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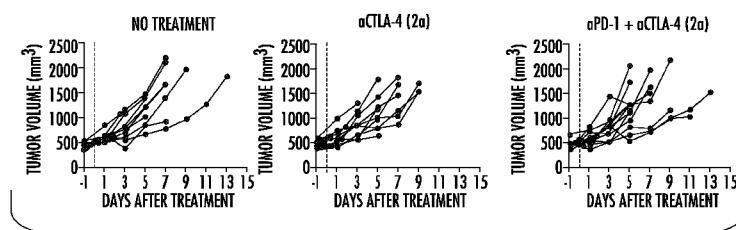


FIG. 7A

(57) Abstract: The inventors have found that androgen deprivation renders the tumor microenvironment in prostate cancer tumors more immunogenic. The combination of androgen deprivation with administration of a depleting anti-CTLA-4 antibody was shown to significantly delay the development of castration resistant prostate cancer. The effect was also observed when anti-CTLA-4 antibody was administered with an additional checkpoint inhibitor, such as anti-PD-1 antibody.



ANDROGEN DEPRIVATION WITH IMMUNE CHECKPOINT BLOCKADE DELAYS
THE DEVELOPMENT OF CASTRATION RESISTANT PROSTATE CANCER

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/134,640, filed on March 18, 2015, which is hereby incorporated by reference for all purposes as if fully set forth herein.

BACKGROUND OF THE INVENTION

[0002] Prostate cancer is the most commonly diagnosed malignancy among men in the USA, and is the second most common cause of cancer mortality in that group. It is estimated that one in six American men will receive a diagnosis of prostate cancer at some point in their lives, at an average age of 68 years. In 2010, there were 217,730 newly diagnosed cases and 32,050 deaths from prostate cancer in the USA alone.

[0003] The management of advanced and metastatic disease, however, is more difficult and requires systemic treatment with either hormonal (i.e., androgen deprivation) therapy (ADT) or chemotherapy. Since androgens are the main regulators of prostate cancer growth, the rationale for ADT is that tumor cells deprived of key hormonal growth regulators will either undergo apoptosis or survive in an arrested state in the G1 phase of the cell cycle. The median duration of response to ADT is approximately 18–24 months, after which, most patients progress to a more aggressive form of disease termed castration (hormone)-resistant prostate cancer (CRPC).

[0004] ADT for advanced prostate cancer is designed to disrupt the androgen receptor (AR) pathway. The intended therapeutic target is the full-length androgen receptor (AR-FL), complete with an intact ligand-binding domain (LBD). Prostate tumors that progress despite first-line ADT (e.g., LHRH analogues), generally termed castration-resistant prostate cancer (CRPC), frequently show continued androgen receptor signaling driven by intratumoral androgens as well as elevated levels of AR-FL. In support of the importance of ligand-driven AR-FL signaling in CRPCs, a number of clinically effective endocrine therapies targeting AR-LBD were recently developed to treat patients with CRPCs (e.g., abiraterone, MDV3100). Nevertheless, the majority of patients progress shortly after treatment, again with reactivated androgen receptor signaling.

[0005] The outlook for patients with CRPC is quite grim. Several investigators have reported that, without treatment, median survival time ranges from 9.1 to 21.7 months. CRPC is now the second most common cause of male cancer-related mortality. However, recent discoveries pertaining to the biology and pathophysiology of the disease over the last two decades have enabled the development of new therapeutic modalities with the hope of improving those statistics. In recent years, a select few chemotherapy, hormonal, immunotherapy and palliative agents have gained US FDA approval for use in patients with CRPC. Additionally, many experimental anticancer drugs are in development, and there are numerous ongoing clinical trials seeking to elucidate optimal treatment regimens using existing modalities that maximize survival time while minimizing side effects.

SUMMARY OF THE INVENTION

[0006] Androgen deprivation therapy induces immune cell infiltration in human prostate cancer. These findings suggest that immunotherapy may be most efficacious when administered concurrently with androgen deprivation, early in disease progression.

[0007] In accordance with an embodiment, the present invention provides a method for treating prostate cancer in a subject comprising administering to a subject a pharmaceutical composition comprising an effective amount of a depleting anti- cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibody.

[0008] In accordance with an embodiment, the present invention provides a method for treating prostate cancer in a subject comprising administering to a subject a pharmaceutical composition comprising an effective amount of a depleting anti- cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibody and an effective amount of an anti PD1 antibody.

[0009] In accordance with another embodiment, the present invention provides a method for prevention or delayed onset of castrate resistant prostate cancer in a subject diagnosed with prostate cancer comprising administering to a subject a pharmaceutical composition comprising an effective amount of a depleting anti-CTLA-4 antibody.

[0010] In accordance with another embodiment, the present invention provides a method for prevention or delayed onset of castrate resistant prostate cancer in a subject diagnosed with prostate cancer comprising administering to a subject a pharmaceutical composition

comprising an effective amount of a depleting anti-CTLA-4 antibody and an effective amount of an anti PD1 antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Figure 1A shows that subcutaneous Myc-Cap tumor model mimics human castration-resistant prostate cancer progression. Eight-week old male FVB mice were subcutaneously implanted with Myc-Cap cells (1×10^6 cells) at right flank. On day 28 after implantation, mice were randomly assigned to either a no treatment group or a surgical castration (bilateral orchiectomy) group. Tumor volumes were measured twice weekly. The representative tumor growth curves of at least 3 independent experiments are shown.

[0012] Figure 1B shows total RNA was extracted from subcutaneous Myc-Cap tumors (Pre-C, pre-castration; post-C D and D7, day 1 and day 7 after castration; C-resistant, castration-resistant; $n \geq 10$ for each group), Myc-Cap culture cells (from 2 independent experiments), murine prostate (pooled from 3 mice), murine spleen (from 2 mice), and 2 murine ovarian cancer cell lines. mRNAs of androgen receptor (AR), prostate stem cell antigen (PSCA) and mesothelin (MSLN) of individual samples was quantified and normalized to the mean level of Pre-C group. Mean expression levels $\pm$ SDs are shown. P-value was determined by unpaired t test or Mann-Whitney test. *, $P < 0.05$; **, $P < 0.001$; NS, not statistically significant.

[0013] Figures 2A-2D depict that castration transiently changes the tumor microenvironment toward more immunogenic. Eight-week old male FVB mice were subcutaneously implanted with Myc-Cap cells (1×10^6 cells) at right flank. On day 28 after implantation, mice were left untreated or surgically castrated. Tumors were dissected for RNA extraction at the indicated time points (Pre-C, pre-castration; Post-C D1 and D7, post-castration day 1 and day 7; C-resistant, castration-resistant; $n \geq 10$ for each group). Total RNA of Myc-Cap culture cells from 2 independent experiments, naïve splenocytes (shown as spleen or Non-stim; $n=3$) and splenocytes stimulated with PMA (20 ng/ml) and ionomycin (1 μ g/ml) for 6h ($n=3$) were extracted. mRNA expression of indicated markers was quantified and normalized to that of Pre-C group. 2A depicts the different marker mRNA levels in each cell type. 2B depicts the levels of cytokines produced at the different time points. 2C depicts

the levels of different transcriptional factors produced at the different time points. 2D depicts the levels of immune checkpoint molecules or ligands produced at the different time points in tumors and spleen. Mean expression levels $\pm$ SDs are shown. P-value was determined by unpaired t test or Mann-Whitney test. *, $P < 0.05$; **, $P < 0.001$; NS, not statistically significant.

[0014] Figure 3 shows graphs of levels of various markers indicating that castration induces intra-tumoral infiltration of immune cells.

[0015] Figures 4A-4B are graphs depicting castration inducing T cell activation. 4A depicts the levels of markers IFN γ , TNF α , IL2 and GrzB $^{+}$ in CD8 $^{+}$ T cells. 4B depicts the levels of markers IFN γ , TNF α , and IL2 in CD4 $^{+}$ T cells.

[0016] Figures 5A-5C show that the combination of degarelix and anti-CTLA-4 (IgG2a) with/without anti-PD-1 prolongs castration resistance-free survival and overall survival of Myc-Cap tumor bearing mice. 8 week-old FVB/NJ male mice were implanted with Myc-Cap (1×10^6 cells) at right flank, and were treated with degarelix alone, degarelix with anti-PD-1, degarelix with anti-CTLA-4 (IgG1 D265A), degarelix with anti-CTLA-4 (IgG2a), degarelix with anti-PD-1 and anti-CTLA-4 (IgG1 D265A), or degarelix with anti-PD-1 and anti-CTLA-4 (IgG2a) when tumor size was $\geq 450 \text{ mm}^3$. Individual tumor growth is shown as Figure 5A. Castration resistance is arbitrarily defined as tumor size larger than that on castration. Castration resistance-free survival and overall survival are shown as Figure 5B and 5C, respectively. * indicates $P < 0.05$: a, compared to degarelix alone; b, compared to degarelix and anti-PD-1; c, compared to degarelix and anti-CTLA-4 (IgG1 D265A); d, compared to degarelix combined with anti-PD-1 and anti-CTLA-4 (IgG1 D265A).

[0017] Figures 6A-6B show graphs of various T cell activation parameters on D7 after administration of degarelix.

[0018] Figures 7A-7B: Combination of anti-PD-1 and anti-CTLA-4 (IgG2a) antibodies, rather than anti-CTLA-4 (IgG2a) alone, suppresses tumor growth but fails to prolong survival of mice bearing castration-resistant Myc-Cap tumors. 8 week-old FVB/NJ male mice were implanted with Myc-Cap (1×10^6 cells) at right flank, and were castrated via bilateral orchiectomy when tumor size was $\geq 450 \text{ mm}^3$. When the castration-resistant tumor reached 400 mm^3 , mice were randomly allocated to no treatment, anti-CTLA-4 (IgG2a) or combination of anti-PD-1 and anti-CTLA-4 (IgG2a). Individual tumor growth is shown. B.

Overall survival of castration-resistant tumor bearing mice is shown. The median survival after treatment is 7 days for each treatment group.

DETAILED DESCRIPTION OF THE INVENTION

[0019] In accordance with an embodiment, the present invention provides a method for treating prostate cancer in a subject comprising administering to a subject a pharmaceutical composition comprising an effective amount of a depleting anti-CTLA-4 antibody.

[0020] In accordance with another embodiment, the present invention provides the use of a depleting anti-CTLA-4 antibody, in an effective amount to treat prostate cancer in a subject in need thereof.

[0021] As used herein, the term "treat," as well as words stemming therefrom, includes preventative as well as disorder remitative treatment. The terms "reduce," "suppress," "prevent," and "inhibit," as well as words stemming therefrom, have their commonly understood meaning of lessening or decreasing. These words do not necessarily imply 100% or complete treatment, reduction, suppression, or inhibition.

[0022] As used herein, the term "a depleting anti-CTLA-4 antibody" means a monoclonal antibody which binds the protein CTLA-4, which is expressed on the surface of activated T lymphocytes and blocks the binding of the antigen-presenting cell blocks the binding of the antigen-presenting cell ligands B7.1 and B7.2 to CTLA-4, resulting in inhibition of B7-CTLA-4-mediated downregulation of T-cell activation, and which has a depleting effect on T_{reg} cells.

[0023] In accordance with an embodiment, the methods of the present invention comprise optionally, administration of one or more other anti-androgen and/or anti-cancer compounds, together with a pharmaceutically acceptable carrier.

[0024] In accordance with another embodiment, the present invention provides the use of a depleting anti-CTLA-4 antibody, and optionally, one or more other anti-androgen and/or anti-cancer compounds, together with a pharmaceutically acceptable carrier in an effective amount to treat prostate cancer in a subject in need thereof.

[0025] In accordance with an embodiment, the method further comprises administering to the subject a pharmaceutical composition comprising an effective amount of an additional checkpoint inhibitor.

[0026] In accordance with another embodiment, the present invention provides the use of a depleting anti-CTLA-4 antibody, and a pharmaceutical composition comprising an effective amount of an additional checkpoint inhibitor in an effective amount to treat prostate cancer in a subject in need thereof.

[0027] In some embodiments, the checkpoint inhibitor is a composition that includes blockade of the checkpoint protein, programmed cell death protein 1 (PD-1). Examples of such inhibitors include, pembrolizumab, nivolumab, MPDL3280A, MEDI4736, AMP-514, and MSB0010718C.

[0028] As defined herein, “anti-androgen compounds” include any compound which can act as an androgen hormone receptor antagonist and/or are capable of preventing or inhibiting the biologic effects of androgens, or male sex hormones on androgen receptors, and functional portions thereof.

[0029] In an embodiment, the present invention provides that the other anti-androgen compounds are selected from the group consisting of degarelix, spironolactone, cyproterone acetate, flutamide, ketoconazole, finasteride, bexlosteride, izonsteride, epristeride, turosteride, R1881 (methyltrienolone), nilutamide, bicalutamide, MDV3100 (4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-methylbenzamide), BMS-641988, YM-580, DIMP, abiraterone acetate, VN/124-1, dutasteride, FCE 28260, SKF 105111, apoptone (HE3235), TAK-700, and ARN-509.

[0030] In an embodiment, the present invention provides that the other anticancer compounds can be, for example, anticancer drugs from the following drug classes, including, but not limited to, antimitotics, antineoplastics, antimetabolites, and alkylating agents. Such classes of anticancer drugs are well known in the art.

[0031] In accordance with another embodiment, the present invention provides a method for prevention or delayed onset of castrate resistant prostate cancer in a subject diagnosed with prostate cancer comprising administering to a subject a pharmaceutical composition comprising an effective amount of an anti-CTLA-4 antibody.

[0032] In accordance with an embodiment, the methods of the present invention comprise optionally, administration of one or more other anti-androgen and/or anti-cancer compounds, together with a pharmaceutically acceptable carrier.

[0033] In accordance with an embodiment, the method further comprises administering to the subject a pharmaceutical composition comprising an effective amount of an additional checkpoint inhibitor.

[0034] With respect to methods described herein, the pharmaceutically acceptable carrier can be any of those conventionally used, and is limited only by physico-chemical considerations, such as solubility and lack of reactivity with the active compound(s), and by the route of administration. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. Examples of the pharmaceutically acceptable carriers include soluble carriers such as known buffers which can be physiologically acceptable (e.g., phosphate buffer) as well as solid compositions such as solid-state carriers or latex beads. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active agent(s), and one which has little or no detrimental side effects or toxicity under the conditions of use.

[0035] The carriers or diluents used herein may be solid carriers or diluents for solid formulations, liquid carriers or diluents for liquid formulations, or mixtures thereof.

[0036] Solid carriers or diluents include, but are not limited to, gums, starches (e.g., corn starch, pregelatinized starch), sugars (e.g., lactose, mannitol, sucrose, dextrose), cellulosic materials (e.g., microcrystalline cellulose), acrylates (e.g., polymethylacrylate), calcium carbonate, magnesium oxide, talc, or mixtures thereof.

[0037] For liquid formulations, pharmaceutically acceptable carriers may be, for example, aqueous or non-aqueous solutions, suspensions, emulsions or oils. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Aqueous carriers include, for example, water, alcoholic/aqueous solutions, cyclodextrins, emulsions or suspensions, including saline and buffered media.

[0038] Examples of oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, mineral oil, olive oil, sunflower oil, fish-liver oil, sesame oil, cottonseed oil, corn oil, olive, petrolatum, and mineral. Suitable fatty acids for use in

parenteral formulations include, for example, oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[0039] Parenteral vehicles (for subcutaneous, intravenous, intraarterial, or intramuscular injection) include, for example, sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's and fixed oils. Formulations suitable for parenteral administration include, for example, aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[0040] Intravenous vehicles include, for example, fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Examples are sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. In general, water, saline, aqueous dextrose and related sugar solutions, and glycols such as propylene glycols or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions.

[0041] In addition, in an embodiment, the compounds of the present invention may further comprise, for example, binders (e.g., acacia, cornstarch, gelatin, carbomer, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone), disintegrating agents (e.g., cornstarch, potato starch, alginic acid, silicon dioxide, croscarmellose sodium, crospovidone, guar gum, sodium starch glycolate), buffers (e.g., Tris-HCl, acetate, phosphate) of various pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), protease inhibitors, surfactants (e.g. sodium lauryl sulfate), permeation enhancers, solubilizing agents (e.g., cremophor, glycerol, polyethylene glycerol, benzalkonium chloride, benzyl benzoate, cyclodextrins, sorbitan esters, stearic acids), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxyanisole), stabilizers (e.g., hydroxypropyl cellulose, hydroxypropylmethyl cellulose), viscosity increasing agents (e.g., carbomer, colloidal silicon dioxide, ethyl cellulose, guar gum), sweeteners (e.g., aspartame, citric acid), preservatives (e.g., thimerosal, benzyl alcohol, parabens), lubricants (e.g., stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate), flow-aids (e.g., colloidal silicon dioxide), plasticizers (e.g., diethyl phthalate, triethyl citrate), emulsifiers

(e.g., carbomer, hydroxypropyl cellulose, sodium lauryl sulfate), polymer coatings (e.g., poloxamers or poloxamines), coating and film forming agents (e.g., ethyl cellulose, acrylates, polymethacrylates), and/or adjuvants.

[0042] The choice of carrier will be determined, in part, by the particular compound, as well as by the particular method used to administer the compound. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the invention. The following formulations for parenteral, subcutaneous, intravenous, intramuscular, intraarterial, intrathecal and interperitoneal administration are exemplary, and are in no way limiting. More than one route can be used to administer the compounds, and in certain instances, a particular route can provide a more immediate and more effective response than another route.

[0043] Suitable soaps for use in parenteral formulations include, for example, fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include, for example, (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl- β -aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

[0044] The parenteral formulations will typically contain from about 0.5% to about 25% by weight of the compounds in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants, for example, having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include, for example, polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol.

[0045] The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for

injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets.

[0046] Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J.B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Trissel, 15th ed., pages 622-630 (2009)).

[0047] For purposes of the invention, the amount or dose of the compounds, salts, solvates, or stereoisomers of any one the compounds included in the methods, as set forth above, administered should be sufficient to effect, e.g., a therapeutic or prophylactic response, in the subject over a reasonable time frame. The dose will be determined by the efficacy of the particular compound and the condition of a human, as well as the body weight of a human to be treated.

[0048] The dose of any one the compounds included in the methods, or salts, solvates, or stereoisomers thereof, as set forth above, of the present invention also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular compound. Typically, an attending physician will decide the dosage of the compound with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, compound to be administered, route of administration, and the severity of the condition being treated. By way of example, and not intending to limit the invention, the dose of the compound can be about 0.001 to about 1000 mg/kg body weight of the subject being treated/day, including about 0.00001 mg, 0.0001 mg, 0.001 mg, 0.005 mg, 0.01 mg, 0.05 mg, 0.1 mg, 0.25 mg, 0.5 mg, 1 mg, 2 mg, 4 mg, 8 mg, 10 mg, 12 mg, 14 mg, 16 mg, 18 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, and 950 mg per kg body weight/day. In some embodiments the dosages of the compounds described herein can be from about 0.01 to about 100 mg/kg body weight/day, about 0.1 mg to about 10 mg/kg body weight/day.

[0049] Alternatively, the compounds of the present invention can be modified into a depot form, such that the manner in which the compound is released into the body to which it is administered is controlled with respect to time and location within the body (see, for

example, U.S. Patent No. 4,450,150). Depot forms of compounds can be, for example, an implantable composition comprising the compound and a porous or non-porous material, such as a polymer, wherein the compound is encapsulated by or diffused throughout the material and/or degradation of the non-porous material. The depot is then implanted into the desired location within the body and the compounds are released from the implant at a predetermined rate.

[0050] In one embodiment, the compositions use in the methods of the present invention provided herein can be controlled release compositions, i.e., compositions in which the one or more compounds are released over a period of time after administration. Controlled or sustained release compositions include formulation in lipophilic depots (e.g., fatty acids, waxes, oils). In another embodiment the composition is an immediate release composition, i.e., a composition in which all or substantially all of the composition is released immediately after administration.

[0051] In yet another embodiment, the compositions used in the methods of the present invention can be delivered in a controlled release system. For example, the agent may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, or other modes of administration. In an embodiment, a pump may be used. In one embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity to the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., *Design of Controlled Release Drug Delivery Systems*, Xiaoling Li and Bhaskara R. Jasti eds. (McGraw-Hill, 2006)).

[0052] The compositions included in the methods of the present invention may also include incorporation of the active ingredients into or onto particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, hydrogels, etc., or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance.

[0053] In accordance with the present invention, the compositions used in the methods of the present invention may be modified by, for example, the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline. The modified compositions are known to exhibit

substantially longer half-lives in blood following intravenous injection, than do the corresponding unmodified compounds. Such modifications may also increase the compositions' solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. As a result, the desired *in vivo* biological activity may be achieved by the administration of such polymer-compound adducts less frequently, or in lower doses than with the unmodified compositions.

EXAMPLES

[0054] The present inventors used a subcutaneous allograft model of murine prostate cancer (Myc-Cap), which mimics the development of human castration-resistant prostate cancer (CRPC) progression to study the anti-tumor effects of concurrent hormonal/immunotherapy. Implanted Myc-Cap tumors initially respond to androgen deprivation (degarelix acetate or bilateral orchiectomy), but mice eventually progress with CRPC. (Figs. 1-2).

[0055] Castration transiently changes the tumor microenvironment toward more immunogenic. Eight-week old male FVB mice were subcutaneously implanted with Myc-Cap cells (1×10^6 cells) at right flank. On day 28 after implantation, mice were left untreated or surgically castrated (Fig. 2).

[0056] To test the hypothesis that the combination of androgen deprivation and immune checkpoint blockade could mediate pre-clinical benefit, mice were treated with either anti-PD-1, a depleting anti-CTLA-4 antibody (IgG2A), a non-depleting anti-CTLA-4 antibody (IgG1 D265A) or antibody combinations in the peri-castration period, then followed mice for the development of castration-resistant disease. Interestingly, the depleting anti-CTLA-4 antibody, both with, and without anti-PD-1 antibody, was strikingly effective in preventing the emergence of castration-resistant disease. The median castration-resistance free survival was 22 days in mice treated with androgen deprivation alone versus 32 days in mice treated with androgen deprivation and depleting anti-CTLA-4 antibody ($P < 0.05$, compared to androgen deprivation alone) versus 30 days in mice treated with androgen deprivation, depleting anti-CTLA-4 and anti-PD-1 ($P < 0.05$, compared to androgen deprivation alone; non-

significant, compared to androgen deprivation and depleting anti-CTLA-4 antibody). (Figs. 3-5).

[0057] Immunologically, it was found that castration increases intratumoral infiltration of helper, cytotoxic and regulatory T cells (T_{regs}), natural killer cells, and macrophages, as well as effector cytokine production of T cells (Fig. 3). Up-regulated expression of CTLA-4 and PD-1 as well as their respective ligands (Fig. 4) was also found. Mechanistic studies showed that androgen deprivation combined with depleting anti-CTLA-4 /anti-PD-1 significantly reduces intratumoral T_{regs} and increases interferon- γ - or tumor necrosis factor- α - producing T cells in the tumor and its draining lymph node (Figs. 5-7).

[0058] In conclusion, while androgen deprivation renders the tumor microenvironment more immunogenic; the combination of androgen deprivation and depleting anti-CTLA-4 antibody with/without anti-PD-1 can significantly delay or prevent the development of CRPC.

[0059] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0060] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0061] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

Claims:

1. Use of an effective amount of a depleting anti-CTLA-4 antibody for treating prostate cancer in a subject in need thereof.
2. Use of a pharmaceutical composition comprising an effective amount of a depleting anti-CTLA-4 antibody and a pharmaceutically acceptable carrier for treating prostate cancer in a subject in need thereof.
3. The use of either claims 1 or 2, wherein the use further comprises an effective amount of an additional checkpoint inhibitor.
4. The use of either claims 1 or 2, wherein the subject is undergoing androgen deprivation therapy.
5. The use of either claims 1 or 2, wherein the use further comprises an effective amount of an anticancer agent.
6. The use of claim 3, wherein the additional checkpoint inhibitor is selected from the group consisting of pembrolizumab, nivolumab, MPDL3280A, MEDI4736, AMP-514, and MSB0010718C.
7. The use of claim 6, wherein the checkpoint inhibitor is an anti-PD1 antibody.
8. Use of an effective amount of a depleting anti-CTLA-4 antibody for treating or prevention of delayed onset of castrate resistant prostate cancer in a subject diagnosed with prostate cancer.
9. Use of a pharmaceutical composition comprising an effective amount of a depleting anti-CTLA-4 antibody and a pharmaceutically acceptable carrier for treating or prevention of delayed onset of castrate resistant prostate cancer in a subject diagnosed with prostate cancer.
10. The use of either claims 8 or 9, wherein the use further comprises an effective amount of an additional checkpoint inhibitor.

11. The use of either claims 8 or 9, wherein the subject is undergoing androgen deprivation therapy.

12. The use of either claims 8 or 9, wherein the use further comprises an effective amount of an anticancer agent.

13. The use of claim 10, wherein the additional checkpoint inhibitor is selected from the group consisting of pembrolizumab, nivolumab, MPDL3280A, MEDI4736, AMP-514, and MSB0010718C.

14. The use of claim 13, wherein the checkpoint inhibitor is an anti-PD1 antibody.

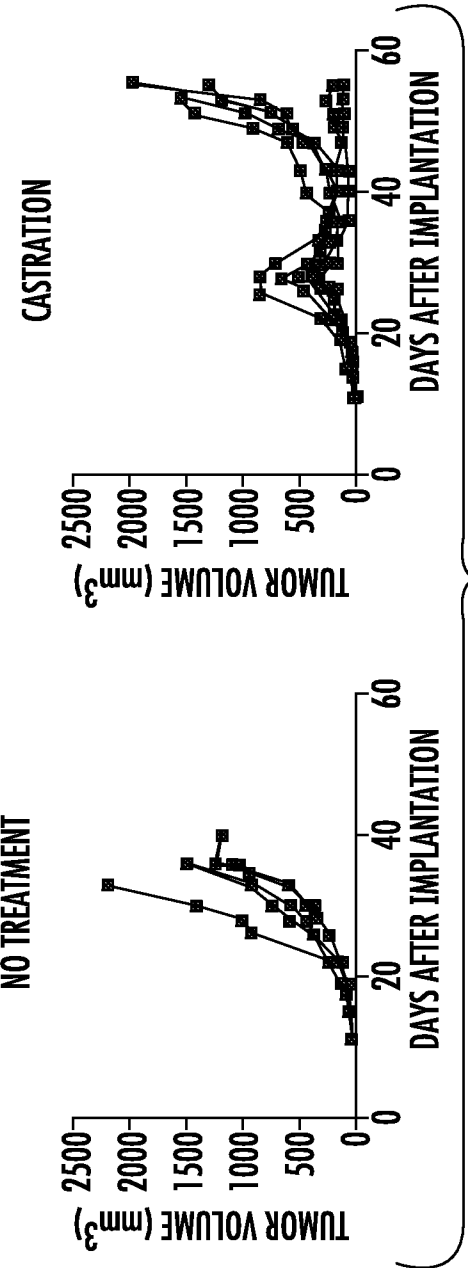
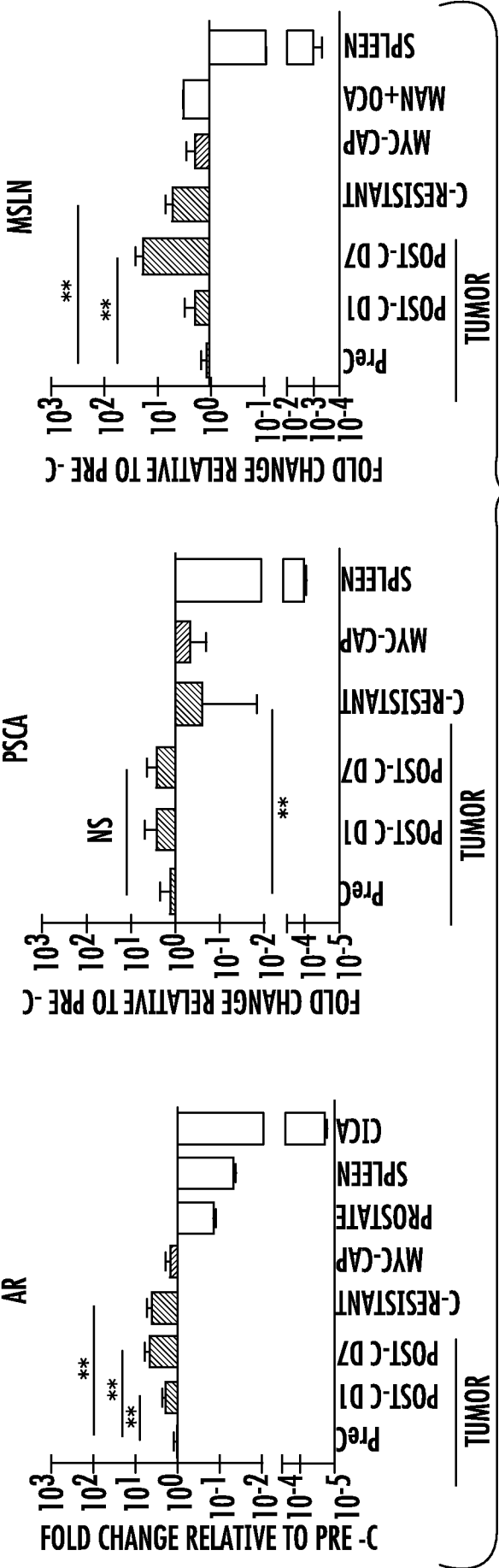


FIG. 1A



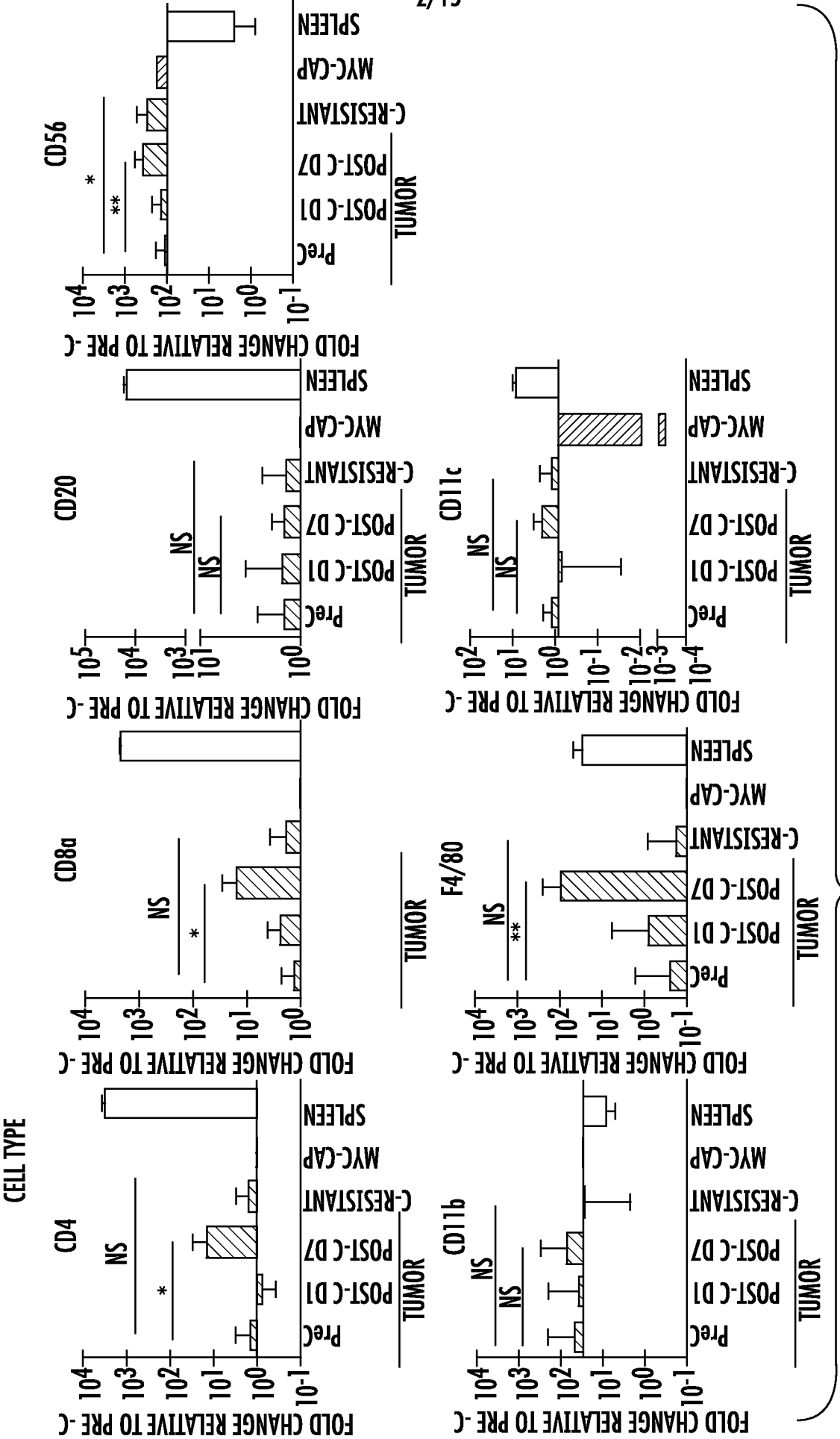


FIG. 2A

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CYTOKINES

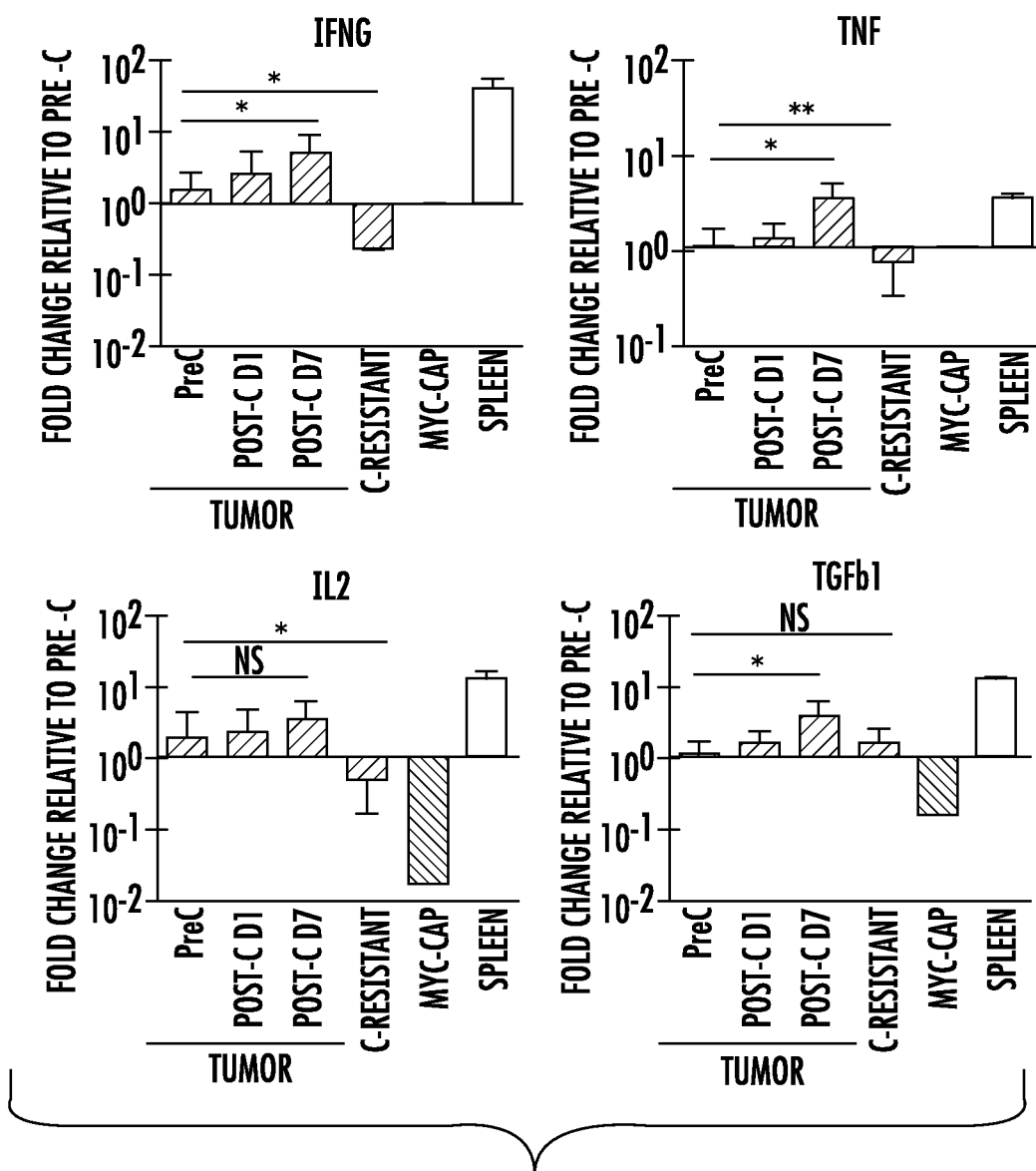
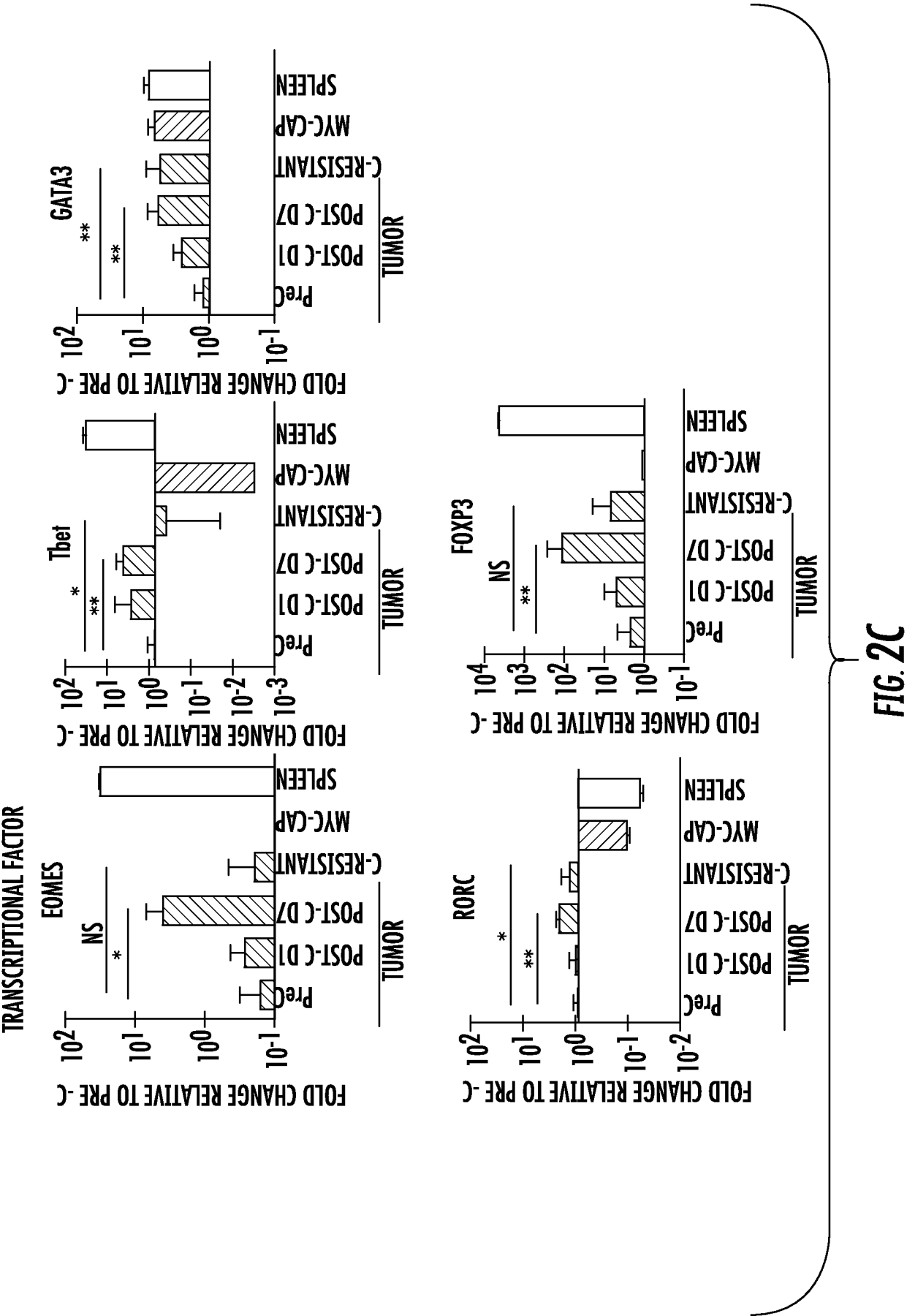


FIG. 2B



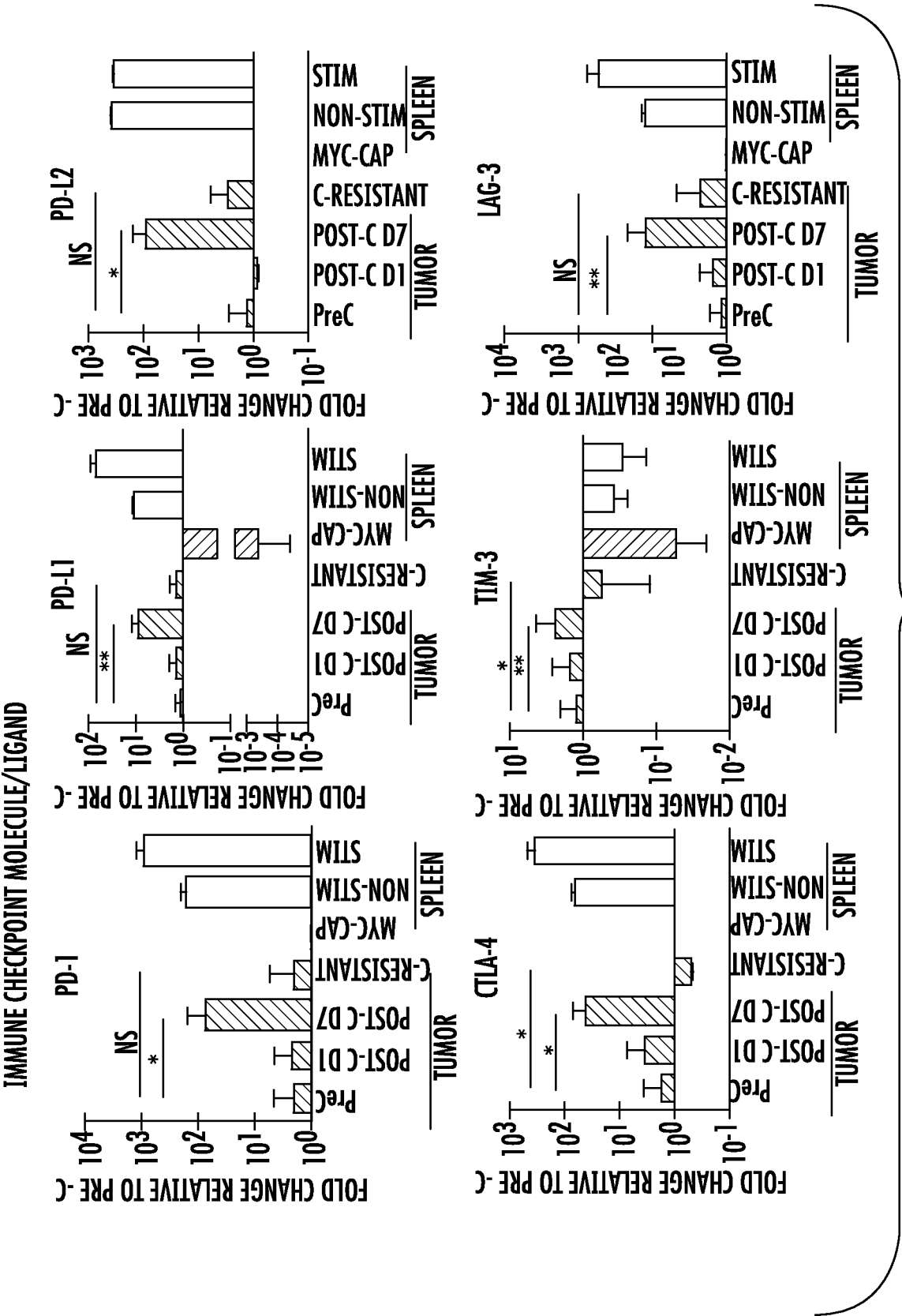


FIG. 2D

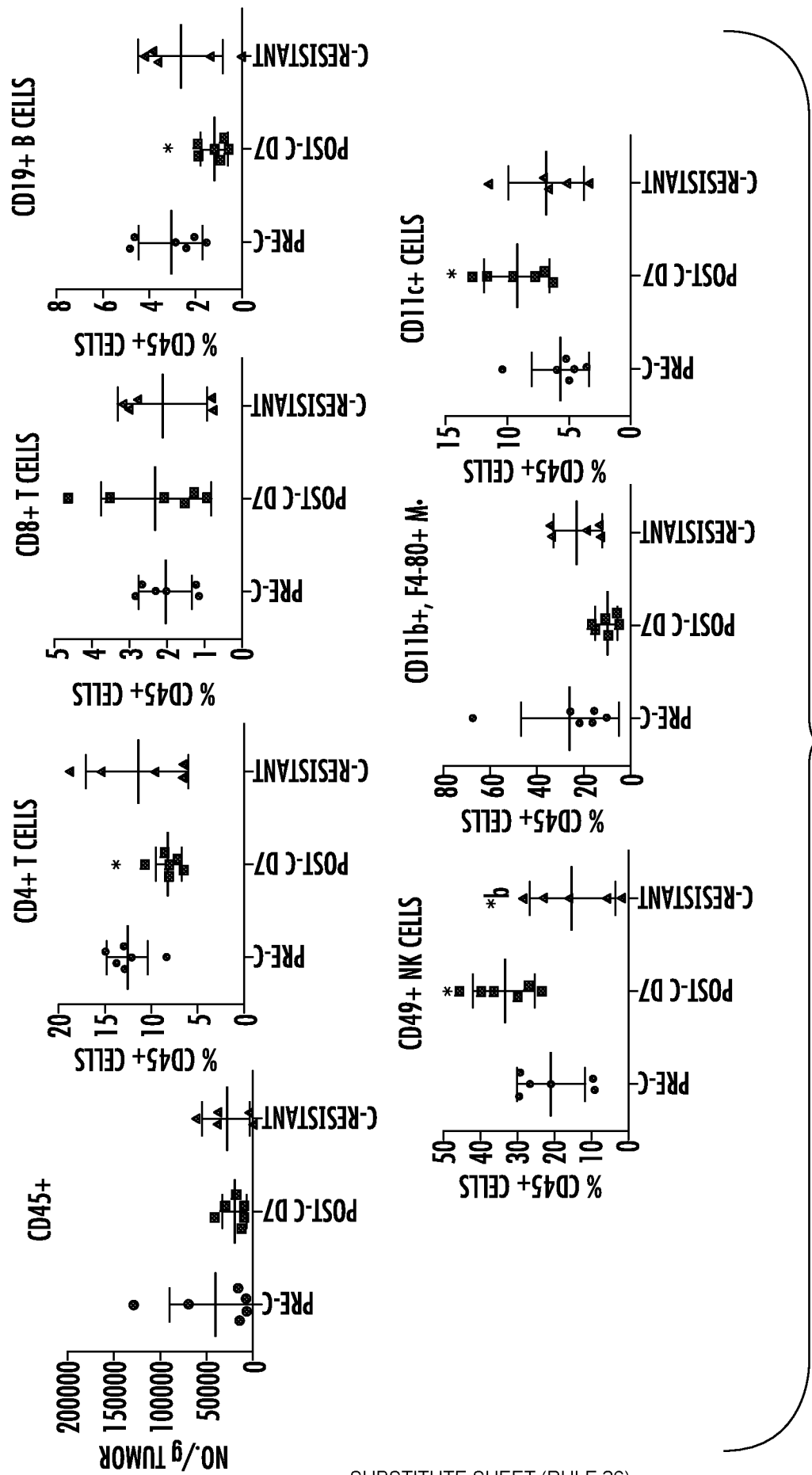


FIG. 3

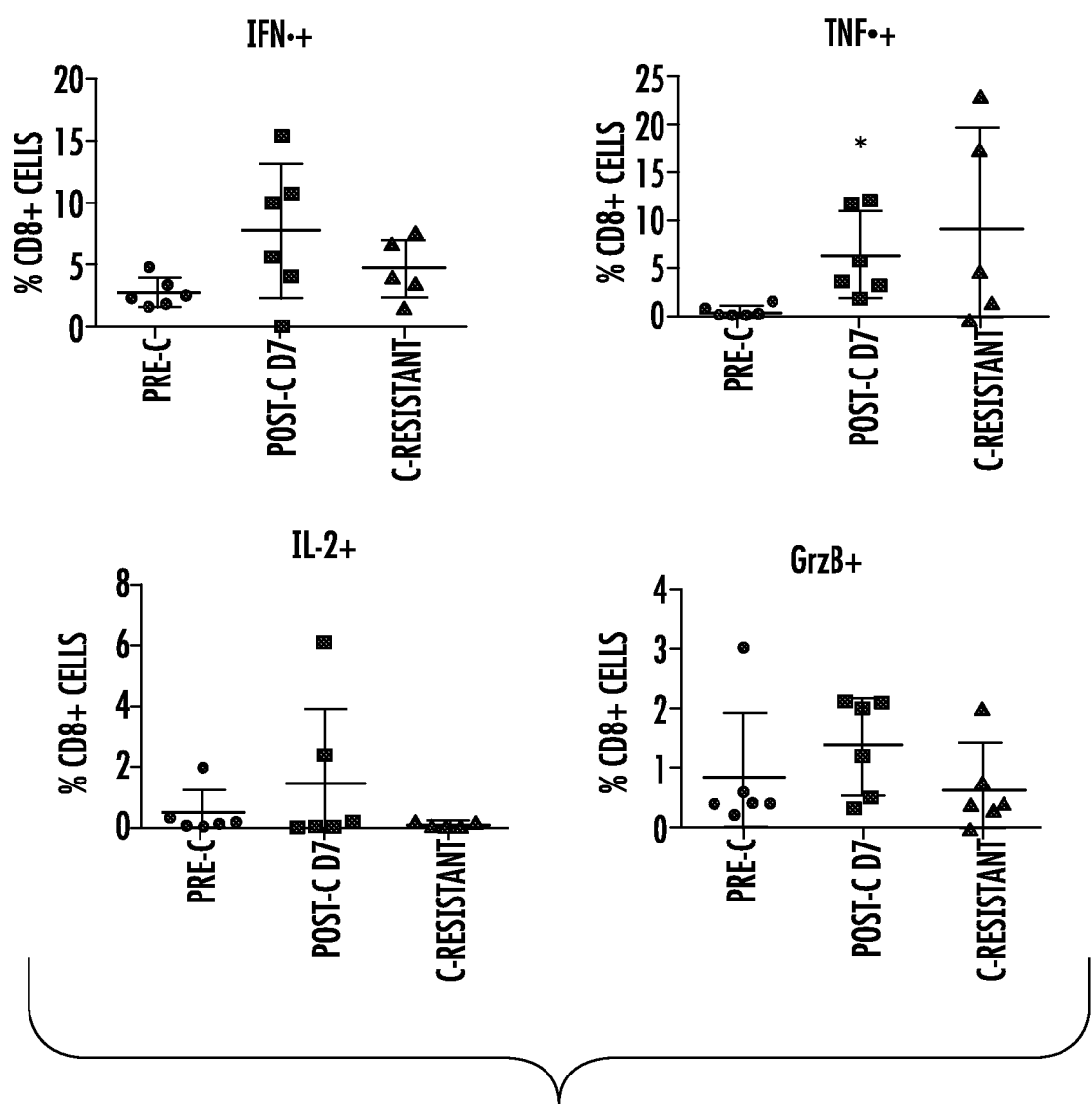
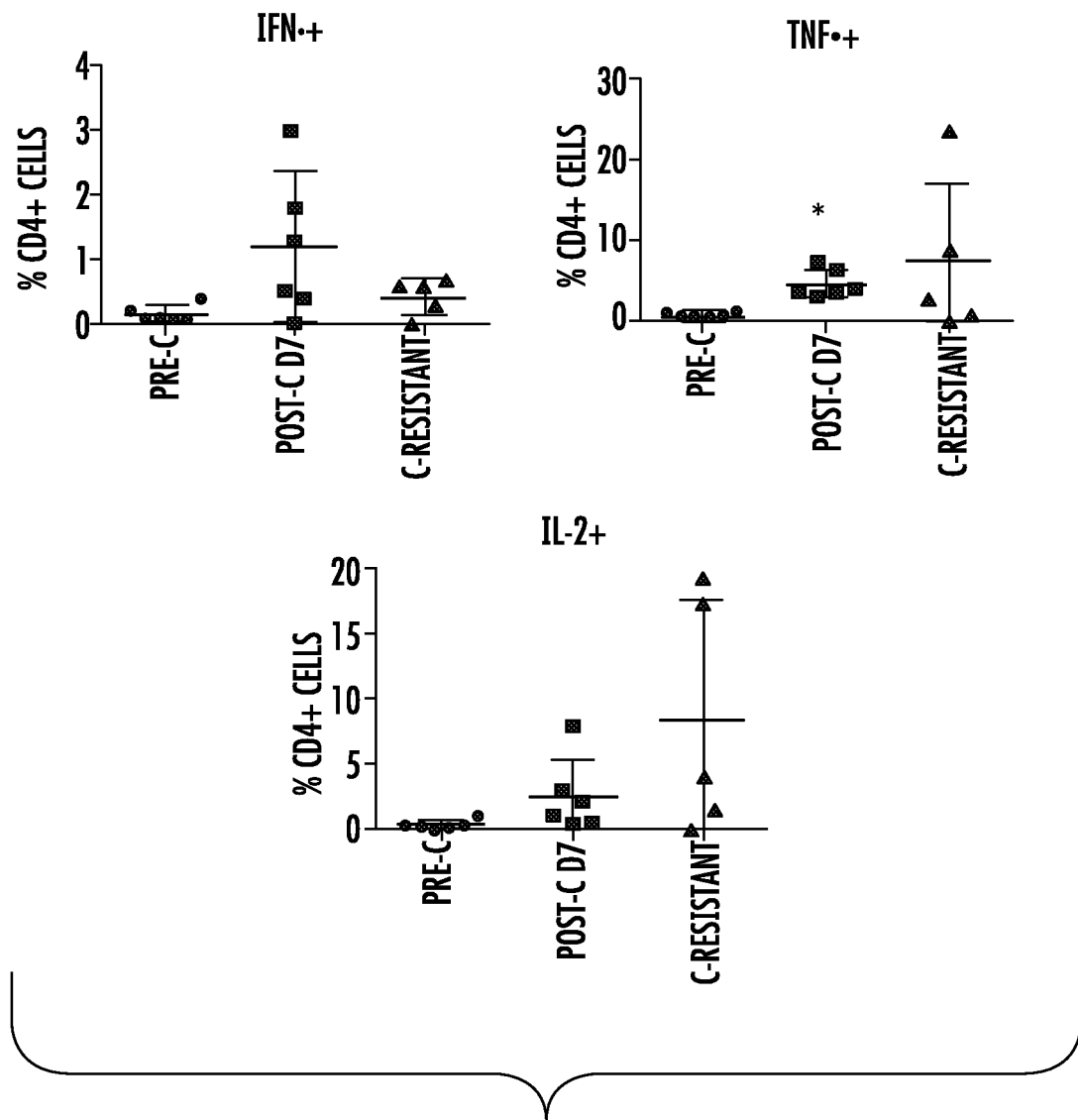


FIG. 4A

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**FIG. 4B**

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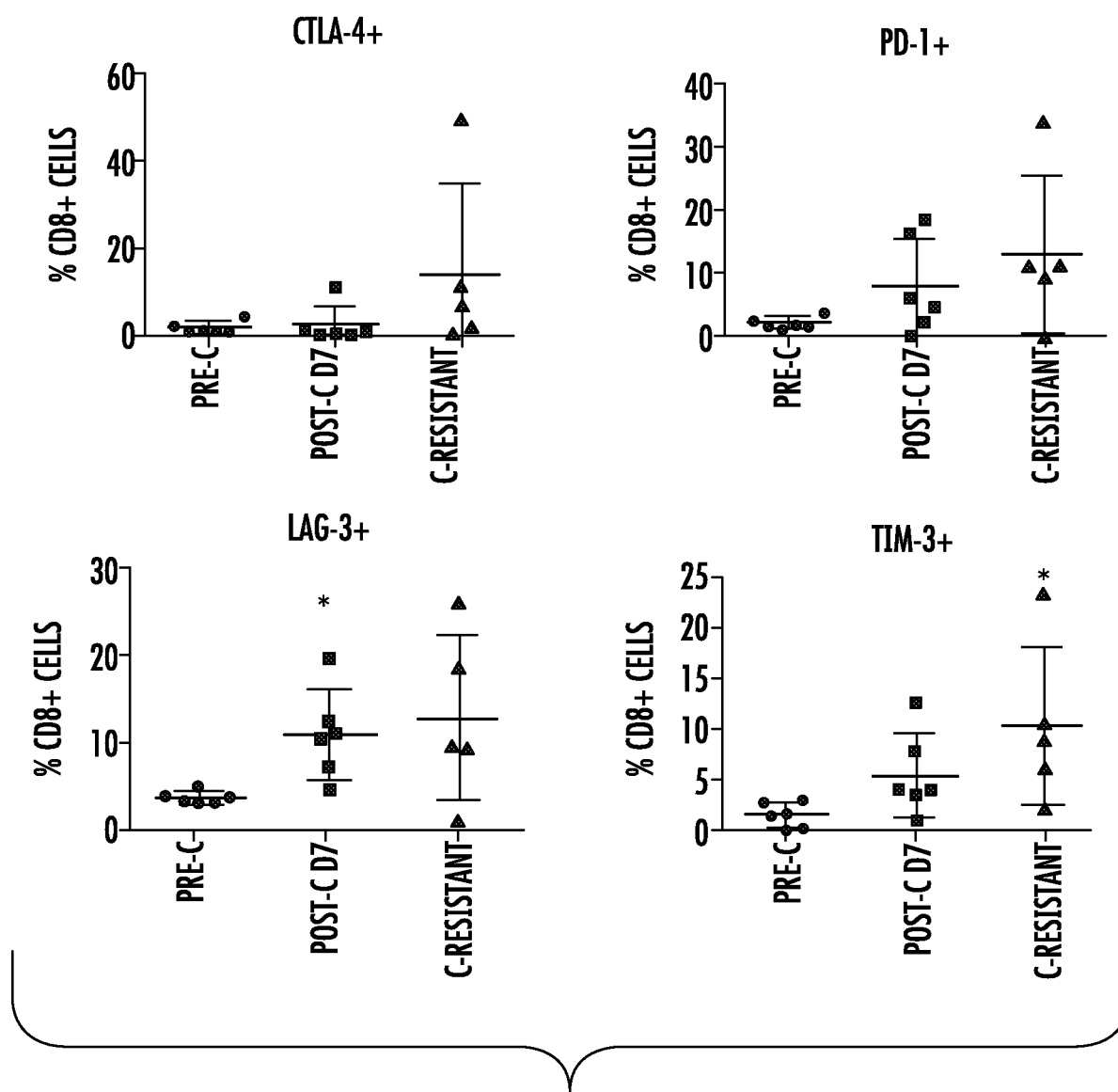


FIG. 4C

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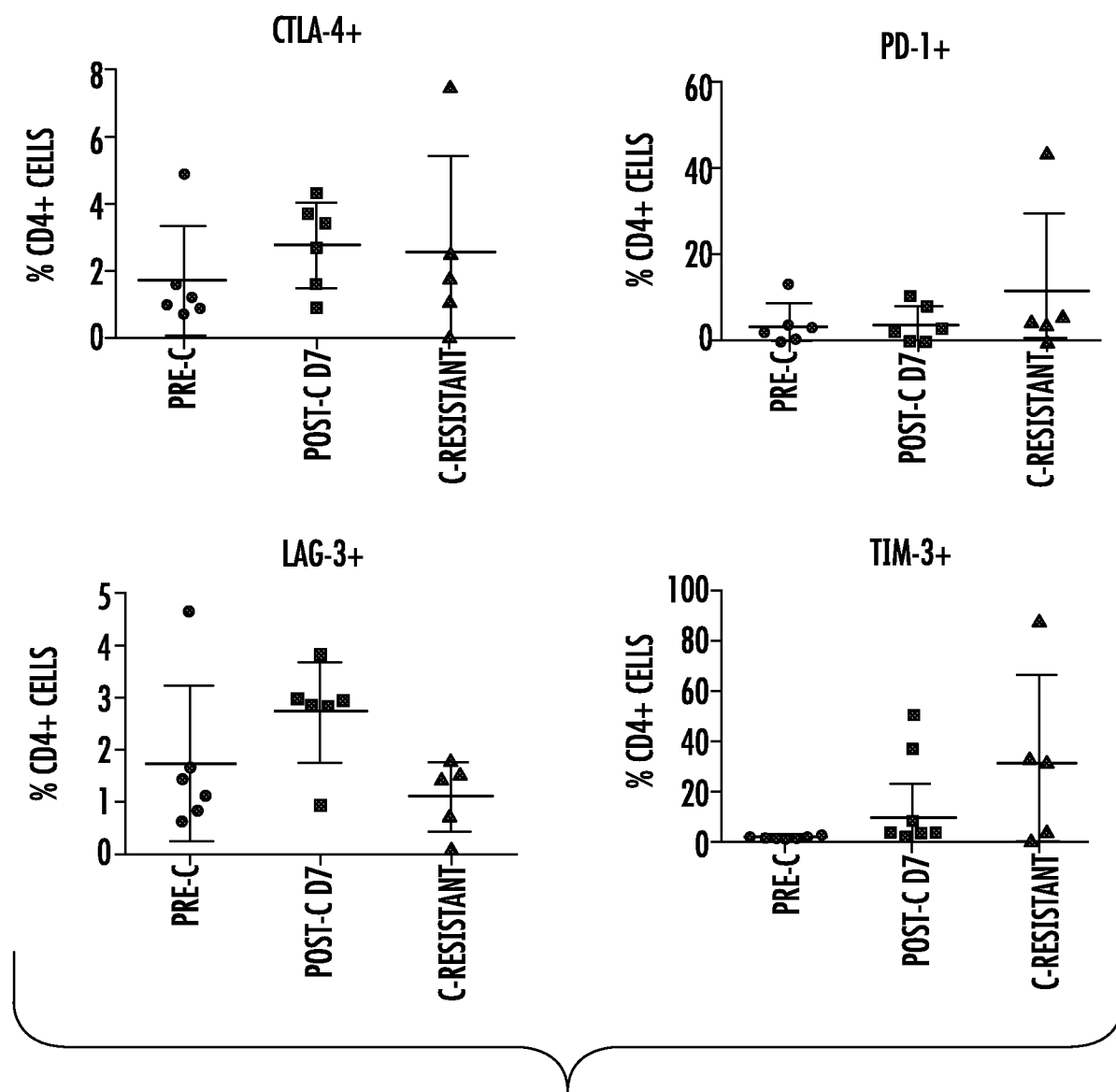


FIG. 4D

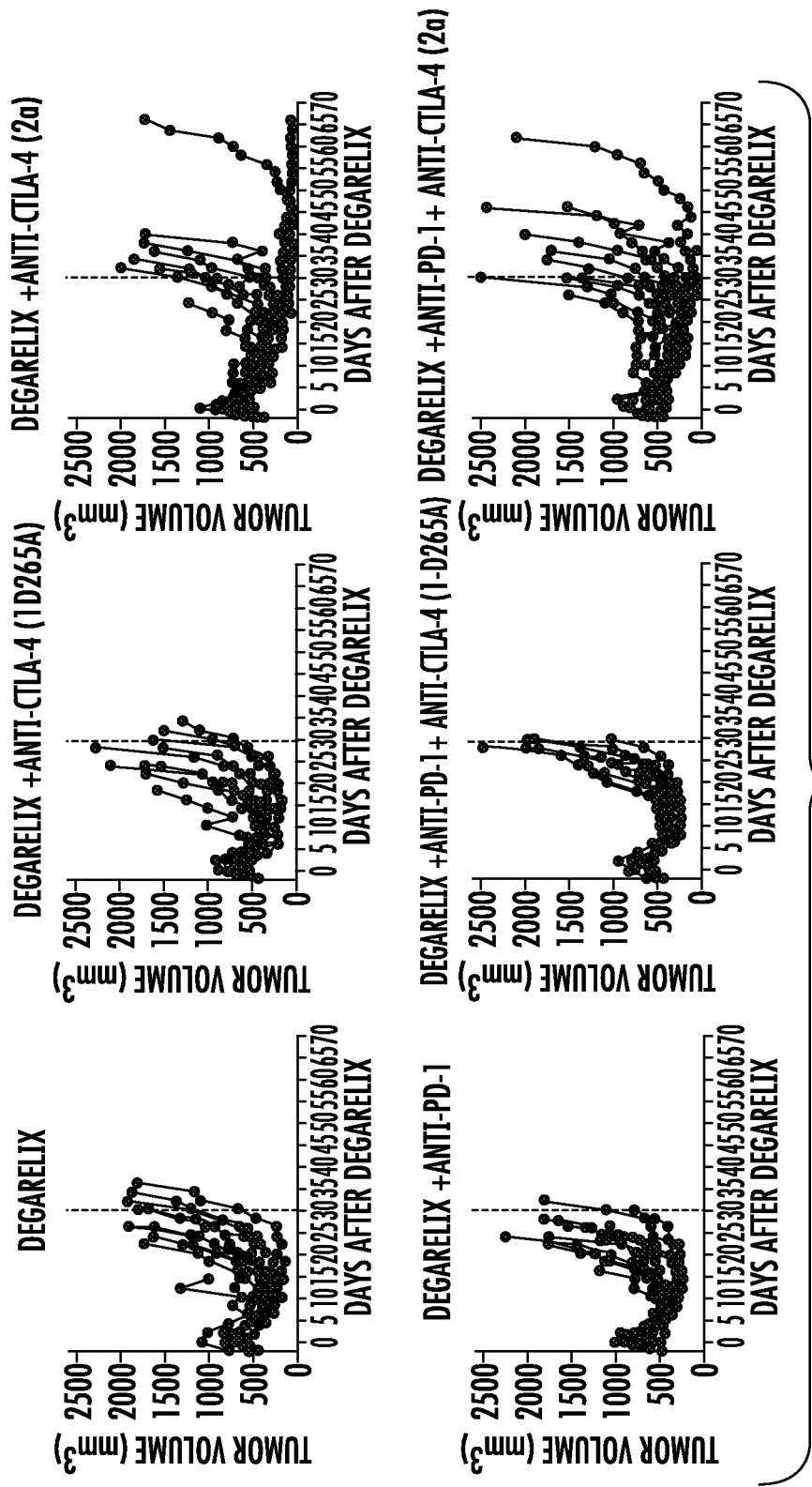
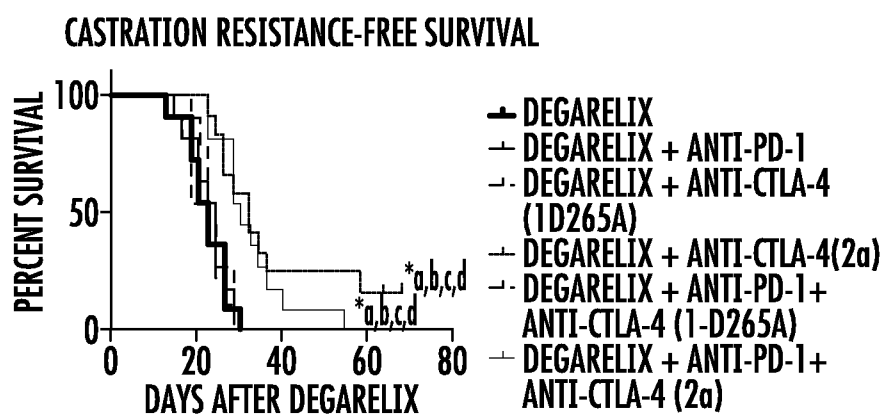
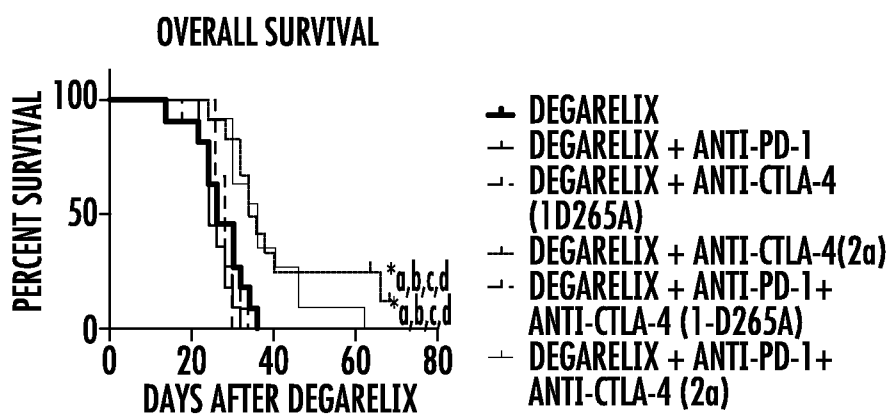


FIG. 5A

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**FIG. 5B****FIG. 5C**

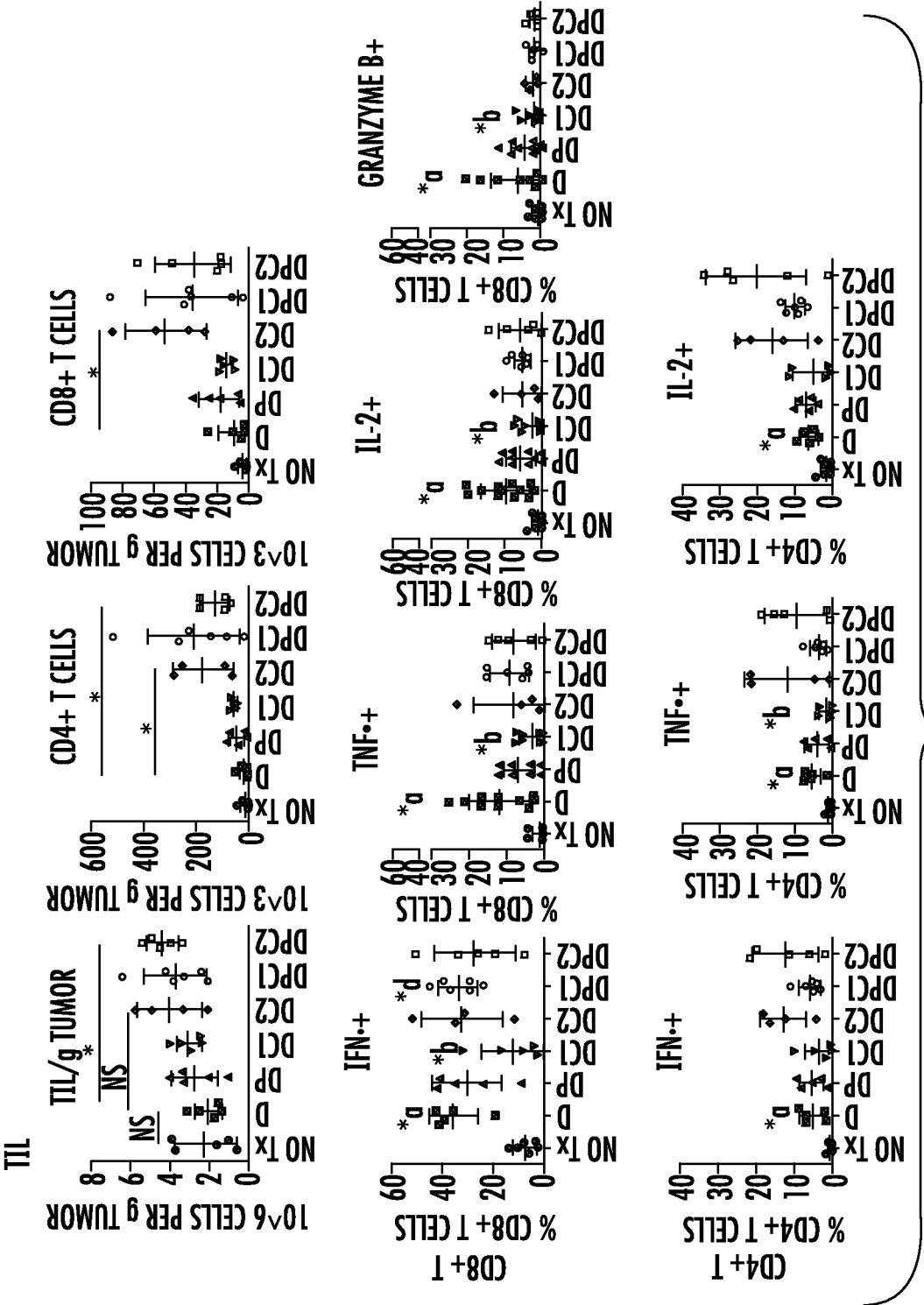


FIG. 6A

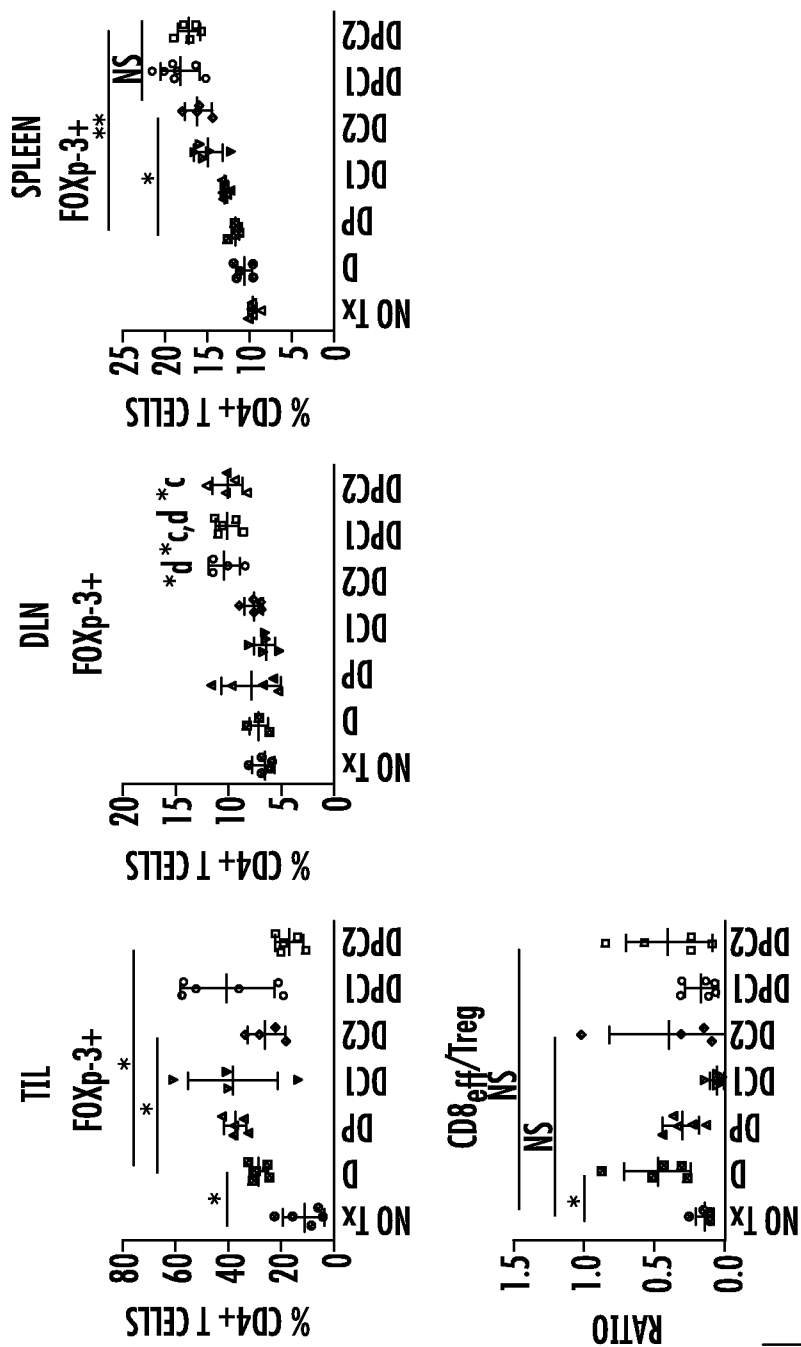


FIG. 6B

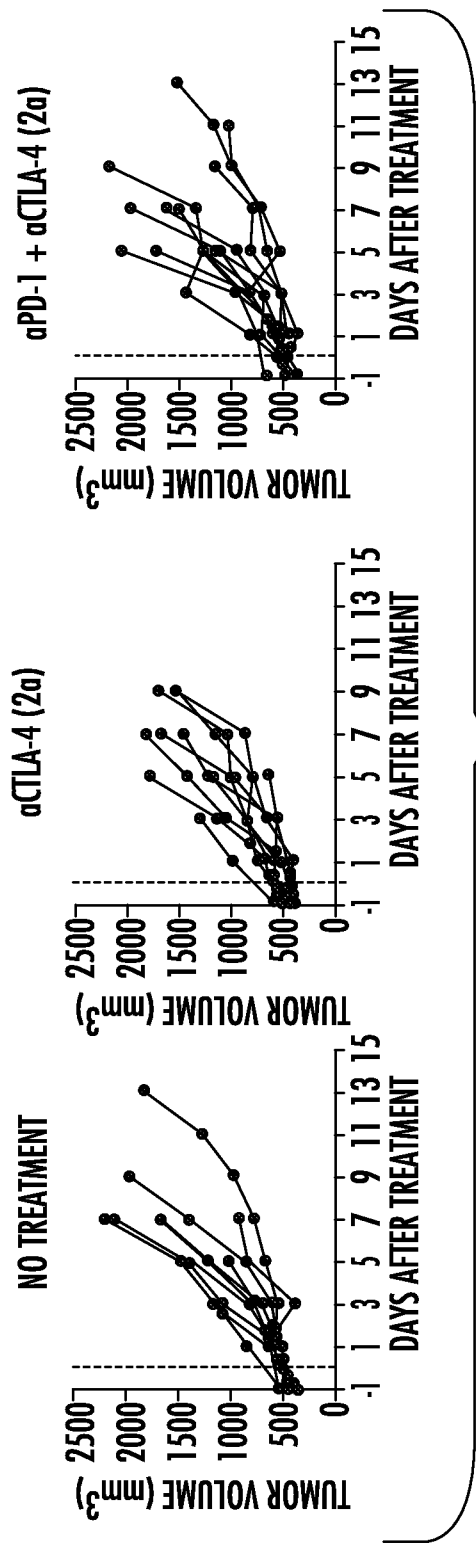


FIG. 7A

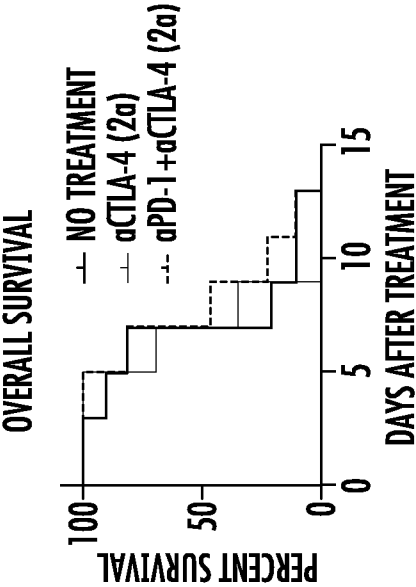


FIG. 7B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/022668**A. CLASSIFICATION OF SUBJECT MATTER****A61K 39/395(2006.01)i, A61P 35/00(2006.01)i**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 39/395; C07K 16/28; A61P 35/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: depleting anti-CTLA-4 antibody, prostate cancer, androgen deprivation therapy, PD1, castrate resistant prostate cancer**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KR 10-2015-0020189 A (BRISTOL-MYERS SQUIBB COMPANY) 25 February 2015 See claims 1, 2, 14, 15 and 17; and paragraphs [0022], [0103], [0188].	1-14
X	US 8685394 B2 (JURE-KUNKEL, MARIA) 01 April 2014 See claims 1, 4, 5; and column 1, lines 36-50.	1,2,5
X	WADA, SATOSHI et al., "Sequencing CTLA-4 blockade with cell-based immunotherapy for prostate cancer," Journal of Translational Medicine, 04 April 2013, Vol. 11 (internal pages 1-14) See conclusion.	1,2
X	HURWITZ, ARTHUR A. et al., "Combination immunotherapy of primary prostate cancer in a transgenic mouse model using CTLA-4 blockade," Cancer Research, 01 May 2000, Vol. 60, No. 9, pp. 2444-2448 See abstract.	1,2
X	KWON, EUGENE D. et al., "Elimination of residual metastatic prostate cancer after surgery and adjunctive cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) blockade immunotherapy," Proceedings of the National Academy of Sciences, 21 December 1999, Vol. 96, No. 626, pp. 15074-15079 See abstract.	1,2



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

"P" document published prior to the international filing date but later than the priority date claimed

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

02 September 2016 (02.09.2016)

Date of mailing of the international search report

02 September 2016 (02.09.2016)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2016/022668

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