansion of local T cells. Moreover, AMV564 delivery or entry into draining lymph nodes in addition to the periphery, for example, doses of up to 50, 75 and 100 mcg by CIV route, could achieve de-repression of anti-tumor T cells and restore antigen presentation and immune homeostasis. Subcutaneous delivery of AMV564, for example at doses of 5, 15, or 50 mcg, provides a direct mechanism of initial distribution in the lymphatic system, including tumor draining lymph nodes. With subcutaneous administration AMV564 is effective at lower doses, likely due to the access to the lymph system.

AMV564 can both relieve immune suppression and activate T cell effector function in cancer patients. AMV564 can do so by relieving immunosuppression via targeted depletion of myeloid derived suppressor cells (MDSC) and by directly activating/repolarizing T cells and improved T effector function.

Importantly, subcutaneous administration of AMV564 facilitates immune activation by targeting the lymphatic system.

In one aspect of the methods of treatment described herein the target cell is a leukemic blast, and a dosing regimen that establishes a steady-state exposure of 30 – 70 pM AMV564 is used to reduce leukemic blasts in AML, Myelodysplastic Syndrome (MDS) and other cancers with high CD33

expression. Such a regimen could be achieved by dosing at 50 – 125 mcg (“micrograms”) by continuous intravenous infusion (CIV), for example, 50 mcg, 75 mcg, 100 mcg.

Also described herein is a method of for controlling both MDSC by administering 5 – 125 mcg (for example, 50 mcg, 75 mcg, 100 mcg) of AMV564 by intravenous infusion. In some cases, for example, MDS patients with a lower blast burden MDSC likely play a more critical role in disease progression. In such cases, a 5, 15, 30, 50 mcg AMV564 can be administered, e.g., by intravenous infusion.

Described herein is method for reducing myeloid-derived suppressor cells and activating T cells in a patient, the method comprising administering AMV564 (a polypeptide having the amino acid sequence of SEQ ID NO: 1) to the patient. In various embodiments: AMV564 is administered by subcutaneous injection; the dose of AMV564 injected is 5 – 150 mcg (micrograms); the AMV564 is administered on a least 7 days (8, 9, 10, 11, 12, 13 or 14 days) over a 14 day period; the AMV564 is administered daily (e.g., at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 mcg/day) subcutaneously; the AMV564 is administered on 10 days over a 14 day period; the AMV564 is administered on 5 consecutive days on two occasions over a 14 day period; the AMV564 is administered on 5 consecutive days, is not administered on the following two days and is administered on the following 5 consecutive days; the AMV564 is administered over a 21 day cycle in which AMV564 is administered on at least 7 days over a 14 day period and is not administered over the subsequent 7 day period; the 21 day cycle is repeated at least two times; AMV564 is administered on at least 10 days over a 14 day period with administration on 5 consecutive days followed by 2 days of no administration followed by administration on 5 consecutive days; the dose of AMV564 administered is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 mcg on each day when administered; the patient is being treated with a therapy that activates T cells (e.g., the therapy is a CAR T cell therapy; the therapy is a CTL therapy; the therapy is an antibody therapy; the therapy is treatment with a T cell engager that comprises a CD3 binding domain and activates T cells); the patient is suffering from or being treated for being treated for a leukemia (acute myeloid leukemia or myelodysplastic syndrome); the patient is suffering or being treated for a solid tumor; the solid tumor is selected from the group consisting of: pancreatic cancer, ovarian cancer, colon cancer, rectal cancer, non-small cell lung carcinoma, urothelial cancer, squamous cell carcinoma, rectal cancer, penile cancer, endometrial cancer, small bowel cancer, cancer of the appendix; the administration of AMV564 achieves a steady-state exposure of 30 – 70 pM AMV564.

Also described is a method for treating a patient suffering from a solid tumor, the method comprising administering AMV564 a polypeptide having the amino acid sequence of SEQ ID NO: 1) to the patient. In various embodiments: AMV564 is administered by subcutaneous injection; the dose of AMV564 injected is 5 – 150 mcg (micrograms); the AMV564 is administered on at least 7 days (8, 9, 10, 11, 12, 13 or 14 days) over a 14 day period; the AMV564 is administered daily (e.g., at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 mcg/day) subcutaneously; the AMV564 is administered on 10 days over a 14 day period; the AMV564 is administered on 5 consecutive days on two occasions over a 14 day period; the AMV564 is administered on 5 consecutive days, is not administered on the following two days and is administered on the following 5 consecutive days; the AMV564 is administered over a 21 day cycle in which AMV564 is administered on at least 7 days over a 14 day period and is not administered over the subsequent 7 day period; the 21 day cycle is repeated at least two times; AMV564 is administered on at least 10 days over a 14 day period with administration on 5 consecutive days followed by 2 days of no administration followed by administration on 5 consecutive days; the dose of AMV564 administered is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 mcg on each day when administered; the patient is being treated with a therapy that activates T cells (e.g., the therapy is a CAR T cell therapy; the therapy is a CTL therapy; the therapy is an antibody therapy; the therapy is treatment with a T cell engager that comprises a CD3 binding domain and activates T cells); the patient is suffering from or being treated for being treated for a leukemia (acute myeloid leukemia or myelodysplastic syndrome); the patient is suffering or being treated for a solid tumor; the solid tumor is selected from the group consisting of: pancreatic cancer, ovarian cancer, colon cancer, rectal cancer, non-small cell lung carcinoma, urothelial cancer, squamous cell carcinoma, rectal cancer, penile cancer, endometrial cancer, small bowel cancer, cancer of the appendix; the administration of AMV564 achieves a steady-state exposure of 30 – 70 pM AMV564; the solid tumor is selected from the group consisting of: Small Cell Lung Cancer (NSCLC) (e.g., metastatic nonsquamous NSCLC, III NSCLC, metastatic NSCLC expressing PD-L1), melanoma, Merkel cell, Microsatellite Instability-High Cancer (e.g., unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient); the patient has progressed on checkpoint blockade; the administration of AMV564 achieves a steady-state exposure of 30 – 70 pM AMV564; the method comprises administering 50 – 125 mcg AMV564 by continuous intravenous infusion.

Also described is a method for treating AML by administering AMV564 to reach a steady-state exposure of 30 – 70 pM AMV564.

CD33, also known as Siglec-3, is a transmembrane protein expressed on cells of myeloid lineage. CD33 has long been regarded as an appealing target for acute myeloid leukemia (AML), due to both high prevalence and high expression on leukemic blasts. The function of CD33 is not well understood, but activation of CD33 signaling on early lineage myeloid cells, such as immune-suppressive myeloid-derived suppressor cells (MDSC), has been shown to result in expansion of MDSC and production of suppressive cytokines and factors. CD33 expression is used as one component of cell surface marker sets to identify these immune-suppressive monocytic and granulocytic cells (for example, by use of flow cytometry for cellular immune phenotyping). It is unclear what role, if any, that CD33 plays on more differentiated myeloid lineage cells such as mature monocytes, neutrophils, macrophages and dendritic cells. Indeed, knockout of CD33 in human cells using CRISPR technology has demonstrated that CD33 is not required for lineage differentiation, but it nonetheless continues to be expressed at varying levels on most of these cells, rendering it as a challenging target for a non-selective T cell engager, with respect to both safety and efficacy.

T cell engagers function by acting as a bridge between an antigen on a target cell and a different antigen on a T cell, forming a ternary complex between the drug and two different cell types, thereby mimicking the formation of a natural T cell-target cell synapse that is generated in the course of an adaptive immune response. It is thought that between 10 – 100 molecules of drug must be appropriately bound in order to activate T cell killing, and T cells must be available also. This active state thus brings additional considerations for receptor occupancy and dosing beyond a standard model of, for example, inhibiting a ligand or receptor via biologic drug binding, where dosing strategies aim for maximum target coverage until unacceptable toxicity is reached. There are yet more factors to consider for T cell engagers that target a broadly expressed antigen, such as CD33. Apart from the potential safety risks associated with broad depletion of the myeloid lineage cells (which confer valuable protection against infection), this cellular population can be very large (e.g. there are up to 100 billion neutrophils generated daily in a human body) and efficacy could thus be compromised due to insufficiently suitable drug distribution and an inadequate supply of T cells to accomplish killing. The targeting of other populations of cells such as leukemic blasts or the even rarer immune-suppressive MDSC could thus be hampered by a broad distribution of drug across CD33-positive normal myeloid cells and the need for sufficient associated T cells to achieve killing. For these reasons, determining the appropriate dose, dose schedule and route of administration for a T cell engager is unusually challenging, far more so than for monovalent, monospecific agents.

Binding properties of AMV564 and dose selection

The bivalent design of AMV564 is reflected in its physical properties. AMV564 is very potent and demonstrates cellular killing at low receptor occupancy, with elimination of CD33 target cells demonstrated ex vivo or in vitro with picomolar or sub-picomolar EC50 values. AMV564 is a potent agonist, which can elicit biological activity at low receptor occupancy or low target binding levels. Binding studies using flow cytometry show that there is no binding of neutrophils, polymorphonuclear (PMN) leukocytes or monocytes at 1 or 10 pM AMV564 as compared to MDSC and leukemic blast cell line KG1, which are both potently bound at 1 and 10 pM (FIGS 1F-1G). At concentrations of 1 and 10 pM, AMV564 is thus highly selective for MDSC as compared to other abundant CD33 expressing cells. The

Thus, an optimal therapeutic window for both safety and anti-tumor (leukemic blasts) and/or anti-suppressor (MDSC) cell activity might be obtained when engagement of target cells by AMV564 is sufficient for activity, but binding of other myeloid cells is minimal. Apart from safety considerations, excessive engagement of the large population of cells encompassing the normal myeloid lineage could result in reduction in efficacy due to suboptimal drug distribution and inadequate available T cells to achieve formation of the necessary ternary complexes that mimic a natural T cell synapse to facilitate cell killing.

Depletion of MDSC and dose selection

Overcoming the suppressive tumor microenvironment is a major challenge in immune therapy. The critical cellular effectors of the suppressive tumor microenvironment are MDSC, which are associated with immune dysfunction, repression of anti-tumor immunity and poor response to immunotherapy. MDSC suppress T cell and NK cell responses via a variety of cytokines, active species and pathways. In addition, they repress effective antigen presentation by dendritic cells in tumor draining lymph nodes. In another aspect of this disclosure, AMV564 depletes MDSC in the periphery and bone marrow of AML patients at low doses. The rapid decline observed at low, lead-in doses of AMV546 indicates potent binding and depletion of both monocytic and granulocytic MDSC populations. MDSC are rare in the periphery of a healthy adult and become significantly elevated in cancer patients. However, they are nonetheless relatively rare cells as compared to the mature myeloid lineage in general, and the efficacy of their depletion and control could be reduced at higher doses when overall receptor occupancy is unfavorable for selectivity for a bivalent T cell engager such as AMV564.

Depletion of MDSC in addition to leukemic blasts is beneficial in AML with respect to maintaining T cell activation and proliferation, and may promote more durable benefits such as sustained responses and restoration or induction of T cell memory.

Doses of 50 – 125 mcg of AMV564 by CIV route, for example, 50 mcg, 75 mcg, 100 mcg doses, could maintain control of both MDSC and leukemic blasts. This dosing regimen could also be effective for MDS. In MDS patients with a lower blast burden, where MDSC likely play a more critical role in disease progression, an even lower dose may be effective (e.g. 5, 15, 30, 50 mcg by CIV route).

In solid tumor patients, administration of AMV564 at doses of 5-50 mcg, generating approximate steady state exposures ranging from 0.1 – 5 pM, can be effective in depleting MDSC and promoting a favorable CD4 and CD8 T cell activation profile and cytokine milieu to promote restoration of anti-tumor immunity. Higher doses of 50-75 mcg or 75 – 150 mcg would also yield exposures that remain within the selective range for MDSC depletion.

Circulating MDSC are a pharmacodynamic biomarker of AMV564 and T cell responses. As MDSC are known to be induced by T cell activation, they are induced as a consequence of the T cell activation stimulated by AMV564. The MDSC reflect engagement of AMV564 with target cells (MDSC) and depletion of such cells, and in relationship to dose for a bivalent, bispecific T cell engager such as AMV564, they reflect effective dosing within the optimal therapeutic index of the drug, to enable effective depletion of these comparatively rare cells as compared to the rest of the CD33 positive myeloid lineage.

Because AMV564 depletes MDSC, treatment with AMV564, for example, under the dosing regimens described herein, can be useful for depleting MDSC in a variety of contexts. For example, AMV564 can be used to deplete MDSC in patients being treated with a therapy that activates T cells or involves administration of activated T cells.

Management of Cytokine Release Syndrome (CRS)

CRS, while not fully understood, appears to be related to T cell activation and subsequent activation of macrophages and other myeloid cells to generate and secrete IL-6, IL-1 β and other cytokines. CRS is commonly associated with T cell engaging therapies such as T cell engaging bispecific antibodies and CAR-T therapy. CRS is most apparent at initiation of dosing. As shown below, administration of AMV564 by subcutaneous route in solid tumor patients at doses, for example, of 15 – 50 mcg results in robust T cell activation as assessed by various metrics including up to 10 – 40X increases from baseline in detectable peripheral Interferon gamma (IFN γ) in the first cycle of dosing. However, the increase in IL-6 is comparatively modest (the two cytokines are around 1:1 or favor higher IFN γ) (see

FIGS 7A-7E) and IL-1B is not detected at significant levels. This favorable profile is consistent with the absence of CRS observed in patients in this clinical study. This favorable profile of demonstrably strong T cell activation with lack of CRS could reflect a combination of features including the depletion of MDSC (which can produce inflammatory cytokines), bivalent T cell engagement by AMV564 (which may more closely resemble a more native T cell receptor engagement) and the lymphatic delivery and distribution kinetics associated with subcutaneous injection of AMV564. AMV564 has a favorable therapeutic index that is amenable to chronic dosing, these properties should also assist in the mitigation of CRS after lead in dosing to target dose is completed.

Combination Therapies

Effective treatment of tumorigenesis is often achieved via combination therapy. The functional therapeutic index of AMV564, with a dosage range that maximizes both efficacy and safety (notably lack of significant depletion of normal myeloid cells) positions it well for combination therapy approaches. Combination strategies include but are not limited to, in solid tumors, checkpoint blockade (PD-1 or PDL-1 blocking agents), T cell activators and expanders such as cytokines IL-2, IL-10 and IL-15, dual targeting agents such as those targeting checkpoint (e.g. PD-1 or PDL-1) and immune repression (e.g. TGF β), CAR-T therapies, NK activating therapies, or standard of care chemotherapy. In AML and MDS, the previously listed therapies could also be used in combination, along with other established agents in AML such as hypomethylating agents (e.g. azacytidine, decitabine), differentiation agents (e.g. targeting IDH1/2), targeted agents (e.g. against FLT3), agents targeting anti-apoptotic proteins such as BCL2 (e.g. venetoclax), BCL-XL, or MCL1, or lenalidomide.

AMV564 can be used alone or in combination to treat melanoma (e.g., patients with unresectable or metastatic melanoma, melanoma with involvement of lymph node(s) following complete resection); non-Small Cell Lung Cancer (NSCLC) (e.g., metastatic non-squamous NSCLC, III NSCLC, metastatic NSCLC expressing PD-L1); head and Neck Squamous Cell Cancer (HNSCC); Classical Hodgkin Lymphoma (cHL); Primary Mediastinal Large B-Cell Lymphoma (PMBCL); urothelial Carcinoma (e.g., locally advanced or metastatic urothelial carcinoma expressing PD-L1); Microsatellite Instability-High Cancer (e.g., unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient; solid tumors that have progressed following prior treatment; Gastric Cancer (e.g., recurrent locally advanced or metastatic gastric or gastroesophageal junction adenocarcinoma expressing PD-L1); Cervical Cancer; Hepatocellular Carcinoma (HCC); Merkel Cell Carcinoma (MCC); and Renal Cell Carcinoma (RCC).

BRIEF DESCRIPTION OF THE FIGURES

FIGS 1A-1G present data showing that AMV264 depletes MDSC and activates T cells ex vivo and that AMD564 binds MSC and KG-1 cells at 1 and 10 pm, but does not bind monocytes at these concentrations.

FIGS 2A-2F present data showing that that AMV264 depletes MDSC and activates T cells in patients. The lighter bar indicates the days of the lead in dose and the darker bar indicates the days of the target dose.

FIGS 3A-3C present data showing the impact of AMV264 on peripheral blood MDSC, bone marrow MDSC, peripheral blood T cells. The lighter bar indicates the days of the lead in dose and the darker bar indicates the days of the target dose.

FIGS 4A-4D present data showing that AMV564 is a selective an potent conditional agonist. The single open circles and triangles show the result of a CD3/CD28 stimulation in the absence of AMV564.

FIGS 5A-5E present data showing the MDSC control is associated with Treg control in solid tumor patients. The filled squared are G-MDSC and the filled circles are M-MDSC. The bars of the axis indicate AMV564 dosing days.

FIGS 6A-6G present data showing that CD8:Treg ratio improves on AMV564 therapy in solid tumor patients. The dotted line indicates the baseline ratio; bars along the x-axis indicate dosing days and the broad bar indicates the ratio for healthy controls.

FIGS 7A-7E present data showing that AMV564 promotes favorable CD4 and CD8 T cell polarization in an ovarian cancer patient. The dotted line indicates the baseline ratio; bars along the x-axis indicate dosing days and the broad bar indicates the ratio for healthy controls.

FIGS 8A-8F show IFN γ Cycle 1, IFN γ Cycle 2, IL-6 Cycle 1 and IL-6 Cycle 2 levels in six solid tumor patients treated with AMV564.

FIGS 9A-9D show the results of M-MDSC and G-MDSC measurements in four solid tumor patients treated with AMV564 in combination pembrolizumab. The filled squared are G-MDSC and the filled circles are M-MDSC. The bars of the axis indicate AMV564 dosing days.

FIGS 10A-10D show the impact of AMV564 in combination pembrolizumab on T-Bet and granzyme B positive CD8 cells and CD8/Treg ratio in two solid tumor patients. The dotted line indicates the baseline ratio; bars along the x-axis indicate AMV564 dosing days and the broad bar indicates the ratio for healthy controls.

FIGS 11A-11B show the impact of AMV564 in combination pembrolizumab treat on CD8 cell proliferation and activation in two solid tumor patients.

FIGS 12A-12C show differences in the binding of AMV564 and a CD33 antibody to KG-1 and KG-1a cells.

DETAILED DESCRIPTION

AMV564

AMV564 is a homodimer of SEQ ID NO: 1. AMV564 is described in US 9212225 (Diabody 16; SEQ ID NO: 113 without the 6 His tag at the amino terminus) and WO 2016/196230 (SEQ ID NO:139). A pharmaceutical composition of AMV564 comprises a polypeptide having the amino acid sequence of SEQ ID NO:1 and a pharmaceutically acceptable carrier or excipient.

AMV564

DQMTQSPSS LSASVGDRVT ITCRSSTGAV TTSNYANWVQ QKPGKAPKAL
 IGGTNKRAPG VPSRFGSLI GDKATLTISS LQPEDFATYY CALWYSNLWV
 FGQGTKVEIK GSGSGSQVQL VQSGAEVKKP GASVKVSCA SGYTFTSYDI
 NWVRQAPGQG LEWMGWMNPN SGNTGFAQKF QGRVTMTRDT STSTVYMELS
 SLRSEDVAV YCARDRANTD YSLGMDVWGQ GTLVTVSSGG SGQSVLTQPP
 SASGTPGQRV TISCSGSRN IGSNTVNWYQ QLPGTAPKLL IYGNNQRPSG
 VPDRFSGSKS GTSASLAISG LQSEDEADYY CATWDDSLIG WVFGGGTKLT
 VLGGSGGSEV QLVESSGGLV QPGGSLRLSC AASGFTFSTY AMNWVRQAPG
 KGLEWVGRIR SKYNNYATYY ADSVKDRFTI SRDDSKNSLY LQMNSLKTED
 TAVYYCARHG NFGNSYVSFY AYWGQGTTLV VSS (SEQ ID NO: 1)

Example 1: AMV564 Depletes MDSC and Activates T Cells ex vivo

In this study it was found that AMV564 treatment of primary cells (PBMC, MDS bone marrow, tumor PBMC) ex vivo both depletes MDSC and activates T cells. FIG 1A shows that treatment of PBMC with CD33 ligand S100A9 results in expansion of MDSC and increase in CD33 expression. In FIGS 1B-1E it can be seen that exposure to AMV564: negates reactive oxygen species (ROS) produced in response to S100A9 stimulation of PBMC to expand MDSC (FIG 1B), causes selective depletion of MDSC (FIG 1C), and increases in CD8 T cell (FIG 1D) and CD4 T cell (FIG 1E) numbers and activation state (as assessed by IFN γ positive fraction).

Thus, treatment ex vivo of patient-derived peripheral blood mononuclear cells (PBMC) resulted in selective depletion of MDSCs ($p < 0.01$) and a decrease in the production of reactive oxygen species. AMV564 induced a significant increase in activated T cells only in the presence of CD33+ target cells,

with >2-fold increase in the proliferation of CD4+ and CD8+ T cells. The increase in proliferation was dose-dependent and accompanied by a significant increase in IFN γ production.

As showing in FIG 1F, AMV564 binds to MDSC (and leukemic blast line KG1) at 1 and 10 pM. However, at these concentrations, there is essentially no evidence of binding to monocytes, neutrophils and polymorphonuclear leukocytes (PMN). These concentrations are within the range of exposure observed for dosing of AMV564 by subcutaneous route at doses of 5 - 15 – 50 mcg (about 0.1 – 5 pM). As shown in FIG 1G.

Example 2: AMV564 Depletes MDSC and Activates T Cells in Patients

In this clinical study, it was found that AMV564 treatment leads to depletion of peripheral blood MDSC and bone marrow MDSC. The results of this analysis are shown in FIGS 2A-2F where it can also be seen that AMV564 treatment resulted in depletion of AML blasts with no decrease in neutrophils. As can be seen, rapid depletion of both monocytic and granulocytic MDSCs is apparent with little or no impact on circulating neutrophil or monocytes populations. Evidence of early T cell activation is apparent with rapid re-distribution/margination of T cells (this apparent transient lymphopenia is a consequence of T cell activation and migration to lymph nodes and tissues). In FIGS, 2A-2F the period of lead-in AMV564 dosing (15 mcg/kg; 3 days) is indicated by the lighter colored bar and the target AMV564 dosing (100 mcg/kg) is indicated by the darker colored bar). In FIGS. 2A and 2B it can be seen the peripheral blood MDSC are depleted. In FIG 2C it can be seen the bone marrow MDSC are depleted. FIGS 2D-2E, respectively, show the impact of AMV564 treatment on peripheral blood T cells, peripheral blood neutrophils, and peripheral blood blasts.

Example 3: AMV564 Depletes MDSC in solid tumor patients

.As shown in FIGS 3A-C, an initial increase in peripheral blood MDSC in response to T cell activation is observed in lead in dosing (Days 1-3 in some patients). FIG 3C shows the rapid redistribution/margination of peripheral blood T cells, consistent with T cell activation, as T cells transmigrate to lymph nodes and tissues. However, at the target dose, peripheral MDSC are controlled. Bone marrow MDSC are also substantially decreased when assessed at day 15 relative to baseline. However, both bone marrow and peripheral blood MDSC can rebound once AMV564 treatment is stopped.

Example 4: AMV564 is a Selective and Potent Conditional Agonist

Primary human T cells and KG-1 cells were exposed to AMV564. Target-dependent cytotoxicity (FIG 4A), target dependent T cell proliferation (FIG 4B), viability of differentiated monocytes and neutrophils (FIG 4C), viability of differentiated monocytes and neutrophils (FIG 4D) were measured,

all with CD3/CD28 used as reference T cell stimulation. As KG1 expresses CD33 and AMV564 shows similar binding to KG1 as it does to MDSC, KG1 was used as a surrogate for MDSC in these assays. As can be seen in FIGS 4A-4D, AMV564 induces potent dose-dependent cell death of KG1 (FIG 4A), at a maximum level similar to a CD3-CD28 stimulation. This is accompanied by an increase in daughter cells, reflective of T cell proliferation (FIG 4B) at levels equivalent to or exceeding the CD3-CD28 reference stimulation. However, there is no evidence of AMV564 promoting significant cell death for autologous monocytes or neutrophils (FIG 4C), and similarly, no evidence of any induction of T cell proliferation (FIG 4D) with these cell populations, unlike general T cell stimulation using CD3-CD28.

Example 5: Phase 1 Clinical Study of AMV564 in Patients with Solid Tumors

This study enrolled adult patients having non-resectable, advanced metastatic solid tumors that are recurrent and progressing since the last anti-tumor therapy and for which no recognized standard therapy exists. The patients had ECOG performance status of ≤ 2 and adequate organ function. Patients were treated with AMV564 alone (15, 50, or 75 mcg/day) or AMV564 (5, 15, or 50 mcg/day) in combination Pembrolizumab administered intravenously at 200 mg every 3 weeks (Q3W). In both cases, AMV564 was administered once a day by subcutaneous injection on days 1-5 and days 8-12 of a 21-day cycle. AMV564 was well tolerated and pharmacodynamic analyses showed evidence of relief of immune suppression (decreased MDSC and Treg) and promotion of effector CD8 and Th1 CD4 responses in this Phase 1 population of heterogeneous cancer patients previously treated with other therapies.

In general, patients treated with AMV564 exhibited a cytokine profile consistent with activation of T cells including CD4 Th1 helper cells, antigen presenting cells, and improved T cell trafficking to tissues such as tumor tissues (increase in IFN γ , IL-15, IL-18, soluble granzyme B and CXCL10). While strong T cell activation was observed, there were no episodes of cytokine release syndrome.

Example 6: MDSC Control is Associated with Treg Control in Solid Tumor Patients

FIGS. 5A-5D show that M-MDSC and G-MDSC are controlled in patients with solid tumors (FIG 5A: ovarian - 15 mcg AMV564; FIG 5B: cutaneous 50 mcg AMV564; FIG 5C: small bowel- 15 mcg AMV564; FIG 5D: gastroesophageal junction - 15 mcg AMV564) that were treated with AMV564 (once a day by subcutaneous injection on days 1-5 and days 8-12 of a 21-day cycle). FIG 5E depicts the change in Treg from baseline (B) through two cycles (C1 and C2) of therapy (squares = ovarian cancer; pink circles = small bowel; green circles = cutaneous carcinoma; upper triangles = gastroesophageal junction).

Example 7: CD8:Treg Ratio Improves on AMV564 Therapy in Solid Tumor Patients

FIGS 6A-6G show that there an increase in the CD8/Treg ratio (for most subjects) while solid tumor patients (FIG 6A: Small Bowel - Stable Disease; FIG 6B: Ovarian - Complete Response; FIG 6C: GE Junction - Progressive Disease; FIG 6D: Endometrial - Stable Disease; FIG 6E: Colorectal - Progressive Disease; FIG 6F: Cutaneous - Stable Disease; FIG 6G: Appendiceal- Stable Disease) were on AMV564 therapy (once a day by subcutaneous injection on days 1-5 and days 8-12 of a 21-day cycle (FIGS 6A-6C 15 mcg; FIG 6D-6G 50 mcg). The dotted line indicates the baseline ratio; bars along the x-axis indicate dosing days and the broad bar indicates the ratio for healthy controls whose peripheral blood samples were processed in the same flow based assay.

Example 8: AMV564 Promotes Favorable CD4 and CD8 T Cell Polarization in an Ovarian Cancer Patient

An ovarian cancer patient who had previously undergone multiple lines of platinum-based chemotherapy, surgery, radiation, pembrolizumab (best response stable disease, completed 6 months prior to study start), and therapy with niraparib and letrozole, was treated with 15 mcg AMV564 (once a day by subcutaneous injection on days 1-5 and days 8-12 of a 21-day cycle). This patient exhibited an ongoing increase in CD8/Treg, increase in % CD8, maintained or increased effector CD8 (TBX21 and/or granzyme B positive) and memory CD8 cells, a dynamic increases in TBX21-positive CD4 T helper cells and dynamic modulation of PD1 positive CD8 fraction but without substantial overall increase as shown in FIGS 7A-7E. This patient progressed from stable disease, to partial response to complete response as assessed by CT scans at 6-8 week intervals.

Example 9: Patients Treated with AMV564 Exhibit Signs of T Cell Activation without CRS

FIGS 8A-8F (Patients 1, 2, 3, 11, 9 and 14, respectively) show the results of IFN γ and IL-6 measurements in six solid tumor patients treated with AMV564 (once a day by subcutaneous injection on days 1-5 and days 8-12 of a 21-day cycle). As can be seen there is clear evidence of systemic IFN γ production without excessive IL-6 production (ratio of IFN γ : IL-6 was about 1:1 or better for most patients).

Over 5 cycles of treatment, a solid tumor patient (data not shown) exhibited increases in IFN γ , TNF α and IL18 and other factors at later time points (e.g., end of cycle 2 and cycles 3, 4) consistent with MHC class I upregulation, dendritic cell activation and T cell trafficking (decrease in MDSC-inducing factor G-CSF). This same patient entered the study with poor CD8/Treg at baseline. Improvement was observed throughout treatment, particularly around cycle 3, where increases in CD8 T cell proliferation and activation (Ki67 and CD38 fractions) were observed, consistent with improvement

in CD8 effector function (T-bet and granzyme B positive fractions). This mirrors timing of observations for increases in cytokines and factors consistent with improved dendritic cell activation and Th1 response, suggesting that AMV564 was, over time, driving a more favorable immune polarization even in this late-stage patient.

Example 10: Treatment with AMV564 and Pembrolizumab

FIGS 9A-9D show the results of M-MDSC and G-MDSC measurements in four solid tumor patients treated with AMV564 once a day by subcutaneous injection on days 1-5 and days 8-12 of a 21-day cycle (5 mcg/day (FIG 9A and FIG 9B) or 15 mcg/day (FIG 9C and FIG 9D)) in combination pembrolizumab administered intravenously at 200 mg every 3 week (Q3W). The AMV564 administration days are indicated by a bar along the x-axis and the pembrolizumab treatment days are indicated with an asterisk. As can be seen, very good MDSC control was observed.

FIGS. 10A-10D shows data from two patients (FIG 10A and FIG 10C: Patient 15; FIG 10B and FIG 10D: Patient 16) treated with AMV 564 (15 mcg once a day by subcutaneous injection on days 1-5 and days 8-12 of a 21-day cycle in combination pembrolizumab (administered intravenously at 200 mg Q3W). This data shows evidence of a substantial increase in CD8 effector cell fraction in cycles 1 – 2 and a substantial increase in CD8/Treg ratio. The data also show expansion of T-Bet and granzyme B positive CD8 cells. These effects were not apparent in 5 mcg AMV564 combination cohort in this study.

FIGS 11A-11B shows data from two patients (FIG 11A: Patient 15; FIG 11B: Patient 16) treated with AMV 564 (15 mcg once a day by subcutaneous injection on days 1-5 and days 8-12 of a 21-day cycle in combination pembrolizumab (administered intravenously at 200 mg Q3W). This data shows evidence of significant increase in CD8 proliferation (assessed by CD8 Ki67) and activation (assessed by CD 8 CD38). The substantial and rapid increases in 2 of 3 patients dosed in combination, from a poor baseline level, suggests potential combination benefit if AMV564 and pembrolizumab.

Example 11: AMV564 Exhibits Selectivity Against Different Leukemic Blast Cells

As noted above, the structure of AMV564 may allow it to bind clusters of dimerized CD33. Importantly, the bivalent structure of AMV564 promotes more potent binding to clustered CD33 compared to a monovalent CD33 binding molecule, such as a CD33 antibody. Clustered CD33 can be found in lipid raft regions or active signaling configurations. While CD33 is broadly expressed by leukemic blasts, the degree and homogeneity of expression can vary. The binding of AMV564 to leukemic blasts and primary human myeloid cells and impact on T cell mediated cytotoxicity was thus further explored, and novel assays for evaluation of patient samples were developed.

Various AML cell lines, primary human cells, and patient samples were analyzed using flow cytometry with appropriate marker panels including AMV564, which was directly labeled (phycoerythrin) or detected with labeled anti-AMV564 antibodies. T cell cytotoxicity assays were conducted using primary human T cells and leukemic blasts or other target cells (3:1 ratio) for 48 or 72 hours.

Results from flow cytometry assays of cell binding by AMV564 were similar whether using phycoerythrin-labeled AMV564 or a labeled anti-AMV564 antibody. AMV564 showed consistent binding characteristics for many leukemic blast lines (largely of M2 FAB subtype); however, substantial differences in binding were detected between the KG1 myeloid cell line (M2 FAB subtype) and the KG1a cell line (M0 FAB subtype) that represents a morphologically and functionally less mature state than the parental KG1 cell line. At saturating concentrations of M254 (10 nM), there was >10-fold less binding of AMV564 to KG1a cells than KG1 cells (Figure 12B). In contrast, a CD33 antibody (WM53) had a similar binding profile for each cell line (Figure 12A). The difference in AMV564 binding between these two cell lines is reflected in the cytotoxic potency of AMV564, with a 17.6-fold difference in EC50 (0.42 pM for KG1 vs 7.39 pM for KG1a, Figure 12C).

AMV564 can engage CD8 and CD4 T cells to drive target-dependent killing and the difference in potency against KG1 vs KG1a was observed using both CD8 and CD4 T cells. Similarly, whereas purified naïve CD8 T cells killed KG1 effectively with AMV564, there was almost no cytotoxicity against KG1a. T cell proliferation was proportional to cytotoxicity in these assays, with much larger increases in proliferating cells apparent with KG1 target cells. There was little or no detectable AMV564 binding or killing of autologous monocytes or neutrophils. The AMV564 binding assay is compatible with primary human samples.

The assay described above can be used to select patients in whom blasts are expressing CD33 in a predominantly clustered configuration, and thus identify patients most likely to experience deeper and more durable responses with AMV564 monotherapy. In addition, it may be possible to AML identify patients likely to have a better response to AMV564 therapy by determining the subclass of the AML according to the French-American-British (FAB) classification system identifies immunotypes based on morphology (Table 1). Subtypes in which CD33 is expressed in a predominantly clustered configuration, e.g., M2, M3 or M4, may be more responsive to therapy with M254 than subtypes in which the CD33 is not expressed in a predominantly clustered configuration, e.g., M1.

Table 1

FAB subtype	Name	Adult AML patients (%)
M0	Undifferentiated acute myeloblastic leukemia	5%
M1	Acute myeloblastic leukemia with minimal maturation	15%
M2	Acute myeloblastic leukemia with maturation	25%
M3	Acute promyelocytic leukemia	10%
M4	Acute myelomonocytic leukemia	20%
M4eos	Acute myelomonocytic leukemia with eosinophilia	5%
M5	Acute monocytic leukemia	10%
M6	Acute erythroid leukemia	5%
M7	Acute megakaryocytic leukemia	5%

Claims

1. A method for treating a patient with a bispecific T cell engager having two CD33 binding domains and two CD3 binding domains, the method comprising:
 - (a) providing a patient sample comprising CD33 expressing tumor cells;
 - (b) measuring the binding of a CD33 antibody to the CD33 expressing cells;
 - (c) measuring the binding of the bispecific T cell engager to the CD33 expressing cells;
 - (d) selecting the patient for treatment with the bispecific T cell engager if the binding of the T cell engager to the CD33 expressing cells is equivalent to the binding of the CD33 antibody to the CD33 expressing cells; and
 - (e) treating the CD33 expressing cells with the bispecific T cell engager.
2. A method for treating a patient suffering from acute myeloid leukemia (AML) with a bispecific T cell engager having two CD33 binding domains and two CD3 binding domains, the method comprising:
 - (a) determining the FAB subtype of the AML;
 - (b) treating the patient with a bispecific T cell engager having two CD33 binding domains and two CD3 binding domains if the FAB subtype is M2.
3. A method for stratifying patients for entry into a clinical trial for assessment of bispecific T cell engager having two CD33 binding domains and two CD3 binding domains for treatment of AML, the method comprising:
 - (a) providing a patient sample comprising CD33 expressing tumor cells;
 - (b) measuring the binding of a CD33 antibody to the CD33 expressing cells;
 - (c) measuring the binding of the bispecific T cell engager to the CD33 expressing cells;
 - (d) selecting the patient for inclusion in the clinical trial if the binding of the T cell engager to the CD33 expressing cells is equivalent to the binding of the CD33 antibody to the CD33 expressing cells otherwise excluding the patient from the clinical trial.
4. The method of any of claims 1-3 wherein the bispecific T cell engager having two CD33 binding domains and two CD3 binding domains is AMV564.

5. The method of claim 1 or claim 2, wherein the bispecific T cell engager having two CD33 binding domains and two CD3 binding domains is AMV564 and AMV564 is administered by subcutaneous injection.
6. The method of claim 5, wherein the dose of AMV564 injected is 5 – 150 mcg.
7. The method of claim 5, wherein AMV564 is administered on a least 7 days over a 14 day period.
8. The method of claim 5, wherein AMV564 is administered on 10 days over a 14 day period.
9. The method of claim 5, wherein AMV564 is administered on 5 consecutive days on two occasions over a 14 day period.
10. The method of claim 5, wherein the AMV564 is administered on 5 consecutive days, is not administered on the following two days and is administered on the following 5 consecutive days.
11. The method of claim 5, wherein the AMV564 is administered over a 21 day cycle in which AMV564 is administered on at least 7 days over a 14 day period and is not administered over the subsequent 7 day period.
12. The method of claim 11, wherein the 21 day cycle is repeated at least two times.
13. The method of claim 11, wherein AMV564 is administered on at least 10 days over a 14 day period with administration on 5 consecutive days followed by 2 days of no administration followed by administration on 5 consecutive days.
14. The method of claim 6, wherein the dose of AMV564 administered is 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 µgm on each day when administered.
15. The method of any of claims 5-14, wherein the patient is being treated with a therapy that activates T cells.
16. The method of claim 15, wherein the therapy is a CAR T cell therapy.
17. The method of claim 15, wherein the therapy is a CTL therapy.
18. The method of claim 15, wherein the therapy is an antibody therapy.

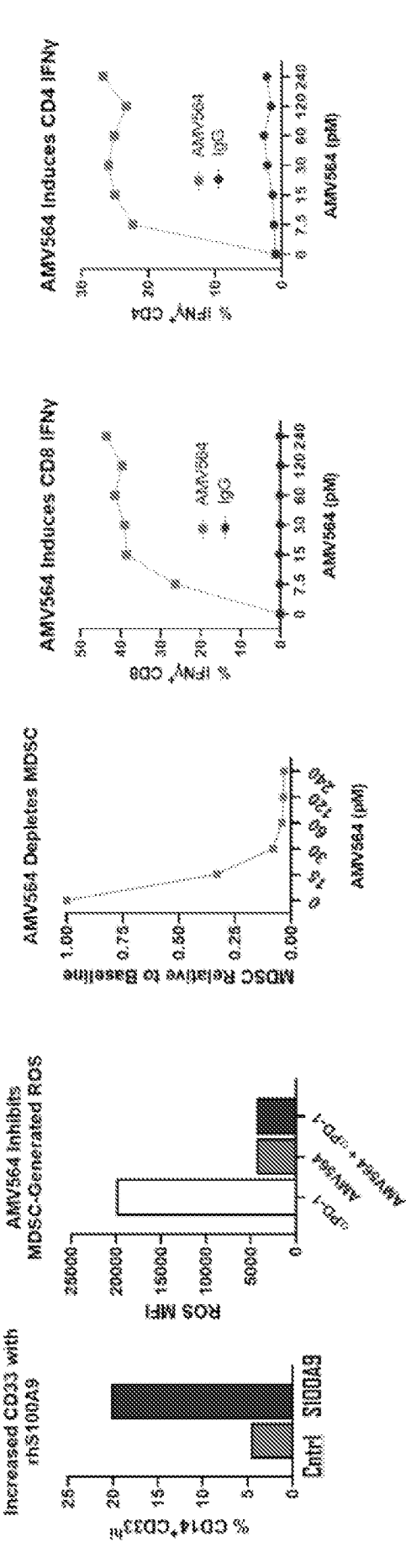


FIG. 1A

FIG. 1B

FIG. 1C

FIG. 1D

FIG. 1E

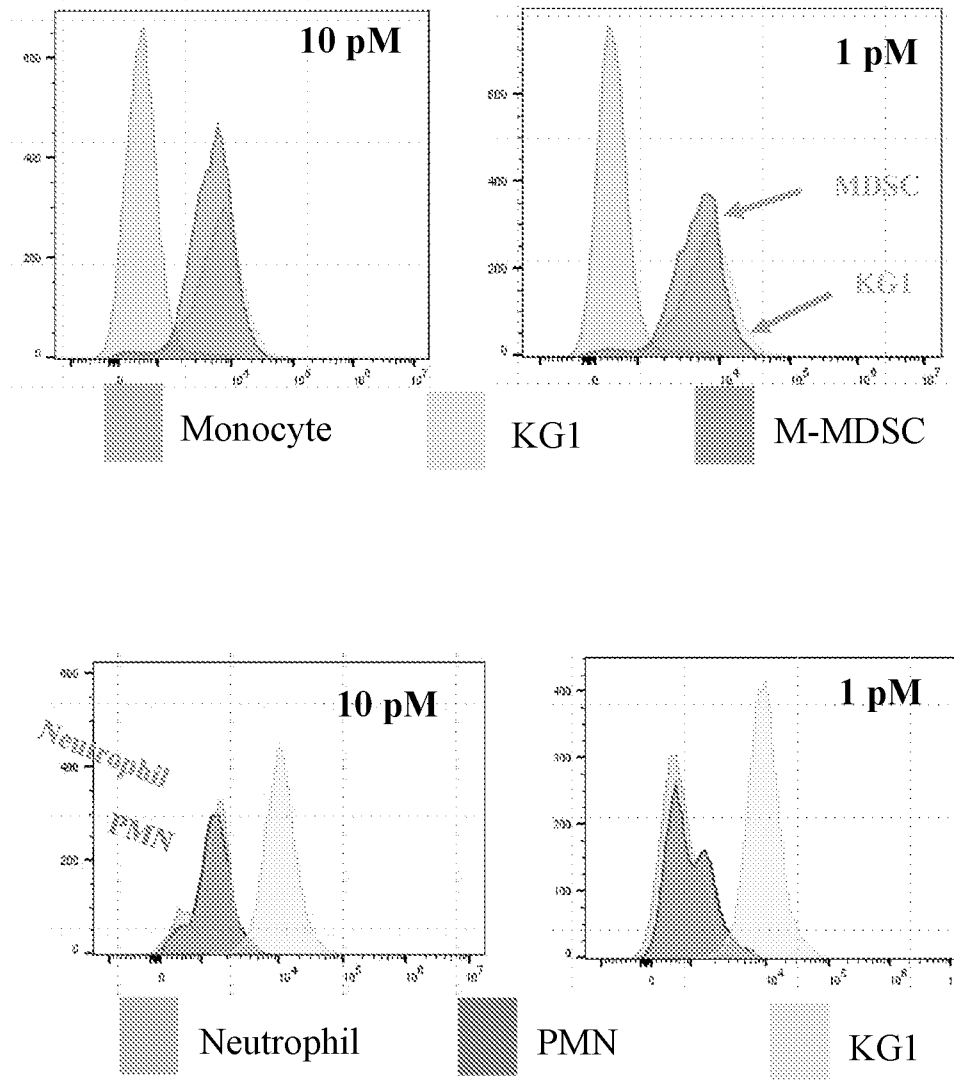


FIG. 1F

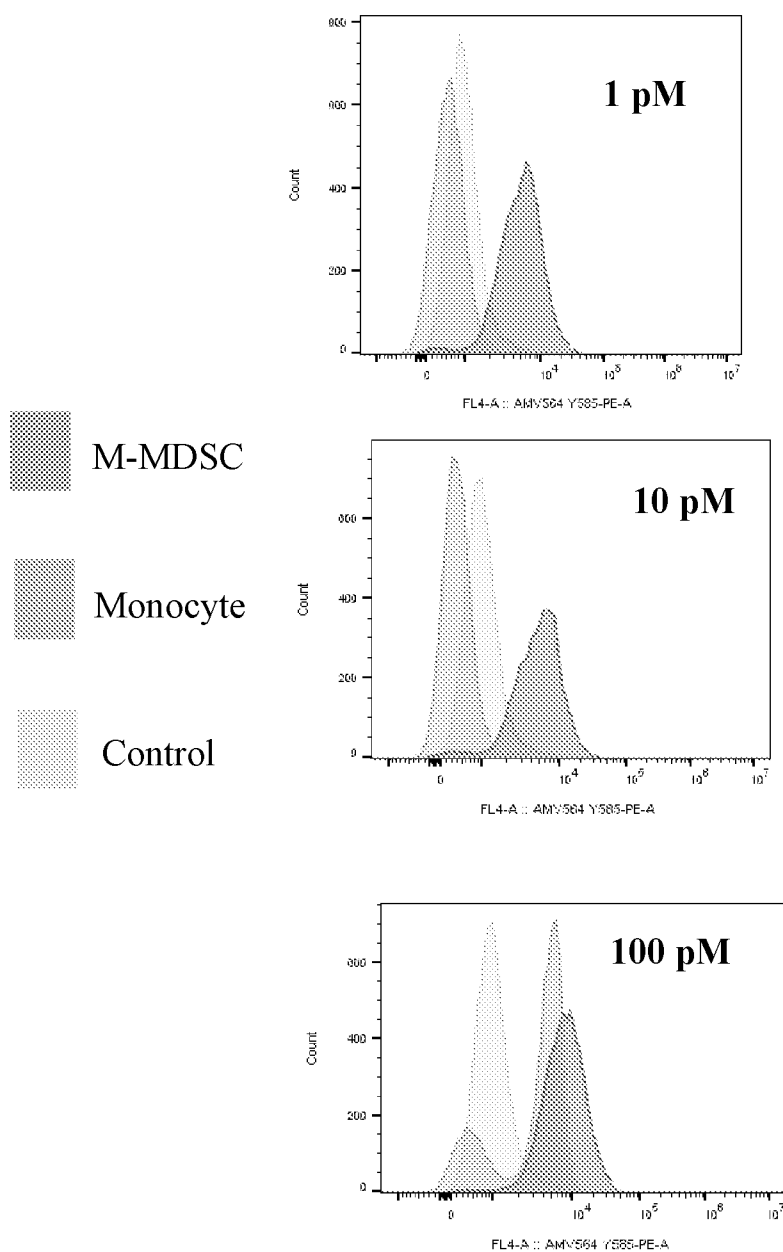


FIG. 1G

FIG. 2A

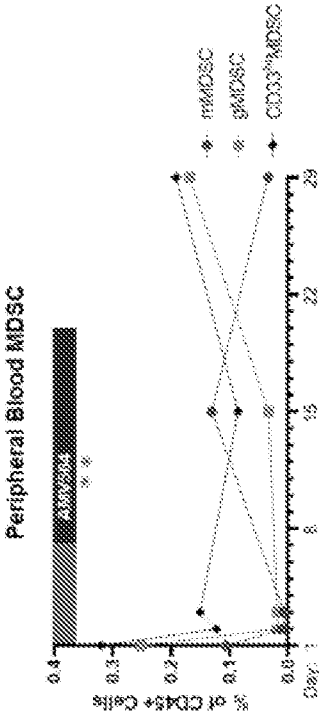


FIG. 2B

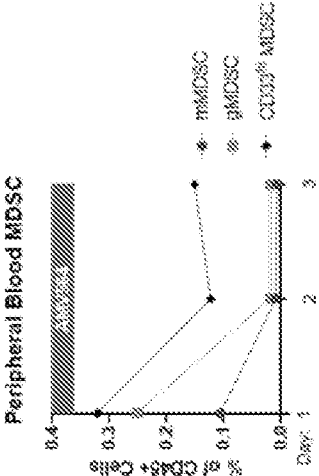


FIG. 2C

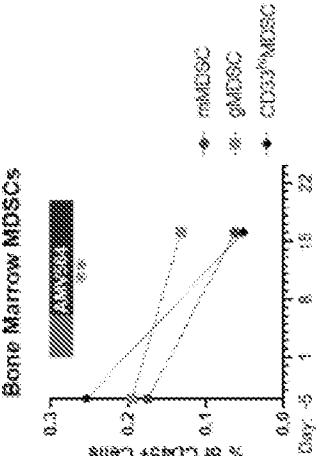


FIG. 2D

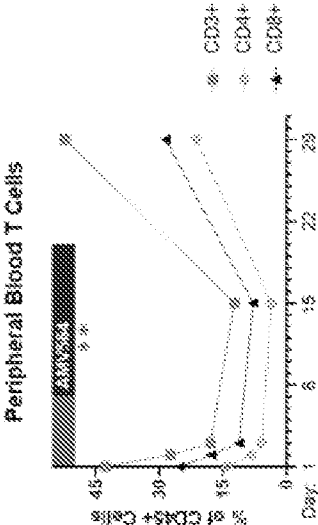


FIG. 2E

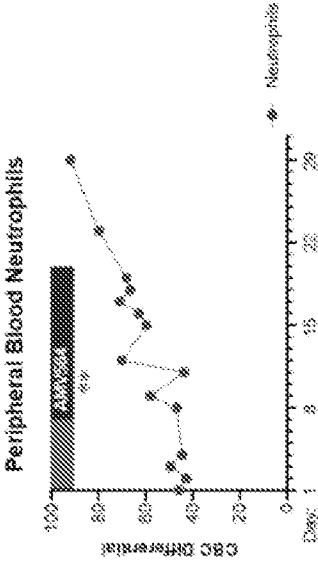
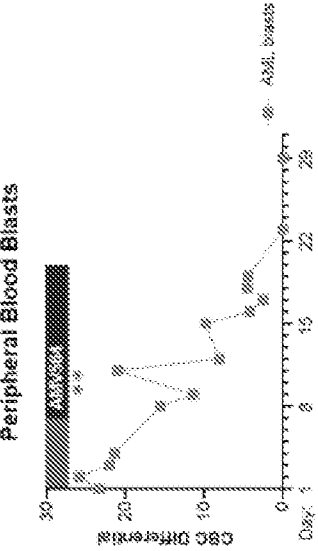


FIG. 2F



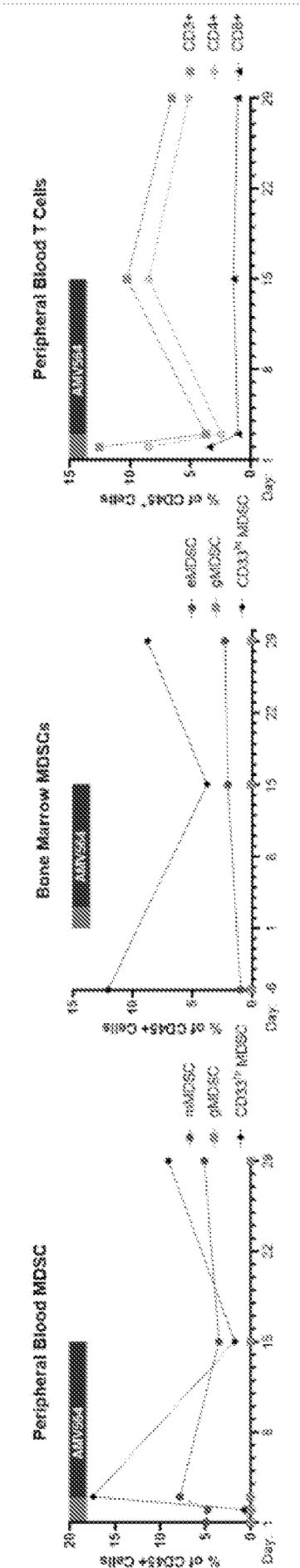


FIG. 3A

FIG. 3B

FIG. 3C

FIG. 4A

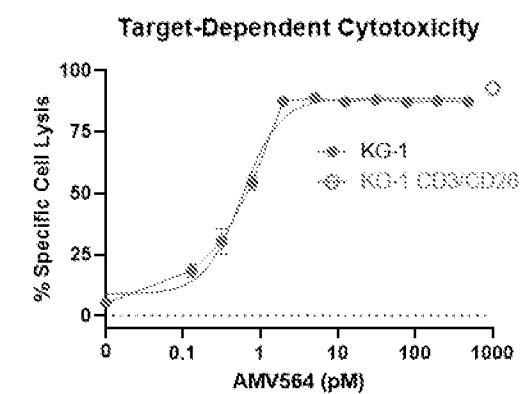


FIG. 4B

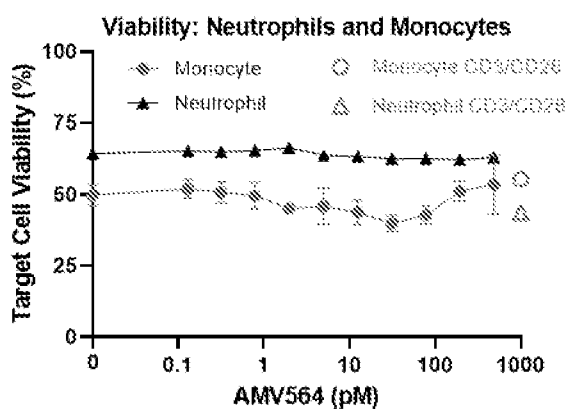
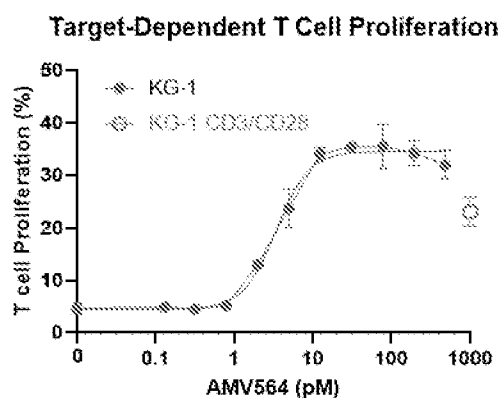


FIG. 4C

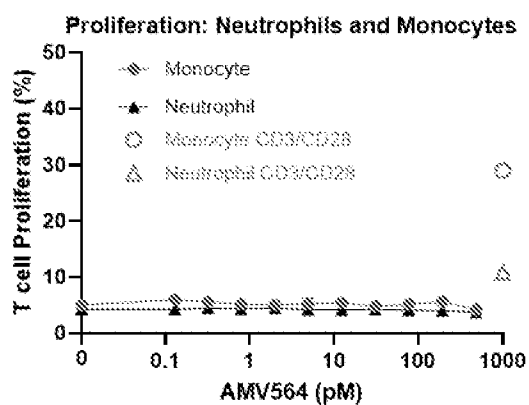


FIG. 4D

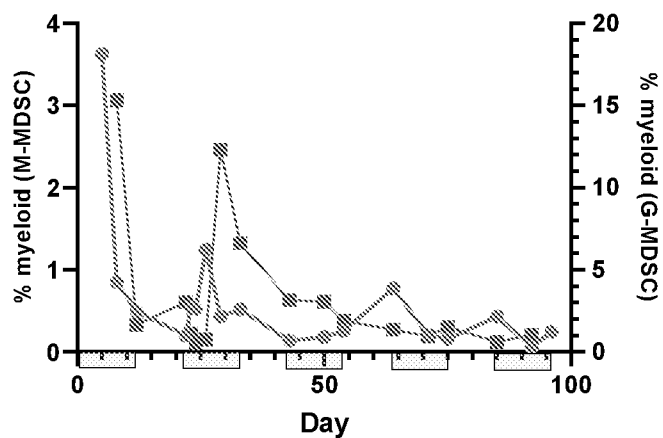


FIG. 5A

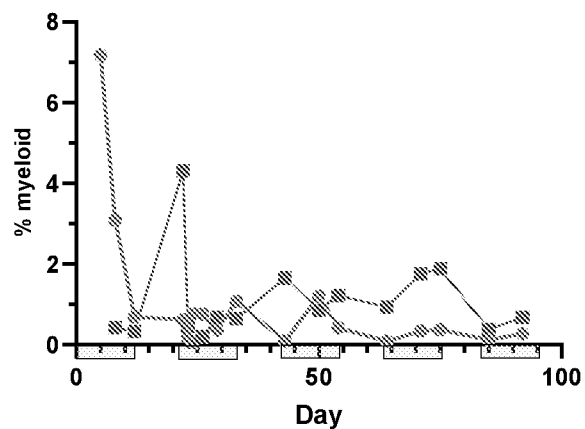


FIG. 5B

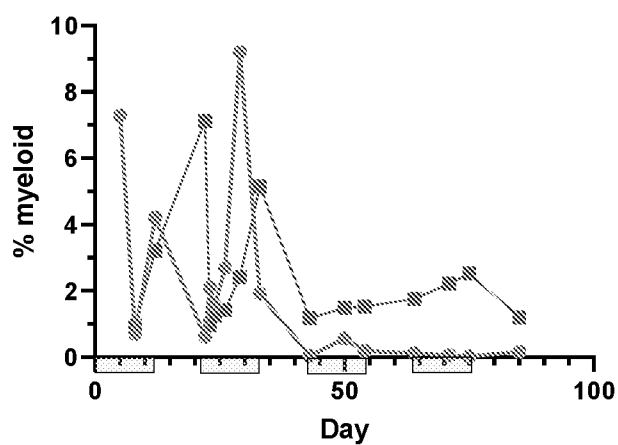


FIG. 5C

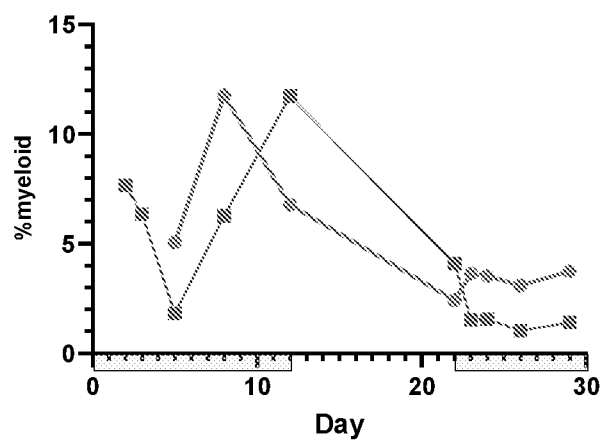


FIG. 5D

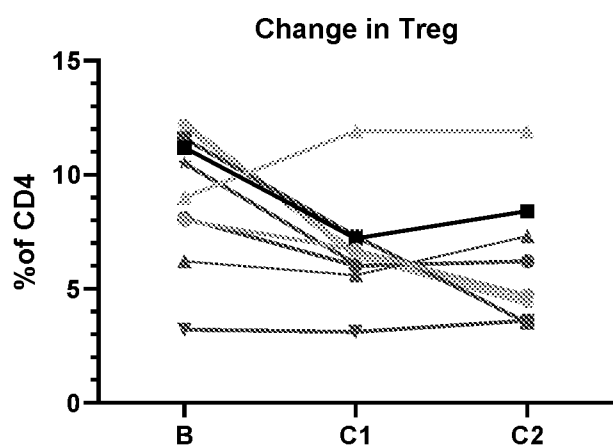


FIG. 5E

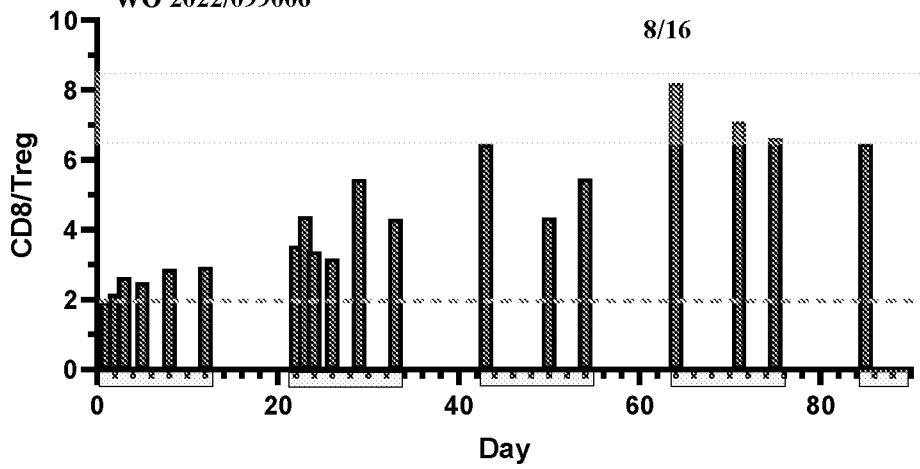


FIG. 6A

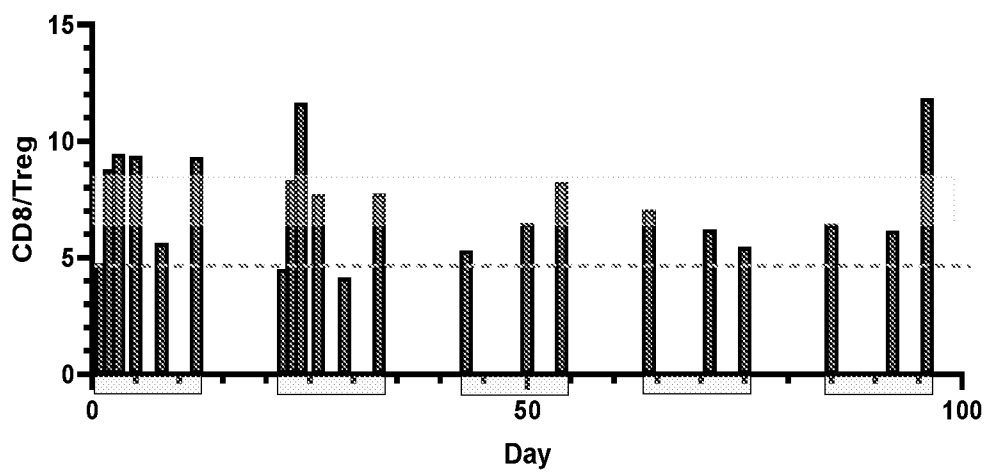


FIG. 6B

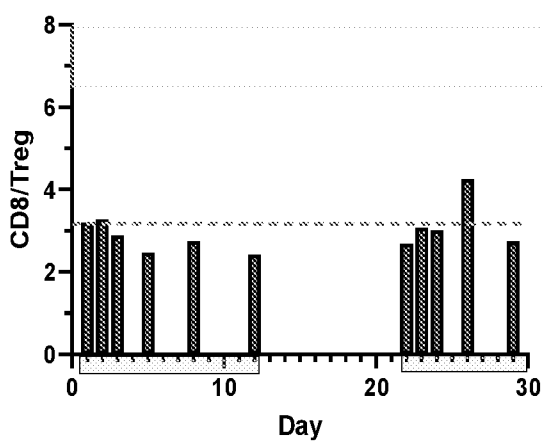


FIG. 6C

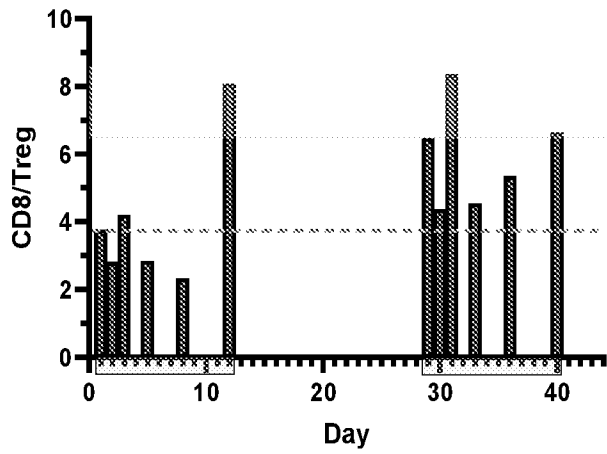


FIG. 6D

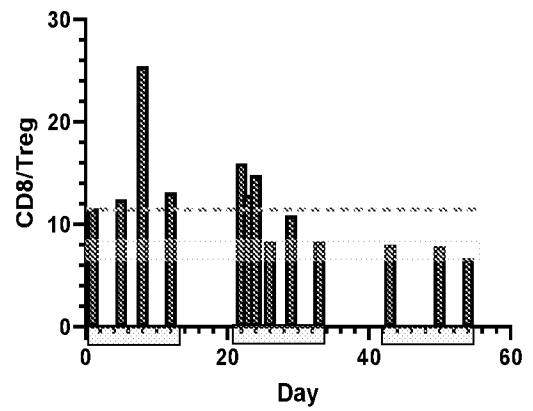


FIG. 6E

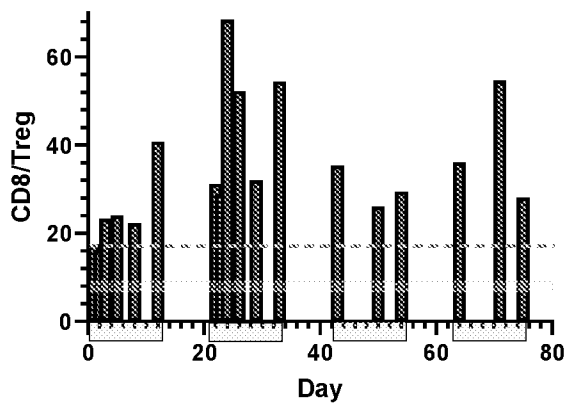


FIG. 6F

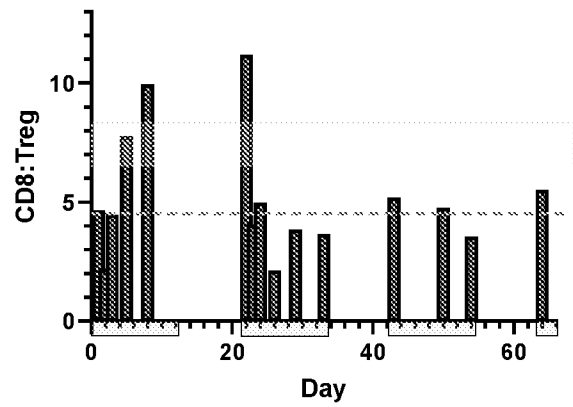


FIG. 6G

10/16

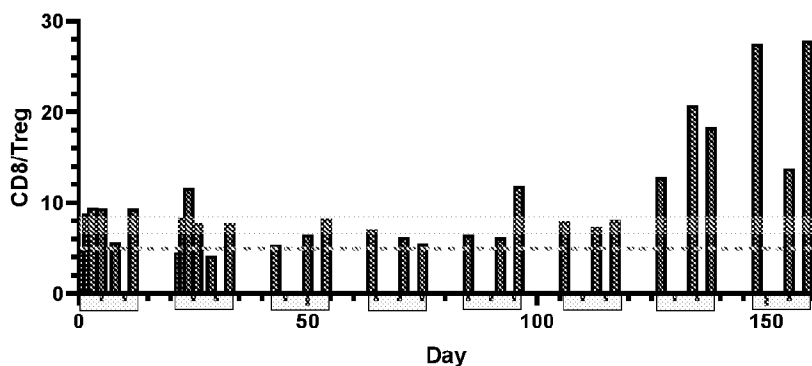


FIG. 7A

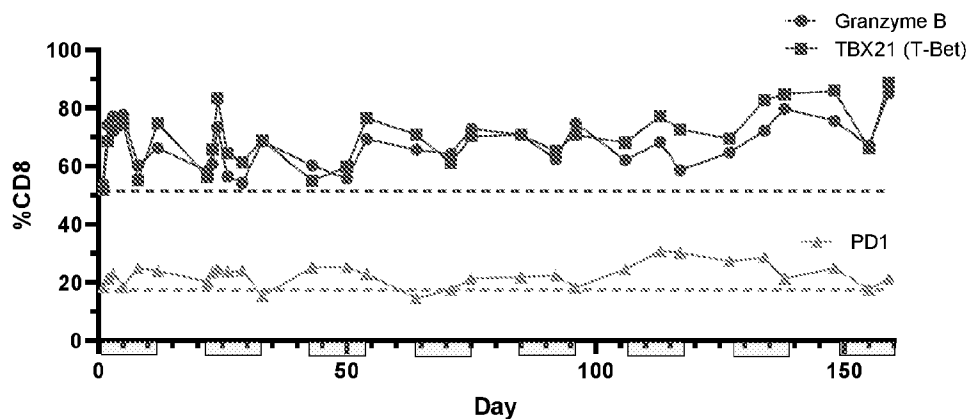


FIG. 7B

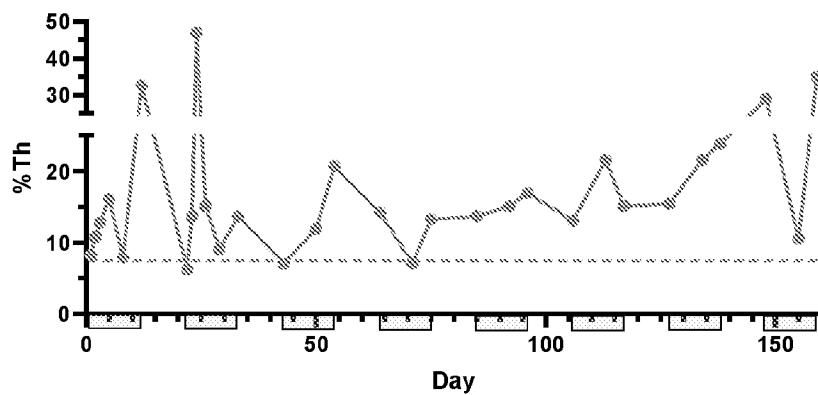


FIG. 7C

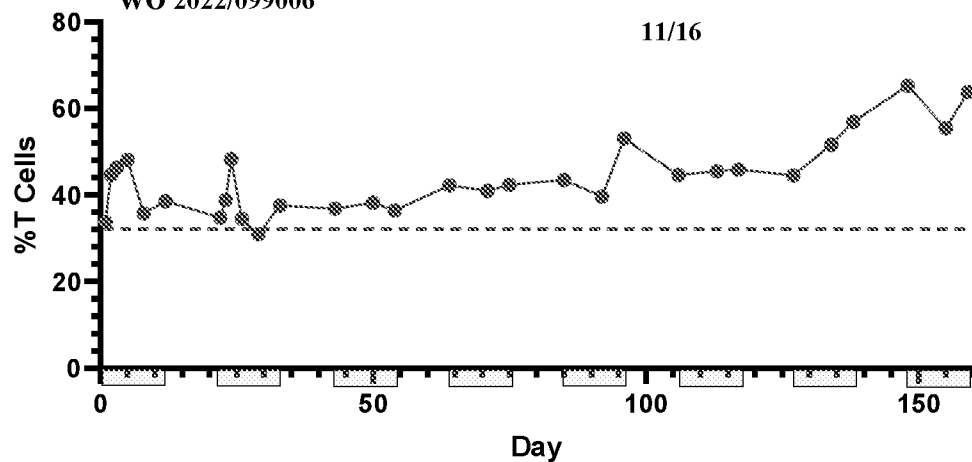


FIG. 7D

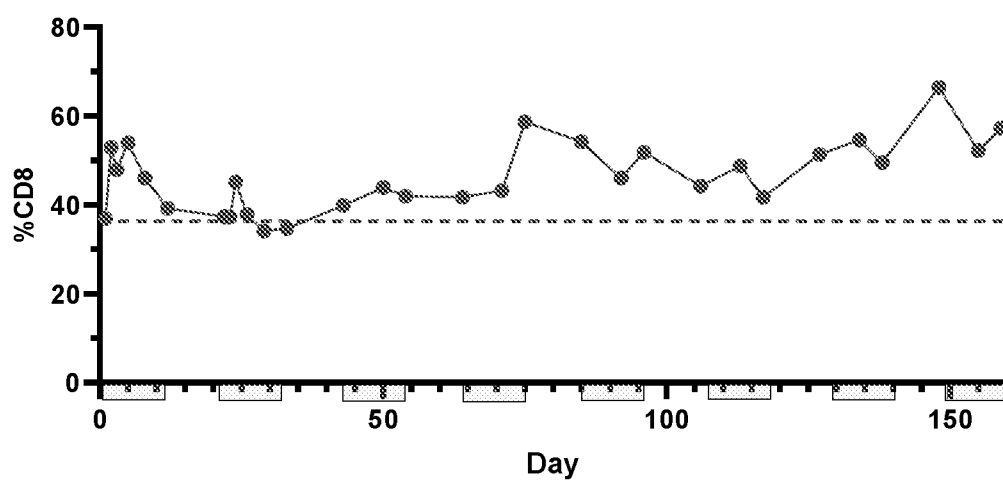


FIG. 7E

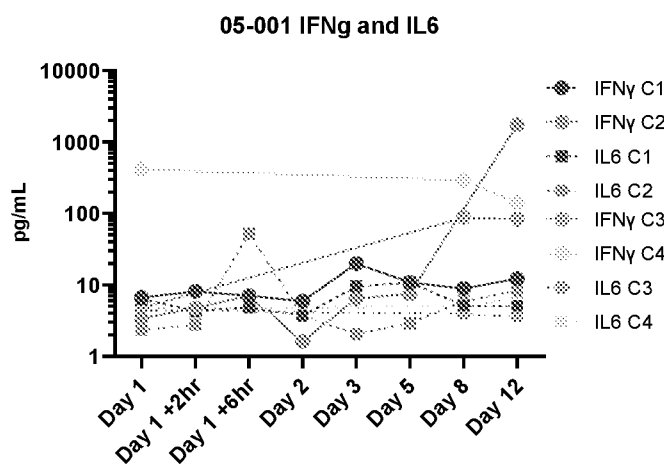


FIG. 8A

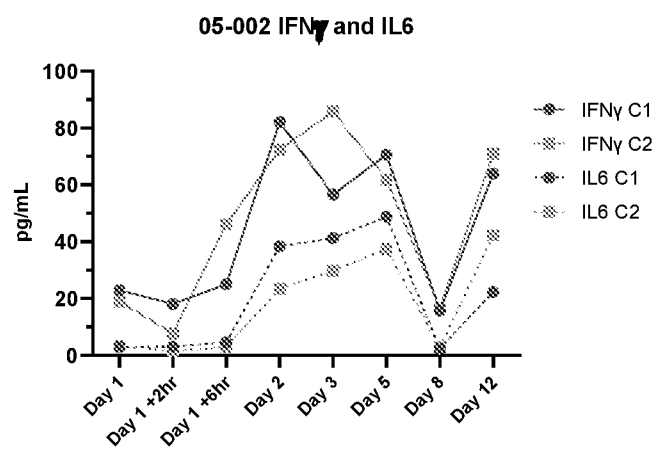


FIG. 8B

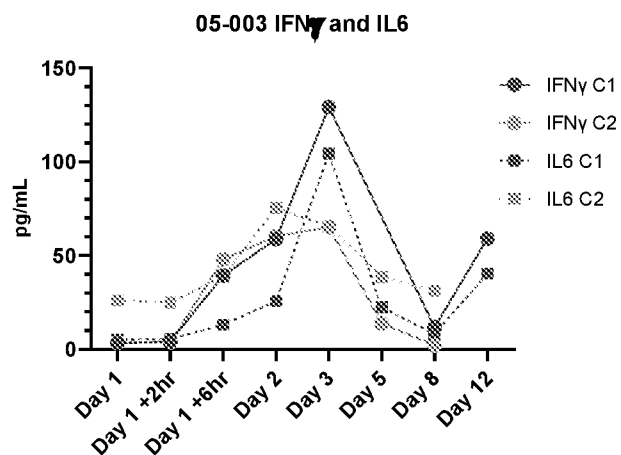


FIG. 8C

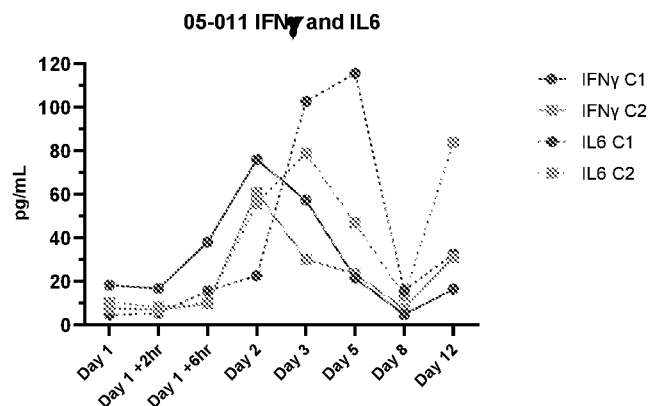


FIG. 8D

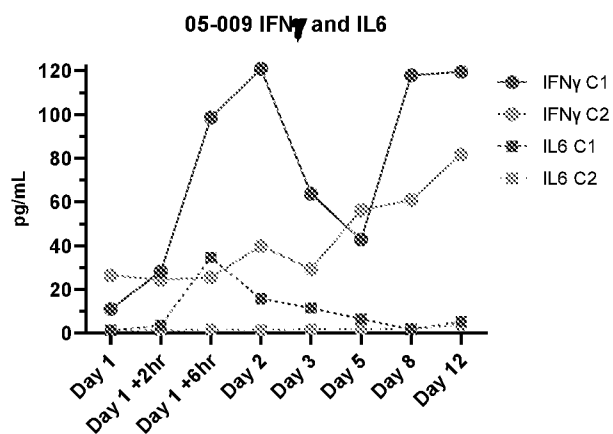


FIG. 8E

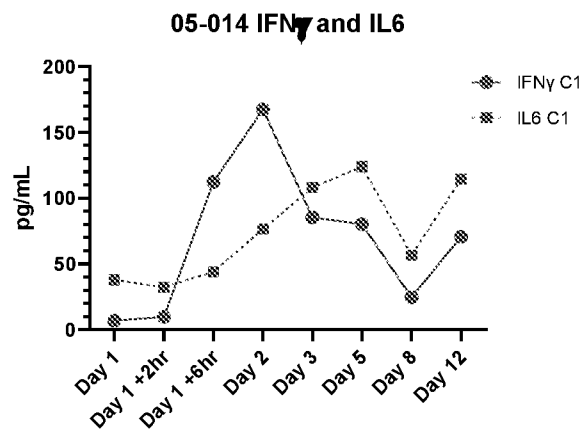


FIG. 8F

05-009 MDSC

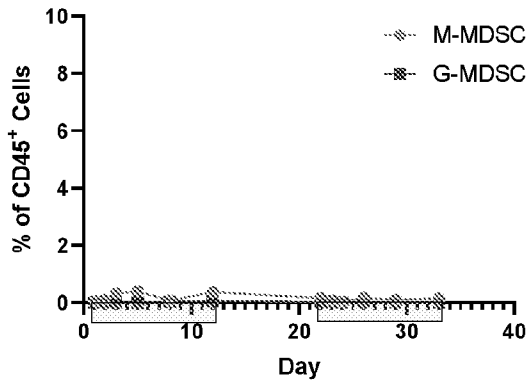


FIG. 9A

05-010 MDSC

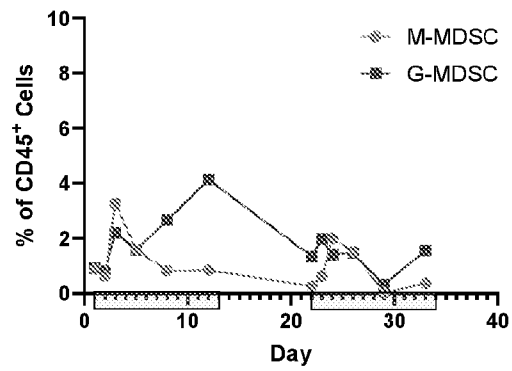


FIG. 9B

01-015 MDSC

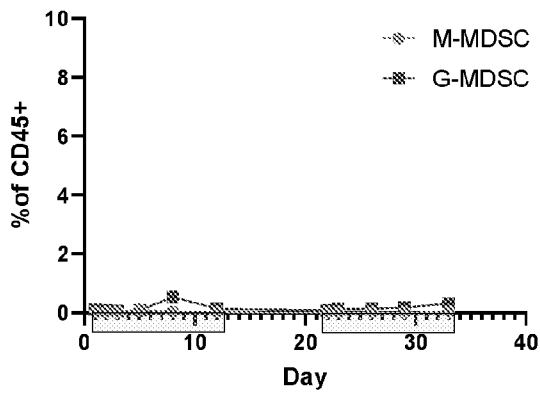


FIG. 9C

05-016 MDSC

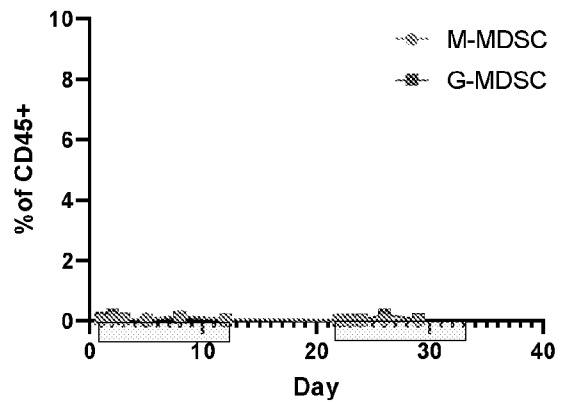


FIG. 9D

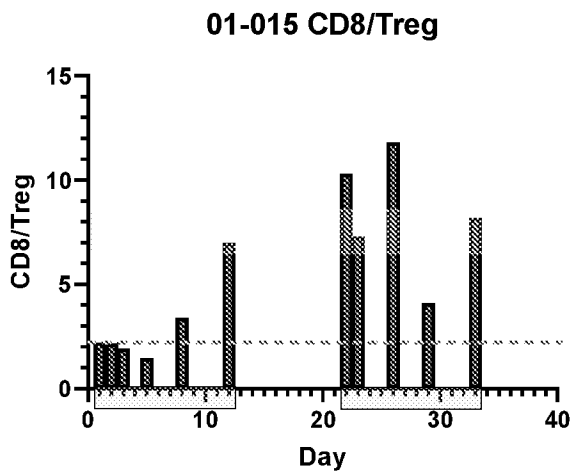


FIG. 10A

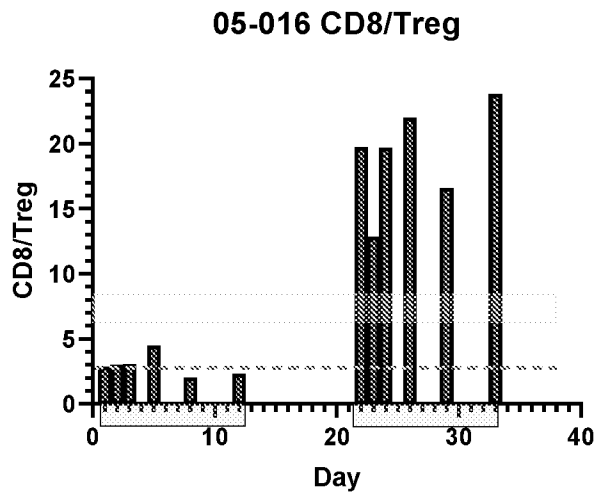


FIG. 10B

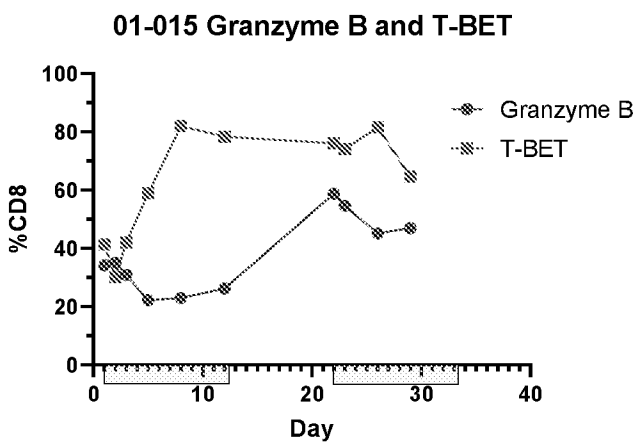


FIG. 10C

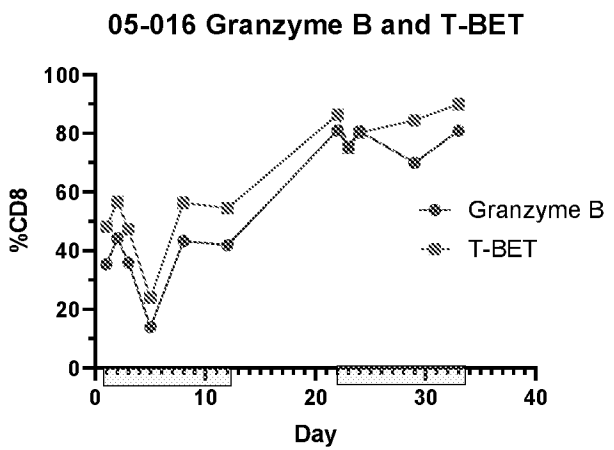


FIG. 10D

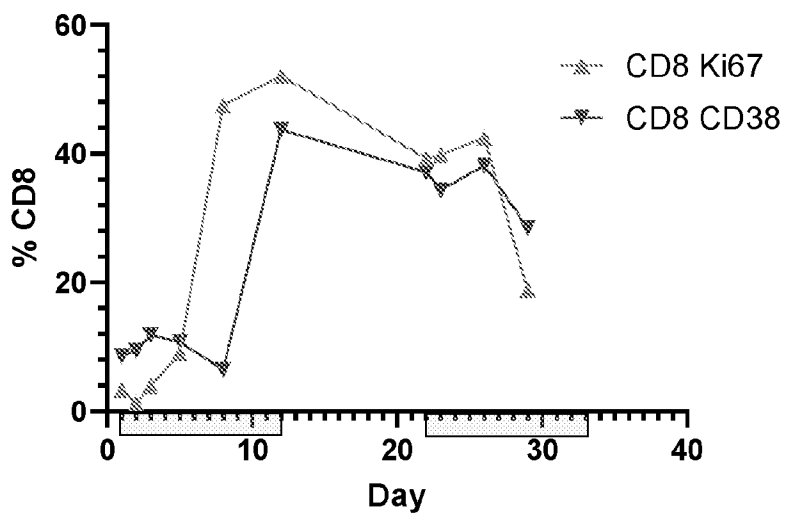
01-015 CD8 Proliferation, Activation

FIG. 11A

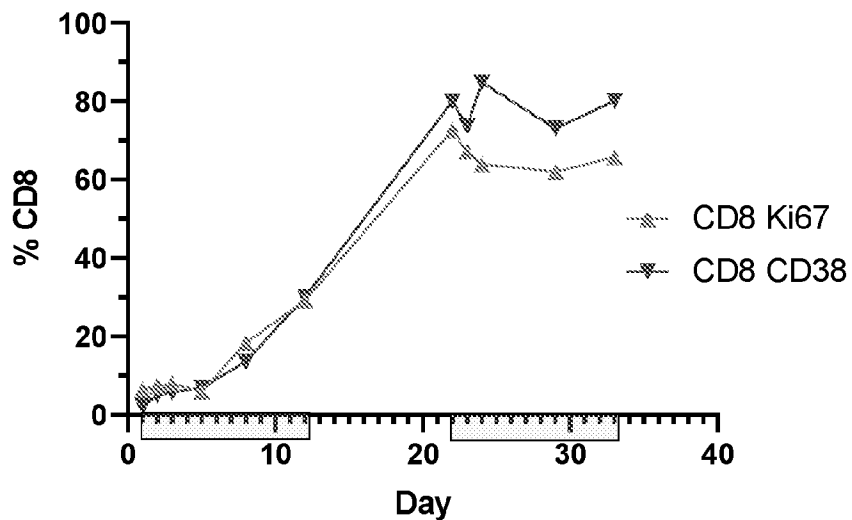
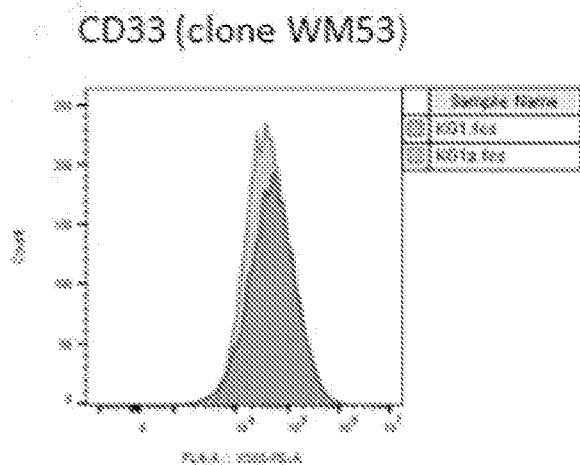
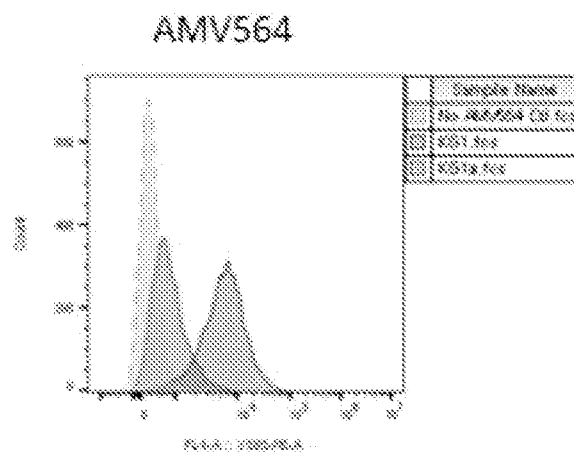
05-016 CD8 Proliferation, Activation

FIG. 11B



Both KG1 and KG1a express
CD33 at a similar level



Distinct KG1 and
KG1a binding

FIG. 12A

FIG. 12B

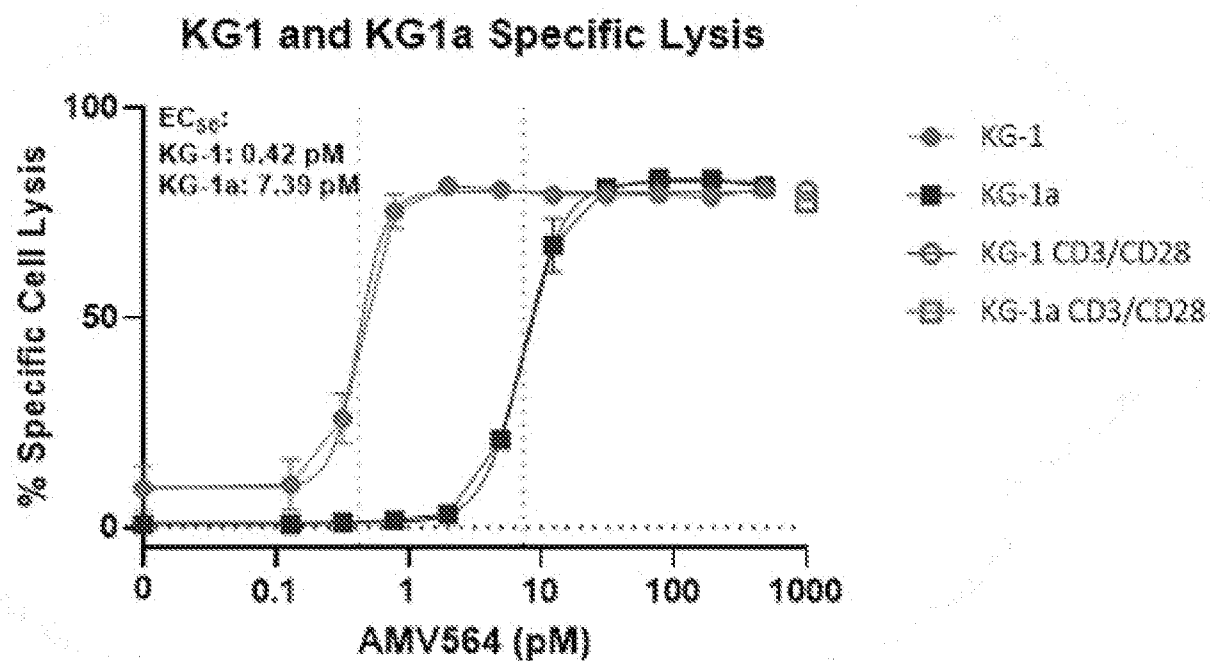


FIG. 12C

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2021/058254

A. CLASSIFICATION OF SUBJECT MATTERINV. **A61K39/00 A61P35/02 C07K16/28**

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)

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, BIOSIS, EMBASE, WPI Data**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	U. REUSCH ET AL: "Characterization of CD33/CD3 Tetraivalent Bispecific Tandem Diabodies (TandAbs) for the Treatment of Acute Myeloid Leukemia", CLINICAL CANCER RESEARCH, vol. 22, no. 23, 17 May 2016 (2016-05-17), pages 5829-5838, XP055333349, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-16-0350 page 5833, right-hand column, paragraph 2; figures 2,3C,4; table 1 page 5834, right-hand column, paragraph 2 - page 5835, right-hand column, paragraph 1 page 5836, left-hand column, paragraph 3 - right-hand column, paragraph 1 ----- -/--	1-18



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

10 February 2022

Date of mailing of the international search report

18/02/2022

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2

NL - 2280 HV Rijswijk

Tel. (+31-70) 340-2040,

Fax: (+31-70) 340-3016

Authorized officer

Cilensek, Zoran

INTERNATIONAL SEARCH REPORT

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PCT/US2021/058254

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X,P	SARDE AURELIEN ET AL: "Selectivity of T Cell Engager AMV564 Against Different Leukemic Blast Populations and Potential Application for Patient Selection", BLOOD, vol. 136, no. Supplement 1, 5 November 2020 (2020-11-05), pages 25-26, XP055889914, US ISSN: 0006-4971, DOI: 10.1182/blood-2020-141475 Retrieved from the Internet: URL:https://ashpublications.org/blood/article/136/Supplement%201/25/471708/Selectivity-of-T-Cell-Engager-AMV564-Against> the whole document -----	1-18
X,P	Eckard Sterling ET AL: "AMV564, A CLINICALLY ACTIVE T CELL ENGAGER, INDUCES A TARGET-DEPENDENT ADAPTIVE IMMUNE RESPONSE", J Immunother Cancer, 10 December 2020 (2020-12-10), XP055889915, Retrieved from the Internet: URL:https://jitc.bmj.com/content/jitc/8/Suppl_3/A416.2.full.pdf [retrieved on 2022-02-09] the whole document -----	1-18
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2021/058254

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOSEPH E. MAAKARON ET AL: "CD33-Targeted Therapies: Beating the Disease or Beaten to Death?", THE JOURNAL OF CLINICAL PHARMACOLOGY, 6 August 2020 (2020-08-06), XP055732032, US ISSN: 0091-2700, DOI: 10.1002/jcph.1730 the whole document -----	1-18

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Information on patent family members

International application No

PCT/US2021/058254

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