



(51) International Patent Classification:

A61K 38/16 (2006.01) *A61K 48/00* (2006.01)
A61K 38/17 (2006.01) *A61P 35/00* (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:

PCT/US2012/035586

(22) International Filing Date:

27 April 2012 (27.04.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/480,311 28 April 2011 (28.04.2011) US
 61/567,042 5 December 2011 (05.12.2011) US

(71) **Applicant** (for all designated States except US): **UNIVERSITY OF SOUTHERN CALIFORNIA** [US/US]; 1150 South Olive Street, Suite 2300, Los Angeles, California 90015 (US).

(72) Inventors; and

(75) **Inventors/Applicants** (for US only): **EPSTEIN, Alan L.** [US/US]; c/o University of Southern California, 1150 South Olive Street, Suite 2300, Los Angeles, California 90015 (US). **LECHNER, Melissa G.** [US/US]; c/o University of Southern California, 1150 South Olive Street, Suite 2300, Los Angeles, California 90015 (US).

(74) **Agents:** **KONSKI, Antoinette, F.** et al.; **Foley & Lardner LLP**, 975 Page Mill Road, Palo Alto, California 94304 (US).

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

(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) **Title:** HUMAN MYELOID DERIVED SUPPRESSOR CELL CANCER MARKERS

(57) **Abstract:** Provided herein are methods for determining the presence of cancer (malignant versus benign), monitoring the progression of cancer, monitoring cancer relapse, monitoring the response to cancer therapy, or cancer staging in a subject, by evaluating CD33⁺/HLA-DR^{low}, CD14⁺/HLA-DR^{low}, CD66b⁺/HLA-DR^{low} or, CD11b⁺/HLA-DR^{low} MDSC for activation of a transcription factor. Transcription factors include, but are not limited to, STAT3, pSTAT3, HIF1 α , or C/EBP β . The MDSC phenotype can be CD33⁺/HLA-DR^{low}/HIF1 α ⁺/STAT3⁺, CD14⁺/HLA-DR^{low}/HIF1 α ⁺/STAT3⁺/pSTAT3⁺/C/EBP β ⁺, CD66b⁺/HLA-DR^{low}/HIF1 α ⁺/STAT3⁺/pSTAT3⁺/C/EBP β ⁺, CD33⁺/HLA-DR^{low}/HIF1 α ⁺/STAT3⁺/pSTAT3⁺/C/EBP β ⁺, CD11b⁺/HLA-DR^{low}/HIF1 α ⁺/STAT3⁺/pSTAT3⁺/C/EBP β ⁺, or CD11b⁺/HLA-DR^{low}/C/EBP β ⁺. Also provided herein are methods for inducing human MDSC from healthy donor peripheral blood mononuclear cells (PBMC) by co-culturing PBMC with human solid tumor cell lines and subsequently measuring their suppressive ability.



HUMAN MYELOID DERIVED SUPPRESSOR CELL CANCER MARKERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 61/480,311 filed April 28, 2011 and U.S. Provisional Application Serial No. 61/567,042 filed December 5, 2011, the contents of which are each incorporated by reference into the present disclosure.

BACKGROUND

[0002] Early detection of cancer or its recurrence is tantamount to a good health outcome. Identifying markers that surface at an early stage allow the oncologist to more successfully treat the cancer patient.

[0003] Immune suppressor cells, such as myeloid-derived suppressor cells (MDSC), contribute to tumor immune tolerance and the failure of immunotherapy regimens in cancer patients and experimental tumor models (1). MDSC reportedly inhibit T cell effector functions through a range of mechanisms, including: arginase 1 (ARG-1)-mediated depletion of L-arginine (2); inducible nitric oxide synthase (iNOS) and NADPH oxidase (NOX2) production of reactive nitrogen and oxygen species (3, 4); vascular endothelial growth factor over-expression (5); cysteine depletion (6); and the expansion of T-regulatory (Treg) cell populations (7, 8). While rare or absent in healthy subjects, MDSC accumulate in the settings of trauma, severe infection or sepsis, and cancer (9), possibly as a result of the hypoxic environment and hypoxia-inducible factor (HIF)-1 α expression (10). MDSC have been described in patients with colon carcinoma (11), melanoma (11), hepatocellular carcinoma (12), head and neck squamous cell carcinoma (4), non-small cell lung carcinoma (13), renal cell carcinoma (14), pancreatic adenocarcinoma (15) and breast carcinoma (16). In cancer patients, Diaz, *et al.* (16) propose that accumulation of MDSC correlates with more advanced disease and poor prognosis. The ability to detect characteristic MDSC in cancer patients would allow the oncologist to determine the effectiveness of therapy and if tumor recurrence has occurred after treatment. Furthermore, MDSC characterization in peripheral blood could be used to differentiate between benign and malignant lesions and masses which can be difficult to discern by routine imaging, fine needle aspiration biopsies, or existing serum assays. Thus, there is a need in the art for methods of identifying MDSC populations so that the

appropriate treatment regime can be prescribed. This invention satisfies this need and provides related advantages as well.

SUMMARY

[0004] Provided herein are methods for identifying MDSC populations from subject samples and treating a subject with cancer comprising, or alternatively consisting essentially of, or yet further consisting of: a) detecting, in a cell-containing sample from said subject, myeloid-derived suppressor cells (MDSC), wherein said MDSC have a phenotype of the group $CD33^+HLA-DR^{low}HIF1\alpha^+/STAT3^+$, $CD14^+HLA-DR^{low}HIF1\alpha^+/STAT3^+ / pSTAT3^+ / C/EBPb^+$, $CD66b^+HLA-DR^{low}HIF1\alpha^+ / STAT3^+ / pSTAT3^+ / C/EBPb^+$, $CD33^+HLA-DR^{low}HIF1\alpha^+ / STAT3^+ / pSTAT3^+ / C/EBPb^+$, $CD11b^+HLA-DR^{low}HIF1\alpha^+ / STAT3^+ / pSTAT3^+ / C/EBPb^+$, or $CD11b^+HLA-DR^{low}C/EBP\beta^+$; and, b) if said MDSC are detected, administering a cancer therapeutic and not treating the patient if such a phenotype is not detected. In another aspect, a method for treating a cancer patient determined to be likely responsive to the methods provided, the method comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the patient an effective amount of a cancer therapy, wherein the patient was determined to be likely responsive by screening a cell or tissue isolated from the patient for a myeloid-derived suppressor cells (MDSC), wherein said MDSC have a phenotype of the group $CD33^+HLA-DR^{low}HIF1\alpha^+/STAT3^+$, $CD14^+HLA-DR^{low}HIF1\alpha^+ / STAT3^+ / pSTAT3^+ / C/EBPb^+$, $CD66b^+HLA-DR^{low}HIF1\alpha^+ / STAT3^+ / pSTAT3^+ / C/EBPb^+$, $CD33^+HLA-DR^{low}HIF1\alpha^+ / STAT3^+ / pSTAT3^+ / C/EBPb^+$, $CD11b^+HLA-DR^{low}HIF1\alpha^+ / STAT3^+ / pSTAT3^+ / C/EBPb^+$, or $CD11b^+HLA-DR^{low}C/EBP\beta^+$.

[0005] A further aspect relates to a method of treating a cancer subject determined to be responsive to the treatment, comprising administering to the subject an effective amount of a cancer therapeutic, wherein the cancer subject is determined to be responsive to the therapy by a method comprising detecting, in a cell-containing sample from said subject, myeloid-derived suppressor cells (MDSC) having a phenotype of the group $CD33^+HLA-DR^{low}HIF1\alpha^+/STAT3^+$, $CD14^+HLA-DR^{low}HIF1\alpha^+ / STAT3^+ / pSTAT3^+ / C/EBPb^+$, $CD66b^+HLA-DR^{low}HIF1\alpha^+ / STAT3^+ / pSTAT3^+ / C/EBPb^+$, $CD33^+HLA-DR^{low}HIF1\alpha^+ / STAT3^+ / pSTAT3^+ / C/EBPb^+$, $CD11b^+HLA-DR^{low}HIF1\alpha^+ / STAT3^+ / pSTAT3^+ / C/EBPb^+$, and $CD11b^+HLA-DR^{low}C/EBP\beta^+$.

[0006] Another aspect relates to a method for detecting the presence of cancer or aiding in the detecting of cancer in a subject comprising, or alternatively consisting essentially of, or yet further consisting of detecting, in a cell-containing sample from the subject, myeloid-derived suppressor cells (MDSC), wherein the MDSC have a phenotype of the group $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}$, $CD14^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD66b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD11b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, and $CD11b^{+}HLA-DR^{low}C/EBP\beta^{+}$; and wherein the presence of the MDSC indicates the likely presence of cancer and the absent of MDSC indicates that the presence of the cancer is not likely.

[0007] A further aspect relates to a method for determining the efficacy of a cancer treatment in a subject having cancer, said method comprising, or alternatively consisting essentially of, or yet further consisting of detecting, in a cell-containing sample from the subject, myeloid-derived suppressor cells (MDSC), wherein said MDSC have a phenotype of the group $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}$, $CD14^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD66b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD11b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, and $CD11b^{+}HLA-DR^{low}C/EBP\beta^{+}$; and wherein a decrease or absence of said MDSC during cancer therapy indicates an effective therapeutic response and an increase or presence of the MDSC during cancer therapy indicates an ineffective therapeutic response.

[0008] A further aspect relates to a method for generating human MDSC from a source of myeloid lineage cells comprising, or alternatively consisting essentially of, or yet further consisting of: (a) contacting the source of myeloid lineage cells with a tumor sample under conditions sufficient to induce MDSC; and, (b) isolating the MDSC. The isolated MDSC and compositions containing the cells are further provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] **Figure 1. Schematic of Co-culture and MDSC Suppression Assays for the *in vitro* generation of tumor-associated myeloid suppressor cells.** *Induction:* Normal donor peripheral blood mononuclear cell (PBMC) are co-cultured with human solid tumor cell lines for one week. *MDSC Isolation:* $CD33^{+}$ cells are isolated from PBMC-tumor co-cultures by anti- $CD33$ microbead labeling and magnetic column separation.

Suppression Assay: Tumor-educated CD33⁺ cells are subsequently co-cultured with fresh, autologous CFSE-labeled CD8⁺ T cells at a 1:4 ratio in the presence of T cell stimuli. After 3 days, T cell proliferation is measured as carboxyfluorescein succinimidyl ester (CFSE) -dilution using flow cytometry. Suppressive function is evaluated as the ability of CD33⁺ cells to inhibit autologous CD8⁺ T cell proliferation.

[0010] Figure 2. CD33⁺ MDSC induced by subset MDSC-inducing cell lines produce increased IL-1 β , IL-6 (A), TNF α (B), VEGF (C) and GM-CSF (D). Protein secretion of these cytokines by head and neck squamous cell carcinoma (HNSCC) cell lines was measured in supernatants using ELISA techniques to confirm gene expression findings. Mean protein levels shown (n=2, run in triplicate), $\pm$ SEM. * indicates statistical significance, $p < 0.05$.

[0011] Figure 3. HNSCC-induced MDSC inhibit autologous T cell proliferation and IFN γ production. A subset of HNSCC cell lines induces a CD33⁺ population with suppressive function characteristic of MDSC, including inhibition of autologous CD8⁺ T cell proliferation (A) and IFN γ secretion (B). Tumor cell lines are grouped by strength of MDSC induction: strong (black), weak (gray), and non-inducing (white). For both graphs, mean shown (n $\geq$ 2 donors) $\pm$ SEM. * indicates statistical significance, $p < 0.05$.

[0012] Figure 4. Human breast carcinoma, lung carcinoma, and glioma cell lines preferentially induce CD11b⁺ MDSC over CD33⁺ MDSC and identify CD11b⁺ MDSC as a second subset. A. CD11b⁺ cells from breast cancer, lung cancer, or glioma cell line-PBMC co-cultures were evaluated for suppressive function against CD3/CD28 stimulated autologous T cells. Mean (n=2) T cell proliferation $\pm$ SEM or T cell proliferation (n=1) is shown from Suppression Assays of CD33⁺ or CD11b⁺ cells with autologous T cells, respectively. B. CD11b⁺ MDSC subset can be induced from normal donor PBMC by cytokines FLT3L and TGF β . Mean shown (n=3) $\pm$ SEM. * indicates statistically significant in mean T cell proliferation compared with T cells alone ($p < 0.05$).

[0013] Figure 5. Characterization of CD33⁺ and CD11b⁺ tumor cell line-induced MDSC. A. Phenotype of HNSCC cell line-induced CD33⁺ and breast cancer cell line-induced CD11b⁺ MDSC compared with medium only, non-suppressive CD33⁺ and CD11b⁺ cells as measured by flow cytometry. Mean percent positive cells (n $\geq$ 2) $\pm$ SD shown. B. Expression of antigen presenting cell (left) and suppressor cell (right) markers on strongly suppressive (induced by HNSCC cell lines SCCL-MT1, SCC-4, CAL-27)

versus non-suppressive (induced by SW 2224, RPMI 2650, or medium only) CD33⁺ myeloid cells as measured by flow cytometry. Mean fluorescence above isotype control (data from 3 unique donors; mean shown for all three induction conditions (n = 9) ±SEM). For comparison of mean values between suppressive cell and non-suppressive cells * indicates statistical significance, p<0.05 and † indicates p=0.59.

[0014] Figure 6. Human MDSC mediate suppression through up-regulation of ARG-1, NOX2, iNOS, VEGF, and TGFβ. A. Expression of putative suppressive genes ARG-1, iNOS, NOX2-component NCF1, VEGF, and TGFβ in a subset of tumor cell line-induced CD33⁺ MDSC. * indicates statistical significance, p<0.05, by ANOVA followed by Dunnett test for pairwise comparisons to medium only CD33⁺ controls for expression measured for each gene. B. Tumor cell line-induced CD33⁺ MDSC inhibit proliferation of autologous, CD3/CD28-stimulated T cells. Specific inhibitors of MDSC suppressive mechanisms ARG-1 and NOX2 mediate partial but incomplete reversal of suppression. * indicates statistical significant difference in mean T cell proliferation (mean shown + SEM, n≥7 for each inhibitor, data from 2 independent experiments with similar results), p<0.05, by ANOVA followed by Tukey test for pairwise comparisons. C. Comparison of ARG-1, iNOS, and NOX2-component NCF1 gene expression in CD33⁺ and CD11b⁺ human MDSC revealed similar levels of expression between these subsets. Mean fold change shown relative to medium only controls (n = 3 unique donors for MDSC from co-cultures with each of three inducing tumor models) ±SEM. No statistically significant difference between mean expression between subsets as determined by Student's t test for each gene observed.

[0015] Figure 7. Transcription factors promoting human MDSC suppressive function. A. HIF1α and STAT3 expression in tumor cell line-induced CD33⁺ or CD11b⁺ MDSC compared with medium only controls as measured by qRT-PCR. Mean shown (data from six unique donors, two independent experiments) ±SEM; * indicates statistical significance, p<0.05, † indicates p=0.06 for pairwise comparisons of CD33⁺ versus CD11b⁺ MDSC. B. Immunohistochemistry of p-STAT3 and C/EBPβ in CD33⁺ (*top panels*) and CD11b⁺ (*bottom panels*) MDSC. Representative images shown from multiple samples stained (200x original magnification). C. Activation of human MDSC subsets by toll-like receptor agonist lipopolysaccharide produces up-regulation of HIF1α, C/EBPβ, and STAT3, concurrent with increased expression of suppressive mediators

ARG-1, iNOS, and NOX2. Mean shown (data from three unique donors) $\pm$ SEM.

D. Transcriptional changes in MDSC subsets associated with inactivation of suppressive function. Reversal of CD33⁺ MDSC suppressive function by ATRA, sunitinib, and CXB correlated with decreased STAT3 and HIF1 α expression (arrows). Functional inhibition of human CD11b⁺ MDSC by ATRA and Sunitinib correlated with decreased C/EBP β levels (arrow), but no change in STAT3 and HIF1 α mRNA levels. CXB was not found to have inhibitory actions on CD11b⁺ MDSC and it was not observed to decrease C/EBP β levels in this population. Mean shown (data from three unique donors) $\pm$ SEM, * indicates statistically significant decrease ($p < 0.05$) in transcript level in drug-treated MDSC compared with untreated MDSC (ANOVA with Dunnett post-test).

[0016] **Figure 8. Amino acid sequence of STAT3 (SEQ. ID. NO. 1).**

[0017] **Figure 9. Amino acid sequence of HIF1 α (SEQ. ID. NO. 2).**

[0018] **Figure 10. Amino acid sequence of C/EBP β (SEQ. ID. NO. 3).**

[0019] **Figure 11. Clinical assay for cancer detection and monitoring. A.**

Schematic showing an embodiment of a minimally-invasive clinical assay for cancer detection and monitoring. Patient peripheral blood cells are analyzed by flow cytometry for the presence of myeloid suppressor cells (MDSC) as a marker for tumor presence. Active MDSC are distinguished from normal blood cells by a 3-marker phenotype that correlates directly with suppressive function. Accumulation of active MDSC correlates directly with disease stage and tumor burden, allowing tracking of disease stage, tumor response to therapy, and tumor recurrence or progression by a simple blood test. B and C. Demonstration of MDSC in the peripheral blood of cancer patients having the phenotype: CD33⁺HLA-DR^{low} HIF1 α ⁺. Twenty milliliters of peripheral blood was collected from normal, healthy volunteers (B) or HNSCC cancer patients (C) and PBMC were isolated by density gradient separation. PBMC were stained for CD33⁺ and HLA-DR⁺ using fluorescence-labeled monoclonal antibodies, then cells were fixed and permeabilized for intracellular staining of HIF1 α by a third antibody. Stained sample PBMC and isotype controls were analyzed on a FACSCalibur flow cytometer using CellQuestPro software and collecting at least 50,000 live leukocyte events (gated as shown in the middle panels). HLA-DR^{low} HIF1 α ⁺ cells were found to be 0.12-1.99% of myeloid cells in normal donor controls (n=3) compared with 16.23-15.78% of myeloid cells in head and neck cancer

patients (n=2); representative dot plots are shown. The following tabulated data depicts the results from the FACS analysis in Figure 11:

Normal healthy donor A

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	1307	38.27	2.50	20.40	18.12	81.78	60.37
UR	197	5.77	0.38	51.69	50.51	152.62	108.42
LL	1907	55.84	3.64	13.31	11.41	9.06	7.32
LR	4	0.12	0.01	44.66	44.51	10.69	9.69

Normal healthy donor B

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	331	44.61	0.62	23.34	21.63	175.48	70.12
UR	126	16.98	0.24	64.28	60.69	180.48	104.88
LL	271	36.52	0.51	20.36	18.45	11.00	9.19
LR	14	1.89	0.03	53.08	51.96	14.18	12.24

Normal healthy donor C

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	3407	31.08	5.70	24.13	22.73	47.64	39.61
UR	1289	13.15	2.41	58.52	56.24	67.76	55.08
LL	5272	53.78	9.86	20.33	18.78	11.17	9.62
LR	195	1.99	0.36	46.39	45.89	14.73	13.91

HNSCC Patient A

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	898	29.25	0.93	25.10	24.72	151.56	96.11
UR	628	14.16	0.65	95.85	82.39	175.14	90.75
LL	2100	41.36	2.27	13.77	17.82	4.52	3.58
LR	720	15.23	0.75	64.59	61.24	8.99	7.15

HNSCC Patient B

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	620	20.05	1.24	24.63	23.50	138.60	80.95
UR	378	10.28	0.64	102.56	89.11	222.24	87.96
LL	1655	53.00	3.33	17.12	15.39	4.67	3.55
LR	488	15.78	0.98	69.30	65.12	9.13	7.43

[0020] For each FACS analysis run, at least 50,000 live events were counted per sample on a BD FACS Calibur. Data was analyzed using BD CellQuestPro software.

DETAILED DESCRIPTION

[0021] As used in the specification and claims, the singular form “a,” “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes a plurality of cells, including mixtures thereof.

[0022] As used herein, the term “comprising” is intended to mean that the compositions and methods include the recited elements, but not excluding others. “Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. “Consisting of” shall mean excluding more than trace elements of other ingredients. Embodiments defined by each of these transition terms are within the scope of this invention.

[0023] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1 or 1.0 as is appropriate. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term “about” which includes a standard deviation of about 15 %, or alternatively about 10% or alternatively about 5 %. It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0024] An “effective amount” is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages. Such delivery is dependent on a number of variables including the time period for which the individual dosage unit is to be used, the bioavailability of the therapeutic agent, the route of administration, etc. It is understood, however, that specific dose levels of the therapeutic agents of the present invention for any particular subject depends upon a variety of factors including the activity of the specific compound employed, bioavailability of the compound, the route of administration, the age of the animal and its body weight, general health, sex, the diet of the animal, the time of administration, the rate of excretion, the drug combination, and the severity of the particular disorder being treated and form of administration. Treatment dosages generally

may be titrated to optimize safety and efficacy. Typically, dosage-effect relationships from in vitro and/or in vivo tests initially can provide useful guidance on the proper doses for patient administration. Studies in animal models generally may be used for guidance regarding effective dosages for treatment of diseases. In general, one will desire to administer an amount of the compound that is effective to achieve a serum level commensurate with the concentrations found to be effective in vitro. Thus, where a compound is found to demonstrate in vitro activity, for example as noted in the Tables discussed below one can extrapolate to an effective dosage for administration in vivo. These considerations, as well as effective formulations and administration procedures are well known in the art and are described in standard textbooks. Consistent with this definition and as used herein, the term “therapeutically effective amount” is an amount sufficient to treat a specified disorder or disease or alternatively to obtain a pharmacological response treating a glioblastoma.

[0025] As used herein, “treating” or “treatment” of a disease in a patient refers to (1) preventing the symptoms or disease from occurring in an animal that is predisposed or does not yet display symptoms of the disease; (2) inhibiting the disease or arresting its development; or (3) ameliorating or causing regression of the disease or the symptoms of the disease. As understood in the art, “treatment” is an approach for obtaining beneficial or desired results, including clinical results. For the purposes of this invention, beneficial or desired results can include one or more, but are not limited to, alleviation or amelioration of one or more symptoms, diminishment of extent of a condition (including a disease), stabilized (i.e., not worsening) state of a condition (including disease), delay or slowing of condition (including disease), progression, amelioration or palliation of the condition (including disease), states and remission (whether partial or total), whether detectable or undetectable. Preferred are compounds that are potent and can be administered locally at very low doses, thus minimizing systemic adverse effects.

[0026] Provided herein are methods comprising , or alternatively consisting essentially of, or yet further consisting of determining the presence of cancer, monitoring the progression of cancer, aiding in the diagnosis of a cancer, monitoring cancer relapse, monitoring the response to cancer therapy, or cancer staging in a subject, by screening or detecting, in a cell-containing sample from the individual, myeloid-derived suppressor cells (MDSC), said MDSC being identified by their expression of CD33, CD14, CD66b, or CD11b and HLA-DR and wherein said identified MDSC are defined by the phenotype

CD33⁺, CD14⁺, CD66b⁺, or CD11b⁺ and HLA-DR^{low}. The presence of MDSC with the identified phenotype indicates the presence of cancer or cancer relapse in said individual. A decrease or absence of MDSC during cancer therapy indicates a successful response to therapy in a subject. An increase in MDSC from a baseline cancer diagnosis indicates an advance in cancer stage in a subject. Surprisingly found herein, the following defined phenotypes of human MDSC: CD33⁺HLA-DR^{low}HIF1 α ⁺/STAT3⁺, CD14⁺HLA-DR^{low}HIF1 α ⁺/STAT3⁺/pSTAT3⁺/C/EBPb⁺, CD66b⁺HLA-DR^{low}HIF1 α ⁺/STAT3⁺/pSTAT3⁺/C/EBPb⁺, CD33⁺HLA-DR^{low}HIF1 α ⁺/STAT3⁺/pSTAT3⁺/C/EBPb⁺, CD11b⁺HLA-DR^{low}HIF1 α ⁺/STAT3⁺/pSTAT3⁺/C/EBPb⁺, and CD11b⁺HLA-DR^{low}C/EBPb⁺), collectively account for the subsets of human MDSC induced by solid tumors and human malignant lymphomas, thereby providing reliable markers for cancer detection and monitoring.

[0027] The indicated protein may be expressed on the surface of the cell or within the cell. Additionally, the expression can include all or a portion of the protein.

[0028] CD11b is expressed on the surface of many leukocytes involved in the innate immune system, including monocytes, granulocytes, macrophages, and natural killer cells. The following GenBank DNA accession numbers represent the CD11b protein sequence: NP_001139280.1, NP_000623.2, and P11215.2. The sequences represented by each of these GenBank accession numbers is incorporated by reference for all purposes.

[0029] CD33 or Siglec-3 is a transmembrane receptor expressed on cells of myeloid lineage. CD33 is usually considered myeloid-specific, but it can also be found on some lymphoid cells. The following GenBank DNA accession numbers represent the CD33 protein sequence: NP_001763.3, NP_001171079.1, NP_001076087.1, P20138.2, CAD36509.1, AAH28152.1, AAK83654.1, EAW71996.1, EAW71995.1, and EAW71994.1. The sequences represented by each of these GenBank accession numbers is incorporated by reference for all purposes.

[0030] CD14 is a marker of monocytes in the body, another myeloid-derived population that circulates in peripheral blood. The following GenBank DNA accession numbers represent the CD14 protein sequence: CAG33297.1, AAA51930.1, P08571.2, NP_001167576.1, NP_001167575.1, NP_001035110.1, NP_000582.1, ADX31876.1, AAC83816.1, EAW62037.1, AAH10507.1, and BAG55282.1. The sequences

represented by each of these GenBank accession numbers in incorporated by reference for all purposes.

[0031] CD66b is a marker of granulocytes in the body, another myeloid-derived population that circulates in peripheral blood. In cancer these myeloid populations may be induced to become suppressive MDSC. The following GenBank DNA accession numbers represent the CD66b protein sequence: AAH26263.1, P31997.2, NP_001806.2, AAC13659.1, and CAB08298.1. The sequences represented by each of these GenBank accession numbers in incorporated by reference for all purposes.

[0032] The methods also include determining if a tumor is malignant or benign, aiding in the determination of such diagnosis, cancer prognosis, monitoring the progression of cancer, monitoring cancer relapse, monitoring the response to cancer therapy, or cancer staging in a subject, by evaluating $CD33^+/HLA-DR^{low}$, $CD14^+/HLA-DR^{low}$, $CD66b^+/HLA-DR^{low}$ or, $CD11b^+/HLA-DR^{low}$ MDSC for activation of a transcription factor wherein the presence of a MDSC with a cancer-related phenotype of transcription factor activation indicates the presence of cancer or cancer relapse or tumor malignancy in said subject, a decrease or absence of the MDSC during cancer therapy indicates a successful response to therapy in said subject, an increase or persistence in level of said MDSC indicates a reduced survival or early disease recurrence, and an increase in the MDSC indicates an advance in cancer stage in the subject. Transcription factors include, but are not limited to, STAT3, pSTAT3, HIF1 α , or C/EBP β . In an embodiment, the MDSC phenotype is $CD33^+HLA-DR^{low}HIF1\alpha^+/STAT3^+$. In another embodiment, the MDSC phenotype is $CD14^+HLA-DR^{low}HIF1\alpha^+/STAT3^+/pSTAT3^+/C/EBPb^+$. In another embodiment, the MDSC phenotype is $CD66b^+HLA-DR^{low}HIF1\alpha^+/STAT3^+/pSTAT3^+/C/EBPb^+$. In another embodiment, the MDSC phenotype is $CD33^+HLA-DR^{low}HIF1\alpha^+/STAT3^+/pSTAT3^+/C/EBPb^+$. In another embodiment, the MDSC phenotype is $CD11b^+HLA-DR^{low}HIF1\alpha^+/STAT3^+/pSTAT3^+/C/EBPb^+$. In another embodiment the MDSC phenotype is $CD11b^+HLA-DR^{low}C/EBP\beta^+$. While not wishing to be held by theory, because the induction of MDSC is an early event in tumor growth, the methods can be used to evaluate tumor occurrence, recurrence or regression before detection by standard radiologic imaging methods.

[0033] Activation of transcription indicates the presence of cancer. In a subject previously diagnosed with cancer, increased activation indicates further progression of the

cancer and advanced stage of cancer. In a subject undergoing cancer therapy, an increased activation indicates a failing therapy. Methods of detecting gene expression are well known in the art.

[0034] STAT3: STATs (signal transducers and activators of transcription) are a family of 7 transcription factors that form part of the JAK-STAT signaling cascade. STAT3 is a transcription factor that binds to the interleukin-6 (IL-6)-responsive elements identified in the promoters of various acute-phase protein genes. STAT3 is activated by IL31 through IL31RA. pSTAT3 is the activated and phosphorylated form of STAT3. The STAT3 protein forms a homodimer or a heterodimer with a related family member (at least STAT1). Interacts with IL31RA, NCOA1, PELP1, SIPAR, SOCS7, STATIP1 and TMF1. STAT3 protein interacts with HCV core protein, IL23R in presence of IL23, and, via SH2 domain, with NLK. STAT3 protein interacts with KPNA4 and KPNA5; KPNA4 may be the primary mediator of nuclear import. STAT3 protein interacts with CAV2; the interaction is increased on insulin-induced tyrosine phosphorylation of CAV2 and leads to STAT3 activation. Constitutive nuclear presence of STAT3 is independent of tyrosine phosphorylation. pSTAT3 refers to the phosphorylated form of STAT3. STAT3 is expressed in heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. STAT3 is activated in many cancers and transformed cell lines and is required in cell culture for transformation and blocking of apoptosis. The following GenBank DNA accession numbers represent the STAT3 protein sequence: AAK17196.1, NP_644805.1, NP_003141.2, NP_998827.1, P40763.2, CAA10032.1, AAB84254.1, AAH14482.1, AAH00627.1, AAS66986.1, EAW60826.1, and EAW60824.1. The sequences represented by each of these GenBank accession numbers is incorporated by reference for all purposes.

[0035] HIF1 α . Hypoxia-inducible factor-1 (HIF1) is a transcription factor found in mammalian cells cultured under reduced oxygen tension that plays an essential role in cellular and systemic homeostatic responses to hypoxia. HIF1 is a heterodimer composed of an alpha subunit and a beta subunit. The beta subunit has been identified as the aryl hydrocarbon receptor nuclear translocator (ARNT). HIF1 α encodes the alpha subunit of HIF-1. Overexpression of a natural antisense transcript (aHIF) of this gene has been shown to be associated with nonpapillary renal carcinomas. Two alternative transcripts encoding different isoforms have been identified. HIF1 α functions as a master transcriptional regulator of the adaptive response to hypoxia. Under hypoxic conditions

HIF1 α activates the transcription of over 40 genes, including, erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia. HIF1 α plays an essential role in embryonic vascularization, tumor angiogenesis and pathophysiology of ischemic disease. HIF1 α binds to core DNA sequence 5'-[AG]CGTG-3' within the hypoxia response element (HRE) of target gene promoters. Activation of HIF1 α requires recruitment of transcriptional coactivators such as CREBBP and EP300. Activity is enhanced by interaction with both, NCOA1 or NCOA2. Interaction with redox regulatory protein APEX seems to activate CTAD and potentiates activation by NCOA1 and CREBBP.

[0036] HIF1 α is cytoplasmic in normoxia, but accumulates and translocates to the nucleus with HIF1 β in response to hypoxia. In addition, under normoxic conditions HIF1 α transcription can be up-regulated by inflammatory cytokines and signaling through JAK/STAT or NF κ B pathways. HIF1 α colocalizes with SUMO1 in the nucleus. HIF1 α is expressed in most tissues with highest levels in kidney and heart. HIF1 α is overexpressed in the majority of common human cancers and their metastases, due to the presence of intratumoral hypoxia and as a result of mutations in genes encoding oncoproteins and tumor suppressors. The following GenBank DNA accession numbers represent the HIF1 α protein sequence: Q16665.1, NP_001230013.1, NP_851397.1, NP_001521.1, and AAH12527.1. The sequences represented by each of these GenBank accession numbers is incorporated by reference for all purposes.

[0037] C/EBP β or C/EBP β . The protein encoded by this intronless gene is a bZIP transcription factor which can bind as a homodimer to certain DNA regulatory regions. It can also form heterodimers with the related proteins CEBP-alpha, CEBP-delta, and CEBP-gamma. The encoded protein is important in the regulation of genes involved in immune and inflammatory responses and has been shown to bind to the IL-1 response element in the IL-6 gene, as well as to regulatory regions of several acute-phase and cytokine genes. In addition, the encoded protein can bind the promoter and upstream element and stimulate the expression of the collagen type I gene. C/EBP β is an important transcriptional activator in the regulation of genes involved in immune and inflammatory responses and can work coordinately with inflammation-related transcription factor NF κ B. C/EBP β specifically binds to an IL-1 response element in the IL-6 gene. NF-IL6 also binds to regulatory regions of several acute-phase and cytokines

genes. C/EPB- β therefore plays a role in the regulation of acute-phase reaction, inflammation and hemopoiesis. The consensus recognition site is 5'-T[TG]NNGNAA[TG]-3'. C/EPB- β functions in brown adipose tissue (BAT) differentiation. C/EPB- β is located in the nucleus and expressed at low levels in the lung, kidney and spleen. The following GenBank DNA accession numbers represent the C/EBP β protein sequence: P17676.2, NP_005185.2, AAH05132.2, AAH21931.1, and AAH07538.1. The sequences represented by each of these GenBank accession numbers are incorporated by reference for all purposes.

[0038] HLA-DR is a MHC class II cell surface receptor encoded by the human leukocyte antigen complex. The following GenBank DNA accession numbers represent the HLA-DR protein sequence: CAA23788.1, AAA58655.1, and AAO49820.1. The sequences represented by each of these GenBank accession numbers are incorporated by reference for all purposes.

[0039] In some embodiments, the detection agent can be an antibody. Antibodies may be prepared using an intact polypeptide or fragments containing smaller polypeptides as the immunizing antigen. For example, it may be desirable to produce antibodies that specifically bind to the N- or C-terminal or any other suitable domains of a protein. The polypeptide used to immunize an animal can be derived from translated cDNA or chemical synthesis and can be conjugated to a carrier protein, if desired. Such commonly used carriers which are chemically coupled to the polypeptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA) and tetanus toxoid. The coupled polypeptide is then used to immunize the animal.

[0040] If desired, polyclonal or monoclonal antibodies can be further purified, for example by binding to and elution from a matrix to which the polypeptide or a peptide to which the antibodies were raised is bound. Those skilled in the art know various techniques common in immunology for purification and/or concentration of polyclonal as well as monoclonal antibodies (Coligan et al, Unit 9, Current Protocols in Immunology, Wiley Interscience, 1994).

[0041] Methods for making antibodies of all types, including fragments, are known in the art (See for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1988)). Methods of immunization, including all necessary steps of preparing the immunogen in a suitable adjuvant, determining antibody

binding, isolation of antibodies, methods for obtaining monoclonal antibodies are all known to the skilled artisan

[0042] The monoclonal antibodies as defined include antibodies derived from one species (such as murine, rabbit, goat, rat, human, etc.) as well as antibodies derived from two (or more) species, such as chimeric and humanized antibodies. Movahedi et al., Blood vol. 111 no.8 (2008) 4233-44 provides commercial source identification of antibodies to CD11b, (BD Biosciences) and provides various markers for MDSC by flow cytometry. Other commercially available antibodies include monoclonal antibody to CD33 (PE-CyTM7) (BD Biosciences, Catalog Number 333946), monoclonal antibody to HIF-1 α (BD Biosciences, Catalog Number 610958, monoclonal antibody to C/EBP β (Thermo Scientific Pierce Antibodies, Catalog Number MA1-826 (MA1-826 detects C/EBP β protein in human, mouse, and rat samples. MA1-826 only detects the LAP* isoform of C/EBP β).

[0043] Some embodiments disclosed herein concern diagnostic and prognostic methods for the detection of inflammation and/or cancer. Such detection methods may be used, for example, for early diagnosis of the disease, to determine whether a tumor is malignant or benign, to monitor the progress of the disease or the progress of treatment protocols, or to assess the grade of the cancer. The detection can occur *in vitro* or *in vivo*. The presence of a cancer is determined when the percentage of myeloid cells is at least 2% MDSC, at least 3% MDSC, at least 4% MDSC, at least 5% MDSC, at least 6% MDSC, at least 7% MDSC, at least 8% MDSC, at least 9% MDSC, at least 10% MDSC, at least 11% MDSC, at least 12% MDSC, at least 13% MDSC, at least 14% MDSC, or at least 15% MDSC. Useful methods include, but are not limited to, flow cytometry, an enzyme immune assay (EIA) such as enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay, or Western blot detection.

[0044] The methods disclosed herein are applicable to any cancer. Cancers include, but are not limited to HNSCC, breast cancer, cervical cancer, ovarian cancer, colorectal carcinoma, brain cancer, melanoma, sarcoma, endometrial cancer, bladder cancer, renal cancer, gastric cancer, thyroid cancer, small cell lung cancer, non-small cell lung cancer, prostate cancer, liver cancer, and pancreatic cancer.

[0045] Cancers can also include malignant lymphoma. Malignant lymphomas can include mature cell neoplasm, mature T cell or natural killer cell neoplasm, and Hodgkins lymphoma.

[0046] A tumor is literally a swelling of any type, such as an inflammatory or other swelling, but modern usage generally denotes a neoplasm. The suffix "-oma" means tumor and usually denotes a benign neoplasm, as in fibroma, lipoma, and so forth, but sometimes implies a malignant neoplasm, as with so-called melanoma, hepatoma, and seminoma, or even a non-neoplastic lesion, such as a hematoma, granuloma, or hamartoma. The suffix "-blastoma" denotes a neoplasm of embryonic cells, such as neuroblastoma of the adrenal or retinoblastoma of the eye.

[0047] Histogenesis is the origin of a tissue and is a method of classifying neoplasms on the basis of the tissue cell of origin. Adenomas are benign neoplasms of glandular epithelium. Carcinomas are malignant tumors of epithelium. Sarcomas are malignant tumors of mesenchymal tissues. One system to classify neoplasia utilizes biological (clinical) behavior, whether benign or malignant, and the histogenesis, the tissue or cell of origin of the neoplasm as determined by histologic and cytologic examination. Neoplasms may originate in almost any tissue containing cells capable of mitotic division. The histogenetic classification of neoplasms is based upon the tissue (or cell) of origin as determined by histologic and cytologic examination.

[0048] "Suppressing" tumor growth indicates a growth state that is curtailed compared to growth without any therapy. Tumor cell growth can be assessed by any means known in the art, including, but not limited to, measuring tumor size, determining whether tumor cells are proliferating using a ^3H -thymidine incorporation assay, or counting tumor cells. "Suppressing" tumor cell growth means any or all of the following states: slowing, delaying, and "suppressing" tumor growth indicates a growth state that is curtailed when stopping tumor growth, as well as tumor shrinkage.

[0049] The term "culturing" refers to the in vitro propagation of cells or organisms on or in media of various kinds. It is understood that the descendants of a cell grown in culture may not be completely identical (morphologically, genetically, or phenotypically) to the parent cell. By "expanded" is meant any proliferation or division of cells.

[0050] Mature cell neoplasm can include Chronic lymphocytic leukemia/Small lymphocytic lymphoma, B-cell prolymphocytic leukemia, Lymphoplasmacytic lymphoma

(such as Waldenström macroglobulinemia), Splenic marginal zone lymphoma, Plasma cell myeloma, Plasmacytoma, Monoclonal immunoglobulin deposition diseases, Heavy chain diseases, Extranodal marginal zone B cell lymphoma (MALT lymphoma), Nodal marginal zone B cell lymphoma (NMZL), Follicular lymphoma, Mantle cell lymphoma, Diffuse large B cell lymphoma, Mediastinal (thymic) large B cell lymphoma, Intravascular large B cell lymphoma, Primary effusion lymphoma, and Burkitt lymphoma/leukemia.

[0051] Mature T or natural killer cell neoplasm can include T cell large granular lymphocytic leukemia, Aggressive NK cell leukemia, Adult T cell leukemia/lymphoma, Extranodal NK/T cell lymphoma (nasal type), Enteropathy-type T cell lymphoma, Hepatosplenic T cell lymphoma, Blastic NK cell lymphoma, Mycosis fungoides / Sezary syndrome, Primary cutaneous CD30-positive T cell lymphoproliferative disorder, Primary cutaneous anaplastic large cell lymphoma, Lymphomatoid papulosis, Angioimmunoblastic T cell lymphoma, Peripheral T cell lymphoma (unspecified), and Anaplastic large cell lymphoma.

[0052] The sample can be peripheral blood or any other source of myeloid lineage cells, including, but not limited to, peripheral blood leukocytes, red blood cell lysate of whole peripheral blood, tumor, lymph, lymph node tissue, spleen cells, cerebrospinal fluid, ascities, and pleural effusions. The sample can be fresh, previously frozen, or preserved.

[0053] Provided herein are also methods for inducing human MDSC from healthy donor peripheral blood mononuclear cells (PBMC) by co-culturing PBMC with human solid tumor cell lines and subsequently measuring their suppressive ability. Culture methods are well-known in the art. These co-culturing methods generate purified human MDSC in quantities sufficient for morphology, phenotype, gene expression, and functional studies and pre-clinical testing of MDSC inhibitors.

[0054] In certain embodiments, provided herein are methods for generating a tumor cell line-educated- CD33⁺ human MDSC, CD14⁺ human MDSC, CD66b⁺ human MDSC, or CD11b⁺ human MDSC by a) contacting peripheral blood mononuclear cells (PBMCs) with tumor cells, and b) isolating CD33⁺ cells, CD14⁺ cells, CD66b⁺ cells, or CD11b⁺ human MDSC, respectively. PBMC-tumor cell line co-cultures are established in tissue culture flasks. The tumor can be any solid tumor or malignant lymphoma listed above. Tumor-educated CD33⁺, CD14⁺ cells, CD66b⁺ cells, or CD11b⁺ cells are isolated from

established PBMC-tumor cell line co-cultures. The tumor-educated $CD33^+$, $CD14^+$ cells, $CD66b^+$ cells, or $CD11b^+$ cells can be checked for viability and can be tested for suppressive function by co-culture with fresh, autologous $CD8^+$ T cells in the presence of T cell stimuli. Methods for detecting viability and suppressive function are well-known in the art. The method of generating *in vitro* human MDSC disclosed herein can be used to generate human MDSC standards and control myeloid cells (such as by culture of PBMC alone or with non-induced tumor or fibroblast cell lines) for use in diagnostic assays and kits thereof.

[0055] Also provided herein are methods for identifying a candidate compound as a suppression agonist by comparing suppression activity function of tumor cell line-educated- $CD33^+$ cells, $CD14^+$ cells, $CD66b^+$ cells, or $CD11b^+$ human MDSC in the presence and in the absence of the candidate compound, wherein the relative reduction of T cell proliferation in the presence of the candidate compound indicates pro-suppressive activity.

[0056] Further provided is a method for identifying a candidate compound as a suppression antagonist comprising comparing suppression activity function of tumor cell line-educated- $CD33^+$ cells, $CD14^+$ cells, $CD66b^+$ cells, or $CD11b^+$ human MDSC in the presence and in the absence of said candidate compound, wherein the relative increase of T cell proliferation in the presence of said candidate compound indicates anti-suppressive activity.

[0057] Also provided herein are methods for treating a subject with cancer by evaluating a cell containing sample from the subject for the presence of myeloid-derived suppressor cells (MDSC), the MDSC being identified by their phenotype of any of $CD33^+HLA-DR^{low}HIF1\alpha^+/STAT3^+$, $CD14^+HLA-DR^{low}HIF1\alpha^+/STAT3^+/pSTAT3^+/C/EBPb^+$, $CD66b^+HLA-DR^{low}HIF1\alpha^+/STAT3^+/pSTAT3^+/C/EBPb^+$, $CD33^+HLA-DR^{low}HIF1\alpha^+/STAT3^+/pSTAT3^+/C/EBPb^+$, $CD11b^+HLA-DR^{low}HIF1\alpha^+/STAT3^+/pSTAT3^+/C/EBPb^+$, and $CD11b^+HLA-DR^{low}C/EBP\beta^+$ and if any of these phenotypes of MDSC is present, administering a cancer therapeutic. In addition, the success of the cancer treatment can be monitored by monitoring the increase or decrease of $CD33^+HLA-DR^{low}HIF1\alpha^+/STAT3^{++}$, $CD14^+HLA-DR^{low}HIF1\alpha^+/STAT3^+/pSTAT3^+/C/EBPb^+$, $CD66b^+HLA-DR^{low}HIF1\alpha^+/STAT3^+/pSTAT3^+/C/EBPb^+$, $CD33^+HLA-DR^{low}HIF1\alpha^+/$

STAT3⁺ / pSTAT3⁺ /C/EBPb⁺, CD11b⁺HLA-DR^{low} HIF1α⁺ / STAT3⁺ / pSTAT3⁺ /C/EBPb⁺, or CD11b⁺HLA-DR^{low}C/EBPβ⁺ after initiation of therapy.

[0058] Cancer therapeutics are known in the art and depend on the cancer being treated. Cancer therapeutics include any treatment regime typical for the cancer type. Typical cancer therapeutics include radiation therapy, chemotherapy, surgery, and transplantation. It is within the skill of an attending doctor to select the appropriate cancer treatment regime depending on the specific condition of the subject.

[0059] Suitable therapeutic agents include, but are not limited to chemotherapeutic compounds such as DNA alkylating agents, topoisomerase inhibitors, endoplasmic reticulum stress inducing agents, a platinum compound, an antimetabolite, vincalkaloids, taxanes, epothilones, enzyme inhibitors, receptor antagonists, therapeutic antibodies, tyrosine kinase inhibitors, antibiotics, boron radiosensitizers (i.e. velcade) and chemotherapeutic combination therapies. Non-limiting examples of chemotherapeutic agents and therapeutic agents are provided here. Chemical and biological equivalents of these agents are within the scope of this invention.

[0060] In one aspect of the invention, the anticancer drug is a DNA alkylating agent which attaches an alkyl group to DNA. Such agents are well known in the art and are used to treat a variety of tumors. Non-limiting examples of a DNA alkylating agents are Nitrogen mustards, such as Mechlorethamine, Cyclophosphamide (Ifosfamide, Trofosfamide), Chlorambucil (Melphalan, Prednimustine), Bendamustine, Uramustine and Estramustine; Nitrosoureas, such as Carmustine (BCNU), Lomustine (Semustine), Fotemustine, Nimustine, Ranimustine and Streptozocin; Alkyl sulfonates, such as Busulfan (Mannosulfan, Treosulfan); Aziridines, such as Carboquone, ThioTEPA, Triaziquone, Triethylenemelamine; Hydrazines (Procarbazine); Triazines such as Dacarbazine and Temozolomide; Altretamine and Mitobronitol.

[0061] In another aspect of the invention, the anticancer drug is a platinum based compound which is a subclass of DNA alkylating agents. Such agents are well known in the art and are used to treat a variety of cancers, such as, lung cancers, head and neck cancers, ovarian cancers, colorectal cancer and prostate cancer. Non-limiting examples of such agents include Carboplatin, Cisplatin, Nedaplatin, Oxaliplatin, Triplatin tetranitrate, Satraplatin, Aroplatin, Lobaplatin, and JM-216. (see McKeage et al. (1997) J. Clin. Oncol. 201:1232-1237 and in general, CHEMOTHERAPY FOR GYNECOLOGICAL

NEOPLASM, CURRENT THERAPY AND NOVEL APPROACHES, in the Series Basic and Clinical Oncology, Angioli et al. Eds., 2004).

[0062] “Oxaliplatin” (Eloxatin®) is a platinum-based chemotherapy drug in the same family as cisplatin and carboplatin. It is typically administered in combination with fluorouracil and leucovorin in a combination known as FOLFOX for the treatment of colorectal cancer. Compared to cisplatin the two amine groups are replaced by cyclohexyldiamine for improved antitumour activity. The chlorine ligands are replaced by the oxalato bidentate derived from oxalic acid in order to improve water solubility. Equivalents to Oxaliplatin are known in the art and include without limitation cisplatin, carboplatin, aroplatin, lobaplatin, nedaplatin, and JM-216 (see McKeage et al. (1997) J. Clin. Oncol. 201:1232-1237 and in general, CHEMOTHERAPY FOR GYNECOLOGICAL NEOPLASM, CURRENT THERAPY AND NOVEL APPROACHES, in the Series Basic and Clinical Oncology, Angioli et al. Eds., 2004).

[0063] In one aspect of the invention, the anticancer drug is a topoisomerase inhibitor which is an agent that interferes with the action of topoisomerase enzymes (topoisomerase I and II). Topoisomerases are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA. Such agents are well known in the art. Non-limiting examples of Topoisomerase I inhibitors include Camptothecine derivatives including CPT-11/Irinotecan, SN-38, APC, NPC, camptothecin, topotecan, exatecan mesylate, 9-nitrocamptothecin, 9-aminocamptothecin, lurtotecan, rubitecan, silatecan, gimatecan, diflomotecan, extatecan, BN-80927, DX-8951f, and MAG-CPT as described in Pommier (2006) Nat. Rev. Cancer 6(10):789-802 and U.S. Patent Appl. No. 2005/0250854; Protoberberine alkaloids and derivatives thereof including berberrubine and coralyn as described in Li et al. (2000) Biochemistry 39(24):7107-7116 and Gatto et al. (1996) Cancer Res. 56(12):2795-2800; Phenanthroline derivatives including Benzo[i]phenanthridine, Nitidine, and fagaronine as described in Makhey et al. (2003) Bioorg. Med. Chem. 11(8):1809-1820; Terbenzimidazole and derivatives thereof as described in Xu (1998) Biochemistry 37(10):3558-3566; and Anthracycline derivatives including Doxorubicin, Daunorubicin, and Mitoxantrone as described in Foglesong et al. (1992) Cancer Chemother. Pharmacol. 30(2):123-125, Crow et al. (1994) J. Med. Chem. 37(19):3191-3194, and (Crespi et al. (1986) Biochem. Biophys. Res. Commun. 136(2):521-8.

[0064] In one aspect of the invention, the topoisomerase I inhibitors can be selected from the group of, but not limited to, Camptothecine derivatives including CPT-11/Irinotecan, SN-38, APC, NPC, camptothecin, topotecan, exatecan mesylate, 9-nitrocamptothecin, 9-aminocamptothecin, lurtotecan, rubitecan, silatecan, gimatecan, diflomotecan, extatecan, BN-80927, DX-8951f, and MAG-CPT as described in Pommier (2006) *Nat. Rev. Cancer* 6(10):789-802 and US Patent Appl. No. 2005/0250854; Protoberberine alkaloids and derivatives thereof including berberrubine and coralyne as described in Li et al. (2000) *Biochemistry* 39(24):7107-7116 and Gatto et al. (1996) *Cancer Res.* 15(12):2795-2800; Phenanthroline derivatives including Benzo[i]phenanthridine, Nitidine, and fagaronine as described in Makhey et al. (2003) *Bioorg. Med. Chem.* 11(8):1809-1820; Terbenzimidazole and derivatives thereof as described in Xu (1998) *Biochemistry* 37(10):3558-3566; and Anthracycline derivatives including Doxorubicin, Daunorubicin, and Mitoxantrone as described in Foglesong et al. (1992) *Cancer Chemother. Pharmacol.* 30(2):123-125, Crow et al. (1994) *J. Med. Chem.* 37(19):3191-3194, and (Crespi et al. (1986) *Biochem. Biophys. Res. Commun.* 136(2):521-8, will be used in combination therapy with antibody based chemotherapy described above to treat patients identified with the appropriate genetic markers.

[0065] Irinotecan (CPT-11) is sold under the tradename of Camptosar®. It is a semi-synthetic analogue of the alkaloid camptothecin, which is activated by hydrolysis to SN-38 and targets topoisomerase I. Chemical equivalents are those that inhibit the interaction of topoisomerase I and DNA to form a catalytically active topoisomerase I-DNA complex. Chemical equivalents inhibit cell cycle progression at G2-M phase resulting in the disruption of cell proliferation.

[0066] In another aspect, some agents inhibit Topoisomerase II and have DNA intercalation activity such as, but not limited to, Anthracyclines (Aclarubicin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Amrubicin, Pirarubicin, Valrubicin, Zorubicin) and Antracenediones (Mitoxantrone and Pixantrone).

[0067] In one aspect of the invention, Topoisomerase II inhibitors include, but are not limited to Etoposide and Teniposide.

[0068] In another aspect of the invention, dual topoisomerase I and II inhibitors selected from the group of, but not limited to, Saintopin and other Naphthecenediones, DACA and other Acridine-4-Carboxamides, Intoplicine and other Benzopyridoindoles, TAS-103

and other 7H-indeno[2,1-c]Quinoline-7-ones, Pyrazoloacridine, XR 11576 and other Benzophenazines, XR 5944 and other Dimeric compounds, and Anthracenyl-amino Acid Conjugates as described in Denny and Baguley (2003) Curr. Top. Med. Chem. 3(3):339-353. In one aspect, they can be used in combination therapy with antibody based chemotherapy described above to treat patients identified with the appropriate genetic markers.

[0069] “Lapatinib” (Tykerb®) is an oncolytic dual EGFR and erbB-2 inhibitor. Lapatinib has been investigated as an anticancer monotherapy, as well as in combination with trastuzumab, capecitabine, letrozole, paclitaxel and FOLFIRI (irinotecan, 5-fluorouracil and leucovorin), in a number of clinical trials. It is currently in phase III testing for the oral treatment of metastatic breast, head and neck, lung, gastric, renal and bladder cancer.

[0070] A chemical equivalent of lapatinib is a small molecule or compound that is a tyrosine kinase inhibitor or alternatively a HER-1 inhibitor or a HER-2 inhibitor. Several TKIs have been found to have effective antitumor activity and have been approved or are in clinical trials. Examples of such include, but are not limited to Zactima (ZD6474), Iressa (gefitinib) and Tarceva (erlotinib), imatinib mesylate (STI571; Gleevec), erlotinib (OSI-1774; Tarceva), canertinib (CI 1033), semaxinib (SU5416), vatalanib (PTK787/ZK222584), sorafenib (BAY 43- 9006), sutent (SU11248) and leflunomide (SU101).

[0071] A biological equivalent of lapatinib is a peptide, antibody or antibody derivative thereof that is a HER-1 inhibitor and/or a HER-2 inhibitor. Examples of such include but are not limited to the humanized antibody trastuzumab and Herceptin.

[0072] In one aspect of the invention, the therapeutic agent is an endoplasmic reticulum stress inducing agent. Examples of such agents include, but are not limited to, Celecoxib, dimethyl-celecoxib and boron radiosensitizers (i.e. valcade (Bortezomib)).

[0073] In another aspect of the invention, the anticancer drug is an antimetabolite agent which inhibits the use of a metabolite, i.e. another chemical that is part of normal metabolism. In cancer treatment, antimetabolites interfere with DNA production, thus cell division and growth of the tumor. Non-limiting examples of these agents are Folic acid based, i.e. dihydrofolate reductase inhibitors, such as Aminopterin, Methotrexate and Pemetrexed; thymidylate synthase inhibitors, such as Raltitrexed, Pemetrexed; Purine

based, i.e. an adenosine deaminase inhibitor, such as Pentostatin, a thiopurine, such as Thioguanine and Mercaptopurine, a halogenated/ribonucleotide reductase inhibitor, such as Cladribine, Clofarabine, Fludarabine, or a guanine/guanosine: thiopurine, such as Thioguanine; or Pyrimidine based, i.e. cytosine/cytidine: hypomethylating agent, such as Azacitidine and Decitabine, a DNA polymerase inhibitor, such as Cytarabine, a ribonucleotide reductase inhibitor, such as Gemcitabine, or a thymine/thymidine: thymidylate synthase inhibitor, such as a Fluorouracil (5-FU).

[0074] Fluorouracil (5-FU) belongs to the family of therapy drugs call pyrimidine based anti-metabolites. 5-FU is transformed into different cytotoxic metabolites that are then incorporated into DNA and RNA thereby inducing cell cycle arrest and apoptosis. It is a pyrimidine analog, which is transformed into different cytotoxic metabolites that are then incorporated into DNA and RNA thereby inducing cell cycle arrest and apoptosis. Chemical equivalents are pyrimidine analogs which result in disruption of DNA replication. Chemical equivalents inhibit cell cycle progression at S phase resulting in the disruption of cell cycle and consequently apoptosis. Equivalents to 5-FU include prodrugs, analogs and derivative thereof such as 5'-deoxy-5-fluorouridine (doxifluoridine), 1-tetrahydrofuryl-5-fluorouracil (ftorafur), Capecitabine (Xeloda), S-1 (MBMS-247616, consisting of tegafur and two modulators, a 5-chloro-2,4-dihydroxypyridine and potassium oxonate), raltitrexed (tomudex), nolatrexed (Thymitaq, AG337), LY231514 and ZD9331, as described for example in Papamicheal (1999) The Oncologist 4:478-487.

[0075] Capecitabine and Tegafur are examples of chemical equivalents of 5-FU. It is a prodrug of (5-FU) that is converted to its active form by the tumor-specific enzyme PynPase following a pathway of three enzymatic steps and two intermediary metabolites, 5'-deoxy-5-fluorocytidine (5'-DFCR) and 5'-deoxy-5-fluorouridine (5'-DFUR). Capecitabine is marketed by Roche under the trade name Xeloda®.

[0076] Leucovorin (Folinic acid) is an adjuvant used in cancer therapy. It is used in synergistic combination with 5-FU to improve efficacy of the chemotherapeutic agent. Without being bound by theory, addition of Leucovorin is believed to enhance efficacy of 5-FU by inhibiting thymidylate synthase. It has been used as an antidote to protect normal cells from high doses of the anticancer drug methotrexate and to increase the antitumor effects of fluorouracil (5-FU) and tegafur-uracil. It is also known as citrovorum

factor and Wellcovorin. This compound has the chemical designation of L-Glutamic acid N[4[[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl] -, calcium salt (1:1).

[0077] Examples of vincalkaloids, include, but are not limited to vinblastine, Vincristine, Vinflunine, Vindesine and Vinorelbine.

[0078] Examples of taxanes include, but are not limited to docetaxel, Larotaxel, Ortataxel, Paclitaxel and Tesetaxel. An example of an epothilone is iabepilone.

[0079] Examples of enzyme inhibitors include, but are not limited to farnesyltransferase inhibitors (Tipifarnib); CDK inhibitor (Alvocidib, Seliciclib); Proteasome inhibitor (Bortezomib); Phosphodiesterase inhibitor (Anagrelide); IMP dehydrogenase inhibitor (Tiazofurine); and Lipoxigenase inhibitor (Masoprocol).

[0080] Examples of tyrosine kinase inhibitors include, but are not limited to ErbB: HER1/EGFR (Erlotinib, Gefitinib, Lapatinib, Vandetanib, Sunitinib, Neratinib); HER2/neu (Lapatinib, Neratinib); RTK class III: C-kit (Axitinib, Sunitinib, Sorafenib); FLT3 (Lestaurtinib); PDGFR (Axitinib, Sunitinib, Sorafenib); and VEGFR (Vandetanib, Semaxanib, Cediranib, Axitinib, Sorafenib); bcr-abl (Imatinib, Nilotinib, Dasatinib); Src (Bosutinib) and Janus kinase 2 (Lestaurtinib).

[0081] PTK/ZK is a "small" molecule tyrosine kinase inhibitor with broad specificity that targets all VEGF receptors (VEGFR), the platelet-derived growth factor (PDGF) receptor, c-KIT and c-Fms. Dreves (2003) *Idrugs* 6(8):787-794. PTK/ZK is a targeted drug that blocks angiogenesis and lymphangiogenesis by inhibiting the activity of all known receptors that bind VEGF including VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1) and VEGFR-3 (Flt-4). The chemical names of PTK/ZK are 1-[4-Chloroanilino]-4-[4-pyridylmethyl]phthalazine Succinate or 1-Phthalazinamine, N-(4-chlorophenyl)-4-(4-pyridinylmethyl)-, butanedioate (1:1). Synonyms and analogs of PTK/ZK are known as Vatalanib, CGP79787D, PTK787/ZK 222584, CGP-79787, DE-00268, PTK-787, PTK-787A, VEGFR-TK inhibitor, ZK 222584 and ZK.

[0082] Examples of chemotherapeutic agents and combination therapies include, but are not limited to amsacrine, Trabectedin, retinoids (Alitretinoin, Tretinoin), Arsenic trioxide, asparagine depleter (Asparaginase/Pegaspargase), Celecoxib, Demecolcine, Elesclomol,

Elsamitrucin, Etoposide, Lonidamine, Lucanthone, Mitoguanine, Mitotane, Oblimersen, Temsirolimus, and Vorinostat.

[0083] “FOLFOX” is an abbreviation for a type of combination therapy that is used to treat colorectal cancer. It includes 5-FU, oxaliplatin and leucovorin. Information regarding this treatment is available on the National Cancer Institute’s web site, cancer.gov, last accessed on January 16, 2008.

[0084] “FOLFOX/BV” is an abbreviation for a type of combination therapy that is used to treat colorectal cancer. This therapy includes 5-FU, oxaliplatin, leucovorin and Bevacizumab. Furthermore, “XELOX/BV” is another combination therapy used to treat colorectal cancer, which includes the prodrug to 5-FU, known as Capecitabine (Xeloda) in combination with oxaliplatin and bevacizumab. Information regarding these treatments are available on the National Cancer Institute’s web site, cancer.gov or from the National Comprehensive Cancer Network’s web site, nccn.org, last accessed on May 27, 2008.

[0085] Examples of therapeutic antibodies include, but are not limited to anti-HER1/EGFR (Cetuximab, Panitumumab); Anti-HER2/neu (erbB2) receptor (Trastuzumab); Anti-EpCAM (Catumaxomab, Edrecolomab) Anti-VEGF-A (Bevacizumab); Anti-CD20 (Rituximab, Tositumomab, Ibritumomab); Anti-CD52 (Alemtuzumab); and Anti-CD33 (Gemtuzumab), as well as biological equivalents thereof.

[0086] Bevacizumab is sold under the trade name Avastin by Genentech. It is a humanized monoclonal antibody that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF). Biological equivalent antibodies are identified herein as modified antibodies and those which bind to the same epitope of the antigen, prevent the interaction of VEGF to its receptors (Flt01, KDR a.k.a. VEGFR2) and produce a substantially equivalent response, e.g., the blocking of endothelial cell proliferation and angiogenesis.

[0087] In one aspect, the “chemical equivalent” means the ability of the chemical to selectively interact with its target protein, DNA, RNA or fragment thereof as measured by the inactivation of the target protein, incorporation of the chemical into the DNA or RNA or other suitable methods. Chemical equivalents include, but are not limited to, those agents with the same or similar biological activity and include, without limitation a pharmaceutically acceptable salt or mixtures thereof that interact with and/or inactivate the same target protein, DNA, or RNA as the reference chemical.

[0088] In one aspect, the “biological equivalent” means the ability of the antibody to selectively bind its epitope protein or fragment thereof as measured by ELISA or other suitable methods. Biologically equivalent antibodies include, but are not limited to, those antibodies, peptides, antibody fragments, antibody variant, antibody derivative and antibody mimetics that bind to the same epitope as the reference antibody. An example of an equivalent Bevacizumab antibody is one which binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF).

Formulations

[0089] The pharmaceutical compositions can be administered by any one of the following routes: ocular, oral, systemic (e.g., transdermal, intranasal or by suppository), or parenteral (e.g., intramuscular, intravenous or subcutaneous) administration. In some embodiments, the manner of administration is oral using a convenient daily dosage regimen that can be adjusted according to the degree of affliction. Compositions can take the form of tablets, pills, capsules, semisolids, powders, sustained release formulations, solutions, suspensions, elixirs, aerosols, or any other appropriate compositions. Another manner for administering compounds of described herein is inhalation.

[0090] The choice of formulation depends on various factors such as the mode of drug administration and bioavailability of the drug substance. For delivery via inhalation the compound can be formulated as liquid solution, suspensions, aerosol propellants or dry powder and loaded into a suitable dispenser for administration. There are several types of pharmaceutical inhalation devices-nebulizer inhalers, metered dose inhalers (MDI), mouth mask and dry powder inhalers (DPI). Nebulizer devices produce a stream of high velocity air that causes the therapeutic agents (which are formulated in a liquid form) to spray as a mist that is carried into the patient’s respiratory tract. MDI’s typically are formulation packaged with a compressed gas. Upon actuation, the device discharges a measured amount of therapeutic agent by compressed gas, thus affording a reliable method of administering a set amount of agent. DPI can dispense therapeutic agents in the form of a free flowing powder that can be dispersed in the patient’s inspiratory air-stream during breathing by the device. In order to achieve a free flowing powder, the therapeutic agent is formulated with an excipient such as lactose. A measured amount of the therapeutic agent is stored in a capsule form and is dispensed with each actuation.

[0091] Recently, pharmaceutical formulations have been developed especially for drugs that show poor bioavailability based upon the principle that bioavailability can be increased by increasing the surface area i.e., decreasing particle size. For example, U.S. Patent No. 4,107,288 describes a pharmaceutical formulation having particles in the size range from 10 to 1,000 nm in which the active material is supported on a crosslinked matrix of macromolecules. U.S. Patent No. 5,145,684 describes the production of a pharmaceutical formulation in which the drug substance is pulverized to nanoparticles (average particle size of 400 nm) in the presence of a surface modifier and then dispersed in a liquid medium to give a pharmaceutical formulation that exhibits remarkably high bioavailability.

[0092] For ocular administration, the compositions described herein can be formulated as a solution, emulsion, suspension, etc., suitable for administration to the eye. A variety of vehicles suitable for administering compounds to the eye are known in the art. Specific non-limiting examples are described in U.S. Patent No. 6,261,547; U.S. Patent No. 6,197,934; U.S. Patent No. 6,056,950; U.S. Patent No. 5,800,807; U.S. Patent No. 5,776,445; U.S. Patent No. 5,698,219; U.S. Patent No. 5,521,222; U.S. Patent No. 5,403,841; U.S. Patent No. 5,077,033; U.S. Patent No. 4,882,150; and U.S. Patent No. 4,738,851.

[0093] The compositions can additionally contain solid pharmaceutical excipients such as starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk and the like. Liquid and semisolid excipients may be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, etc. Liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols.

[0094] Compressed gases may be used to disperse a composition described herein in aerosol form. Inert gases suitable for this purpose are nitrogen, carbon dioxide, etc. Other suitable pharmaceutical excipients and their formulations are described in Remington's Pharmaceutical Sciences, edited by E. W. Martin (Mack Publishing Company, 18th ed., 1990).

[0095] The amount of the composition in a formulation can vary within the full range employed by those skilled in the art. Typically, the formulation will contain, on a volume percent (v/v %) basis, from about 0.01-99.99 v/v% of a composition described herein based on the total formulation, with the balance being one or more suitable pharmaceutical excipients. In some embodiments, the composition is present at a level of about 1-80 v/v %.

[0096] Thus, the composition of this invention can be formulated for administration intranasally as a spray or in a drop; transdermally via a transdermal patch or iontophoresis and by inhalation using a nebulizer, MDI or similar device. As such, the compositions can further comprise, or alternatively consist essentially of, or yet further consist of, a permeation or penetration enhancer, a plasticizer, or the like making the composition suitable for transdermal administration. In one aspect, the invention is a transdermal reservoir having within it an effective amount of a composition of this invention for transdermal administration of the composition. In a further aspect, the invention provides a transdermal device containing the transdermal reservoir. The transdermal reservoir and/or device can be used to administer an effective amount of the composition of this invention to a subject in need of treatment. These devices are suitable to administer pain medications such as analgesics and narcotics. Examples of these therapeutic agents are provided supra.

[0097] This invention also provides the compositions as described above for intranasal administration. As such, the compositions can further comprise, or alternatively consist essentially of, or yet further consist of, a permeation or penetration enhancer or the like making the composition suitable for intranasal administration. In one aspect, the invention is an intranasal formulation having within it an effective amount of a composition of this invention for intranasal administration of the composition. These formulations are suitable to cancer drugs or drugs to treat neurological disorders. Examples of these drugs are provided below.

[0098] This invention also provides the compositions as described above for administration by inhalation. As such, the compositions can further comprise, or alternatively consist essentially of, or yet further consist of, a permeation or penetration enhancer or the like making the composition suitable for administration by inhalation. In one aspect, the invention is formulation for administration by inhalation having within it

an effective amount of a composition of this invention for inhalation of the composition. These formulations are suitable to administer cancer drugs or drugs to treat neurological disorders. Examples of these drugs are provided supra.

[0099] When practiced in vivo in a subject other than a human patient such as a mouse, the method provides an animal model for use in discovering alternative agents, compositions and therapies. In a human patient, the method treats pathologies as described above or as characterized by hyperproliferative cells, e.g., cancer. Methods for detecting clinical and sub-clinical evidence of effective therapy are known in the art and described in U.S. Patent Appl. No. 2004/0087651, (published May 6, 2004), Balassiano et al. (2002) Intern. J. Mol. Med. 10:785-788; Thorne et al. (2004) Neuroscience 127:481-496; Fernandes et al. (2005) Oncology Reports 13:943-947; da Fonseca et al. (2006) 66:611-615; da Fonseca et al. (2008) Surgical Neurology 70:259-267; da Fonseca et al. (2008) Arch. Immunol. Ther. Exp. 56:267-276 and Hashizume et al. (2008) Neuroncology 10:112-120, each incorporated by reference. In each of these methods, an effective amount of a composition of this invention is delivered or administered to the subject, e.g., mouse or human patient.

[0100] This invention also provides a method for treating a disease in a subject in need of such treatment comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the subject an effective amount of the composition as described herein to the subject, thereby treating the disease.

[0101] The compositions can be administered to an animal or mammal by a treating veterinarian.

[0102] Also provided herein are methods for determining a prognosis of a subject with cancer by evaluating a cell containing sample from the subject for the presence of myeloid-derived suppressor cells (MDSC), the MDSC being identified by their phenotype of any of CD33⁺HLA-DR^{low}HIF1 α ⁺/STAT3⁺, CD14⁺HLA-DR^{low} HIF1 α ⁺/ STAT3⁺ / pSTAT3⁺ /C/EBP β ⁺, CD66b⁺HLA-DR^{low} HIF1 α ⁺/ STAT3⁺ / pSTAT3⁺ /C/EBP β ⁺, CD33⁺HLA-DR^{low} HIF1 α ⁺/ STAT3⁺ / pSTAT3⁺ /C/EBP β ⁺, CD11b⁺HLA-DR^{low} HIF1 α ⁺/ STAT3⁺ / pSTAT3⁺ /C/EBP β ⁺, and CD11b⁺HLA-DR^{low}C/EBP β ⁺ and if any of these phenotypes of MDSC is present, altering treatment. Prognosis includes determining overall survival, early disease recurrence, and treatment failure.

[0103] The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits, such as those described below, comprising at least one probe or primer nucleic acid described herein, which may be conveniently used, e.g., to determine whether a subject is likely to experience tumor recurrence following therapy as described herein or has or is at risk of developing disease such as colon cancer.

[0104] Diagnostic procedures can also be performed in situ directly upon tissue sections (fixed and/or frozen) of primary tissue such as biopsies obtained from biopsies or resections, such that no nucleic acid purification is necessary. Nucleic acid reagents can be used as probes and/or primers for such in situ procedures (see, for example, Nuovo, G. J. (1992) PCR IN SITU HYBRIDIZATION: PROTOCOLS AND APPLICATIONS, RAVEN PRESS, NY).

[0105] In addition to methods which focus primarily on the detection of one nucleic acid sequence, profiles can also be assessed in such detection schemes. Fingerprint profiles can be generated, for example, by utilizing a differential display procedure, Northern analysis and/or RT-PCR.

[0106] Probes can be affixed to surfaces for use as “gene chips.” Such gene chips can be used to detect genetic variations by a number of techniques known to one of skill in the art. In one technique, oligonucleotides are arrayed on a gene chip for determining the DNA sequence of a by the sequencing by hybridization approach, such as that outlined in U.S. Patent Nos. 6,025,136 and 6,018,041. The probes of the disclosure also can be used for fluorescent detection of a genetic sequence. Such techniques have been described, for example, in U.S. Patent Nos. 5,968,740 and 5,858,659. A probe also can be affixed to an electrode surface for the electrochemical detection of nucleic acid sequences such as described by Kayem et al. U.S. Patent No. 5,952,172 and by Kelley et al. (1999) Nucleic Acids Res. 27:4830-4837.

[0107] This disclosure also provides for a panel of genetic markers selected from, but not limited to the probes and/or primers to determine gene expression as identified herein. The probes or primers can be attached or supported by a solid phase support such as, but not limited to a gene chip or microarray. The probes or primers can be detectably labeled.

[0108] In one aspect, the panel contains the herein identified probes or primers as wells as other probes or primers. In a alternative aspect, the panel includes one or more of the

above noted probes or primers and others. In a further aspect, the panel consist only of the above-noted probes or primers.

[0109] Primers or probes can be affixed to surfaces for use as “gene chips” or “microarray.” Such gene chips or microarrays can be used to detect genetic variations by a number of techniques known to one of skill in the art. In one technique, oligonucleotides are arrayed on a gene chip for determining the DNA sequence of a by the sequencing by hybridization approach, such as that outlined in U.S. Patent Nos. 6,025,136 and 6,018,041. The probes of the disclosure also can be used for fluorescent detection of a genetic sequence. Such techniques have been described, for example, in U.S. Patent Nos. 5,968,740 and 5,858,659. A probe also can be affixed to an electrode surface for the electrochemical detection of nucleic acid sequences such as described by Kayem et al. U.S. Patent No. 5,952,172 and by Kelley et al. (1999) *Nucleic Acids Res.* 27:4830-4837.

[0110] Various “gene chips” or “microarray” and similar technologies are know in the art. Examples of such include, but are not limited to LabCard (ACLARA Bio Sciences Inc.); GeneChip (Affymetric, Inc); LabChip (Caliper Technologies Corp); a low-density array with electrochemical sensing (Clinical Micro Sensors); LabCD System (Gamera Bioscience Corp.); Omni Grid (Gene Machines); Q Array (Genetix Ltd.); a high-throughput, automated mass spectrometry systems with liquid-phase expression technology (Gene Trace Systems, Inc.); a thermal jet spotting system (Hewlett Packard Company); Hyseq HyChip (Hyseq, Inc.); BeadArray (Illumina, Inc.); GEM (Incyte Microarray Systems); a high-throughput microarraying system that can dispense from 12 to 64 spots onto multiple glass slides (Intelligent Bio-Instruments); Molecular Biology Workstation and NanoChip (Nanogen, Inc.); a microfluidic glass chip (Orchid biosciences, Inc.); BioChip Arrayer with four PiezoTip piezoelectric drop-on-demand tips (Packard Instruments, Inc.); FlexJet (Rosetta Inpharmatic, Inc.); MALDI-TOF mass spectrometer (Sequenome); ChipMaker 2 and ChipMaker 3 (TeleChem International, Inc.); and GenoSensor (Vysis, Inc.) as identified and described in Heller (2002) *Annu. Rev. Biomed. Eng.* 4:129-153. Examples of “Gene chips” or a “microarray” are also described in U.S. Patent Publ. Nos.: 2007/0111322, 2007/0099198, 2007/0084997, 2007/0059769 and 2007/0059765 and US Patent 7,138,506, 7,070,740, and 6,989,267.

[0111] In one aspect, “gene chips” or “microarrays” containing probes or primers for the gene of interest are provided alone or in combination with other probes and/or primers. A suitable sample is obtained from the patient extraction of genomic DNA, RNA, or any combination thereof and amplified if necessary. The DNA or RNA sample is contacted to the gene chip or microarray panel under conditions suitable for hybridization of the gene(s) of interest to the probe(s) or primer(s) contained on the gene chip or microarray. The probes or primers may be detectably labeled thereby identifying the polymorphism in the gene(s) of interest. Alternatively, a chemical or biological reaction may be used to identify the probes or primers which hybridized with the DNA or RNA of the gene(s) of interest. The genetic profile of the patient is then determined with the aid of the aforementioned apparatus and methods.

EXAMPLES

MATERIALS AND METHODS

Cell Lines and Cell Culture

[0112] Tumor cell lines were obtained from the American Type Culture Collection (ATCC) or gifted to the Epstein laboratory. Tumor cell line authenticity was performed by cytogenetics and surface marker analysis performed at ATCC or in our laboratory. All cell lines were maintained at 37°C in complete medium [(RPMI-1640 with 10% fetal calf serum (characterized FCS, Hyclone, Inc., Logan, UT), 2mM L-Glutamine, 100U/mL Penicillin, and 100µg/mL Streptomycin)] in tissue culture flasks in humidified, 5% CO₂ incubators and passaged 2-3 times per week by light trypsinization.

Tumor Associated MDSC Generation Protocol

i. Induction

[0113] Human PBMC were isolated from healthy volunteer donors by venipuncture (60mL total volume) followed by differential density gradient centrifugation (Ficoll Hypaque, Sigma, St. Louis, MO). PBMC were cultured in complete medium (6×10^5 cells/mL, supplemented with rhGM-CSF (10ng/mL, R&D Systems, Minneapolis, MN) to support viability) in T-25 culture flasks with human tumor cell lines for one week. Tumor cells were seeded to achieve confluence by day 7 (approximately 1:100 ratio with PBMC), and samples in which tumor cells overgrew were excluded from analysis and

were repeated with adjusted ratios. Alternatively, irradiated tumor cells (3500 rad) were initially seeded at 1:10 ratio in co-cultures to examine whether induction was dependent upon actively dividing tumor cells. PBMC cultured in medium alone were run in parallel as an induction negative control for each donor to control for any effects of FCS. For neutralization experiments, PBMC-tumor cell line co-cultures were repeated in the presence or absence of neutralizing monoclonal antibodies for a subset of HNSCC cell lines: anti-VEGF (Avastin, Genetech, San Francisco, CA), anti-TNF α (Humira, Abbott, Abbott Park, IL), anti-IL-1 β (clone AB-206-NA, Abcam, Cambridge, MA), anti-IL-6 (clone AB-201-NA, Abcam), or anti-GM-CSF (clone BVD2). CD33⁺ or CD11b⁺ cells were subsequently isolated and tested for suppressive function as described below. For these studies 61 healthy volunteer donors (39 male, 22 female) ages 20 to 62 were used under USC Institutional Review Board-approved protocol HS-06-00579. Data were derived from at least two individuals and no inter-donor differences in MDSC induction or function were observed.

ii. MDSC Isolation

[0114] After one week, all cells were collected from tumor-PBMC co-cultures. Adherent cells were removed using the non-protease cell detachment solution Detachin (GenLantis, San Diego, CA). Myeloid (CD33⁺) cells were then isolated from the co-cultures using anti-CD33 magnetic microbeads and LS column separation (Miltenyi Biotec, Germany) as per manufacturer's instructions. Purity of isolated cell populations was found to be greater than 90% by flow cytometry and morphological examination. Viability of isolated cells was confirmed using trypan blue dye exclusion and samples with viability less than 80% were excluded from analysis and repeated in subsequent experiments. Yields of CD33⁺ cells from co-cultures averaged 8.75% of PBMC (range 6-12%), while yields from medium only cultures averaged 10%. For isolation of CD11b⁺ suppressor cells from breast carcinoma co-cultures, anti-CD11b microbeads were used instead of anti-CD33 microbeads.

iii. Suppression Assay

[0115] The suppressive function of tumor-educated CD33⁺ or CD11b⁺ cells was measured by their ability to inhibit the proliferation of autologous T cells in the following Suppression Assay: CD8⁺ T cells isolated from 30mL of PBMC from returning

healthy donors were CFSE-labeled (3 μ M, Sigma) and seeded in 96-well plates at 2x10⁵ cells/well. CD33⁺ cells isolated previously (*ii. MDSC isolation*, above) were added to the 96-well plates at a ratio of 1:4 relative to the T cells. T cell stimulation was provided by anti-CD3/CD28 stimulation beads (Invitrogen, Carlsbad, CA). Suppression Assay wells were analyzed by flow cytometry for T cell proliferation after three days and supernatants were analyzed for IFN γ levels (below). Controls included a positive T cell proliferation control (T cells alone) and an induction negative control of CD33⁺ or CD11b⁺ cells isolated from PBMC cultured in medium only. Samples were run in duplicate and data were collected as percent proliferation for 15,000 cells. Samples were run on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) and data acquisition and analysis were performed using CellQuestPro software (BD) at the USC Flow Cytometry core facility.

Characterization of myeloid suppressor cells

i. Morphology of MDSC

[0116] Wright-Giemsa staining (Protocol Hema 3, Fisher, Kalamazoo, MI) of CD33⁺ or CD11b⁺ cell cytospin preparations was performed to assess the morphology of tumor-educated myeloid cells. Freshly isolated PBMC and CD33⁺ (or CD11b⁺) cultured in medium only or induced by cytokines GM-CSF + IL-6 (26) were prepared in parallel for comparison. Observation, evaluation, and image acquisition were performed using a Leica DM2500 microscope (Leica Microsystems, www.leica-microsystems.com) connected to an automated, digital SPOT RTke camera and SPOT Advanced Software (SPOT Diagnostic Instrument Inc., www.diaginc.com). Images were resized, brightened, and adjusted for color for publication using Adobe Photoshop software (Adobe, www.adobe.com).

ii. Flow cytometry analyses of cell phenotypes

[0117] The phenotype of *in vitro*-generated MDSC was examined for expression of myeloid, antigen-presenting, and suppressor cell markers and compared to non-suppressive tumor-educated CD33⁺ or CD11b⁺ cells. For staining, cells were collected from flasks using Detachin to minimize cell surface protein digestion, and washed twice with FACS buffer (2% FCS in PBS) before resuspending 10⁶ cells in 100 μ l FACS buffer. Cells were stained for 1 hr on ice with cocktails of fluorescently-conjugated monoclonal

antibodies or isotype-matched controls, washed twice with FACS buffer, and resuspended in FACS buffer for analysis. For intracellular staining, cells were fixed and permeabilized using Fixation/Permeabilization Kit (eBioscience, San Diego, CA) after surface staining. Antibodies used were purchased either from BD Biosciences: CD11c (B-ly6), CD33 (HIM3-4), HLA-DR (L243), CD11b (ICRF44), CD66b (G10F5), CD14 (M5E2), CD68 (Y1/82A), 41 BBL (C65-485), OX40L (Ik-1); or eBioscience (San Diego, CA): CD30 (Ber-H2), CD103 (B-Ly7), GITRL (eBioAITR-L), CD56 (MEM-188). Samples were run on a BD FACSCalibur flow cytometer and data acquisition and analysis were performed as above. Data are shown from three unique donors and expressed as a fraction of labeled cells within a live-cell gate set for 15,000 events. CD33⁺ or CD11b⁺ cells from PBMC cultured in medium alone were run in parallel for comparison.

iii. Real-time RT-PCR for gene expression of myeloid suppressor cells and tumor cell lines

[0118] For gene expression studies, tumor-educated CD33⁺ or CD11b⁺ cells were isolated from tumor-PBMC co-cultures by fluorescence activated cell sorting after induction (*i. Induction*, above) and RNA was isolated from MDSC and DNase-treated using Qiagen's RNeasy micro kit (Valencia, CA). RNA was isolated from cultured tumor cells and DNase-treated using Qiagen RNeasy mini kit. For real-time RT-PCR, 100ng of DNase-treated RNA was amplified with gene specific primers using one-step Power SYBR green RNA-to-Ct kit (Applied Biosystems) and run in an MX3000P Stratagene thermocycler (La Jolla, CA). Data were acquired and analyzed using MxPro software (Stratagene). Gene expression was normalized to housekeeping gene GAPDH and fold change determined relative to expression levels in medium only-cultured cells. Primer sequences were obtained from the NIH qRT-PCR database (<http://primerdepot.nci.nih.gov>) and were synthesized by the USC Core Facility (29).

iv. Immunohistochemistry

[0119] Immunohistochemistry studies were performed on cytospin preparations of suppressive and non-suppressive myeloid cells using antibodies against p-STAT3 (clone 6D779, dilution 1:400) and C/EBP β (clone H-7, dilution 1:100) (Santa Cruz Biotech).

v. Measurement of tumor-derived factors by ELISA

[0120] Supernatants were collected from cell line cultures, passed through a 0.2µm syringe filter unit to remove cell debris, and stored in aliquots at -20°C. Levels of IL-1β, IL-6, TNFα, VEGF and GM-CSF in supernatant samples were measured using ELISA DuoSet kits (R&D) per manufacturer's instructions. Plate absorbance was read on an ELX-800 plate reader (Bio-Tek, Winooski, VT) and analyzed using KC Junior software (Bio-Tek).

vi. Statistical analysis

[0121] Changes in mean T cell proliferation and mean IFNγ production in the presence or absence of tumor-educated MDSC were tested for statistical significance by one-way ANOVA followed by Dunnett test for pairwise comparisons of experimental samples to T cells alone. Changes in mean T cell proliferation in suppression assays in the presence or absence of single inhibitors of suppressive mechanisms were evaluated by ANOVA followed by Tukey's test for pairwise comparisons between all groups. Differences in mean expression of phenotypic markers between pooled groups of suppressive and non-suppressive CD33⁺ or CD11b⁺ cells were tested for significance by Student's t test for independent samples. Differences in mean transcription factor or suppressive gene expression between CD11b⁺ and CD33⁺ MDSC were tested for significance by Student's t test. Statistical tests were performed using GraphPad Prism software (La Jolla, CA) with a significance level of 0.05. Graphs and figures were produced using GraphPad Prism, Microsoft Excel, and Adobe Illustrator and Photoshop software (San Jose, CA).

RESULTS***Induction of tumor-associated human myeloid suppressor cells***

[0122] A protocol for the generation of tumor cell line educated-CD33⁺ human MDSC from normal donor PBMC was developed (FIG. 1). As provided in detail above, PBMC-tumor cell line co-cultures were established in tissue culture flasks for one week. Tumor-educated CD33⁺ cells were then isolated, checked for viability, and tested for suppressive function by co-culture with fresh, autologous CD8⁺ T cells in the presence of T cell stimuli. Controls run for each donor in these studies included T cells with CD33⁺ from PBMC cultured in medium alone (induction negative control) and T cells alone (proliferation positive control). Use of irradiated tumor cells in co-cultures yielded

comparable suppressor cell induction, suggesting that tumor cells need not be actively dividing to mediate the observed induction of suppressive function. Unfractionated PBMC preparations were used in evaluating the ability of human solid tumor cell lines to generate myeloid suppressor cells to best approximate an *in vivo* setting, but CD33⁺ suppressor cells were also generated successfully from T cell-depleted PBMC by co-culture with 4-998 osteogenic sarcoma or SCCL-MT1 head and neck squamous cell carcinoma (HNSCC) cells. The same protocol was used for the generation of tumor cell line educated-CD11b⁺ human MDSC from normal donor PBMC.

Identification of a subset of human tumor cell lines with strong myeloid suppressor cell induction capability

[0123] One-hundred-one human solid tumor cell lines were tested for their ability to induce MDSC using the tumor co-culture assay. MDSC could be generated by at least one cell line of every human tumor type examined (cervical/endometrial, ovarian, pancreatic, lung, head and neck, renal cell, liver, colorectal, prostate, thyroid, gastric, bladder, sarcoma, and glioblastoma), with the exception of breast carcinoma (Tables 1). CD33⁺ cells from tumor co-cultures were categorized as strong MDSC (ability to suppress autologous T cell proliferation by greater than 50%), weak MDSC (50-20%, meeting statistical significance), or non-suppressive myeloid cells ($n \geq 2$ biologic samples). Of 101 tumor cell lines examined, 45 consistently generated MDSC with 30 of those generating strongly suppressive MDSC. A range of suppressor cell ability appeared to exist within histologic types for the majority of tumor cell lines examined, with the notable exception that no human breast cancer cell lines (0/9) tested generated suppressive CD33⁺ cells.

TABLE 1

Inducing Tumor Cell Line	Mean Percent Suppression	SEM
Controls		
T cells alone	0.00	
Medium only	-2.35	0.86
Fetal Lung Fibroblasts	-1.03	0.96
Ditt Fibroblasts	-0.13	2.91
**GM-CSF + IL-6	56.30	5.01
HNSCC (6/8)		
**SCCL-MT1	91.83	0.82
**Irradiated	89.18	0.20
**Tcell Depl.	81.49	4.98

**USC-HN2	87.97	ND
**SCC-4	65.72	2.08
**CAL-27	66.26	6.21
**SW 451	59.49	9.59
*FaDu	30.98	4.45
RPMI 2650	17.46	5.01
SW 2224	-13.48	11.21
Thyroid (1/2)		
**SW 579	68.97	3.41
SW 1949	43.90	13.68
Brain (2/9)		
**NU-04	69.41	4.02
**U118MG	51.96	1.48
SW 598	14.29	4.14
A172	2.26	4.97
IMR-5	-1.23	3.09
IMR-32	-3.16	7.48
TE 671	-12.23	4.29
Y79	-72.63	5.58
BM-166	-83.22	0.05
Melanoma (1/3)		
**A375	56.16	0.64
CaC174-36	17.26	6.83
Colo 38	15.83	1.49
Cervical/Endometrial (4/5)		
**HeLa	68.35	5.36
**ME-180	75.24	3.83
**SIHA	54.49	8.66
**RL95-2	52.11	3.84
SW 756	-83.60	2.18
Ovarian (6/9)		
**A2780	64.46	5.33
**ES-2	63.62	5.17
**TOV-21G	52.86	11.37
**SK-OV-3	51.44	9.81
*NIHOVCAR-3	47.89	1.08
*SW 626	46.54	4.07
HOC-7	41.77	19.15
HEY	22.20	3.87
Caov-3	-146.53	2.69
Breast (0/9)		
MCF-7	16.95	0.39
734B	16.72	2.32
T47D	8.47	1.23
BT-474	0.83	11.53
SKBR3	-0.09	13.53
MDA-MB-468	-3.46	0.25
GI-101	-6.41	0.92

SV-BR-1	-8.00	1.75
MDA231	-16.21	2.60
Bladder (1/3)		
**T24	53.89	3.97
SW 780	8.10	10.01
SW 733	-54.63	0.45
Sarcomas (4/9)		
**4-998	58.31	0.82
** <i>Irradiated</i>	52.10	0.44
** <i>Tcell Depl</i>	65.23	8.17
*Rh30	44.63	2.51
*HOS	42.58	4.86
*SW 1353	42.22	4.42
HT 1080	19.37	5.92
SA-4	12.53	1.05
HS 919	3.01	5.31
SW 80	-5.00	3.93
HS 913T	-56.35	1.45
Lung		
Non-small cell (2/7)		
*A427	27.71	6.87
*SW 1573	21.47	1.64
H292	8.23	2.89
NCI-H1650	6.67	4.18
SK-MES-1	4.31	6.03
125	1.54	3.69
NCI-H1975	0.28	2.89
Small Cell (2/3)		
**464	63.96	6.00
*60	47.79	7.71
HUT69C	-24.28	16.75
Mesothelioma (0/1)		
SW 1503	1.93	2.02
Liver (2/5)		
*HA 22T	44.01	4.22
*HEP 3B	23.52	7.44
PLC	22.05	5.22
MAH	11.87	4.72
HEP-G2	-2.10	9.40
Renal (3/6)		
**786-O	75.91	6.06
**CAKI-1	64.94	3.70
**CAKI-2	63.62	5.17
SW 156	36.51	10.69
ACHN	9.85	0.20
SK-NEP-1	0.00	1.82
Prostate (2/3)		
**DU 145	54.73	2.07
*LNCaP	29.09	2.78

PC3	15.12	9.09
Gastric (1/2)		
**SW 1961	64.55	3.04
KATO-III	7.65	2.16
Colorectal (5/6)		
**SW 732	69.19	1.29
**DLD-1	65.59	3.19
**SW 608	53.11	5.15
**SW 707	52.38	0.64
HT-29	38.37	4.91
LS147T	13.62	3.87
Pancreatic (3/10)		
**SW 1990	78.15	1.21
*Panc 2.03	22.28	4.37
*Panc 4.14	21.82	2.60
Panc 9.6.94	27.28	8.47
Panc-1	7.82	3.69
Panc 3.27	6.98	5.44
ASPC-1	3.09	2.36
CAPAN-1	-1.34	2.27
Panc 2.5	-1.79	6.08
MIA PaCa-2	-4.38	1.94
Epidermoid (0/1)		
A431	-31.12	8.55

*Tumor cell line generating MDSC with statistically significant mean suppression of autologous T cells ($p < 0.05$)

**Tumor cell line generating MDSC with strong suppression of T cells (mean suppression $\geq 50\%$)

[0124] Table 1 shows that forty-five of 100 human solid tumor cell lines induce functionally suppressive CD33⁺ myeloid suppressor cells from volunteer normal human PBMC after one-week co-culture *in vitro*. Shown in table 1 are results for Control, HNSCC, Thyroid, Brain, Melanoma, Cervical/Endometrial, Ovarian, Breast, and Bladder cell types, Sarcomas, Lung Non-small cell, Small Cell, Mesothelioma, Liver, Renal, Prostate, Gastric, Colorectal, Pancreatic, and Epidermoid cell types. Tumor cell lines inducing CD33⁺ with statistically significant suppressive function are indicated with the symbol “*”, and those with strong MDSC inducing capacity (mean T cell suppression by CD33⁺ cells $\geq 50\%$) are indicated by “***”. CD33⁺ cells from PBMC cultured in complete medium alone (non-suppressive control) and cytokine-induced MDSC (GM-CSF + IL-6, suppressive control) were run in parallel for comparison.

MDSC-induction capacity correlates with tumor cell line expression of IL-16, IL-6, TNF α , VEGF, and GM-CSF

[0125] To elucidate the potential mechanisms underlying the observed differences in myeloid suppressor cell induction by tumor cell lines, the expression of previously reported MDSC-inducing immune modulatory factors (c-kitL, COX2, FLT3L, GM-CSF, IL-1 β , IL-4, IL-6, IL-10, IDO, iNOS, M-CSF, TGF β , TNF α , VEGF) was examined for a group of eight HNSCC cell lines using quantitative RT-PCR techniques (18,24,26). CD33⁺ MDSC-induction capacity correlated directly with tumor cell line expression of IL-1 β , IL-6, TNF α , VEGF, and GM-CSF (Table 2). Differential gene expression of IL-6, TNF α , VEGF, and GM-CSF was confirmed at the protein level by ELISA techniques (FIG. 2A-D), but IL-1 β levels were below the sensitivity of the assay.

Table 2

	Average % Suppression by induced MDSC		Tumor-derived factors				
			IL-1 β	IL-6	TNF α	VEGF	GM-CSF
MDSC-Induction Capacity ↑	92	SCCL-MT1	362.791	10.963	0.639	0.355	0.018
	88	USC-HN2	295.583	7.818	4.040	0.798	0.506
	66	SCC-4	136.442	4.887	4.263	0.100	0.063
	66	CAL-27	100.526	10.257	3.104	0.536	0.014
	59	SW 451	1.935	4.253	0.979	0.780	0.295
	31	FaDu	38.225	0.535	0.036	0.190	0.002
	17	RPMI 2650	0.165	0.017	0.104	0.148	0.010
	-13	SW 2224	0.009	3.442	0.208	0.088	0.026
p-value			0.0001	0.0032	0.0138	0.0008	0.0472

[0126] Expression of ten putative MDSC-inducing factors was measured in MDSC-inducing and non-inducing head and neck squamous cell carcinoma (HNSCC) cell lines by qRT-PCR. As shown in Table 2, increased MDSC-induction capacity was associated with greater expression of IL-1 β , IL-6, TNF α , and VEGF ($p < 0.05$). [Mean fold change ($n=2$) relative to reference RNA (gray = increased = fold change >1 , white = decreased expression = fold change <1).]

Suppressive CD33⁺ myeloid cells inhibit both CD8⁺ T cell proliferation and IFN γ production

[0127] To characterize further CD33⁺ suppressor cells generated by tumor cell line co-culture, IFN γ production was evaluated in addition to T cell proliferation in MDSC suppression assays from co-culture with seven different HNSCC cell lines: CAL-27,

FaDu, RPMI 2650, SCC-4, SCCL-MT1, SW 2224, and SW 451 (FIG. 3A and 3B). The suppressive capability of HNSCC induced CD33⁺ MDSC was compared with that of a positive T cell proliferation control (T cells alone), an induction negative control of CD33⁺ cells isolated from PBMC cultured in medium only, and an induction positive control of CD33⁺ cells isolated from PBMC cultured with GM-CSF and IL-6. In comparison with T cells alone, CD33⁺ cells induced by SCCL-MT1, SCC-4, CAL-27, SW 451 and the induction positive control demonstrated statistically significant strong inhibition of autologous T cell proliferation, while FaDu induced weakly suppressive MDSC ($p < 0.05$). Consistent with strong suppression, CD33⁺ cells induced by SCCL-MT1, SCC-4, and the induction positive control significantly inhibited IFN γ production ($p < 0.05$). Although CD33⁺ suppressor cells induced by FaDu did not strongly inhibit T cell proliferation, these co-cultures did demonstrate significantly decreased IFN γ production ($p < 0.05$). Conversely, Cal-27 and SW 451 induced MDSC that strongly suppressed T cell proliferation, but only weakly inhibited IFN γ . CD33⁺ cells induced by RPMI 2650 and SW 2224 did not demonstrate suppression of T cell proliferation or inhibition of IFN γ . These findings suggest that MDSC may impede T cell responses through multiple avenues, including inhibition of proliferation and IFN γ production.

Preferential induction of CD11b⁺ MDSC by human cancer cell lines

[0128] To clarify the apparent absence of CD33⁺ MDSC induction by breast carcinoma cell lines, CD11b⁺ cells from breast cancer cell line-PBMC co-cultures were evaluated for suppressive function against CD3/CD28 stimulated autologous T cells (FIG. 4). In these studies, 4/8 human breast cancer cell lines induced CD11b⁺ suppressor cells. Mean ($n=2$) T cell proliferation or T cell proliferation ($n=1$) is shown from suppression assays of CD33⁺ or CD11b⁺ cells with autologous T cells, respectively (FIG. 4). Consequently, breast carcinoma cell lines preferentially induced CD11b⁺ MDSC, even though no human breast cancer cell lines (0/9) tested generated suppressive CD33⁺ cells, suggesting that this component of the MAC-1 phagocytic complex may be a more specific marker for the subset of MDSC induced by breast cancer tumor cells. Non-small cell lung carcinoma cell lines and glioma cell lines, which had a low frequency of CD33⁺ MDSC induction (3/10 and 2/9, respectively), were found also to induce with moderate frequency this CD11b⁺ MDSC subset (4/10 and 4/9, respectively). (FIG. 4). These experiments identify a second subset of human MDSC arising in the cancer setting. Taken collectively with

the survey of CD33⁺ MDSC induction, these data indicate that the induction of MDSC is a universal feature of human cancers with some variation in the phenotype of induced MDSC subsets observed.

Morphology of human CD33⁺ and CD11b⁺ suppressor cells induced by tumor cell lines

[0129] Two subsets of human myeloid cells (CD11b⁺ or CD33⁺ cells) induced from normal donor PBMC by tumor-cell line co-culture have suppressive capacity. The morphology of suppressive tumor-co-cultured CD33⁺ and CD11b⁺ populations was compared to that of freshly isolated PBMC and myeloid cells cultured in medium only by Wright-Giemsa staining. Healthy donor PBMC showed occasional mononuclear cells with pale and scant cytoplasm, scattered amongst predominant lymphocytes. CD33⁺ cells from PBMC cultured in medium alone (with rhGM-CSF for growth support) for one week were predominantly large, mononuclear cells having abundant basophilic cytoplasm with occasional granulocytes and other myeloid lineage cells (e.g. eosinophils). In contrast to the mature lineages seen in medium only myeloid cells, CD33⁺ and CD11b⁺ suppressor cells isolated from PBMC after tumor co-culture showed an abundance of immature cells, including metamyelocytes or band cells and blast-like cells. No obvious morphologic differences were observed between CD33⁺ and CD11b⁺ MDSC.

Phenotype of MDSC shows CD33⁺ and CD11b⁺ subsets, both HLA-DR^{low} and Lineage⁻

[0130] Further characterization of CD33⁺ and CD11b⁺ MDSC subsets examined their expression of a wide range of proposed MDSC and mature innate immune cell markers (CD33, CD11b, CD66b, CD14, CD11c, HLA-DR, GITRL, OX40L, 41BBL (CD137L), CD56). Human MDSC were isolated by magnetic bead column separation after one-week co-culture with SCCL-MT1 or USC-HN2 HNSCC cell lines (CD33⁺) or MCF-7 breast cancer cell line (CD11b⁺) and non-suppressive CD33⁺ or CD11b⁺ control cells were isolated from medium only PBMC cultures. The purity for column isolated populations was found to be >90% by flow cytometry. Positivity for MDSC and mature myeloid lineage markers was measured by flow cytometry for each population and compared between CD33⁺ and CD11b⁺ MDSC subsets and between suppressive and non-suppressive populations (FIG. 5). CD11b expression levels were inversely correlated with suppressive function in CD33⁺ cells in these studies, and similarly CD33 positivity was inversely correlated with suppressive function in CD11b⁺ cells, suggesting a

divergence in the two populations during induction (FIG. 5A). Both CD33⁺ and CD11b⁺ suppressive populations showed decreased expression of activation marker HLA-DR and mature DC marker CD11c compared with non-suppressive populations of CD11b⁺ and CD33⁺ cells. These data are consistent with an accumulation of immature myeloid lineage cells coincident with the induction of suppressive function in either CD11b⁺ or CD33⁺ cells. Differentiated DC markers and T cell co-stimulatory ligands were further examined on the CD33⁺ subset of MDSC and found to be expressed at similarly low levels between suppressive and non-suppressive CD33⁺ cells isolated from tumor co-cultures (p=NS) (FIG. 5B), suggesting that the maturation and antigen presenting defects of MDSC are not primary in T cell suppression. Macrophage marker CD68 and granulocyte marker CD66b expression were low or absent and not differentially expressed by suppressive and non-suppressive CD33⁺ or CD11b⁺ cells in this study.

Tumor-induced myeloid suppressor cells over-express iNOS, TGF β , NOX2, VEGF, and ARG-1

[0131] Further characterization of tumor-induced canonical CD33⁺ MDSC subset by analysis of the expression of putative MDSC suppression genes was performed. For these studies, expression of putative suppression genes was measured in tumor cell line-induced MDSC, including those induced by SCCL-MT1 HNSCC cell line and those induced by 4-998 osteogenic sarcoma, DU 145 prostate carcinoma, CAKI-1 renal cell carcinoma, SK-OV-3 ovarian carcinoma, and SW 608 and SW 732 colorectal adenocarcinoma cell lines (FIG. 6A), and compared to expression levels in normal, non-suppressive CD33⁺ myeloid cells from medium only cultures. These MDSC consistently showed statistically significant up-regulation of arginase (ARG-1), inducible nitric oxide synthase (iNOS), NADPH oxidase (NOX2), vascular endothelial growth factor (VEGF), and/or transforming growth factor (TGF) β compared with control CD33⁺ cells from medium-only cultures (Figure 6A). Subtle variations were observed in the gene expression patterns of these tumor-induced MDSC. To determine the dominant mechanism of T cell suppression by this canonical CD33⁺ MDSC subset, suppression assays were repeated in the presence or absence of specific inhibitors of ARG-1 (nor-NOHA), iNOS (L-NMMA), NOX2 (apocynin), VEGF (neutralizing antibody Avastin), or TGF β 1 (SB431542 or neutralizing antibody 1D11). In these studies no one inhibitor was found to completely reverse suppression (FIG. 6B), consistent with the pleotropic actions of monocytic

MDSC, but inhibitors of ARG-1 and NOX2 did produce statistically significant decreases in suppression by CD33⁺ MDSC. These results were confirmed by siRNA knockdown of individual suppression genes: ARG-1, iNOS, NCF1 (NOX2 component), TGFβ1, or VEGFA. A comparison of ARG-1, iNOS, and NOX2-component NCF1 gene expression in CD33⁺ and CD11b⁺ human MDSC induced by HNSCC or breast carcinoma cell lines revealed similar levels of expression between these subsets with a trend toward increased ARG-1 and NOX2 expression in CD33⁺ MDSC (FIG. 6C).

Higher Hif1α, STAT3, and C/EBPβ gene expression distinguish tumor cell line-induced human MDSC from normal myeloid cells

[0132] We examined the expression of HIF1α, STAT3, and C/EBPβ in tumor cell line (SCCL-MT1 or USC-HN2)-induced CD33⁺ or (MCF7 breast carcinoma) CD11b⁺ human suppressor cells compared to medium only controls by qRT-PCR (data from six unique donors, two independent experiments) (FIG. 7A) and immunohistochemistry (FIG. 7B). CD33⁺ MDSC showed stronger up-regulation of STAT3 and HIF1α while CD11b⁺ MDSC showed comparably greater up-regulation of C/EBPβ (FIG. 7A). Treatment of either CD33⁺ or CD11b⁺ tumor-cell line-induced MDSC with lipopolysaccharide (LPS), a known activator of MDSC function, caused further up-regulation of STAT3, C/EBPβ, and HIF1α concurrent with increased expression of ARG-1, iNOS, and NOX2-component NCF1 (FIG. 7C).

[0133] Gene expression patterns in ATRA, Sunitinib, or CXB-treated CD33⁺ or CD11b⁺ human MDSC were determined (FIG. 7D). Inhibition of human CD33⁺ MDSC by ATRA, Sunitinib, and Celecoxib correlated with decreased STAT3 and HIF1α transcription. In comparison, inhibition of human CD11b⁺ MDSC by ATRA and Sunitinib correlated with decreased C/EBPβ levels, but no change in STAT3 and HIF1α mRNA levels. Celecoxib was not found to have inhibitory actions on CD11b⁺ MDSC and it was not observed to decrease C/EBPβ levels in this population. These data suggest that HIF1α, STAT3, and C/EBPβ are key transcription factors related to suppressive function in tumor cell line-induced human MDSC.

[0134] It should be understood that although the present disclosure has been specifically disclosed by preferred embodiments and optional features, modification, improvement and variation of the disclosure embodied therein herein disclosed may be resorted to by

those skilled in the art, and that such modifications, improvements and variations are considered to be within the scope of this disclosure. The materials, methods, and examples provided here are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the disclosure.

[0135] The disclosure has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the disclosure with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0136] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0137] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

REFERENCES

1. Stewart, T. J., and S. I. Abrams. 2008. How tumours escape mass destruction. *Oncogene*. 27: 5894-5903.
2. Bak, S.P., A. Alonso, M. J. Turk, and B. Berwin. 2008. Murine ovarian cancer vascular leukocytes require arginase-1 activity for T cell suppression. *Mol Immunol*. 46: 258-268.
3. Nagaraj, S., and D. I. Gabrilovich. 2008. Tumor escape mechanism governed by myeloid-derived suppressor cells. *Cancer Res*. 68: 2561-2563.
4. Corzo, C.A., M. J. Cotter, P. Cheng, F. Cheng, S. Kusmartsev, E. Sotomayor, T. Padhya, T. V. McCaffrey, J. C. McCaffrey, and D. I. Gabrilovich. 2009. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J Immunol*. 182: 5693-5701.
5. Donkor, M. K., E. Lahue, T. A. f Ioke, L. R. Shafer, U. Coskun, J. C. Solheim, D. Gulen, J. Bishay, and J. E. Talmadge. 2009. Mammary tumor heterogeneity in the expansion of myeloid-derived suppressor cells. *Int Immunopharmacol*. 9: 937-948.
6. Srivastava, M. K., P. Sinha, V. K. Clements, P. Rodriguez, S. Ostrand-Rosenberg. 2010. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Res*. 70: 68-77.
7. Serafini, P., S. Mgebroff, K. Noonan, and I. Borrello. 2008. Myeloid-derived suppressor cells promote cross-tolerance in B cell lymphoma by expanding regulatory T cells. *Cancer Res*. 68: 5439-5449.
8. Dumitriu, I.E., D. R. Dunbar, S. E. Howie, T. Sethi, and C. D. Gregory. 2009. Human dendritic cells produce TGF-beta 1 under the influence of lung carcinoma cells and prime the differentiation of CD4+CD25+Foxp3+ regulatory T cells. *J Immunol*. 182: 2795-2807.
9. Gabrilovich, D. I., and S. Nagaraj. 2009. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol*. 9: 162-174.
10. Hellwig-Bürgel, T., D. P. Stiehl, A. E. Wagner, E. Metzen, and W. Jelkmann. 2005. Review: hypoxia-inducible factor-1 (HIF-1): a novel transcription factor in immune reactions. *J Interferon Cytokine Res*. 25: 297-310.

11. Mandruzzato, S., S. Solito, E. Falisi, S. Francescato, V. Chiarion-Sileni, S. Mocellin, A. Zanon, C.R. Rossi, D. Nitti, V. Bronte, and P. Zanovello. 2009. IL4Ralpha+ myeloid-derived suppressor cell expansion in cancer patients. *J Immunol.* 182: 6562-6568.
12. Hoechst, B., T. Voigtlaender, L. Ormandy, J. Gamrekashvili, F. Zhao, H. Wedemeyer, F. Lehner, M. P. Manns, T. F. Greten, and F. Korangy. 2009. Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the NKp30 receptor. *Hepatology.* 50: 799-807.
13. Liu, C.Y., Y. M. Wang, C. L. Wang, P. H. Feng, H. W. Ko, Y. H. Liu, Y. C. Wu, Y. Chu, F. T. Chung, C. H. Kuo, K. Y. Lee, S. M. Lin, H. C. Lin, C. H. Wang, C. F. Yu, and H. P. Kuo. 2009. Population alterations of L: -arginase- and inducible nitric oxide synthase-expressed CD11b(+)/CD14 (-)/CD15 (+)/CD33 (+) myeloid-derived suppressor cells and CD8 (+) T lymphocytes in patients with advanced-stage non-small cell lung cancer. *J Cancer Res Clin Oncol.* 136: 35-45.
14. Ko, J. S., A. H. Zea, B. I. Rini, J. L. Ireland, P. Elson, P. Cohen, A. Golshayan, P. A. Rayman, L. Wood, J. Garcia, R. Dreicer, R. Bukowski, and J. H. Finke. 2009. Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. *Clin Cancer Res.* 15: 2148-2157.
15. Morse, M. A., J. R. Hall, and J. M. Plate. 2009. Countering tumor-induced immunosuppression during immunotherapy for pancreatic cancer. *Expert Opin Biol Ther.* 9: 331-339.
16. Diaz-Montero, C. M., M. L. Salem, M. I. Nishimura, E. Garrett-Mayer, D. J. Cole, and A. J. Montero. 2009. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. *Cancer Immunol Immunother.* 58: 49-59.
17. Peranzoni, E., S. Zilio, I. Marigo, L. Dolcetti, P. Zanovello, S. Mandruzzato, and V. Bronte. 2010. Myeloid-derived suppressor cell heterogeneity and subset definition. *Curr Opin Immunol.* 22: 238-244.
18. Serafini, P., I. Borrello, and V. Bronte. 2006. Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. *Semin Cancer Biol.* 16: 53-65.

19. Zea, A.H., P. C. Rodriguez, M. B. Atkins, C. Hernandez, S. Signoretti, J. Zabaleta, D. McDermott, D. Quiceno, A. Youmans, A. O'Neill, J. Mier, A. C. Ochoa. 2005. Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: a mechanism of tumor evasion. *Cancer Res.* 65: 3044-3048.
20. Filipazzi, P., R. Valenti, V. Huber, L. Pilla, P. Canese, M. Iero, C. Castelli, L. Mariani, G. Parmiani, and L. Rivoltini. 2007. Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. *J Clin Oncol.* 25: 2546-2553.
21. Gordon, I. O., and R. S. Freedman. 2006. Defective antitumor function of monocyte-derived macrophages from epithelial ovarian cancer patients. *Clin Cancer Res.* 12: 1515-1524.
22. Hoechst, B., L. A. Ormandy, M. Ballmaier, F. Lehner, C. Kruger, M. P. Manns, T. F. Greten, and F. Korangy. 2008. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. *Gastroenterology.* 135: 234-243.
23. Ostrand-Rosenberg, S., and P. Sinha. 2009. Myeloid-derived suppressor cells: linking inflammation and cancer. *J Immunol.* 182: 4499-4506.
24. Kusmartsev, S., and D. I. Gabrilovich. 2006. Effect of tumor-derived cytokines and growth factors on differentiation and immune suppressive features of myeloid cells in cancer. *Cancer Metastasis Rev.* 25: 323-331.
25. Xiang, X., A. Poliakov, C. Liu, Y. Liu, Z. B. Deng, J. Wang, Z. Cheng, S. V. Shah, G. J. Wang, L. Zhang, W. E. Grizzle, J. Mobley, and H. G. Zhang. 2009. Induction of myeloid-derived suppressor cells by tumor exosomes. *Int J Cancer.* 124: 2621-2633.
26. Lechner, M. G., D. J. Liebertz, and A. L. Epstein. 2010. Characterization of cytokine-induced myeloid-derived suppressor cells from normal human peripheral blood mononuclear cells. *J Immunol.* 185: 2273-2284.
27. Poschke, I., D. Mougiakakos, J. Liansson, G. V. Masucci, and R. Kiessling. 2010. Immature immunosuppressive CD14+HLA-DR-/low cells in melanoma patients are Stat3hi and overexpress CD80, CD83, and DC-sign. *Cancer Res.* 70: 4335-4345.

28. Munera, V., P. J. Popovic, J. Bryk, J. Pribis, D. Caba, B. M. Matta, M. Zenati, and J. B. Ochoa. 2010. Stat 6-dependent induction of myeloid derived suppressor cells after physical injury regulates nitric oxide response to endotoxin. *Ann Surg.* 251: 120-126.
29. Cui, W., D. D. Taub, and K. Gardner. 2007. qPrimerDepot: a primer database for quantitative real time PCR. *Nucleic Acids Res.* 35(Database issue): D805-809.
30. Rodrigues, J. C., G. C. Gonzalez, L. Zhang, G. Ibrahim, .I. J. Kelly, M. P. Gustafson, Y. Lin, A. B. Dietz, P. A. Forsyth, V. W. Yong, and I. F. Parney. 2009. Normal human monocytes exposed to glioma cells acquire myeloid-derived suppressor cell-like properties. *Neuro Oncol.* 4: 351-365.
31. Dong, R., S. Adams, G. Bouma, A. Eddaouda, P. Chana, D. Moulding, A. Duncan, and J. Anderson. 2010. Newly identified multinuclear cells in cultured human dendritic cells possess the phenotype of regulatory DC. Keystone Symposium Molecular and Cellular Biology and Immune Escape in Cancer, Keystone, CO., February 7-12, 2010; p. 171, abstract 135.
32. de Kleer, I., Y. Vercoulen, M. Klein, J. Meerding, S. Albani, R. van der Zee, B. Sawitzki, A. Hamann, W. Kuis, and B. Prakken. 2010. CD30 discriminates heat shock protein 60-induced FOXP3⁺ CD4⁺ T cells with a regulatory phenotype. *J. Immunol.* 185: 2071-2079.
33. del Rio, M. L., G. Bernhardt, J. I. Rodriguez-Barbosa, and R. Förster R. 2010. Development and functional specialization of CD103⁺ dendritic cells. *Immunol Rev.* 234: 268-281.
34. Hori, S., T. Nomura, and S. Sakaguchi. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* 299: 1057-1061.
35. Corzo, C. A., T. Condamine, L. Lu, M. J. Cotter, J. I. Youn, P. Cheng, H. I. Cho, E. Celis, D. G. Quiceno, T. Padhya, T. V. McCaffrey, J. C. McCaffrey, and D. f. Gabrilovich. 2010. HIF-1 α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J Exp Med.* 207: 2439-2453.
36. Ramji, D. P., and P. Foka. 2002. CCAAT/enhancer-binding proteins: structure, function and regulation. *Biochem J.* 365(Pt 3): 561-575.
37. Bronte, V. 2010. The transcription factor C/EBP β is a master regulator of tumor-induced, myeloid-dependent immune suppression. In *Molecular and Cellular Biology and*

Immune Escape in Cancer, February 7-12. Keystone Symposia on Molecular and Cellular Biology, Silverthorne, CO. p. 182.

38. Ko, J. S., P. Rayman, J. Ireland, S. Swaidani, G. Li, K. D. Bunting, B. Rini, J. H. Finke, and P. A. Cohen. 2010. Direct and differential suppression of myeloid-derived suppressor cell subsets by sunitinib is compartmentally constrained. *Cancer Res.* 70: 3526-3536.
39. Corzo, C. A., M. J. Cotter, P. Cheng, F. Cheng, S. Kusmartsev, E. Sotomayor, T. Padhya, T. V. McCaffrey, J. C. McCaffrey, and D. I. Gabrilovich. 2009. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J Immunol.* 182: 5693-5701.
40. Sica, G. L., I. H. Choi, G. Zhu, K. Tamada, S. D. Wang, H. Tamura, A. I. Chapoval, D. B. Flies, J. Bajorath, and L. Chen. 2003. B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. *Immunity.* 18: 849-861.
41. Sadun, R. E., S. M. Sachsman, X. Chen, K. W. Christenson, W. Z. Morris, P. Hu, and A. L. Epstein. 2007. Immune signatures of murine and human cancers reveal unique mechanisms of tumor escape and new targets for cancer immunotherapy. *Clin Cancer Res.* 13: 4016-4025.
42. Jung, Y. J., J. S. Isaacs, S. Lee, J. Trepel, and L. Neckers. 2003. IL-1 beta-mediated up-regulation of HIF-1alpha via an NFkappaB/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis. *FASEB J.* 17: 2115-2117.
43. Xu, Q., J. Briggs, S. Park, G. Niu, M. Kortylewski, S. Zhang, T. Gritsko, J. Turkson, H. Kay, G. L. Semenza, J. Q. Cheng, R. Jove, and H. Yu. 2005. Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. *Oncogene.* 24: 5552-5560.
44. Ramadori, P., G. Ahmad, and G. Ramadori. 2010. Cellular and molecular mechanisms regulating the hepatic erythropoietin expression during acute-phase response: a role for IL-6. *Lab Invest.* 90: 1306-1324.
45. Rius, J., M. Guma, C. Schachtrup, K. Akassoglou, A. S. Zinkernagel, V. Nizet, R. S. Johnson, G. G. Haddad, and M. Karin. 2008. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. *Nature.* 453: 807-811.

46. Watson, J. A., C. J. Watson, A. M. McCrohan, K. Woodfine, M. Tosoetto, J. McDaid, E. Gallagher, D. Betts, J. Baugh, J. O'Sullivan, A. Murrell, R. W. Watson, and A. McCann. 2009. Generation of an epigenetic signature by chronic hypoxia in prostate cells. *Hum Mol Genet.* 18: 3594-3604.
47. Peyssonnaud, C., P. Cejudo-Martin, A. Doedens, A. S. Zinkernagel, R. S. Johnson, and V. Nizet. 2007. Cutting edge: Essential role of hypoxia inducible factor-1alpha in development of lipopolysaccharide-induced sepsis. *J Immunol.* 178: 7516-7519.

WHAT IS CLAIMED IS:

1. A method of treating a cancer subject determined to be responsive to the treatment, comprising administering to the subject an effective amount of a cancer therapeutic, wherein the cancer subject is determined to be responsive to the therapy by a method comprising detecting, in a cell-containing sample from said subject, myeloid-derived suppressor cells (MDSC) having a phenotype of the group $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}$, $CD14^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD66b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD11b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, and $CD11b^{+}HLA-DR^{low}C/EBP\beta^{+}$.

2. A method for detecting or aiding in the detection of the presence of cancer in a subject comprising

detecting, in a cell-containing sample from the subject, myeloid-derived suppressor cells (MDSC), wherein said MDSC have a phenotype of the group $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}$, $CD14^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD66b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD11b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, and $CD11b^{+}HLA-DR^{low}C/EBP\beta^{+}$; and

wherein the presence of the MDSC indicates the presence of cancer and the absence of the MDSC indicates the absence of cancer.

3. A method for determining the efficacy of a cancer treatment in a subject having cancer, said method comprising

detecting, in a cell-containing sample from the subject, myeloid-derived suppressor cells (MDSC), wherein said MDSC have a phenotype of the group $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}$, $CD14^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD66b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD11b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, and $CD11b^{+}HLA-DR^{low}C/EBP\beta^{+}$; and

wherein a decrease or absence of said MDSC during cancer therapy indicates an effective therapeutic response.

4. The method according to any one of claims 1-3, wherein said cancer is selected from the group consisting of: HNSCC, breast cancer, lymphoma, cervical cancer, ovarian cancer, colorectal carcinoma, brain cancer, melanoma, sarcoma, endometrial cancer, bladder cancer, renal cancer, gastric cancer, thyroid cancer, small cell lung cancer, non-small cell lung cancer, prostate cancer, liver cancer, and pancreatic cancer.
5. The method according to claim 4, wherein said cancer is selected from the group consisting of: HNSCC, breast cancer, cervical cancer, ovarian cancer, and colorectal carcinoma.
6. The method according to claim 5, wherein said cancer is lymphoma.
7. The method according to claim 6, wherein said lymphoma is selected from the group consisting of: Mature cell neoplasm, mature T cell or natural killer cell neoplasm, and Hodgkins lymphoma.
8. The method according to claim 7, wherein said Mature cell neoplasm is selected from the group consisting of: Chronic lymphocytic leukemia/Small lymphocytic lymphoma, B-cell prolymphocytic leukemia, Lymphoplasmacytic lymphoma (such as Waldenström macroglobulinemia), Splenic marginal zone lymphoma, Plasma cell myeloma, Plasmacytoma, Monoclonal immunoglobulin deposition diseases, Heavy chain diseases, Extranodal marginal zone B cell lymphoma (MALT lymphoma), Nodal marginal zone B cell lymphoma (NMZL), Follicular lymphoma, Mantle cell lymphoma, Diffuse large B cell lymphoma, Mediastinal (thymic) large B cell lymphoma, Intravascular large B cell lymphoma, Primary effusion lymphoma, and Burkitt lymphoma/leukemia.
9. The method according to claim 7, wherein said mature T or natural killer cell neoplasm is selected from the group consisting of: T cell large granular lymphocytic leukemia, Aggressive NK cell leukemia, Adult T cell leukemia/lymphoma, Extranodal NK/T cell lymphoma (nasal type), Enteropathy-type T cell lymphoma, Hepatosplenic T cell lymphoma, Blastic NK cell lymphoma, Mycosis fungoides / Sezary syndrome, Primary cutaneous CD30-positive T cell lymphoproliferative disorder, Primary cutaneous anaplastic large cell lymphoma, Lymphomatoid papulosis, Angioimmunoblastic T cell lymphoma, Peripheral T cell lymphoma (unspecified), and Anaplastic large cell lymphoma.

10. The method according to any one of the previous claims, wherein said cell containing sample comprises peripheral blood, peripheral blood leukocytes, red blood cell lysate of whole peripheral blood, tumor, lymph node tissue, lymph, spleen cells, cerebrospinal fluid, pleural effusion, or ascites.

11. The method according to any one of the previous claims, wherein said detecting is performed using antibody detection.

12. The method according to any one of the previous claims, wherein said detecting is performed by fluorescence activated flow cytometry.

13. The method according to any one of claims 1 and 4-12 wherein, after administering said cancer therapeutic, the method further comprises

evaluating a cell containing sample from said subject for the presence of myeloid-derived suppressor cells (MDSC),

wherein the MDSC have a phenotype of the group $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}$, $CD14^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD66b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, $CD11b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBP\beta^{+}$, and $CD11b^{+}HLA-DR^{low}C/EBP\beta^{+}$; and

wherein a reduction in said MDSC indicates therapeutic improvement and an increase or unchanged level of said MDSC indicates therapeutic failure.

14. A method for generating human MDSC from a source of myeloid lineage cells comprising:

- (a) contacting the source of myeloid lineage cells with a tumor sample under conditions sufficient to induce MDSC; and,
- (b) isolating the induced MDSC.

15. The method according claim 14 wherein the source of myeloid lineage cells is selected from the group consisting of blood, tumor, lymph, lymph node tissue, spleen cells, cerebrospinal fluid, ascities, and pleural effusions.

16. The method according to claim 15 wherein the source of myeloid lineage cells is peripheral blood mononuclear cells.

17. The method according to any one of claims 14-16 wherein the source of myeloid lineage cells is from a healthy donor.

18. The method according to any one of claims 14-17 wherein said induced MDSC have a phenotype selected from the group consisting of $CD33^{+}$, $CD14^{+}$, $CD66b^{+}$, and $CD11b^{+}$.

19. The method according to claim 18 wherein said induced MDSC have a phenotype selected from the group consisting of $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}$, $CD14^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBPb^{+}$, $CD66b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBPb^{+}$, $CD33^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBPb^{+}$, $CD11b^{+}HLA-DR^{low}HIF1\alpha^{+}/STAT3^{+}/pSTAT3^{+}/C/EBPb^{+}$, and $CD11b^{+}HLA-DR^{low}C/EBP\beta^{+}$.

20. The method according to any one of claims 14-16 wherein said tumor sample is selected from the group consisting of HNSCC, malignant lymphoma, breast cancer, cervical cancer, ovarian cancer, colorectal carcinoma, brain cancer, melanoma, sarcoma, endometrial cancer, bladder cancer, renal cancer, gastric cancer, thyroid cancer, small cell lung cancer, non-small cell lung cancer, prostate cancer, liver cancer, and pancreatic cancer.

21. The method according to claim 20 wherein said malignant lymphoma is selected from the group consisting of mature cell neoplasm, mature T cell or natural killer cell neoplasm, and Hodgkins lymphoma.

22. The method according to claim 21 wherein said mature cell neoplasm is selected from the group consisting of Chronic lymphocytic leukemia/Small lymphocytic lymphoma, B-cell prolymphocytic leukemia, Lymphoplasmacytic lymphoma (such as Waldenström macroglobulinemia), Splenic marginal zone lymphoma, Plasma cell myeloma, Plasmacytoma, Monoclonal immunoglobulin deposition diseases, Heavy chain diseases, Extranodal marginal zone B cell lymphoma (MALT lymphoma), Nodal marginal zone B cell lymphoma (NMZL), Follicular lymphoma, Mantle cell lymphoma, Diffuse large B cell lymphoma, Mediastinal (thymic) large B cell lymphoma, Intravascular large B cell lymphoma, Primary effusion lymphoma, and Burkitt lymphoma/leukemia.

23. The method according to claim 21 wherein said mature T or natural killer cell neoplasm is selected from the group consisting of T cell large granular lymphocytic leukemia, Aggressive NK cell leukemia, Adult T cell leukemia/lymphoma, Extranodal NK/T cell lymphoma (nasal type), Enteropathy-type T cell lymphoma, Hepatosplenic T cell lymphoma, Blastic NK cell lymphoma, Mycosis fungoides / Sezary syndrome, Primary cutaneous CD30-positive T cell lymphoproliferative disorder, Primary cutaneous anaplastic large cell lymphoma, Lymphomatoid papulosis, Angioimmunoblastic T cell lymphoma, Peripheral T cell lymphoma (unspecified), and Anaplastic large cell lymphoma.

24. A method for identifying a candidate compound as a suppression agonist comprising comparing suppression activity function of tumor cell line-educated- CD33⁺ cells, CD14⁺ cells, CD66b⁺ cells, or CD11b⁺ human MDSC in the presence and in the absence of said candidate compound, wherein the relative reduction of T cell proliferation in the presence of said candidate compound indicates pro-suppressive activity.

25. A method for identifying a candidate compound as a suppression antagonist comprising comparing suppression activity function of tumor cell line-educated- CD33⁺ cells, CD14⁺ cells, CD66b⁺ cells, or CD11b⁺ human MDSC in the presence and in the absence of said candidate compound, wherein the relative increase of T cell proliferation in the presence of said candidate compound indicates anti-suppressive activity.

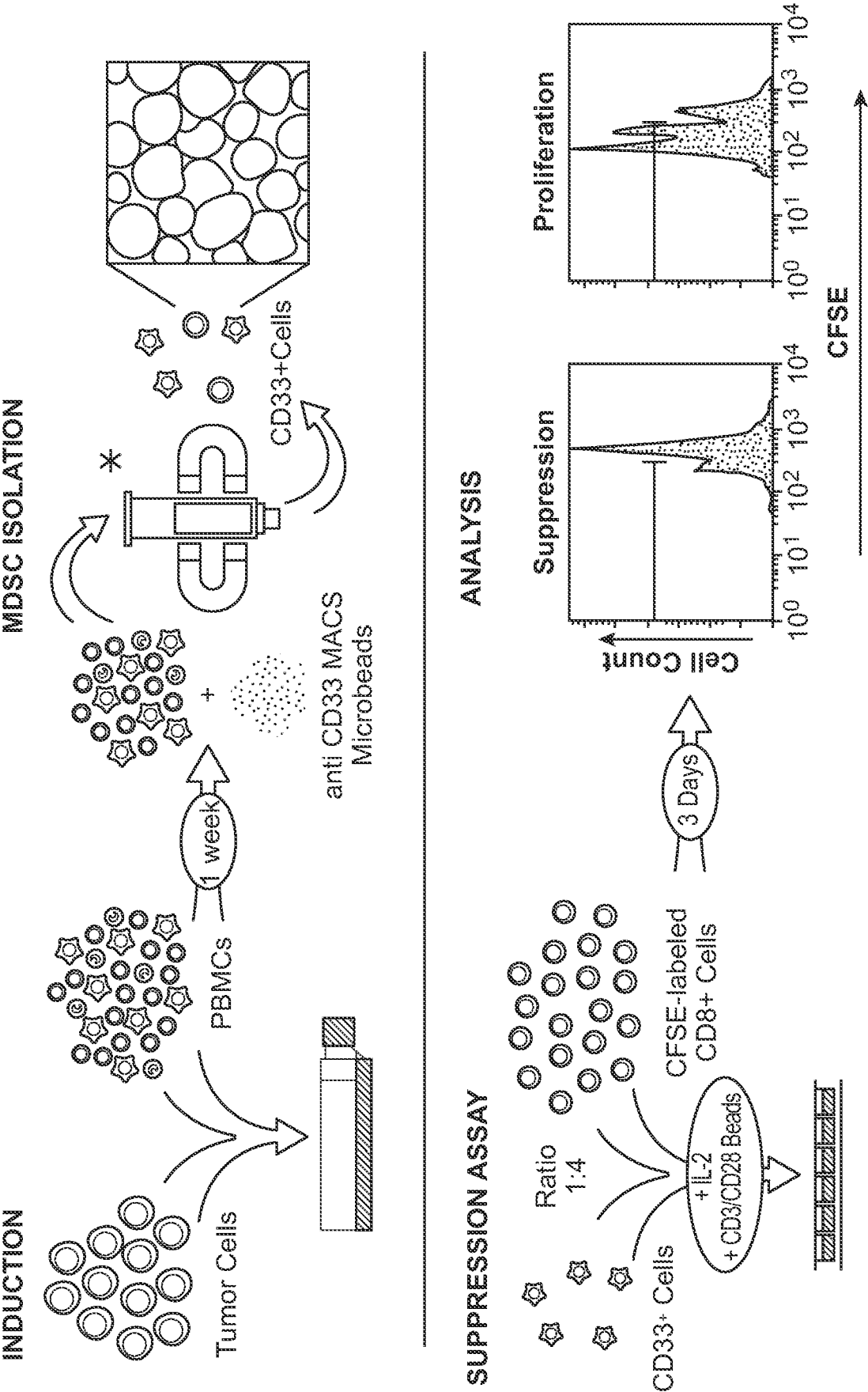


FIG. 1

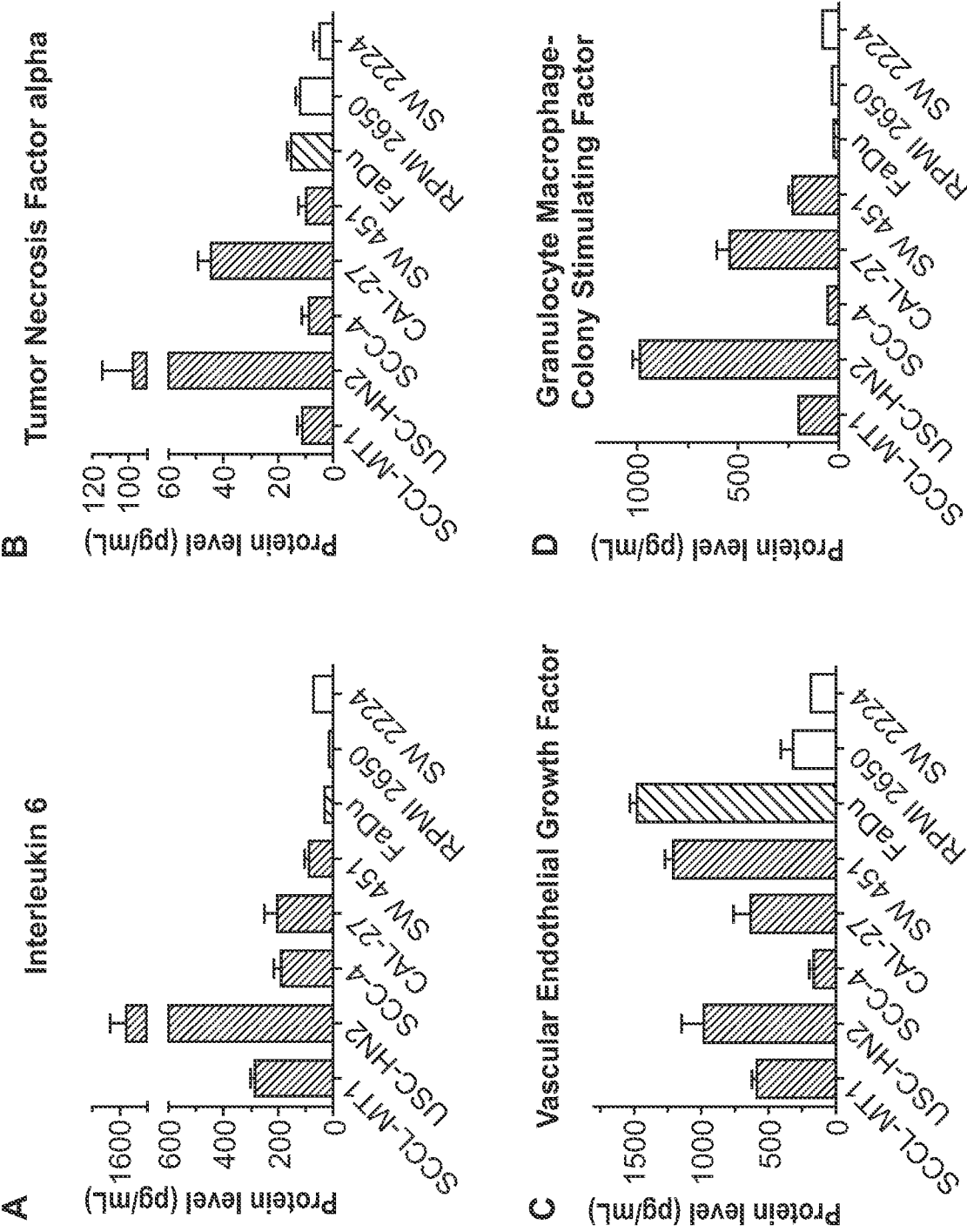


FIG. 2

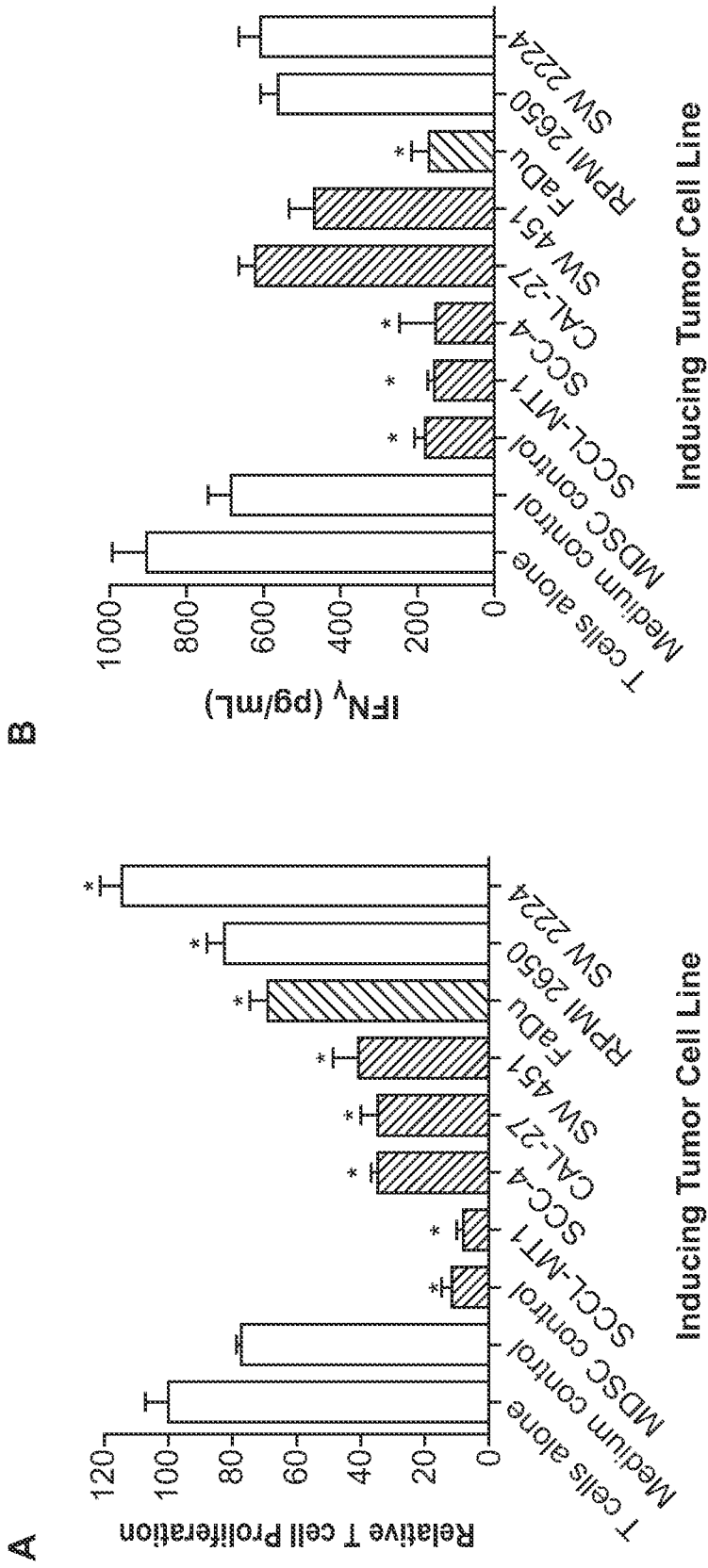


FIG. 3

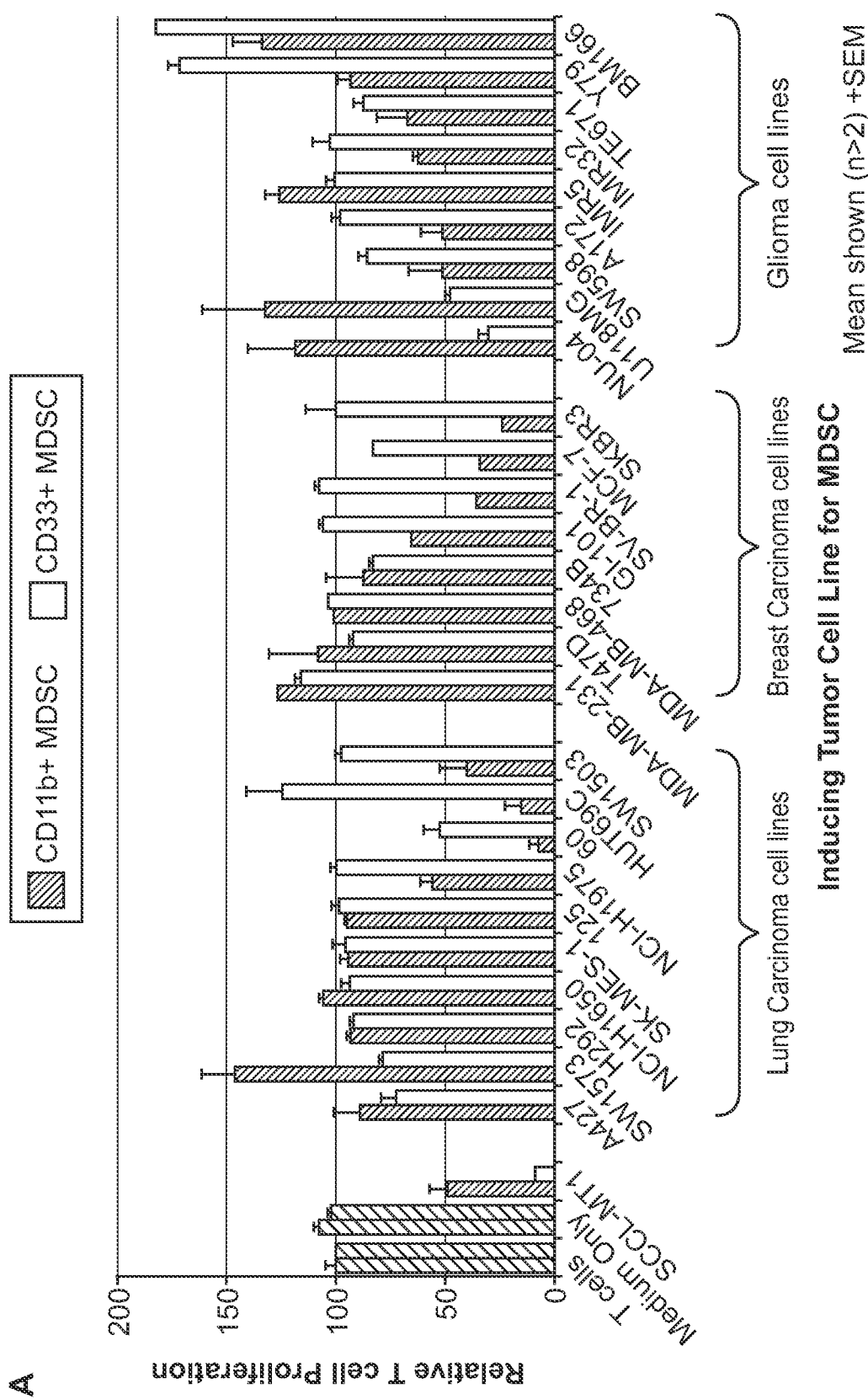
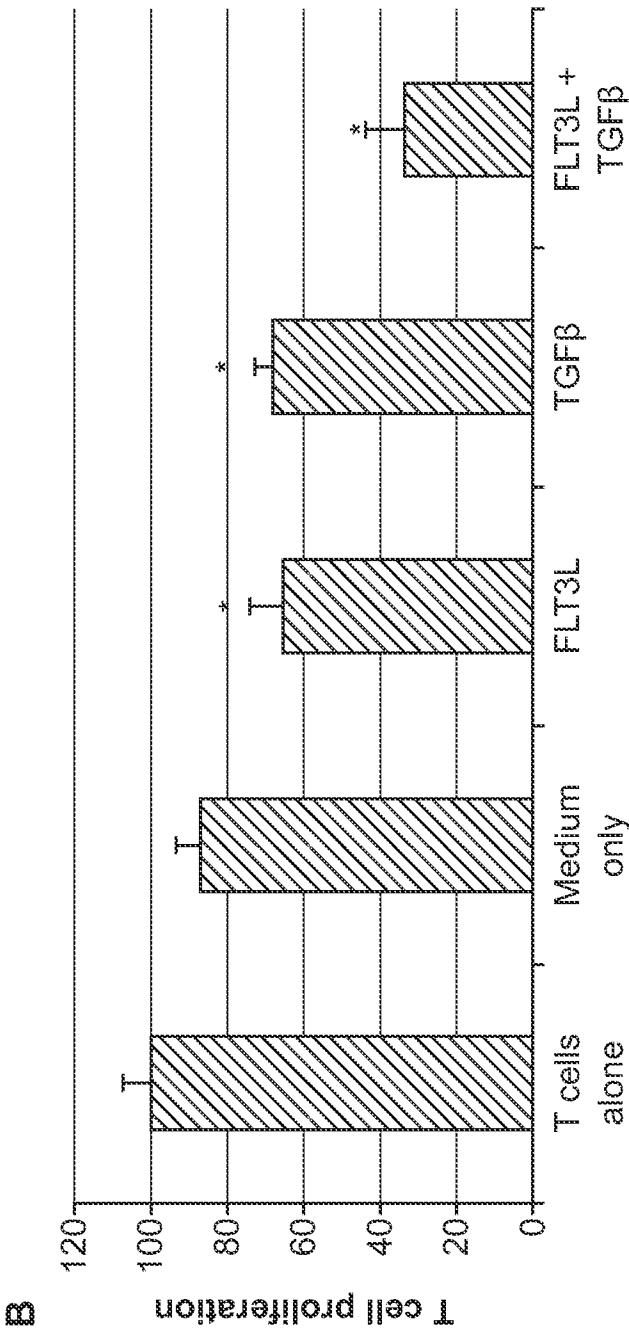


FIG. 4



Cytokine-induction conditions for CD11b+ cells

FIG. 4 (Cont.)

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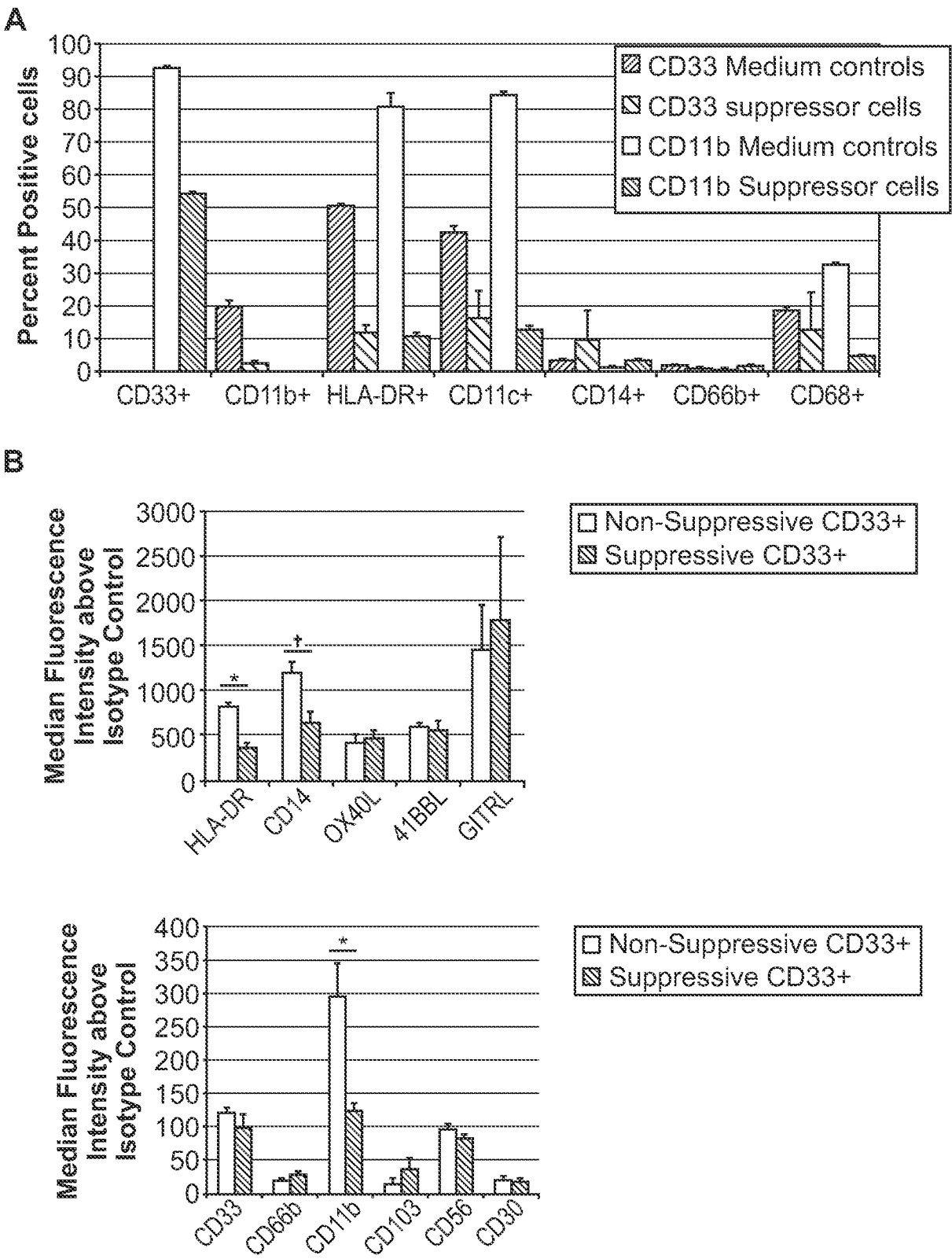


FIG. 5

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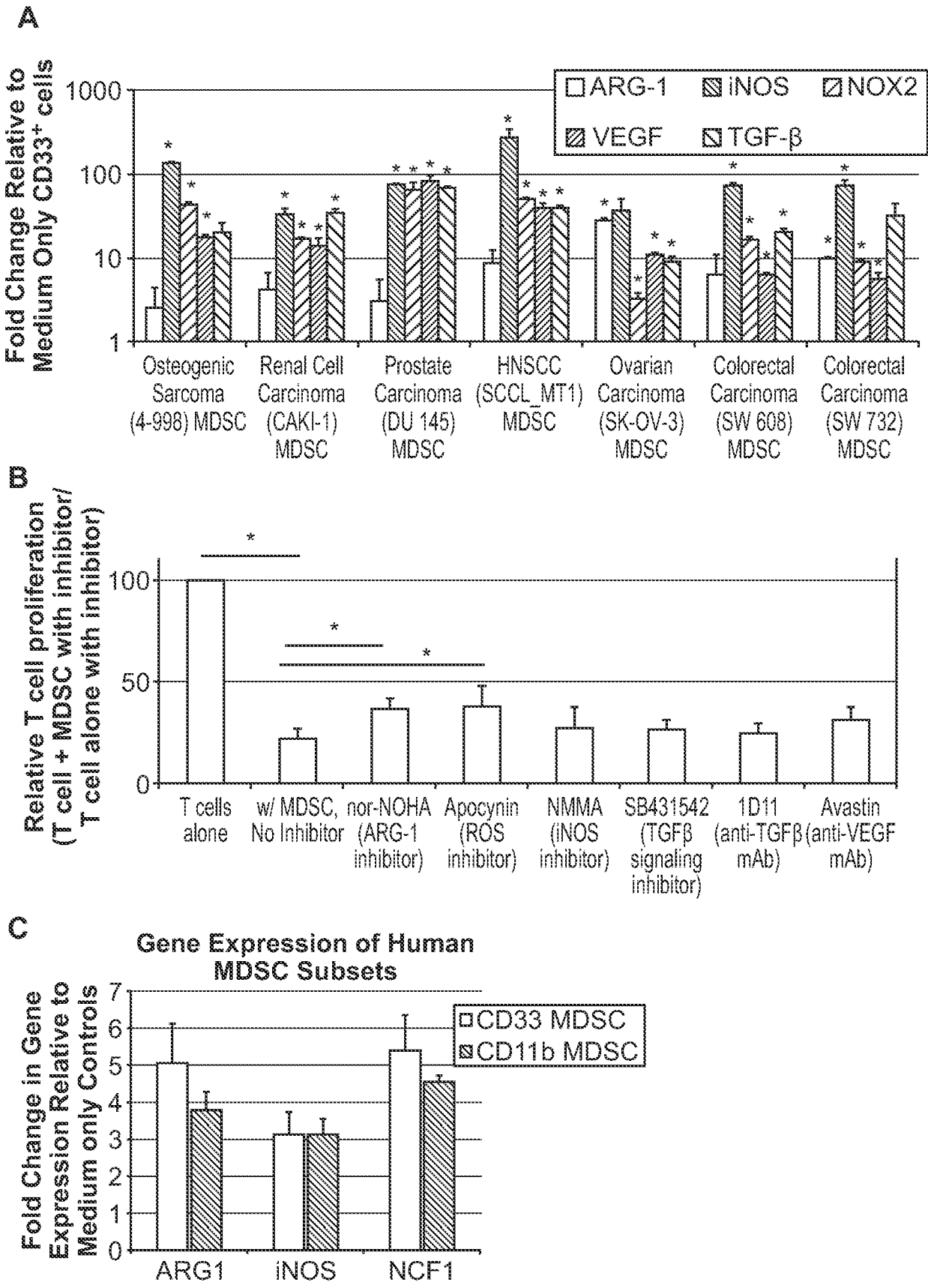
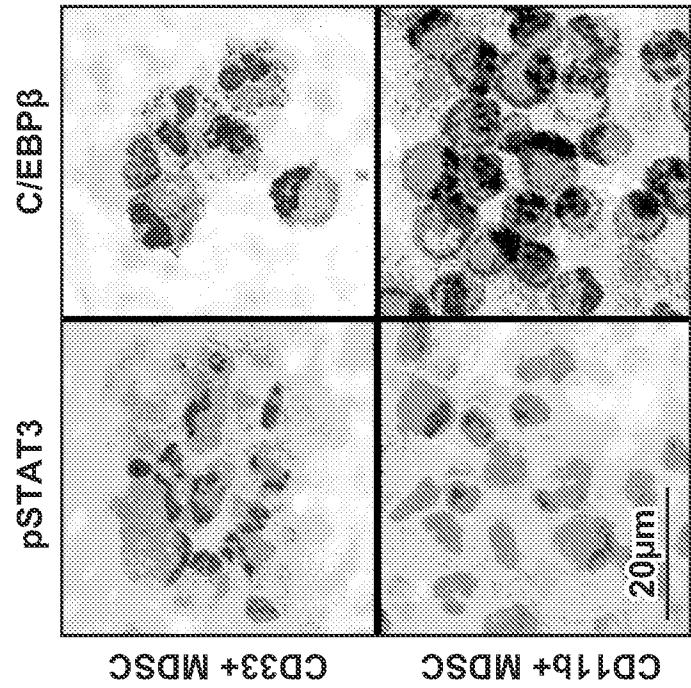
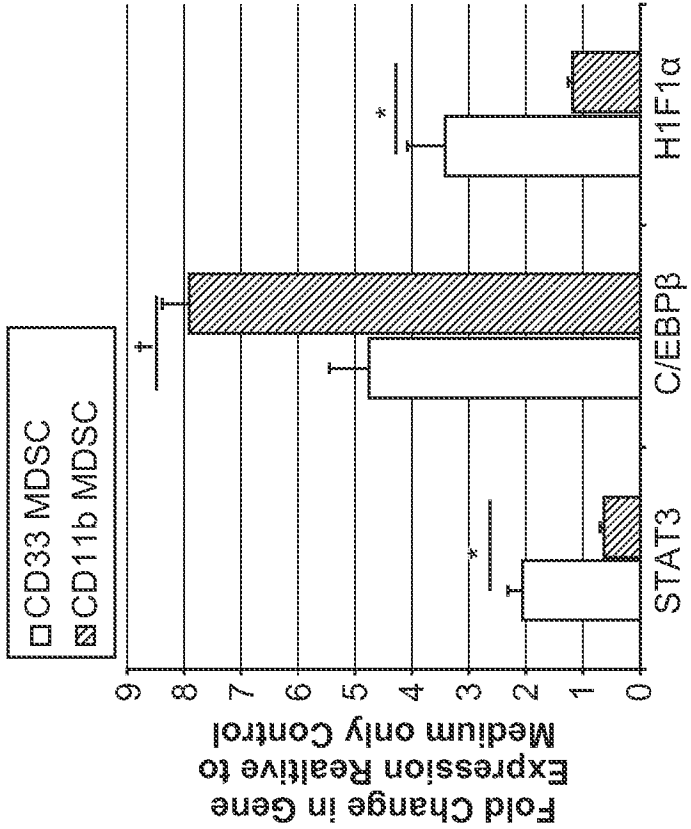


FIG. 6



B



A

FIG. 7

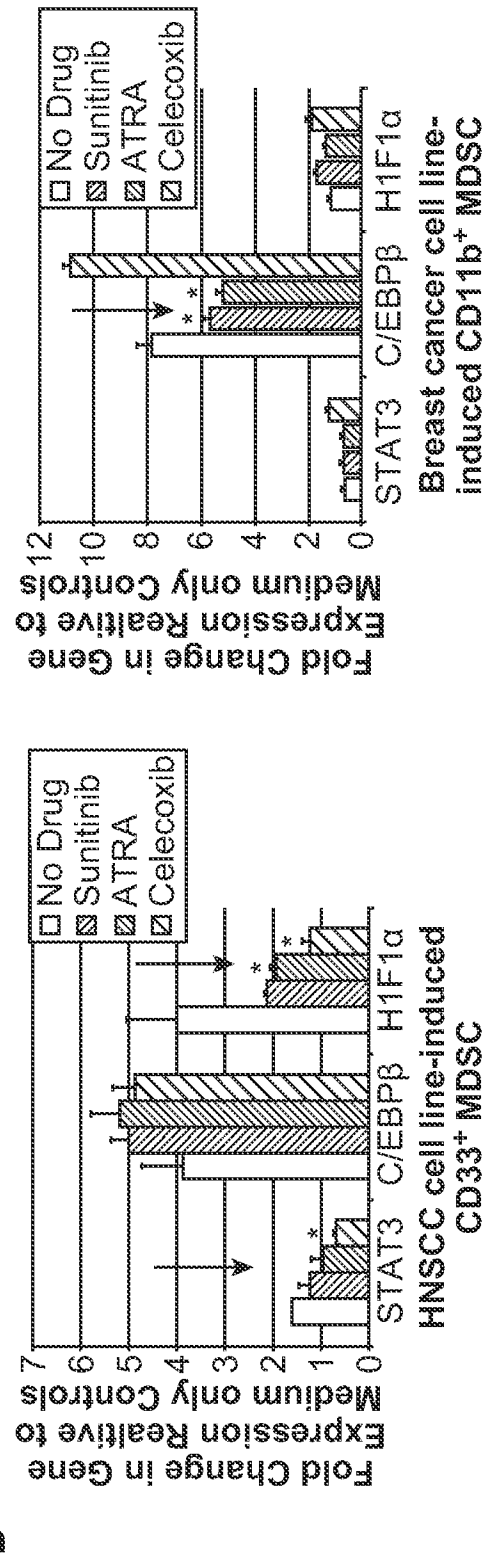
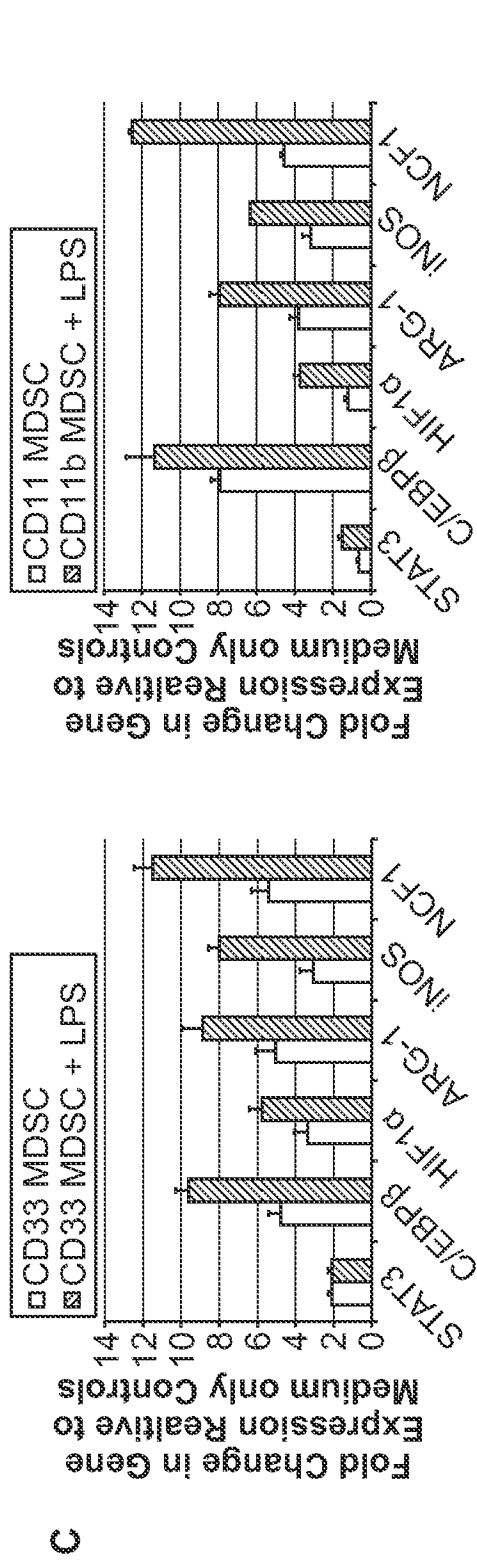


FIG. 7 (Cont.)

10	20	30	40	50	60
MAQWNQLQQL	DTRYLEQLHQ	LYSDSFFMEL	RQFLAPWIES	QDWAYAASKE	SHATLVFHNL
70	80	90	100	110	120
LGEIDQQYSR	FLQESNVLYQ	HNLRRIKQFL	QSRYLEKPMEL	IARIVARCLW	EESRLLQTAA
130	140	150	160	170	180
TAAQQGGQAN	HPTAAVVTEK	QQMLEQHLQD	VRKRVQDLEQ	KMKVVENLQD	DFDFNYKTLK
190	200	210	220	230	240
SQGDMQDLNG	NNQSVTRQKM	QQLEQMLTAL	DQMRRSIVSE	LAGLSAMEY	VQKTLTDEEL
250	260	270	280	290	300
ADWKRRQQIA	CIGGPPNICL	DRLENWITSL	AESQLQTRQQ	IKKLEELQQK	VSYKGDPIVQ
310	320	330	340	350	360
HRPMLERIV	ELFRNLMKSA	FVVERQPCMP	MHPDRPLVIK	TGVQFTTKVR	LLVKFPELNY
370	380	390	400	410	420
QLKIKVCIDK	DSGDVAALRG	SRKFENILGTN	TKVMNMEESN	NGSLSAEFKH	LTTLREQRCGN
430	440	450	460	470	480
GGRANCDASL	IVTEELHLIT	FETEVYHQGL	KIDLETHSLP	VVVISNICQM	PNAWASILWY
490	500	510	520	530	540
NMLTNNPKNV	NFFTCKPPIGT	WDQVAEVLWS	QFSSTTKRGL	SIEQLTTLAE	KLLGPGVNYS
550	560	570	580	590	600
GCQITWAKFC	KENMAGKGFS	FWVWLDNIID	LVKKYILALW	NEGYIMGFIS	KERERAILST
610	620	630	640	650	660
KPPGTFLLR	SESSKEGGVT	FTWVEKDISG	KTQIQSVEPY	TKQQLNNMSF	AEIIMGYKIM
670	680	690	700	710	720
DATNILVSPL	VYLYPDIPKE	EAFGKYCRPE	SQEHPEADPG	SAAPYLKTKF	ICVTPTTCSN
730	740	750	760	770	
TIDLPMSPRT	LDSLMQFGNN	GEGAEPSAGG	QFESLTFDME	LTSECATSPM	

FIG. 8

10	20	30	40	50	60
MEGAGGANDK	KKISSERRKE	KSRDAARSRR	SKESEVFYEL	AHQPLPHNV	SSHLDKASVM
70	80	90	100	110	120
RLTISYLRVR	KLDDAGDLDI	EDDMKAQMNC	FYALKALDGFV	MVLTDDGDMI	YISDNVNKYM
130	140	150	160	170	180
GLTQFELTGH	SVFDFTHPCD	HEEMREMLTH	RNGLVKKGKE	QNTQRSFFLR	MKCTLTSRGR
190	200	210	220	230	240
TMNIKSATWK	VLHCTGHIHV	YDTNSNQPC	GYKKPPMTCL	VLICEPIPH	SNIEIPLDSK
250	260	270	280	290	300
TFLSRHSLDM	KFSYCDERIT	ELMGYEPEEL	IGRSIYEYH	ALDSDHLTKT	HHDMFTKGQV
310	320	330	340	350	360
TTGQYRMLAK	RGYVWVETQ	ATVIYNTKNS	QPQCIVCVNY	VVSGIIQHD	IFSLQQTCEV
370	380	390	400	410	420
LKPVESSDMK	MTQLFTKVES	EDTSSLFDKL	KKEPDALTLL	APAAAGDTIIS	LDFGSGNDTET
430	440	450	460	470	480
DDQQLLEEVPL	YNDVMLPSPN	EKLQININLAM	SPLPTAETPK	PLRSSADPAL	NQEVALKLEP
490	500	510	520	530	540
NPESLELSFT	MPQIQDQTPS	PSDGGSTRQS	PEPNSPSEYC	FYVDSDMVNE	FKLELVEKLF
550	560	570	580	590	600
AEDTEAKNPF	STQDTDLDLE	MLAPYIPMDD	DFQLRSFDQL	SPLESSASP	ESASPQSTVT
610	620	630	640	650	660
VFQQTIQIEP	TANATTTTAT	TDELKTVTKD	RMEDIKILIA	SPSPTHIHK	TTSATSSPYR
670	680	690	700	710	720
DTQSRTASN	RAGKGVIEQT	EKSHPRSPNV	LSVALSQRTT	VPEEELNPKI	LALQNAQRKR
730	740	750	760	770	780
KMEHDGSLFQ	AVGIGTLLQQ	PDDHAATTSL	SWKRVKGCKS	SEQNGMEQKT	IILIPSDLAC
790	800	810	820		
RLLGQSMDES	GLPQLTSYDC	EVNAPIQGSR	NLLQGEELLR	ALDQVN	

FIG. 9

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10 MQLVWDPA 20 CLPLPPPPA 30 FKSMEVANFY 40 YEADCLAAAY 50 GKKAAPAAPF 60 AARPGPRPPA
70 GELGSIGDHE 80 RAIDFSPYLE 90 PLGAPQAPAP 100 ATATDTFEAA 110 PPAPAPAPAS 120 SGQHHDFLSD
130 LFSDDYGGKN 140 CKKPAEYGYV 150 SLGRLGAAKG 160 ALHPGCFAPL 170 HPPPPPPPPP 180 AELKAEPGFE
190 PADCKRKEEA 200 GAPGGGAGMA 210 AGFPYALRAY 220 LGYQAVPSGS 230 SGSLSTSSSS 240 SPPGTFSPAD
250 AKAPPTACYA 260 GAAPAPSQVK 270 SKAKKTVDKH 280 SDEYKIRREK 290 NNIAVRKSRD 300 KAKMRNLETQ
310 HKVLELTAEN 320 ERLQKKVEQL 330 SRELSTLRNL 340 FKQLPEPLLA SSGHC

FIG. 10

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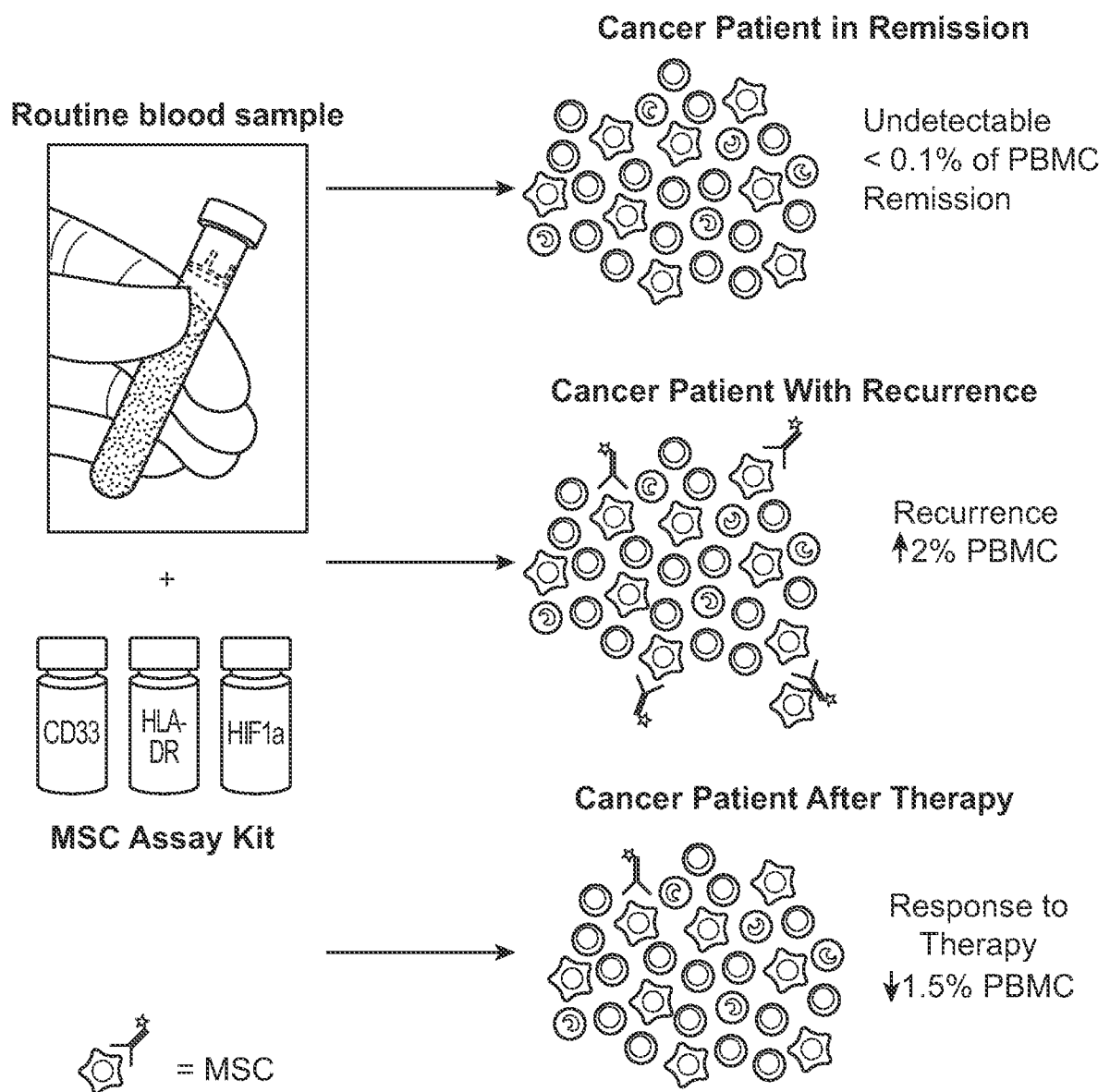


FIG. 11A

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MDSC Clinical Assay Pilot Data on normal healthy donors

Normal healthy donor A

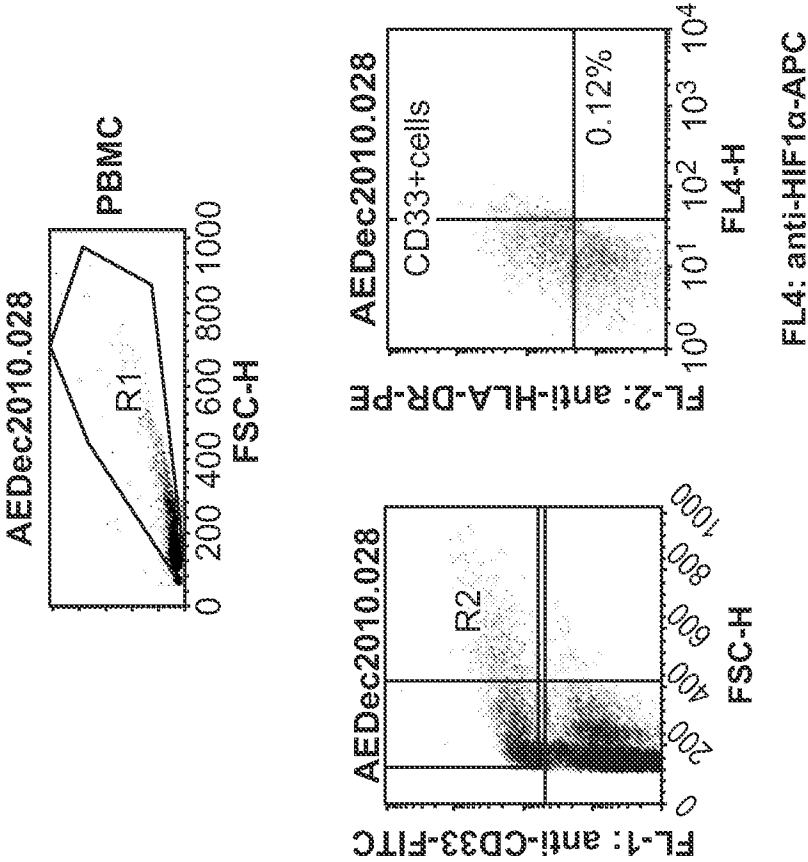


FIG. 11B

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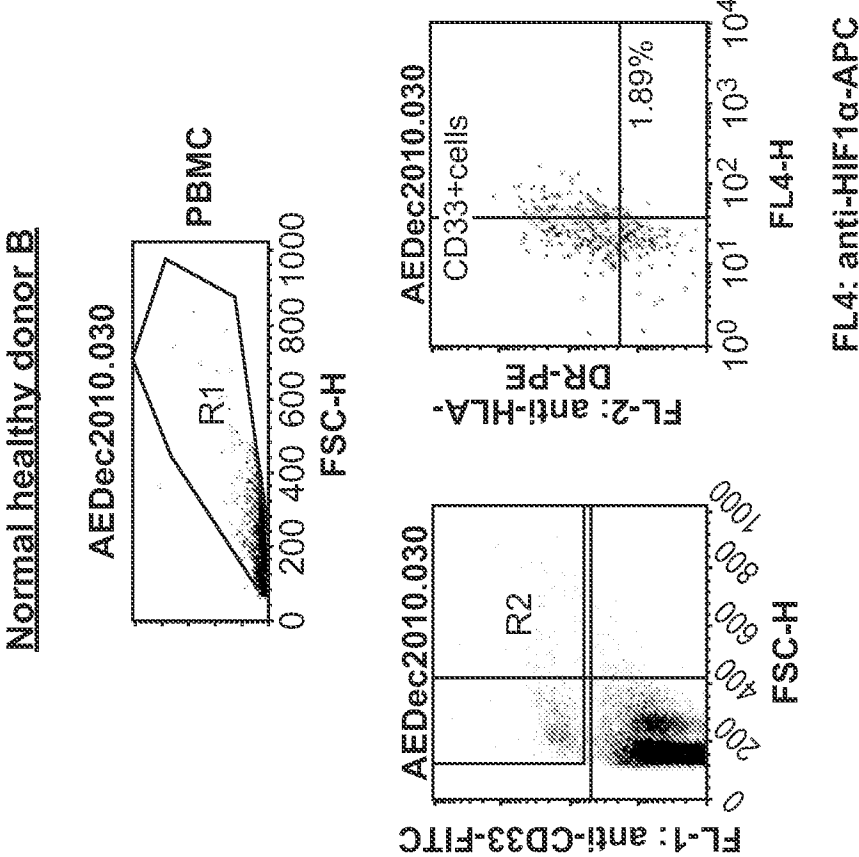


FIG. 11B (Cont. 1)

+

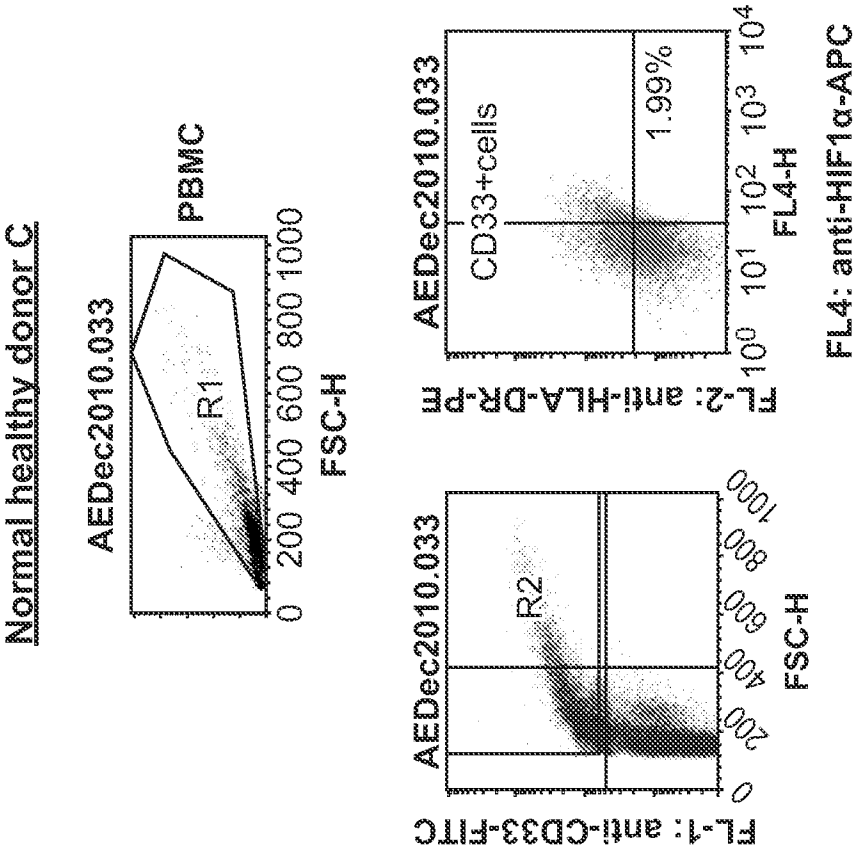


FIG. 11B (Cont. 2)

MDSC Clinical Assay Pilot Data on Head and Neck Cancer Patients

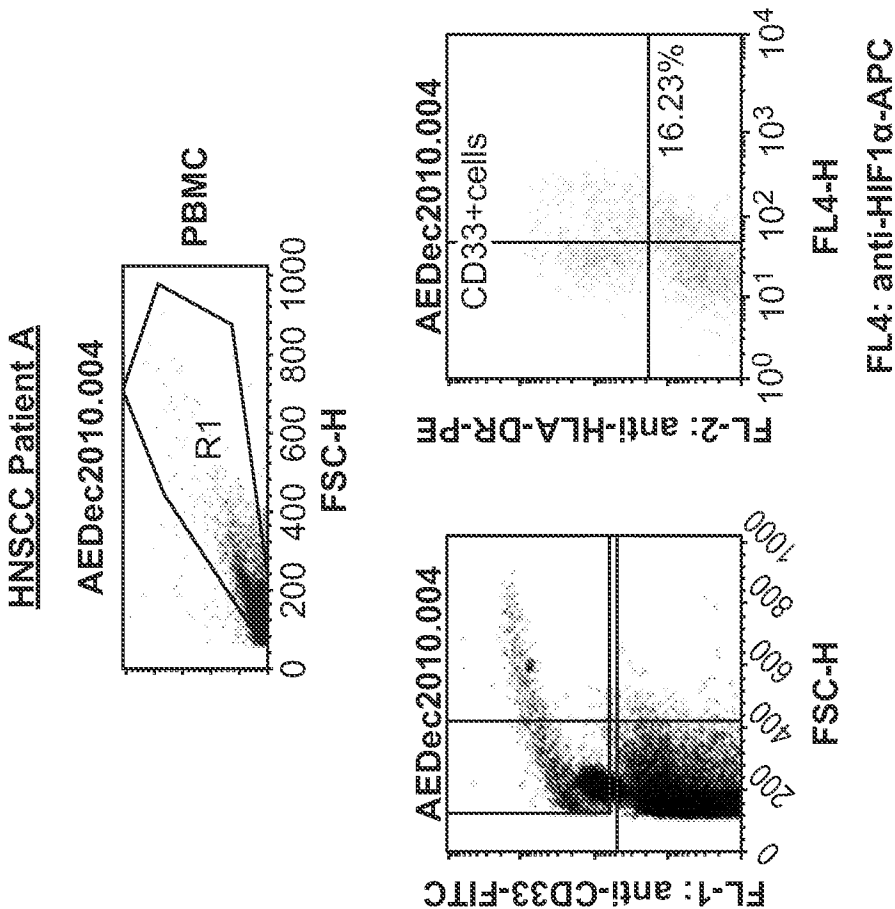


FIG. 11C

+

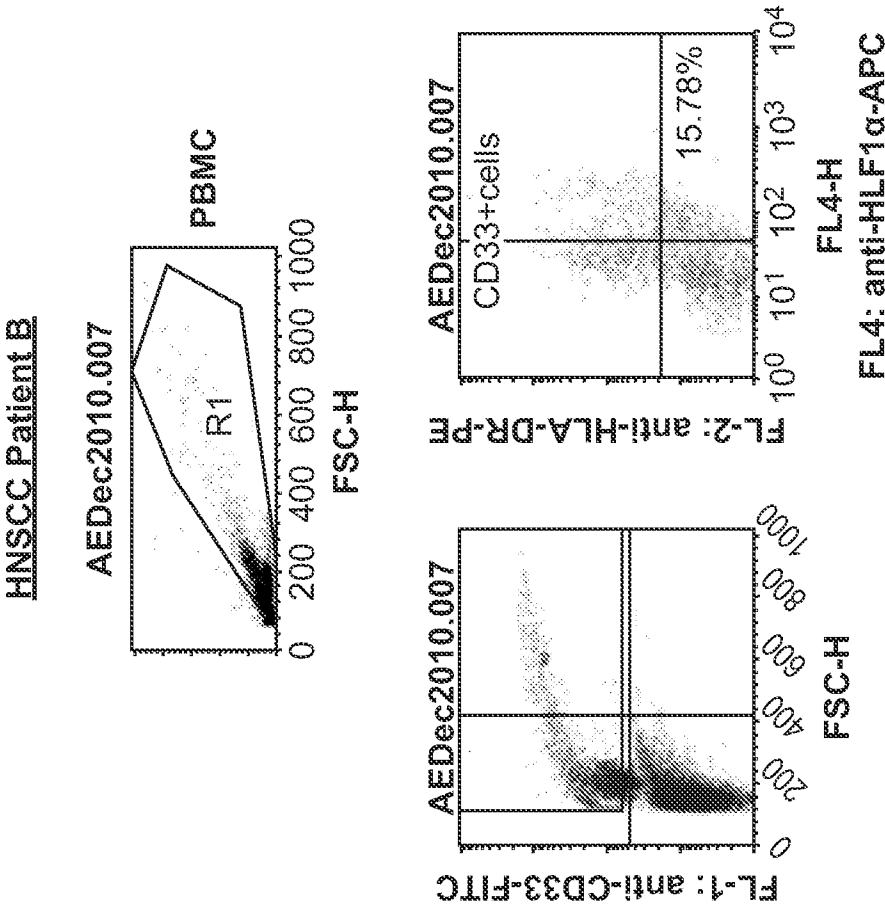


FIG. 11C (Cont.)

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