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(54) Title: BIOMARKERS FOR PREDICTING RESPONSE TO IMMUNOSUPPRESSIVE THERAPY

(57) Abstract: The subject invention concerns methods and materials for assessing a patient's likelihood of responsiveness to an immunosuppressive therapy. The subject invention is contemplated for use with patients having an autoimmune disorder. In an exemplified embodiment, the methods of the invention are used for assessing and/or treating a patient with MDS. In one embodiment, a method of the invention comprises analyzing T cells of a patient for dysregulation of CD4⁺ and/or CD8⁺ T cell subsets, and determining the patient's likelihood of responsiveness to IST based on the level of dysregulation of the patient's CD4⁺ and/or CD8⁺ T cell subsets. In one embodiment, an increased likelihood of patient responsiveness to IST is associated with an increased percentage of CD4⁺ and/or CD8⁺ effector memory T cells and/or terminal effector memory T cells, for a patient. The subject invention also concerns methods for treating a patient with an autoimmune disorder, such as MDS. In one embodiment, a method of the invention comprises determining if a patient is likely to respond to IST, wherein said determination is made using a method of the present invention for assessing likelihood of responsiveness to IST; and if the patient is determined to be one likely to respond to IST, administering an effective regimen of IST to the patient.



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BIOMARKERS FOR PREDICTING RESPONSE TO
IMMUNOSUPPRESSIVE THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. Provisional Application Serial No. 61/038,938, filed March 24, 2008 and U.S. Provisional Application Serial No. 61/046,219, filed April 18, 2008, each of which is hereby incorporated by reference herein in its entirety, including any figures, tables, nucleic acid sequences, amino acid sequences, and drawings.

GOVERNMENT SUPPORT

The subject matter of this application has been supported by a research grant from the National Institutes of Health under grant numbers CA112112 and U54RR019397-05. Accordingly, the government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Myelodysplastic syndromes (MDS) are acquired clonal disorders of hematopoietic progenitors that are characterized by peripheral cytopenias and cytologic dysplasias (Greenberg *et al.*, 1997). There is a strong association between bone marrow failure and autoimmunity that transects classical autoimmune processes such as aplastic anemia, rheumatoid arthritis, Felty's Syndrome, and large granular lymphocyte (LGL) leukemia (Colmegna *et al.*, 2008; Loughran, Jr., 1998; Starkebaum *et al.*, 1997). Similar to these quintessential autoimmune bone marrow failure syndromes, hematopoietic suppression by a T-cell dominant autoimmune process and IST responsiveness has been proposed in younger MDS patients. In MDS cases where hematologic improvement is observed after IST, the response can be durable leading to improved long-term survival (Lim *et al.*, 2007; Molldrem *et al.*, 1997; Biesma *et al.*, 1997). Despite a clear therapeutic benefit and a basic understanding of immune pathology, IST is rarely offered to MDS patients as a

treatment option in the United States due to an uncertainty about the appropriate selection of patients for such therapy (Steensma & Tefferi, 2003). There is an underlying concern that inappropriate use of IST may negatively impact risk for leukemia progression, which occurs in 30-40% of MDS cases. This is of particular concern in younger patients that
5 may benefit from allogeneic stem cell transplantation (List *et al.*, 2004). The identification of immunological factors in addition to younger age to guide appropriate selection of patients for IST based on immune pathobiology is critical to the optimization of MDS treatment.

In MDS patients of younger age, a higher frequency of clinical responsiveness to
10 immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and/or cyclosporine-A (CyA) suggests that unique immune pathobiology contributes to the refractory cytopenias observed in such patients. Factors associated with response to IST included HLA-DR-15 genotype, age, < 5% marrow blasts, low risk for leukemia transformation and in some studies hypocellularity (Lim *et al.*, 2007; Sun *et al.*, 2008).
15 Among these factors, younger age is the most consistently linked clinical feature associated with IST responsiveness in both patients with aplastic anemia and in patients with MDS (Sun *et al.*, 2008; Young, 2002). A model of immune pathogenesis first proposed by Molldrem *et al* suggests that unique or overexpressed antigens stimulate CD8+ T-cells, induce T-cell receptor (TCR) repertoire contraction through expansion of
20 memory cells, and repress hematopoiesis through cross-reactive antigens expressed on normal bone marrow progenitors (Molldrem *et al.*, 1998). In the subset of patients responsive to immunosuppression, effects of the self-reactive T-cell populations are thought to be eliminated by the immunosuppressive actions of these drugs (Molldrem *et al.*, 1998; Kochenderfer *et al.*, 2002).

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BRIEF SUMMARY OF THE INVENTION

The subject invention concerns methods and materials for assessing a patient's likelihood of responsiveness to an immunosuppressive therapy. The subject invention is contemplated for use with patients having an autoimmune disorder. In an exemplified
30 embodiment, the methods of the invention are used for assessing and/or treating a patient with MDS. In one embodiment, a method of the invention comprises analyzing T cells of a patient for dysregulation of CD4⁺ and/or CD8⁺ T cell subsets, and determining the

patient's likelihood of responsiveness to IST based on the level of dysregulation of the patient's CD4⁺ and/or CD8⁺ T cell subsets. In a specific embodiment, an increased likelihood of patient responsiveness to IST is associated with an increased percentage of CD4⁺ and/or CD8⁺ effector memory T cells and/or terminal effector memory T cells for a
5 patient.

The subject invention also concerns methods for treating a patient with an autoimmune disorder, such as MDS. In one embodiment, a method of the invention comprises determining if a patient is likely to respond to IST, wherein said determination is made using a method of the present invention for assessing likelihood of
10 responsiveness to IST; and if the patient is determined to be one likely to respond to IST, administering an effective regimen of IST to the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color.
15 Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figures 1A-1C shows the ratio of CD4:CD8 T-cells. In Figure 1A, a multivariable regression analysis was performed with the log-transformed ratio of CD4:CD8 as the dependent variable and age and disease status (MDS v normal control) as independent variables (MDS=open circles and Control=open triangle). An interaction
20 term between age and disease status was not statistically significant (p-value=0.71) and was dropped from the final model. Disease status was significantly associated with log(CD4:CD8) (p-value<0.0001) while adjusting for age. Regression analyses were also performed separately for CD4 (Figure 1B) and CD8 (Figure 1C) in a log scale. While
25 adjusting for age, %CD4 was significantly lower in MDS cases compared to controls (p-value <0.0001) but there was no difference in %CD8 (p-value = 0.42). No interaction between age and disease status was observed in either model (p=0.31 and p=0.13 for CD4 and CD8, respectively).

Figure 2 shows naïve and memory phenotype analysis in MDS patients and
30 healthy controls. Case-control differences between T-cell subpopulations were compared in 47 MDS patients and 47 healthy controls using the Wilcoxon signed rank test adjusting

for multiple testing using the Holm step down method. P-values for the case-control differences are shown at the top of each panel.

Figures 3A and 3B show age-dependent changes in naïve and memory T-cell subpopulations in MDS patients. Using CD45RA and CD62L staining, naïve CD62L+/CD45RA+ (naïve), central memory CD62L+/CD45RA- (central), effector memory CD62L-/CD45RA+ (effector), and terminal effector memory CD62L-/CD45RA+ (terminal) were determined in 47 MDS patients (black circles, dotted line) and plotted against age in years. The frequency of CD4+ and CD8+ T-cell subpopulations is shown in MDS patients (Figure 3A) and controls (Figure 3B). Associations with age were evaluated using simple linear regression, adjusting for multiple testing with the Holm step down method. Lines indicate data trends in MDS cases and controls, and the slopes with corresponding p-values are presented (p-values < 0.05 are considered statistically significant).

Figures 4A-4C show the change in phenotype and T-cell homeostasis after IST. In Figure 4A, the CD4:CD8 ratio was calculated and compared between responders and non-responders before treatment with anti-Thymocyte globulin immunosuppression (ATG) or ATG in combination with CyA (ATG+CyA). In Figures 4B-1 and 4B-2, homeostatic proliferation was determined by Ki-67 expression (% Ki-67 positive) in CD4+ (Figure 4B-1) and CD8+ (Figure 4B-2) (first gated on CD3+ T-cells) and results were compared between responders (open dots) and non-responders (open triangle). Associations % Ki-67+/CD4+ (Figure 4C-1) and % Ki-67+/CD8+ (Figure 4C-2) T-cells and the CD4:CD8 ratio were evaluated in all patients combined using regression of the logCD4:CD8 ratio vs the Ki-67%/CD4+ and CD8+ adjusted for age. Lines indicate data trends and the corresponding p-values are presented (p-values < 0.05 are considered statistically significant).

Figures 5A-5C show the change in T-cell homeostasis after therapy in IST responsive and non-responsive patients. After labeling T-cell surface markers, the first gate was given to lymphocytes (as shown in Figure 1A). T-cells were identified by anti-CD3-antibody staining (as shown in Figure 1B), gates were then set to distinguish CD8- (CD4+) and CD8+ T-cells populations (as shown in Figure 1C). In Figure 5A, using expression patterns of CD62L (x-axis) and CD45RA (y-axis), the following populations were distinguished in CD4+ and CD8+ T-cells: naïve CD62L+/CD45RA+ (R4), central

memory CD62L+/CD45RA- (R5), effector memory CD62L-/CD45RA- (R6), and terminal effector memory CD62L-/CD45RA+ (R7). The % Ki-67 positive cells and isotype control-PE were concomitantly determined on R4 – R7 gated populations as shown. Results are shown for the percentage of each subpopulation that expressed Ki-67 was determined in individual gates before and after treatment in one exemplary MDS patient. In Figure 5B, the % Ki-67 positive CD4+ (solid lines) and CD8+ (dashed lines) were determined in naïve CD45RA+/CD62L+ (circle), central memory (CD45RA+/CD62L-) (square), effector memory (triangle), and terminal effector memory (hashed mark). For each of these eight cell populations, changes in %Ki-67 from before treatment (pre-TX) to after treatment (post-TX) were compared between responders (open circles) and non-responders (open triangles) using the Wilcoxon rank sum test, adjusting for multiple testing using the Holm step down method ($p < 0.05$ for all eight comparisons). In Figure 5C, the pre-TX versus post-TX changes in percentage of naïve CD62L+/CD45RA+ were also compared between patients that displayed a clinical response (responders) and patients that failed to respond to immunosuppression (non-responders) using the Wilcoxon rank sum test, adjusting for multiple testing using the Holm step down method.

Figures 6A-6E show phenotype using CD45RA/CD62L display. In some experiments, anti-CCR7-PE was also included. Anti-perforin-PE and anti-IFN γ -PE antibodies were used to stain permeabilized cells (BD Biosciences fix and perm kit). Induction of IFN γ release was determined after activation with plate-bound anti-CD3 antibody and anti-CD28 (BD Pharmingen, San Jose, CA USA) cross-linking. Stimulation with anti-CD28 along (5 ng/ml) was used as a negative control. Brefeldin A (10 μ g/ml, Sigma, CA USA) was added during the last 4 hrs of the stimulation period to prevent extracellular secretion. CD45RA/CD62L populations (R4-R7) were defined in both CD4+ and CD8+ T-cell subsets as follows: naïve CD62L+/CD45RA+, central memory CD62L+/CD45RA-, effector memory CD62L-/CD45RA-, and terminal effector memory CD62L-/CD45RA+ as shown in Figures 6A-6D. The percentage of cells that expressed the chemokine receptor CCR7, perforin, and anti-CD3-induced IFN γ in each subpopulation is shown in Figure 6E. Similar to previous reports, IFN γ production and perforin expression were almost exclusively restricted to CD62L negative memory

subsets (R6 and R7), whereas CCR7 expression was strongly associated with a naïve phenotype (CD45RA⁺/CD62L⁺, R4) (Figure 6E) (Sallusto *et al.*, 1999).

DETAILED DESCRIPTION OF THE INVENTION

5 The subject invention concerns methods for assessing or predicting a patient's likelihood of responsiveness to an immunosuppressive therapy. The subject invention is contemplated for use with patients having an autoimmune disorder. In an exemplified embodiment, the methods of the invention are used for assessing and/or treating a patient with MDS. In one embodiment, a method of the invention comprises analyzing T cells of
10 a patient for dysregulation of CD4⁺ and/or CD8⁺ T cell subsets, and determining the patient's likelihood of responsiveness to IST based on the level of dysregulation of the patient's CD4⁺ and/or CD8⁺ T cell subsets. The cells of a patient can be obtained from any suitable biological sample, such as a blood sample. In one embodiment, the blood sample is a peripheral blood sample. The samples can be enriched for lymphocytes,
15 including T cells, using standard methods known in the art. Increases or decreases in percentage of cells, or increases or decreases in percentage of cells expressing a marker protein or nucleic acid of the invention, or increases or decreases in proliferation of cells can be compared to a control sample or to a value obtained from a control sample of patients having the same disorder. For example, in certain embodiments, the control is a
20 value obtained from a sample of patients having the same disorder as the patient that has been determined to be non-responders to IST.

In one embodiment, an increased likelihood of patient responsiveness to IST is associated with an increased percentage of CD4⁺ and/or CD8⁺ effector memory T cells and/or terminal effector memory T cells for a patient. In one embodiment, an increased
25 likelihood of responsiveness to IST is associated with an increased percentage of CD4⁺ effector memory T cells. In a specific embodiment, an increased likelihood of responsiveness to IST is associated with an increased percentage of CD62L⁻ CD4⁺ T cells and/or CD62L⁻ CD8⁺ T cells for the patient. In a more specific embodiment, an increased likelihood of responsiveness to IST is associated with an increased percentage of
30 CD62L⁻/CD45RA⁻ CD4⁺ T cells for the patient having the disorder.

In a further embodiment, an increased likelihood of patient responsiveness to IST is associated with a decreased percentage of CD4⁺ and/or CD8⁺ naïve T cells and/or CD4⁺

and/or CD8⁺ central memory T cells for a patient. In one embodiment, an increased likelihood of patient responsiveness to IST is associated with a decreased percentage of CD4⁺ and/or CD8⁺ central memory T cells for a patient. In a specific embodiment, an increased likelihood of responsiveness to IST is associated with a decreased percentage of CD62L⁺ CD4⁺ T cells and/or CD62L⁺ CD8⁺ T cells for the patient. In a more specific embodiment, an increased likelihood of responsiveness to IST is associated with a decreased percentage of CD62L⁺/CD45RA⁻ CD4⁺ and/or CD62L⁺/CD45RA⁻ CD8⁺ T cells for the patient having the disorder.

In another embodiment, a method of the invention comprises analyzing the level of CD4⁺ T cells present in the patient, wherein a reduced level of CD4⁺ T cells is associated with an increased likelihood of IST responsiveness for the patient. In a still further embodiment, a method of the invention further comprises analyzing the ratio of CD4⁺ to CD8⁺ T cells (CD4⁺:CD8⁺) in a patient, wherein a lower ratio of CD4⁺:CD8⁺ T cells is associated with an increased likelihood of the patient responding to IST.

In a further embodiment, a method of the invention comprises analyzing the proliferation (*e.g.*, proliferative index) of CD4⁺ and/or CD8⁺ T cells of a patient, wherein an increased or higher level of proliferation of CD4⁺ and/or CD8⁺ T cells in a patient is associated with an increased likelihood of the patient responding to IST. In a specific embodiment, a method of the invention comprises analyzing the percentage of Ki-67⁺ CD4⁺ T cells and/or Ki-67⁺ CD8⁺ T cells, wherein an increased percentage of Ki-67⁺ CD4⁺ T cells and/or Ki-67⁺ CD8⁺ T cells is associated with an increased likelihood of the patient responding positively to IST.

In one embodiment of the methods of the invention, marker proteins are detected and/or quantitated by contacting a biological sample with an antibody or other moiety that binds specifically to a target marker protein, and detecting the presence of a complex of the antibody or binding moiety bound to the target marker protein.

The expression levels of markers, such as CD3; CD4; CD8; CD25; protein target bound by Ki-67 antibody (Genbank accession numbers X74107; NM_001145966; and NM_002417); L-selectin (*e.g.*, CD62L) (Genbank accession number NM_000655); and/or tyrosine phosphatase (*e.g.*, CD45) (Genbank accession numbers NM_080922 and NG_007730), in T cells can be detected using antibodies or aptamers that bind specifically to a target marker protein, or using nucleic acid, such as oligonucleotides, that

hybridize with nucleic acid sequences (*e.g.*, mRNA) expressed by genes encoding or associated with the marker protein. An aptamer is an oligonucleotide or peptide molecule that can bind specifically to a target molecule. Aptamers can be prepared from large random pools of oligonucleotides or peptides (Berezovski *et al.*, 2008; Ellington and Szostak, 1990; Hoppe-Seyler and Butz, 2000; Klussmann, 2006). Methods and materials for detecting and/or quantifying expression levels of target nucleic acids are well known in the art and include, for example, Northern blotting and polymerase chain reaction (PCR) methods.

An antibody, or an antibody fragment, or an aptamer that binds specifically to any one of CD3, CD4, CD8, CD25, nuclear or cellular proliferation antigens, L-selectin, and tyrosine phosphatase can be used in the embodiments of the invention. Antibodies or aptamers specific to L-selectin include an antibody or aptamer to the CD62L marker. Antibodies or aptamers specific to tyrosine phosphatase include an antibody or aptamer to one of the CD45RA, CD45RB, and CD45RO markers. An antibody, or antibody fragment, or aptamer that is specific for a cell proliferation antigen can be used in the embodiments of the invention. Antibodies or aptamers specific to a proliferation-associated antigen that can be used according to the present invention include antibodies or aptamers to Ki-67, a nuclear proliferation-associated antigen. In one embodiment, a method of the invention comprises the use of flow cytometry instruments and methods to detect the binding of antibodies or aptamers to antigens on the surface of cells. Flow cytometry instruments and methods of use are well known in the art (Jaroszeski and Heller, 1998; Ormerod, 2008; Radbruch, 2000; Recktenwald and Radbruch, 1997; Shapiro, 2003).

In one aspect, the present invention contemplates the use of nucleic acids as agents for detecting marker proteins in biological samples of patients, wherein the nucleic acids are labeled with a detectable label. The nucleic agents may be labeled with a radioactive label, a fluorescent label, a quantum dot, an enzyme, a chemiluminescent tag, a colorimetric tag or other labels or tags that are discussed above or that are known in the art.

In another aspect, the present invention contemplates the use of Northern blot analysis to detect the presence of marker protein mRNA in a biological sample. The first step of the analysis involves separating a sample containing nucleic acid by gel

electrophoresis. The dispersed nucleic acids are then transferred to a nitrocellulose filter or another filter. Subsequently, the filter is contacted with labeled oligonucleotide under suitable hybridizing conditions, *e.g.*, 50% formamide, 5 x SSPE, 2 x Denhardt's solution, 0.1% SDS at 42° C., as described in *Molecular Cloning: A Laboratory Manual*, Maniatis *et al.* (1982, CSH Laboratory). Other useful procedures known in the art include solution hybridization, dot and slot RNA hybridization, and probe based microarrays. Measuring the radioactivity of hybridized fragments, using standard procedures known in the art quantitates the amount of a particular nucleic acid present in the biological fluid of a patient.

Dot blotting involves applying samples that may contain a nucleic acid of interest to a membrane. The nucleic acid can be denatured before or after application to the membrane. The membrane is incubated with a labeled probe. Dot blot procedures are well known to the skilled artisan and are described more fully in U.S. Patent Nos. 4,582,789 and 4,617,261. Radioimmunoassays, Western blotting, and enzyme linked immunosorbent assay (ELISA) can also be used to detect and quantify marker proteins in a biological sample. RIA, Western blotting, and ELISA methods are well known in the art (Ausubel *et al.*, 1994).

In another aspect, the present invention contemplates the use of polymerase chain reaction (PCR) to detect the presence and/or quantity of a nucleic acid encoding or associated with a marker protein in a biological sample. Polymerase chain reaction is a process for amplifying one or more specific nucleic acid sequences present in a nucleic acid sample using primers and agents for polymerization and then detecting the amplified sequence. The extension product of one primer when hybridized to the other becomes a template for the production of the desired specific nucleic acid sequence, and vice versa, and the process is repeated as often as is necessary to produce the desired amount of the sequence. PCR is routinely used to detect the presence of a desired sequence (U.S. Patent No. 4,683,195).

A specific example of PCR that is routinely performed by the skilled artisan to detect desired sequences is reverse transcription PCR (RT-PCR; Saiki *et al.* (1985) and Scharf *et al.* (1986)). RT-PCR involves isolating total RNA from biological fluid, denaturing the RNA in the presence of primers that recognize the desired nucleic acid sequence, using the primers to generate a cDNA copy of the RNA by reverse

transcription, amplifying the cDNA by PCR using specific primers, and detecting the amplified cDNA by electrophoresis or other methods known to the skilled artisan. The amount of a target nucleic acid sequence in a sample can be quantitated using standard PCR methods.

5 Oligonucleotide primers and probes can be used in PCR methods and other methods involving nucleic acid amplification. In one embodiment, a probe or primer can hybridize to a target polynucleotide of the invention under stringent conditions. Probes and primers of the invention can optionally comprise a detectable label or reporter molecule, such as fluorescent molecules, enzymes, radioactive moiety (*e.g.*, ^3H , ^{35}S , ^{125}I ,
10 *etc.*), and the like. Probes and primers of the invention can be of any suitable length for the method or assay in which they are being employed. Typically, probes and primers of the invention will be 10 to 500 or more nucleotides in length. Probes and primers that are 10 to 20, 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, 71 to 80, 81 to 90, 91 to 100 or more nucleotides in length are contemplated within the scope of the invention. Probes
15 and primers of the invention can have complete (100%) nucleotide sequence identity with the target polynucleotide sequence, or the sequence identity can be less than 100%. For example, sequence identity between a probe or primer and a sequence can be 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75%, 70% or any other percentage sequence identity so long as the probe or primer can hybridize under stringent conditions to a nucleotide
20 sequence of a polynucleotide of the invention.

 In one embodiment of a method of the present invention, the IST that is considered for administration to the patient if the patient is determined to be likely to respond to the IST can be anti-thymocyte globulin (ATG) therapy. In another embodiment, the IST that is considered for administration to the patient if the patient is
25 determined to be likely to respond to the IST can be cyclosporine A (CyA) therapy. In a still further embodiment, the IST that is considered to be administered to a patient is both ATG and CyA therapy. Other IST contemplated by the subject invention include, but is not limited to, use of antibodies to T cell receptors (anti-CD3) and/or IL-2 receptors (anti-CD25); use of interferons; use of Tacrolimus (PROGRAF); use of glucocorticoids; and
30 use of cytostatic compounds such as alkylating agents, methotrexate, and azathioprine.

 The subject invention also concerns methods for treating a patient having an autoimmune disorder, such as MDS, comprising the steps of determining if a patient is

likely to respond to IST, wherein said determination is made using a method of the present invention for assessing likelihood of responsiveness to IST; and if the patient is determined to be one likely to respond to IST, administering an effective regimen of IST to the patient. In one embodiment, the IST administered is anti-thymocyte globulin (ATG) therapy. In another embodiment, the IST administered is cyclosporine A (CyA) therapy. In a still further embodiment, the IST to be administered to a patient is both ATG and CyA therapy. Any other immunosuppressive therapies useful in treating the disorder afflicting the patient are also contemplated within the scope of the invention. In one embodiment, the method further comprises a first step of identifying and/or diagnosing that the patient has or is developing an autoimmune disorder, such as MDS.

Methods of the present invention can also be combined with any other methods or markers known in the art for determining or assessing likelihood of responsiveness to IST for a patient having an autoimmune disorder, such as MDS. For example, wherein the methods are used to assess an MDS patient, the methods of the invention can also incorporate methods utilizing factors such as patient age, HLA-DR2 antigen status of the patient, and/or duration of red cell transfusion dependence (RCTD) (Sauntharajah *et al.*, 2002 and 2003).

The methods of the invention can be used for assessing responsiveness to IST and for treating patients having an autoimmune disorder and/or a disorder in which one of the pathologies is an immune system that is underregulated, or that is overactive, or that is reactive against self, *i.e.*, that recognizes as foreign and mounts an immune response against the patient's own antigens, cells, and tissues. Autoimmune disorders contemplated within the scope of the invention include, but are not limited to, Addison's disease, Crohn's disease, dermatomyositis, diabetes mellitus type 1, Graves' disease, lupus erythematosus, multiple sclerosis (MS), myasthenia gravis, pernicious anemia, polymyositis, rheumatoid arthritis, Sjögren's syndrome, ulcerative colitis, vasculitis, and Wegener's granulomatosis. In an exemplified embodiment, the disorder is MDS.

The subject invention also concerns kits comprising reagents for practicing the methods of the present invention. In one embodiment, a kit of the invention comprises in one or more containers:

i) a reagent for assessing the presence of and/or quantifying the amount of CD4⁺ T cells in a sample; and/or

ii) a reagent for assessing the presence of and/or quantifying the amount of CD8⁺ T cells in a sample; and/or

iii) a reagent for assessing the presence of and/or quantifying the amount of naïve, central memory, effector memory, and/or terminal effector memory T cells in a sample; and/or

iv) a reagent for assessing the proliferation of CD4⁺ and/or CD8⁺ T cells in a sample.

In one embodiment, a kit of the invention further comprises positive or negative controls or standards that the assayed sample can be compared to. In one embodiment, the kit comprises one or more antibody, an antibody fragment, or aptamer specific to CD4⁺ or CD8⁺ T cells. In one embodiment, the kit comprises one or more antibody, antibody fragment, or aptamer specific to CD3 or CD25 markers. In a further embodiment, the kit comprises one or more antibody, an antibody fragment, or aptamer that bind specifically to an L-selectin antigen determinant, such as CD62L and/or one or more antibodies that bind to a tyrosine phosphatase antigen determinant, such as CD45RA, CD45RB, or CD45RO. In another embodiment, the kit comprises one or more antibody, an antibody fragment, or aptamer that bind specifically to Ki-67, a nuclear proliferation-associated antigen. A kit of the invention can comprise any and all of the above embodiments. In another embodiment, the kit comprises one or more nucleic acid or oligonucleotides that selectively hybridize with a nucleic acid encoding or associated with CD3, CD4, CD8, CD25, L-selectin, a tyrosine phosphatase, and/or a cellular or nuclear proliferation-associated marker protein. A reagent of the kit, such as an antibody, aptamer, nucleic acid, or oligonucleotide can optionally be labeled with a detectable label, and/or the kit can optionally comprise a detectable label that can be conjugated or bound to a reagent of the kit, such as an antibody, aptamer, nucleic acid, or oligonucleotide. The kit can also optionally comprise additional reagents for conjugating or binding the detectable label to a reagent of the kit. In one embodiment, a kit of the invention can optionally comprise instructions pertaining to the use of the reagents and/or methods of the invention, packaging materials, sample diluents, buffers, wash reagents, and/or containers.

Detectable labels that can be used with the present invention include, but are not limited to, enzymes, radioisotopes, chemiluminescent and bioluminescent reagents, and

fluorescent moieties. Enzymes that can be used include but are not limited to luciferase, beta-galactosidase, acetylcholinesterase, horseradish peroxidase, glucose-6-phosphate dehydrogenase, and alkaline phosphatase. If the detectable label is an enzyme, then a suitable substrate that can be acted upon by the enzyme can be used for detection and measurement of enzyme activity. In one embodiment, if the detectable label is a peroxidase, the substrate can be hydrogen peroxide (H₂O₂) and 3-3'-diaminobenzidine or 4-chloro-1-naphthol and the like. Other substrates suitable for use with other enzymes are well known in the art. An example of a luminescent material includes luminol. Examples of bioluminescent materials include, but are not limited to, luciferin, green fluorescent protein (GFP), enhanced GFP (Yang *et al.*, 1996), and aequorin. Fluorescent moieties include, but are not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, Cascade Blue, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, Texas Red, Oregon Green, cyanines (*e.g.*, CY2, CY3, and CY5), allophycocyanine, or phycoerythrin. Isotopes that can be used include, but are not limited to, ¹²⁵I, ¹⁴C, ³⁵S, and ³H.

Antibodies contemplated for use in the present invention can be in any of a variety of forms, including a whole immunoglobulin, an antibody fragment such as Fv, Fab, and similar fragments, a single chain antibody that includes the variable domain complementarity determining regions (CDR), and the like forms, all of which fall under the broad term "antibody," as used herein. Antibodies useful in the methods of the present invention can be monoclonal or polyclonal antibodies, and can be from any source including, but not limited to, mouse, rabbit, goat, rat, or human. Antibodies of the invention can be conjugated to a detectable label, such as, for example, a fluorescent moiety. In one embodiment of the present invention, a detectable label can be directly bound to an antibody that binds to a marker of the invention. If the detectable label is to be directly bound, the label may comprise a functional group which is capable of binding to the antibody used with the invention. Alternatively, the detectable label may be indirectly bound, for example, using an avidin-biotin or streptavidin-biotin bridge wherein the avidin or biotin is labeled with a detectable label. In one embodiment, an antibody of the invention is conjugated with avidin and the detectable label is conjugated with biotin.

The term "antibody fragment" refers to a portion of a full-length antibody, generally the antigen binding or variable region. Examples of antibody fragments include Fab, Fab', F(ab')₂ and Fv fragments. Papain digestion of antibodies produces two identical antigen binding fragments, called the Fab fragment, each with a single antigen binding site, and a residual "Fc" fragment, so-called for its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen binding fragments, which are capable of cross-linking antigen, and a residual other fragment (which is termed pFc'). Additional fragments can include diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. As used herein, "functional fragment" with respect to antibodies, refers to Fv, F(ab) and F(ab')₂ fragments.

Antibody fragments can retain an ability to selectively bind with the antigen or analyte and are defined as follows:

(1) Fab is the fragment that contains a monovalent antigen-binding fragment of an antibody molecule. A Fab fragment can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain.

(2) Fab' is the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain. Two Fab' fragments are obtained per antibody molecule. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region.

(3) (Fab')₂ is the fragment of an antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction. F(ab')₂ is a dimer of two Fab' fragments held together by two disulfide bonds.

(4) Fv is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (V_H-V_L dimer). It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or

half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

(5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, 5 linked by a suitable polypeptide linker as a genetically fused single chain molecule. Such single chain antibodies are also referred to as "single-chain Fv" or "sFv" antibody fragments. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains that enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun in *The Pharmacology of Monoclonal* 10 *Antibodies*, vol. 113, Rosenberg and Moore eds. Springer-Verlag, N.Y., pp. 269 315 (1994).

Compounds useful in the treatment methods of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in detail in a number of sources which are well 15 known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin describes formulations which can be used in connection with the subject invention. In general, the compositions of the subject invention will be formulated such that an effective amount of the compound is combined with a suitable carrier in order to facilitate effective administration of the composition. 20 The compositions used in the present methods can also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspension, suppositories, injectable and infusible solutions, and sprays. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional 25 pharmaceutically acceptable carriers and diluents which are known to those skilled in the art. Examples of carriers or diluents for use with the subject compounds include ethanol, dimethyl sulfoxide, glycerol, alumina, starch, and equivalent carriers and diluents. To provide for the administration of such dosages for the desired therapeutic treatment, pharmaceutical compositions of the invention will advantageously comprise between 30 about 0.1% and 99%, and especially, 1 and 15% by weight of the total of one or more of the subject compounds based on the weight of the total composition including carrier or diluent.

As used herein, the term “myelodysplastic syndromes” or “MDS” means any hematopoietic cell disorders characterized by one or more of the following: decreased or ineffective blood cell production, anemia, cytogenetic abnormalities, cytopenias, and risk of progression to acute leukemia or cellular marrow with impaired morphology and maturation. The term “myelodysplastic syndromes” or “MDS” can include any of the following: refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-T) and chronic myelomonocytic leukemia (CMML).

Patient responsiveness to IST for a particular disorder can be based on a measurable parameter that is indicative of patient improvement after receiving a therapeutic treatment. Patient responsiveness to IST in MDS patients includes observing increases in red blood cell production; increases in granulocyte production; and increases in platelet production. In one embodiment, responsiveness to IST in MDS patients is based on hematological improvement as assessed by the International Working Group (IWG) criteria (Cheson *et al.*, 2006; Soand *et al.*, 2008).

MATERIALS AND METHODS

Patients and healthy controls. Seventy-six MDS patients were analyzed in total. Untreated MDS patients (n=47) were recruited from the Malignant Hematology clinic at the H. Lee Moffitt Cancer Center & Research Institute. Diagnoses were confirmed by pathology and classified in accordance with World Health Organization criteria. After obtaining written informed consent, 40 mls of peripheral blood were collected in heparinized tubes from each patient. Samples from 29 patients were obtained in collaboration with Dr. Elaine Sloand of the National Heart Lung and Blood Institute (NHLBI), Bethesda, MD. Twenty patients had been treated with ATG alone or ATG plus cyclosporine, and samples were analyzed before and after therapy. For use as controls, buffy coats from healthy blood donors were obtained from the Southwest Florida Blood Services (SFBS) in St. Petersburg, FL. Peripheral blood mononuclear cells (PBMCs) were isolated from MDS patients and healthy donors by Ficoll-Hypaque (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) gradient centrifugation, as previously described, and frozen in liquid nitrogen (Epling-Burnette *et al.*, 2001).

Detection of naïve and memory T-cell subpopulations. Cryopreserved PBMCs were thawed and incubated with antibodies to distinguish naïve and memory CD4 and CD8 T-cell subtypes were detected after surface staining with anti-CD3-PE Cy7, anti-CD45RA-FITC, anti-CD62L-APC, and either anti-CD4- or CD8-APC Cy7, which were all obtained from BD Biosciences, San Jose, CA USA. Naïve and memory T-cell populations were distinguished by CD45RA and CD62L expression, as described previously by flow cytometry (Maldonado *et al.*, 2003; Rabin *et al.*, 1995). Exclusion of DAPI was used to distinguish cell viability. Results of flow cytometry were analyzed on a LSRII Benchtop analyzer (BD Bioscience).

Flow cytometry to detect proliferation. Cell proliferation was measured by Ki-67 nuclear antigen expression. Ki-67 is expressed by cells in late G1, S, G2, and M phase of the cell cycle (Hazenberg *et al.*, 2000a; Hellerstein *et al.*, 1999). In earlier studies, Ki-67 staining was positively correlated with deuterated glucose labeling of dividing cells *in vivo* (Hellerstein *et al.*, 1999). To determine the percentage of proliferating cells within specific lymphocyte subsets, a gate was used to define CD3+ T-cells that were CD4+ or CD8+ and stained with anti-Ki-67+ antibodies within the lymphocyte gate (R1, in Fig. 1) to determine expression in naïve and memory T-cell subsets based on CD45RA and CD62L surface markers. Anti-Ki-67-PE was used to stain the intracellular nuclear antigen after subsequent fixation and permeabilization (BD Biosciences fix and perm kit) as recommended by the manufacturer.

Statistical Analysis. The statistical methods used included linear regression, the Wilcoxon rank sum test and the Wilcoxon signed rank test (in the instances of paired data). When adjustment for multiple testing was necessary, the Holm step down method was used. A two-sided $P < 0.05$ was considered as statistically significant. The specific statistical tests that were used in each experiment are noted in the figure and table legends.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

5 Example 1—Clinical characteristics of MDS patients

Characteristics of 76 untreated MDS patients (46 from Moffitt and 29 from NHLBI) are summarized in Table 1. Patient ages ranged from 19 to 82 years with a mean age of 64 years. Nearly half of the patients had refractory cytopenia with multilineage dysplasia (RCMD), and two thirds of all patients were low or intermediate-1 based on the
 10 IPSS. Half of the patients had abnormal karyotypes, including del[5q], trisomy 8, monosomy 7, and a complex karyotype with three or more abnormalities.

Table 1. Characteristics of MDS patients

		Total (N)	%
Age (mean=64 y.o.) (n=76)*	< 60 y.o.	26	34.7
	= 60 to = 71 y.o.	24	32.0
	> 71 y.o.	25	33.3
WHO** classification (n=57)	RA	20	35.1
	RCMD	25	43.0
	RAEB	12	21.0
IPSS (n=56)	Lower (low/Int-1)	39	69.6
	Higher (Int-2/High)	17	30.4
Karyotype (n=56) ^{&}	Normal	27	48.2
	Abnormal***	31	55.4
	del 5q	4	
	Trisomy 8	8	
	Monosomy 7	2	
	Complex	13	

15 *A total of 76 patients were included in this study with a mean age of 64 years. All patients met the clinical criteria for myelodysplastic syndrome based on pathological examination. The age was unavailable in 1 patient.

20 **World health organization (WHO) classification was assessed in 57 out of 76 patients. The classification was grouped as Refractory Anemia (RA) and refractory anemia with and refractory anemia with excess blasts 1 and 2 (RAEB).

[#]International prognostic scoring system (IPSS) classification was available in 56 out of 76 patients. Patients were grouped as either lower (low plus intermediate-1) or intermediate-2 and high risk (higher risk). Low risk =IPSS score 0, intermediate-1 (IPSS score 0.5 - 1.0), intermediate-2 (IPSS score 1.5-2.0) and high risk (IPSS score > 2.0).

5 [&]Karotype was performed by standard cytogenetics in 56 out of 76 patients.

^{***}The frequency is shown for the most common abnormalities including deletion of 5q (del 5q), Trisomy 8, monosomy 7, and complex. Complex abnormalities include 3 or greater karyotypic abnormalities.

10 Example 2—Reduced CD4:CD8 Ratio in MDS patients

CD4+ T-cells are normally present in the peripheral blood lymphocyte pool at 2 to 4 times greater than that of CD8+ T-cells, and diminished CD4:CD8 ratio has been previously shown to correlate with poor survival outcome in MDS (Bennett & Komrokji, 2005; Sokol *et al.*, 1989; Kook *et al.*, 2001; Sauntharajah *et al.*, 2001; Wang *et al.*, 15 2006; Okamoto *et al.*, 1997). Similar to previous reports, we found that the CD4:CD8 ratio was reduced in MDS patients compared to healthy controls after adjusting for age (p-value <0.0001) (Fig. 1A). To assess whether the case-control difference in the CD4:CD8 ratio was due to differences in CD4, CD8, or both, percentages of each cell type were individually compared between cases and controls after adjusting for age. This 20 analysis revealed that the percentage of CD4 cells was reduced in the peripheral lymphocyte pool among MDS cases as compared to controls (Fig. 1B, p-value<0.0001), while there was no difference in the percentage of CD8 cells (Fig. 1C, p-value=0.42). There was no statistically significant interaction between age and reduction in CD4:CD8 ratio in this analysis. These results suggest that changes in the CD4+ lymphocyte pool 25 may be responsible for the diminished CD4:CD8 ratio with well-described significance in MDS (Bennett & Komrokji, 2005; Sokol *et al.*, 1989; Kook *et al.*, 2001; Sauntharajah *et al.*, 2001; Wang *et al.*, 2006; Okamoto *et al.*, 1997).

30 Example 3—T-cell subset distribution is defective in CD4+ and CD8+ T-cells in MDS patients

Since we found that CD4+ T-cells are diminished in MDS patients, and CD8+ T-cells have been shown to display TCR repertoire restriction, we examined the phenotype of both CD4+ and CD8+ T-cell populations in the same group of MDS cases and controls. Isoforms of the tyrosine phosphatase CD45 (RB, RA, RO), along with L-selectin (CD62L), have been used to distinguish naïve from memory T-cells (Rabin *et al.*, 35

1995; Sallusto *et al.*, 1999; De Rosa *et al.*, 2001; Champagne *et al.*, 2001). We used multiple color flow staining to distinguish the T-cell subpopulations. An illustration of these markers is shown in supplemental Figure 1 for a healthy control donor. We compared the proportion of circulating naïve and memory CD4⁺ and CD8⁺ T-cell subpopulations in MDS patients (n=47) and healthy control donors, individually age-matched to MDS patients within 4 years (n=47). Data in Figure 2 show that the percentages of naïve CD4⁺ and CD8⁺ T-cells are lower in MDS. In the T-cell pool, both CD4⁺ and CD8⁺ naïve cells were under-represented in cases compared to controls (p<0.0001 for both populations). In addition to reduced naïve cells, the memory CD4⁺ and CD8⁺ subpopulations displaying a CD45RA⁻/CD62L⁻ (effector memory) and CD45RA⁺/CD62L⁻ (terminal effector memory) phenotype were increased in cases compared to controls.

Since the proportion of naïve and memory T-cell distributions change with age due to reduction in thymic function, the age-related differences among the MDS cases, which ranged from 19 to 82 years old (n=47), was compared to control with results shown in Figure 3. Furthermore, T-cell homeostatic mechanisms have been reported to differ among CD4 and CD8 populations with age (Naylor *et al.*, 2005; Czesnikiewicz-Guzik *et al.*, 2008; Goldrath & Bevan, 1999; Schwab *et al.*, 1997). Among these cases, positive correlations were observed between age and both % CD62L positive naïve cells and central memory CD4⁺ T-cells, although the former association was not statistically significant (naïve: slope=0.186, p=0.14; central memory: slope=0.685, p=0.001). Furthermore, the proportions of CD62L⁻ CD4⁺ T-cell populations, including effector memory and terminal effector memory T-cells, were greater in younger MDS patients (slope=-0.433, p=0.128 and slope=-0.364, p=0.128, respectively). Similar patterns with age were observed for naïve, central memory, effector memory and terminal effector memory CD8⁺ T-cells, although the associations were weaker than those observed for CD4⁺ T-cells, and only the reduction in central memory cells in younger patients was statistically significant (Fig. 3B, slope=-.249, p=0.022). These age-related changes in T-cell phenotype were distinctly different from those observed in CD4⁺ or CD8⁺ T-cells from controls, as shown in Figure 3, that ranged in age from 19 to 81 years old (n=54). Although there was a slight tendency for CD4⁺ naïve cells to decrease with age, this trend was not statistically significant (slope=-0.11, p=1.0), and central, effector, and terminal

effector memory CD4⁺ T-cell subpopulations did not change with age within the CD4⁺ subset (Fig. 3C). In these healthy control donors, a dramatic age-related change in T-cell homeostasis occurred within the CD8⁺ T-cell compartment. The percentage of naïve CD8⁺ T-cells decreased 0.61% with each year of age ($p < 0.0001$); whereas, conversion of the peripheral pool to terminal effector memory cells was positively associated with age (0.567% increase with each year of age, $p = 0.001$), as shown in Fig. 3D (left panel). In summary, results show that there is a particular skewing of CD4⁺ and CD8⁺ T-cells toward a CD62L⁻ phenotype with reduction in naïve cells in younger MDS patients.

10 Example 4—Aberrant homeostatic T-cell regulation prior to treatment in patients responsive to IST

Diminished naïve T-cells may result from accelerated turnover characterized by increased cell death or increased conversion of naïve cells into memory cells similar to abnormalities observed in association with other autoimmune syndromes (Krupica *et al.*, 2006; Marleau & Sarvetnick, 2005; Goronzy & Weyand, 2005; Fulop *et al.*, 2003). Since younger age in MDS represents an important characteristic related to IST response, the CD4⁺ and CD8⁺ naïve and memory cell populations and the CD4:CD8 ratio were compared among eight IST responders and fifteen non-responders (ages ranged from 19 to 72 years old with a mean age of 46 and 62 years among responders and non-responders, respectively) to gain a better understanding of how altered T-cell homeostasis impacts bone marrow suppression among younger cases. To understand the relationship between T-cell turnover and IST response, we also determined the expression of Ki-67, a nuclear proliferation-associated antigen, as an indicator of proliferation in both the CD4⁺ and CD8⁺ T-cells. Patients were treated with either equine anti-thymocyte globulin (ATG) or ATG plus cyclosporine A (CyA), and hematologic response was assessed using the International Working Group (IWG) criteria with clinical results reported elsewhere (Cheson *et al.*, 2006; Sloand *et al.*, 2008). First, immune parameters were compared before treatment between patients that demonstrated hematologic response (complete response and partial response) and patients demonstrating no clinical hematologic improvement to this therapy. We found that the CD4:CD8 ratio before treatment was statistically significantly lower in the IST responders compared to non-responders (Fig. 4A) ($p = 0.0003$). Interestingly, we found that the pre-treatment % of Ki-67 positive cells

in both the CD4+ and CD8+ subpopulations was significantly greater in MDS patients demonstrating a response to IST compared to patients who did not respond ($p=0.0038$ and $p=0.0005$, respectively, Fig. 4B-1 and 4B-2). In Figure 4C-1 and 4C-2, we show inverse correlations between the percentage of Ki-67+/CD4+ and Ki-67+/CD8+ T-cells and the CD4:CD8 ratio, which was statistically significant for the CD4+ population ($p=0.0123$ for CD4+ and $p=0.0619$ for CD8+ cells while adjusting for age). These results suggest that cells within both of these compartments are actively cycling when the ratio is diminished in MDS patients.

Example 5—Reconstitution of T-cell homeostasis is associated with recovery of bone marrow hematopoiesis after therapy

Next, we compared the changes in key immune parameters before and after IST in association with clinical hematologic response. The proliferative index is dramatically reduced after treatment specifically in the IST responsive patients as compared to the non-responsive patients in both the CD4+ and CD8+ T-cells as demonstrated by Ki-67 expression. To determine whether the changes in proliferation affected all of the CD4+ and CD8+ subpopulations, individual analyses were performed on naïve and memory cells defined by % Ki67 in association with CD45RA and CD62L staining using the gating strategy shown in Figure 5A. After treatment, a reduction in Ki-67 expression was observed in naïve and all memory subpopulations after IST in patients with a hematological response compared to changes in the non-responders (Figure 5B, $p<0.05$ for all tests). These results strongly suggest that proliferation may be coupled to accelerated T-cell depletion and altered T-cell homeostasis that eventually leads to a reduction in naïve cells. Consistent with this idea, patients demonstrating a hematological response to IST displayed an increase in naïve CD4+ and CD8+ T-cells after treatment compared to non-responders (Figure 5C) ($p=0.001$ and $p=0.0001$, respectively). In summary, results show that proliferation in T-cells is higher and the CD4:CD8 ratio is lower before treatment in younger IST responsive patients. Furthermore, results also demonstrate that hematological response is associated with reconstitution of T-cell homeostasis and reduction in homeostatic proliferation.

Example 6

Since little is known about age as a modifier of immunity in MDS, the current study was conducted to determine whether T-cell homeostasis and function is normal and age-appropriate in MDS patients. Unlike most previously reported findings in CD8+ T-cells, we found that contraction of naïve and central memory cells and accumulation of effector and terminal effector memory cells were also pronounced within the CD4+ T-cell compartment in younger MDS patients and that reduction in the CD4:CD8 ratio was driven by an age-independent decrease in CD4+ T-cells (Molldrem *et al.*, 1998; Sauntharajah *et al.*, 2001; Molldrem *et al.*, 2002; Sloan *et al.*, 2005). To discern how these age-related abnormalities defined in the case-control study may impact bone marrow suppression, we studied T-cell immunity in 20 MDS patients before and after treatment with ATG with or without CyA. Results presented here strongly suggest that reduced CD4+ T-cells not only drive the aberrant CD4:CD8 ratio in a diverse group of MDS patients but is also robustly associated with IST responsiveness in younger patients. In addition to reduced CD4+ T-cells, altered naïve and memory subset distribution was found in CD4+ T-cell and CD8+ cells.

In younger MDS patients, fragility of the CD4+ naïve T-cell compartment is strikingly different than changes associated with a normal aging process where CD4+ naïve cells remain constant, as shown in Fig. 3. In healthy controls, our results are consistent with previous studies showing that regulation of CD4+ and CD8+ homeostasis occurs through distinct mechanisms during the aging process. CD4+ T-cells from patients are clearly more vulnerable to depletion compared to controls (Naylor *et al.*, 2005). There are several examples, including rheumatoid arthritis, infection with human immunodeficiency virus (HIV)-1, and individuals with repeated pathogen exposure, where peripheral CD4+ naïve T-cells are diminished and the CD4:CD8 ratio shifted in association with chronic activation (Hazenber *et al.*, 2000a; Hazenber *et al.*, 2000b; Goronzy & Weyand, 2001). Therefore, these results suggest that CD4+ T-cells in addition to CD8+ T-cells have been activated *in vivo*.

Reduction in naïve cells in MDS may be mediated by either increased apoptotic sensitivity or increased rate of phenotype conversion to memory cells (Hazenber *et al.*, 2000b; Weyand & Goronzy, 1999). A mechanism of increased sensitivity to apoptosis is consistent with previous findings that hypocellularity, in some studies, is associated with

clinical responsiveness to IST (Lim *et al.*, 2007; Sun *et al.*, 2008). Greater susceptibility to Fas-mediated apoptosis and higher levels of TNF α have also been consistently linked to bone marrow failure in not only MDS but also in LGL leukemia (Sloand *et al.*, 2002; Gersuk *et al.*, 1998; Powers *et al.*, 2007; Galili *et al.*, 2007; Reza *et al.*, 1999; Reza *et al.*, 1998). A higher proliferative index, especially affecting naïve cells, in younger IST-responsive MDS cases indicates that T-cells in these patients have been exposed to conditions which promote non-antigen-specific proliferation (*i.e.*, homeostatic proliferation) driven by the IL-2R $\beta\gamma_c$ family of cytokines IL-7, IL-15, and/or IL-21 that are associated with risk for autoimmunity in many situations (Clarke & Rudensky, 2000; Lim & Kim, 2007; Chiu *et al.*, 2006).

An increase in T-cell homeostatic proliferation in response to the IL-2R $\beta\gamma_c$ leads to the indiscriminate expansion of both non-thymic-depleted self-reactive T-cells and antigen-specific T-cells. An increase in homeostatic proliferation alone, however, generally fails to induce autoimmunity. It is the combination of homeostatic proliferation and defects in one or more additional factor(s) circumventing mechanisms of peripheral tolerance that leads ultimately to autoimmunity in this setting (Krupica *et al.*, 2006). It is possible that loss of the CD4 $^{+}$ T-cells in MDS may represent depletion or functional inactivation of a specific subset of CD4 $^{+}$ T-cells, namely regulatory T-cells (Tregs), which are important for peripheral tolerance and prevention of an autoimmune process (Stockinger *et al.*, 2004; Setoguchi *et al.*, 2005; Neujahr *et al.*, 2006; Calzascia *et al.*, 2008). Tregs express high levels of CD25 (IL-2Ra) with low (dim) expression of IL-7R α (CD127) and suppress CD4 $^{+}$ and CD8 $^{+}$ responses to not only TCR stimulation but also homeostatic proliferation (Krupica *et al.*, 2006; Dunham *et al.*, 2008; Vrabelova *et al.*, 2008; Alves *et al.*, 2007). Tregs can develop in the thymus (natural, nTregs) or they can be expanded in the periphery (inducible Tregs, iTregs) (Valencic *et al.*, 2007). Tregs are characterized by the expression of the FOXP3 transcription factor with greater expression of the negative co-stimulatory molecule cytotoxic T lymphocyte-associated antigen (CTLA-4) (Catalfamo *et al.*, 2008). The number of Tregs in the peripheral blood of MDS patients has been shown to positively correlate with risk for disease progression. Further investigation is needed to determine whether the number and/or function of Tregs is lower in IST responsive patients compared to healthy controls with age adjustment. The latter is important since Treg number and/or function may vary with age (Kordasti *et al.*,

2007; Czesnikiewicz-Guzik *et al.*, 2008). Furthermore, elevated levels of IL-21 have recently been shown to suppress Treg function without affecting cell numbers. In addition to Treg reduction, excessive IL-21 (King *et al.*, 2004) or an inflammatory microenvironment independently act as catalyzing factors for homeostatic proliferation-associated autoimmunity (Krupica *et al.*, 2006).

Regardless of the cellular mechanisms that govern the proliferation and turnover in the T-cell compartment, the association between the T-cell abnormalities reported in this study and response to IST provides a strong rationale implicating aberrant T-cell homeostasis as a critical determinant of autoimmunity in MDS that may have positive predictive power for response to IST. In summary, our findings are consistent with a model that IST blocks the proliferative signal that is associated with depletion of CD4+ T-cells and expansion of autoreactive CD8+ T-cells, and restores T-cell homeostasis of the CD4+ and CD8+ T-cell compartment. The presence of altered T-cell homeostasis may represent a marker that is predictive for IST responsiveness.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

REFERENCES

- U.S. Patent No. 4,582,789
- U.S. Patent No. 4,617,261
- 5 U.S. Patent No. 4,683,195
- Alves, N.L. *et al.* (2007) A new subset of human naive CD8⁺ T cells defined by low expression of IL-7R α . *J Immunol* 179, 221-8.
- Ausubel *et al.*, eds (1994) *Current Protocols in Molecular Biology*, Vol. 1, John Wiley and Sons, Inc., New York.
- 10 Bennett, J.M. & Komrokji, R.S. (2005) The myelodysplastic syndromes: diagnosis, molecular biology and risk assessment. *Hematology* 10 Suppl 1, 258-69.
- Berezovski, M.V., Lechmann, M., Musheev, M.U., Mak, T.W., Krylov, S.N. (2008) Aptamer-facilitated biomarker discovery (AptaBiD). *J Am Chem Soc.* 130 (28): 9137-43.
- 15 Biesma, D.H., van den Tweel, J.G. & Verdonck, L.F. (1997) Immunosuppressive therapy for hypoplastic myelodysplastic syndrome. *Cancer* 79, 1548-51.
- Calzascia, T. *et al.* (2008) CD4 T cells, lymphopenia, and IL-7 in a multistep pathway to autoimmunity. *Proc Natl Acad Sci U S A* 105, 2999-3004.
- 20 Catalfamo, M., Tai, X., Karpova, T., McNally, J. & Henkart, P.A. (2008) TcR-induced regulated secretion leads to surface expression of CTLA-4 in CD4(+)CD25(+) T cells. *Immunology* 125(1), 70-79.
- Champagne, P. *et al.* (2001) Skewed maturation of memory HIV-specific CD8 T lymphocytes. *Nature* 410, 106-11.
- 25 Cheson, B.D. *et al.* (2006) Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. *Blood* 108, 419-25.
- Chiu, W.K., Fann, M. & Weng, N.P. (2006) Generation and growth of CD28nullCD8⁺ memory T cells mediated by IL-15 and its induced cytokines. *J Immunol* 177, 7802-10.
- 30 Clarke, S.R. & Rudensky, A.Y. (2000) Survival and homeostatic proliferation of naive peripheral CD4⁺ T cells in the absence of self peptide:MHC complexes. *J Immunol* 165, 2458-64.
- 35 Colmegna, I. *et al.* (2008) Defective proliferative capacity and accelerated telomeric loss of hematopoietic progenitor cells in rheumatoid arthritis. *Arthritis Rheum* 58, 990-1000.

- Czesnikiewicz-Guzik, M. *et al.* (2008) T cell subset-specific susceptibility to aging. *Clin Immunol* 127, 107-18.
- De Rosa, S.C., Herzenberg, L.A., Herzenberg, L.A. & Roederer, M. (2001) 11-color, 13-parameter flow cytometry: identification of human naive T cells by phenotype, function, and T-cell receptor diversity. *Nat Med* 7, 245-8.
- Dunham, R.M. *et al.* (2008) CD127 and CD25 Expression Defines CD4+ T Cell Subsets That Are Differentially Depleted during HIV Infection. *J Immunol* 180, 5582-92.
- Ellington, A.D., and Szostak, J.W. (1990) In vitro selection of RNA molecules that bind specific ligands. *Nature* 346 (6287): 818-22.
- Epling-Burnette, P.K. *et al.* (2001) Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. *J Clin Invest* 107, 351-62.
- Fulop, T., Jr., Larbi, A., Dupuis, G. & Pawelec, G. (2003) Ageing, autoimmunity and arthritis: Perturbations of TCR signal transduction pathways with ageing - a biochemical paradigm for the ageing immune system. *Arthritis Res Ther* 5, 290-302.
- Galili, N., Cerny, J. & Raza, A. (2007) Current treatment options: impact of cytogenetics on the course of myelodysplasia. *Curr Treat Options Oncol* 8, 117-28.
- Gersuk, G.M. *et al.* (1998) A role for tumour necrosis factor-alpha, Fas and Fas-Ligand in marrow failure associated with myelodysplastic syndrome. *Br J Haematol* 103, 176-88.
- Goldrath, A.W. & Bevan, M.J. (1999) Selecting and maintaining a diverse T-cell repertoire. *Nature* 402, 255-62.
- Goronzy, J.J. & Weyand, C.M. (2005) Rheumatoid arthritis. *Immunol Rev* 204, 55-73.
- Goronzy, J.J. & Weyand, C.M. (2001) Thymic function and peripheral T-cell homeostasis in rheumatoid arthritis. *Trends Immunol* 22, 251-5.
- Greenberg, P. *et al.* (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood* 89, 2079-88.
- Hazenber, M.D. *et al.* (2000a) T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART). *Blood* 95, 249-55.
- Hazenber, M.D. *et al.* (2000b) Increased cell division but not thymic dysfunction rapidly affects the T-cell receptor excision circle content of the naive T cell population in HIV-1 infection. *Nat Med* 6, 1036-42.

- Hellerstein, M. *et al.* (1999) Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. *Nat Med* 5, 83-9.
- Hoppe-Seyler, F. and Butz, K. (2000) Peptide aptamers: powerful new tools for molecular medicine. *J Mol Med.* 78 (8): 426-30.
- 5 Jaroszeski, M.J. and Heller, R. (Editors) (1998) Flow Cytometry Protocols. Methods in Molecular Biology, No 91, Humana Press.
- King, C., Ilic, A., Koelsch, K. & Sarvetnick, N. (2004) Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. *Cell* 117, 265-77.
- 10 Klussmann, S. (ed.) (2006) Aptamer Handbook: Functional Oligonucleotides and Their Applications.
- Kochenderfer, J.N., Kobayashi, S., Wieder, E.D., Su, C. & Molldrem, J.J. (2002) Loss of T-lymphocyte clonal dominance in patients with myelodysplastic syndrome responsive to immunosuppression. *Blood* 100, 3639-45.
- 15 Kook, H. *et al.* (2001) Increased cytotoxic T cells with effector phenotype in aplastic anemia and myelodysplasia. *Exp Hematol* 29, 1270-7.
- Kordasti, S.Y. *et al.* (2007) CD4⁺CD25^{high} Foxp3⁺ regulatory T cells in myelodysplastic syndrome (MDS). *Blood* 110, 847-50.
- Krupica, T., Jr., Fry, T.J. & Mackall, C.L. (2006) Autoimmunity during lymphopenia: a two-hit model. *Clin Immunol* 120, 121-8.
- 20 Lim, Z.Y. *et al.* (2007) Low IPSS score and bone marrow hypocellularity in MDS patients predict hematological responses to antithymocyte globulin. *Leukemia* 21, 1436-41.
- 25 Lim, H.W. & Kim, C.H. (2007) Loss of IL-7 receptor alpha on CD4⁺ T cells defines terminally differentiated B cell-helping effector T cells in a B cell-rich lymphoid tissue. *J Immunol* 179, 7448-56.
- List, A.F., Vardiman, J., Issa, J.P. & DeWitte, T.M. (2004) Myelodysplastic syndromes. *Hematology (Am Soc Hematol Educ Program)*, 297-317.
- Loughran, P., Jr. (1998) Anemia in Lymphoproliferative Disorders. *Cancer Control* 5, 51-53.
- 30 Maldonado, A. *et al.* (2003) Decreased effector memory CD45RA⁺ CD62L⁻ CD8⁺ T cells and increased central memory CD45RA⁻ CD62L⁺ CD8⁺ T cells in peripheral blood of rheumatoid arthritis patients. *Arthritis Res Ther* 5, R91-6.
- Marleau, A.M. & Sarvetnick, N. (2005) T cell homeostasis in tolerance and immunity. *J Leukoc Biol* 78, 575-84.

- Molldrem, J.J. *et al.* (1998) Haematological response of patients with myelodysplastic syndrome to antithymocyte globulin is associated with a loss of lymphocyte-mediated inhibition of CFU-GM and alterations in T-cell receptor Vbeta profiles. *Br J Haematol* 102, 1314-22.
- 5 Molldrem, J.J. *et al.* (1997) Antithymocyte globulin for patients with myelodysplastic syndrome. *Br J Haematol* 99, 699-705.
- Molldrem, J.J. *et al.* (2002) Antithymocyte globulin for treatment of the bone marrow failure associated with myelodysplastic syndromes. *Ann Intern Med* 137, 156-63.
- 10 Naylor, K. *et al.* (2005) The influence of age on T cell generation and TCR diversity. *J Immunol* 174, 7446-52.
- Neujahr, D.C. *et al.* (2006) Accelerated memory cell homeostasis during T cell depletion and approaches to overcome it. *J Immunol* 176, 4632-9.
- Okamoto, T. *et al.* (1997) Correlation between immunological abnormalities and prognosis in myelodysplastic syndrome patients. *Int J Hematol* 66, 345-51.
- 15 Ormerod, M.G. (2008) *Flow Cytometry – A Basic Introduction*.
- Powers, M.P. *et al.* (2007) Polymorphisms in TGFbeta and TNFalpha are associated with the myelodysplastic syndrome phenotype. *Arch Pathol Lab Med* 131, 1789-93.
- Rabin, R.L. *et al.* (1995) Altered representation of naive and memory CD8 T cell subsets in HIV-infected children. *J Clin Invest* 95, 2054-60.
- 20 Radbruch, A. (Editor), (2000) *Flow Cytometry and Cell Sorting (Springer Lab Manual)*, Springer Verlag.
- Recktenwald, D. and Radbruch, A. (Editors) (1997) *Cell Separation Methods and Applications*, Marcel Dekker.
- 25 Reza, S. *et al.* (1999) Biologic characteristics of 164 patients with myelodysplastic syndromes. *Leuk Lymphoma* 33, 281-7.
- Reza, S., Shetty, V., Dar, S., Qawi, H. & Raza, A. (1998) Tumor necrosis factor-alpha levels decrease with anticytokine therapy in patients with myelodysplastic syndromes. *J Interferon Cytokine Res* 18, 871-7.
- Saiki *et al.* (1985) *Science*, 230:1350.
- 30 Sallusto, F., Lenig, D., Forster, R., Lipp, M. & Lanzavecchia, A. (1999) Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401, 708-12.
- 35 Sauntharajah, Y. *et al.* (2001) Coincident myelodysplastic syndrome and T-cell large granular lymphocytic disease: clinical and pathophysiological features. *Br J Haematol* 112, 195-200.

- Sauntharajah, Y. *et al.* (2002) HLA-DR15 (DR2) is overrepresented in myelodysplastic syndrome and aplastic anemia and predicts a response to immunosuppression in myelodysplastic syndrome. *Blood* 100:1570-1574.
- 5 Sauntharajah, Y. *et al.* (2003) A simple method to predict response to immunosuppressive therapy in patients with myelodysplastic syndrome. *Blood* 102:3025-3027.
- Scharf *et al.* (1986) *Science*, 233:1076.
- Schwab, R. *et al.* (1997) Expanded CD4+ and CD8+ T cell clones in elderly humans. *J Immunol* 158, 4493-9.
- 10 Setoguchi, R., Hori, S., Takahashi, T. & Sakaguchi, S. (2005) Homeostatic maintenance of natural Foxp3(+) CD25(+) CD4(+) regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. *J Exp Med* 201, 723-35.
- Shapiro, H.M. (2003) *Practical Flow Cytometry*, 4th Edition, Wiley Liss, New York.
- 15 Sloand, E.M., Wu, C.O., Greenberg, P., Young, N. & Barrett, J. (2008) Factors Affecting Response and Survival in Patients With Myelodysplasia Treated With Immunosuppressive Therapy. *J Clin Oncol.*
- Sloand, E.M. *et al.* (2005) Preferential suppression of trisomy 8 compared with normal hematopoietic cell growth by autologous lymphocytes in patients with trisomy 8 myelodysplastic syndrome. *Blood* 106, 841-51.
- 20 Sloand, E.M. *et al.* (2002) Fas-mediated apoptosis is important in regulating cell replication and death in trisomy 8 hematopoietic cells but not in cells with other cytogenetic abnormalities. *Blood* 100, 4427-32.
- Sokol, R.J., Hewitt, S. & Booker, D.J. (1989) Erythrocyte autoantibodies, autoimmune haemolysis, and myelodysplastic syndromes. *J Clin Pathol* 42, 1088-91.
- 25 Starkebaum, G., Loughran, T.P., Jr., Gaur, L.K., Davis, P. & Nepom, B.S. (1997) Immunogenetic similarities between patients with Felty's syndrome and those with clonal expansions of large granular lymphocytes in rheumatoid arthritis. *Arthritis Rheum* 40, 624-6.
- 30 Steensma, D.P. & Tefferi, A. (2003) The myelodysplastic syndrome(s): a perspective and review highlighting current controversies. *Leuk Res* 27, 95-120.
- Stockinger, B., Kassiotis, G. & Bourgeois, C. (2004) Homeostasis and T cell regulation. *Curr Opin Immunol* 16, 775-9.
- 35 Sun, M., Zhang, J., Liu, S., Liu, Y. & Zheng, D. (2008) Sp1 is involved in 8-chloro-adenosine-upregulated death receptor 5 expression in human hepatoma cells. *Oncol Rep* 19, 177-85.

- Valencic, E., Piscianz, E., Tommasini, A. & Granzotto, M. (2007) T cells stimulated in vitro have a suppressive function but do not contain only regulatory T cells. Clin Exp Immunol 150, 561-6.
- 5 Vrabelova, Z. *et al.* (2008) CD 127- and FoxP3+ expression on CD25+CD4+ T regulatory cells upon specific diabetogeneic stimulation in high-risk relatives of type 1 diabetes mellitus patients. Scand J Immunol 67, 404-10.
- Wang, H.Q. *et al.* (2006) Burden of abnormal hematopoietic clone in patients with myelodysplastic syndromes. Chin Med Sci J 21, 99-103.
- 10 Weyand, C.M. & Goronzy, J.J. (1999) T-cell responses in rheumatoid arthritis: systemic abnormalities-local disease. Curr Opin Rheumatol 11, 210-7.
- Young, N.S. (2002) Immunosuppressive treatment of acquired aplastic anemia and immune-mediated bone marrow failure syndromes. Int J Hematol 75, 129-40.

CLAIMS

I claim:

1. A method for assessing or predicting whether a patient having an autoimmune disorder will respond to an immunosuppressive therapy (IST), said method comprising: analyzing T cells of a patient for dysregulation of CD4⁺ and/or CD8⁺ T cells; and determining the patient's likelihood of responding to IST based on the level of dysregulation of the patients CD4⁺ and/or CD8⁺ T cells.

2. The method according to claim 1, wherein the dysregulation analyzed is the level of CD4⁺ T cells in a biological sample from the patient, and wherein a reduced level of CD4⁺ T cells is predictive of an increased likelihood of patient response to IST.

3. The method according to claim 1, wherein the dysregulation analyzed is the ratio of CD4⁺:CD8⁺ T cells in a biological sample from the patient, and wherein a reduced or lower ratio of CD4⁺:CD8⁺ T cells is predictive of an increased likelihood of patient response to IST.

4. The method according to claim 1, wherein the dysregulation analyzed is the level of proliferating of CD4⁺ and/or CD8⁺ T cells in a biological sample from the patient, and wherein an increased or higher level of proliferation of CD4⁺ and/or CD8⁺ T cells is predictive of an increased likelihood of patient response to IST.

5. The method according to claim 4, wherein proliferation is measured by detecting expression of a cellular or nuclear proliferation-associated antigen on a CD4⁺ and/or CD8⁺ T cell.

6. The method according to claim 4, wherein proliferation is measured by detecting the marker Ki-67, wherein an increased percentage of Ki-67⁺ CD4⁺ and/or Ki-67⁺ CD8⁺ T cells is predictive of an increased likelihood of patient response to IST.

7. The method according to claim 6, wherein the Ki-67 marker is detected using an antibody, an antibody fragment, or aptamer specific to the Ki-67 marker.

8. The method according to claim 7, wherein antibody or aptamer binding to the Ki-67 marker of a T cell is detected using flow cytometry.

9. The method according to claim 1, wherein the dysregulation analyzed is the level of CD4⁺ and/or CD8⁺ effector memory T cells and/or terminal effector memory T cells in a biological sample from the patient, and wherein an increased percentage of CD4⁺ and/or CD8⁺ effector memory T cells and/or terminal effector memory T cells is predictive of an increased likelihood of patient response to IST.

10. The method according to claim 1, wherein the dysregulation analyzed is the level of CD4⁺ effector memory T cells and wherein an increase percentage of CD4⁺ effector memory T cells is predictive of an increased likelihood of patient response to IST.

11. The method according to claim 9 or 10, wherein the level of CD4⁺ and/or CD8⁺ effector memory T cells and/or terminal effector memory T cells is measured by detecting expression of an antigen associated with an effector memory T cell and/or an antigen associated with a terminal effector memory T cell.

12. The method according to claim 9 or 10, wherein the level of CD4⁺ effector memory T cells is measured by detecting the marker CD62L, wherein an increased percentage of CD62L⁻ CD4⁺ T cells and/or CD62L⁻ CD8⁺ T cells is predictive of an increased likelihood of patient response to IST.

13. The method according to claim 9 or 10, wherein the level of CD4⁺ effector memory T cells is measured by detecting the marker CD62L and CD45RA, wherein an increased percentage of CD62L/CD45RA⁻ CD4⁺ T cells is predictive of an increased likelihood of patient response to IST.

14. The method according to claim 12 or 13, wherein the CD62L marker is detected using an antibody, an antibody fragment, or aptamer specific to the CD62L marker.

15. The method according to claim 13, wherein the CD45RA marker is detected using an antibody, an antibody fragment, or aptamer specific to the CD45RA marker.

16. The method according to claim 14 or 15, wherein antibody or aptamer binding to the CD62L marker or the CD45RA marker of a T cell is detected using flow cytometry.

17. The method according to claim 1, wherein the dysregulation analyzed is the level of CD4⁺ and/or CD8⁺ naïve T cells and/or central memory T cells, and wherein a decreased percentage of CD4⁺ and/or CD8⁺ naïve T cells and/or central memory T cells is predictive of an increased likelihood of patient response to IST.

18. The method according to claim 1, wherein the dysregulation analyzed is the level of CD4⁺ and/or CD8⁺ central memory T cells, and wherein a decreased percentage of CD4⁺ and/or CD8⁺ central memory T cells is predictive of an increased likelihood of patient response to IST.

19. The method according to claim 17 or 18, wherein the level of CD4⁺ and/or CD8⁺ naïve T cells and/or central memory T cells is measured by detecting expression of an antigen associated with a naïve T cell and/or an antigen associated with a central memory T cell.

20. The method according to claim 17 or 18, wherein the level of CD4⁺ central memory T cells and/or CD8⁺ central memory T cells is measured by detecting the marker CD62L, wherein a decreased percentage of CD62L⁺ CD4⁺ T cells and/or CD62L⁺ CD8⁺ T cells is predictive of an increased likelihood of patient response to IST.

21. The method according to claim 17 or 18, wherein the level of CD4⁺ central memory T cells and/or CD8⁺ central memory T cells is measured by detecting the marker CD62L and CD45RA, wherein a decreased percentage of CD62L⁺/CD45RA⁺ CD4⁺ T cells and/or CD62L⁺/CD45RA⁺ CD8⁺ T cells is predictive of an increased likelihood of patient response to IST.

22. The method according to claim 20 or 21, wherein the CD62L marker is detected using an antibody, an antibody fragment, or aptamer specific to the CD62L marker.

23. The method according to claim 21, wherein the CD45RA marker is detected using an antibody, an antibody fragment, or aptamer specific to the CD45RA marker.

24. The method according to claim 22 or 23, wherein antibody or aptamer binding to the CD62L marker or the CD45RA marker of a T cell is detected using flow cytometry.

25. The method according to any preceding claim, wherein the biological sample is a blood sample.

26. The method according to any preceding claim, wherein the analyzing is conducted using polymerase chain reaction (PCR) methods.

27. The method according to any preceding claim, wherein nucleic acid, such as RNA, encoding or associated with a marker is detected using PCR methods.

28. A method for treating a patient having an autoimmune disorder, the method comprising determining if the patient is likely to respond to IST, wherein said determination is made using a method of any of claims 1-27; and if the patient is determined to be likely to respond to IST, administering an effective treatment regimen to the patient.

29. The method of claim 28, wherein the IST is anti-thymocyte globulin (ATG) therapy.

30. The method of claim 28, wherein the IST is cyclosporine A (CyA) therapy.

31. The method of claim 28, wherein the IST is ATG or CyA therapy or both ATG and CyA therapy.

32. The method according to any preceding claim, wherein the autoimmune disorder is myelodysplastic syndrome (MDS).

33. The method according to any preceding claim, wherein the level of dysregulation of the patient's CD4⁺ and/or CD8⁺ T cells is compared to a control sample or value and correlating the patient's level of dysregulation to a likelihood of responding to IST.

34. A kit comprising in one or more containers:

i) a reagent for assessing the presence of and/or quantifying the amount of CD4⁺ T cells in a sample; and/or

ii) a reagent for assessing the presence of and/or quantifying the amount of CD8⁺ T cells in a sample; and/or

iii) a reagent for assessing the presence of and/or quantifying the amount of naïve, central memory, effector memory, and/or terminal effector memory T cells in a sample; and/or

iv) a reagent for assessing the proliferation of CD4⁺ and/or CD8⁺ T cells in a sample; and/or

v) a reagent for assessing the presence of and/or quantifying the amount of CD3⁺ cells in a sample; and/or

vi) a reagent for assessing the presence of and/or quantifying the amount of CD25⁺ cells in a sample.

35. The kit according to claim 34, wherein the kit optionally comprises positive or negative control or standards.

36. The kit according to claim 34, wherein a reagent of the kit comprises one or more antibody, antibody fragment, or aptamer specific to CD4⁺ or CD8⁺ T cells.

37. The kit according to claim 34, wherein a reagent of the kit comprises one or more antibody, antibody fragment, or aptamer specific to an L-selectin antigen determinant.

38. The kit according to claim 34, wherein the antibody or aptamer binds to CD62L.

39. The kit according to claim 34, wherein a reagent of the kit comprises one or more antibody, antibody fragment, or aptamer specific to a tyrosine phosphatase antigen determinant.

40. The kit according to claim 39, wherein the antibody or aptamer binds to CD45RA, CD45RB, or CD45RO.

41. The kit according to claim 34, wherein a reagent of the kit comprises one or more antibody, antibody fragment, or aptamer specific to a nuclear proliferation associated antigen determinant.

42. The kit according to claim 41, wherein the antibody or aptamer binds to Ki-67.

43. The kit according to claim 34, wherein a reagent of the kit comprises one or more antibody, antibody fragment, or aptamer specific to CD3⁺ cells.

44. The kit according to claim 34, wherein a reagent of the kit comprises one or more antibody, antibody fragment, or aptamer specific to CD25⁺ cells.

45. The kit according to any of claims 34-44, wherein a reagent of the kit can optionally be labeled with a detectable label.

46. The kit according to claim 34-44, wherein the kit optionally comprises a detectable label that can be conjugated or bound to a reagent of the kit.

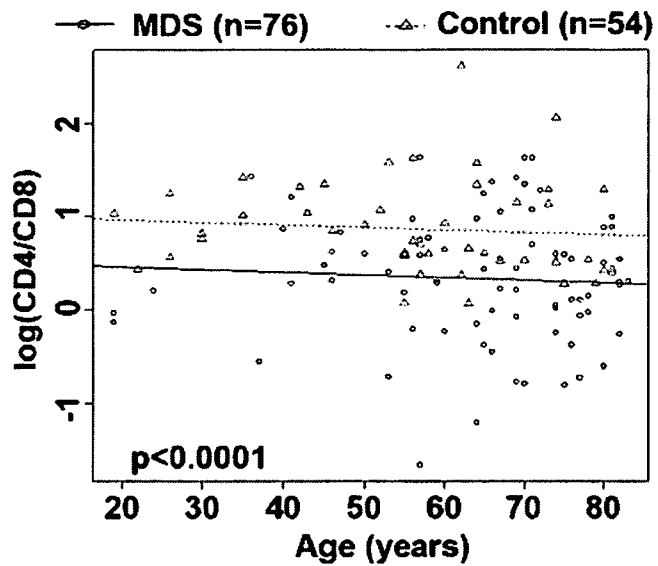


FIG. 1A

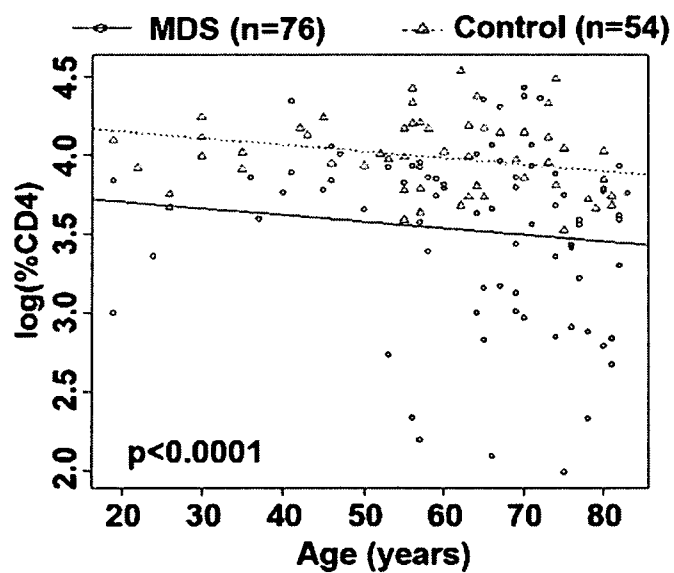


FIG. 1B

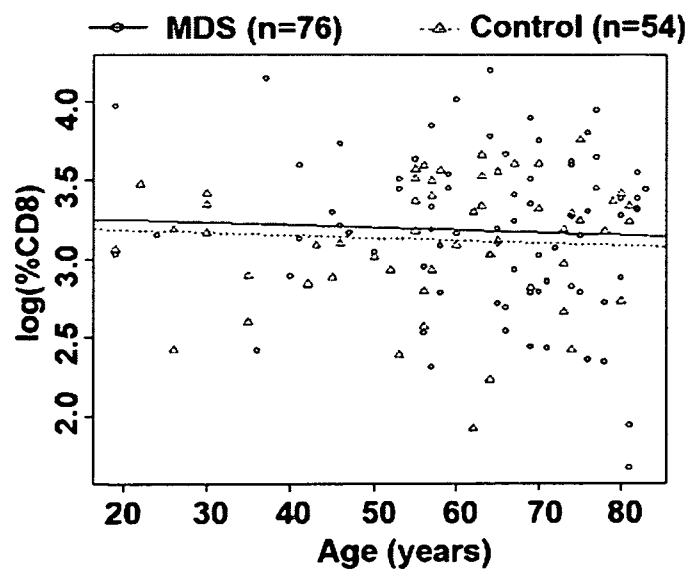


FIG. 1C

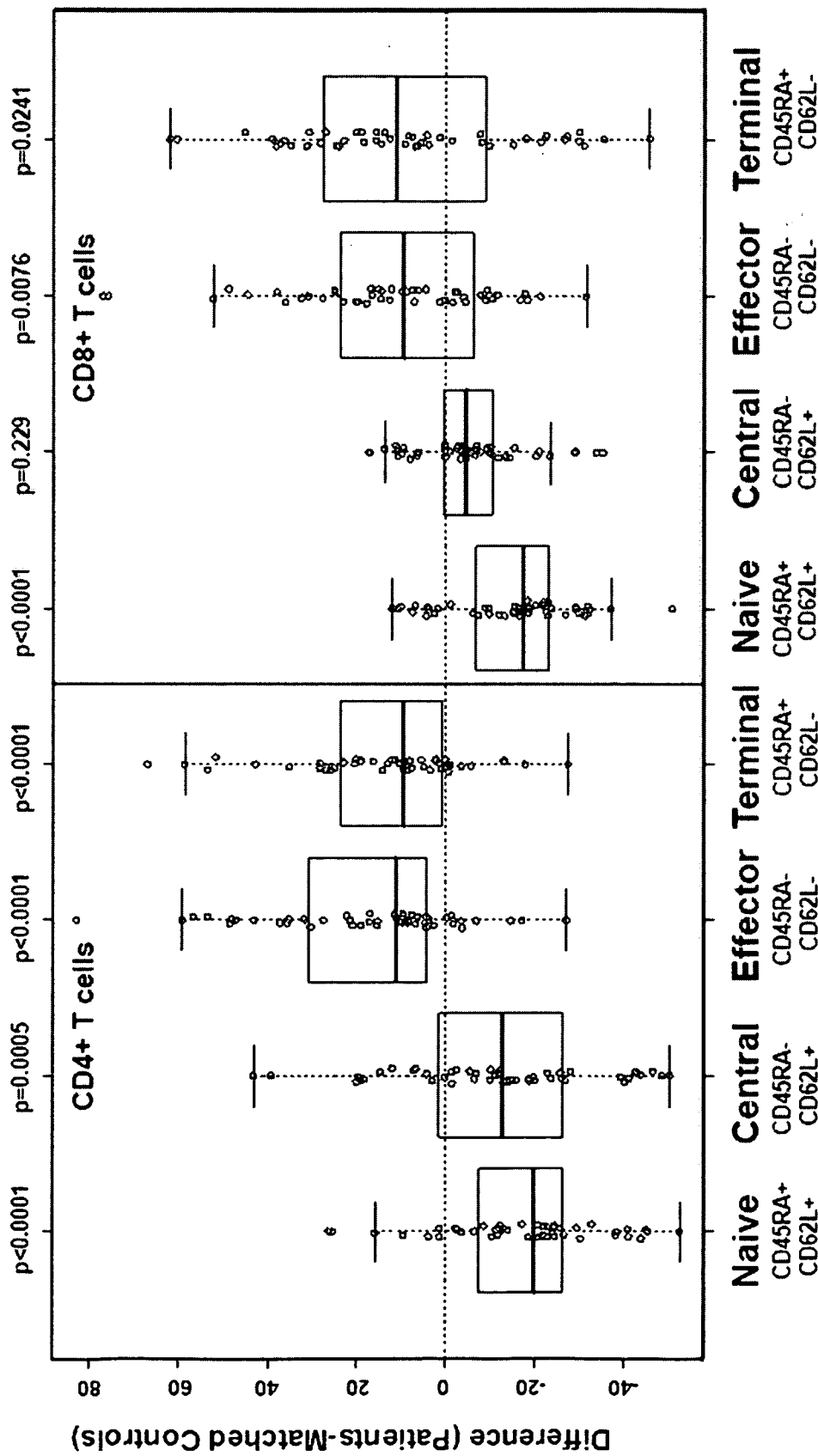
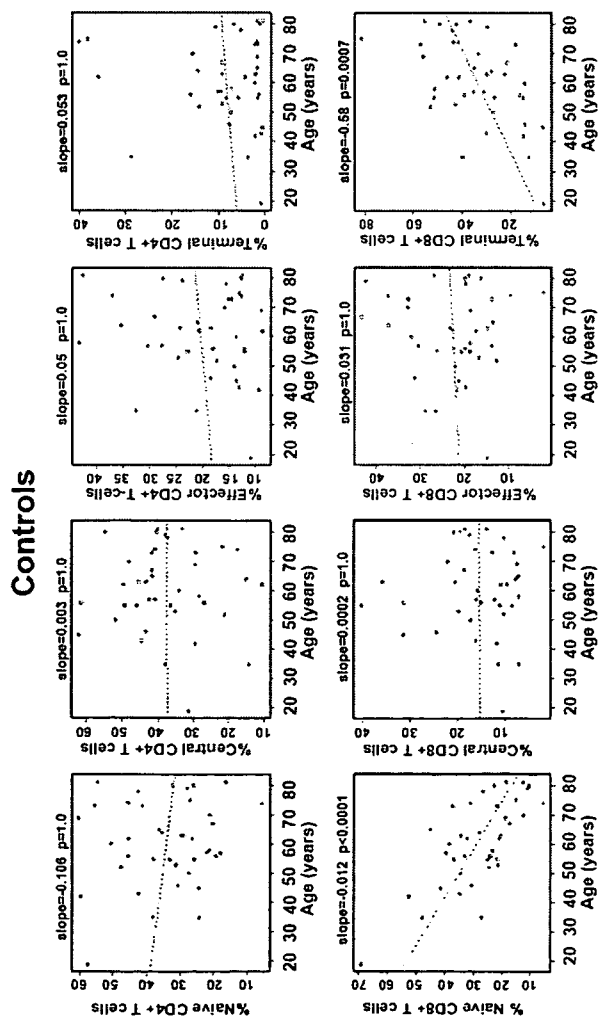
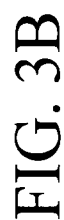


FIG. 2



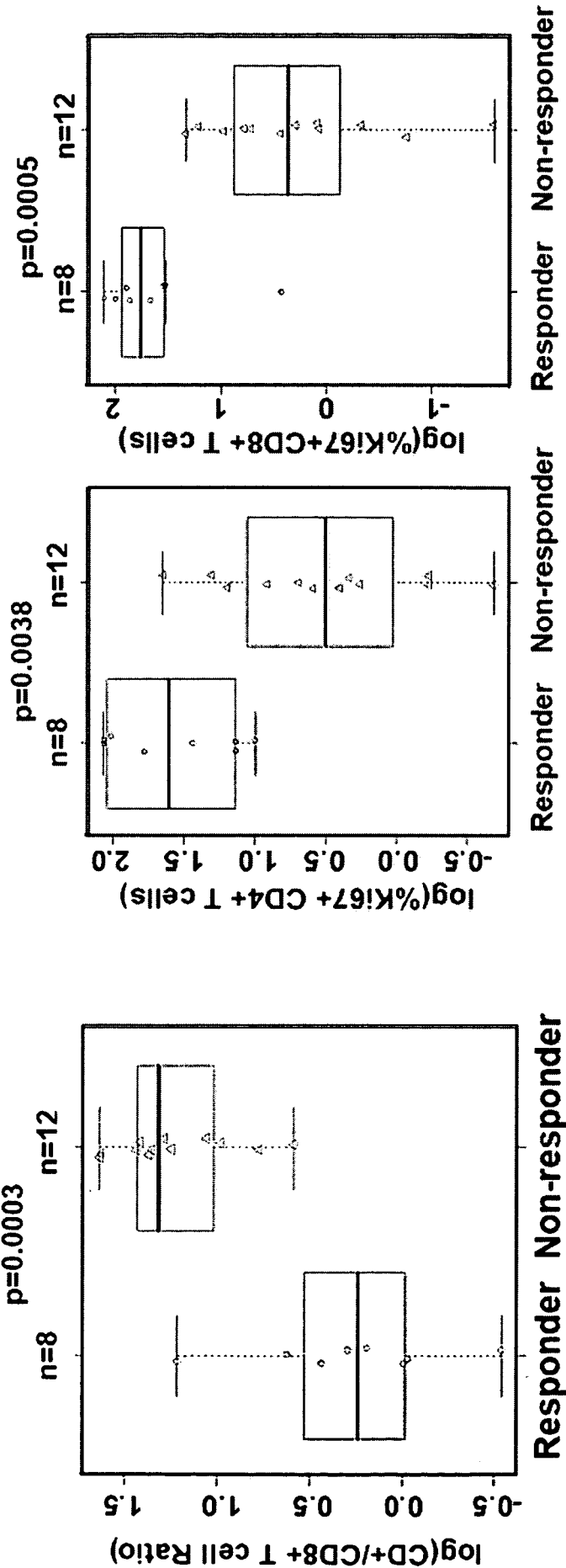


FIG. 4A

FIG. 4B-1

FIG. 4B-2

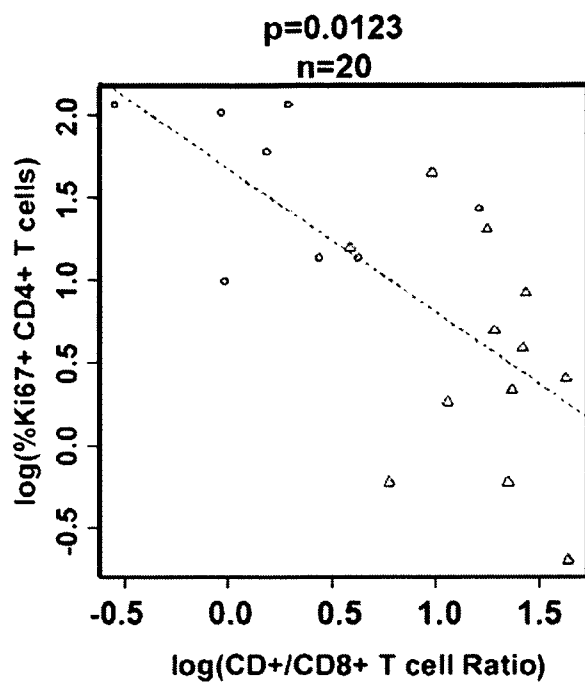


FIG. 4C-1

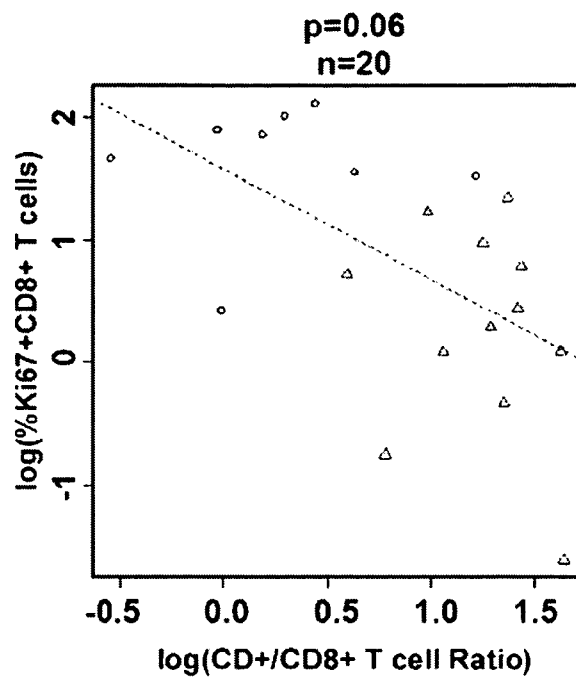


FIG. 4C-2

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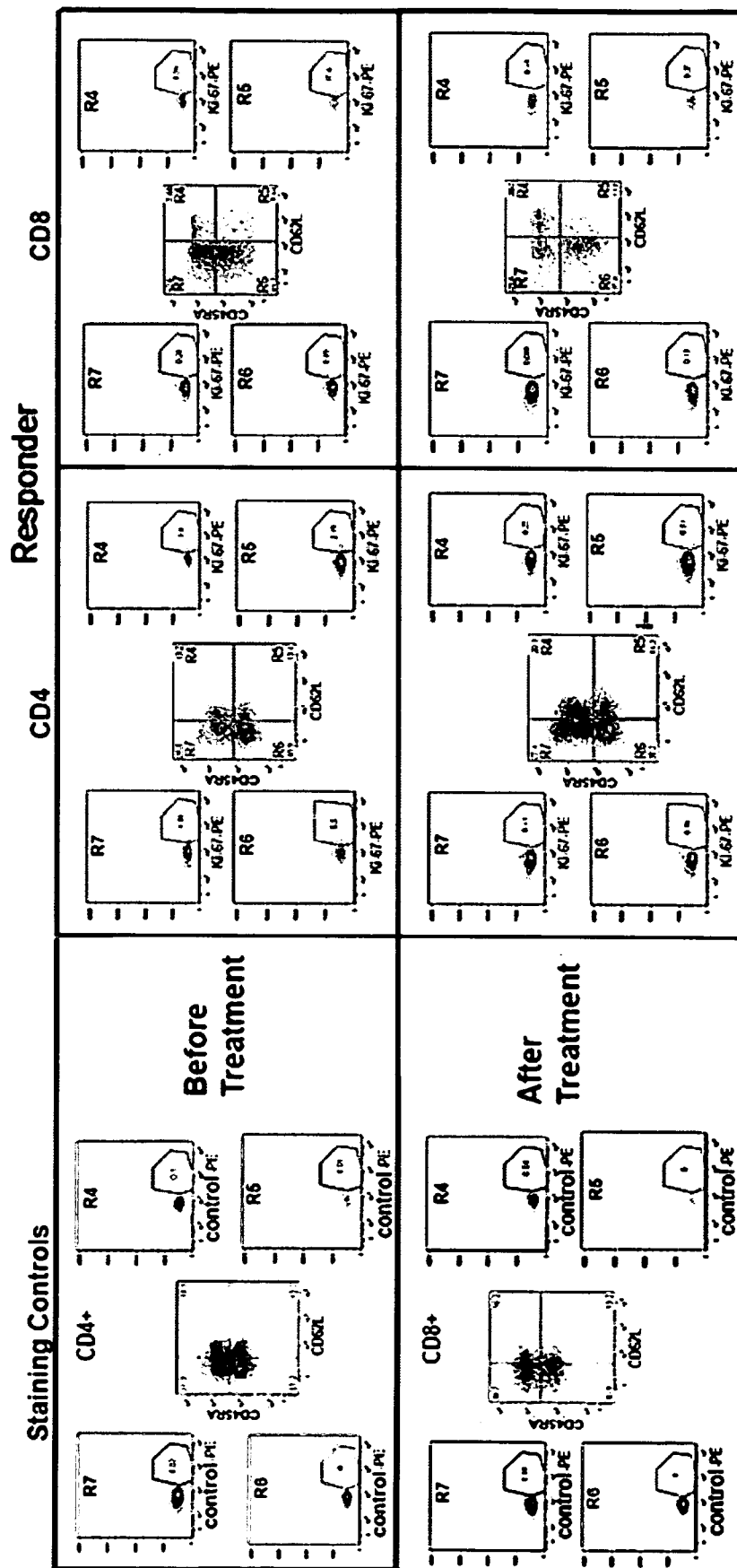


FIG. 5A

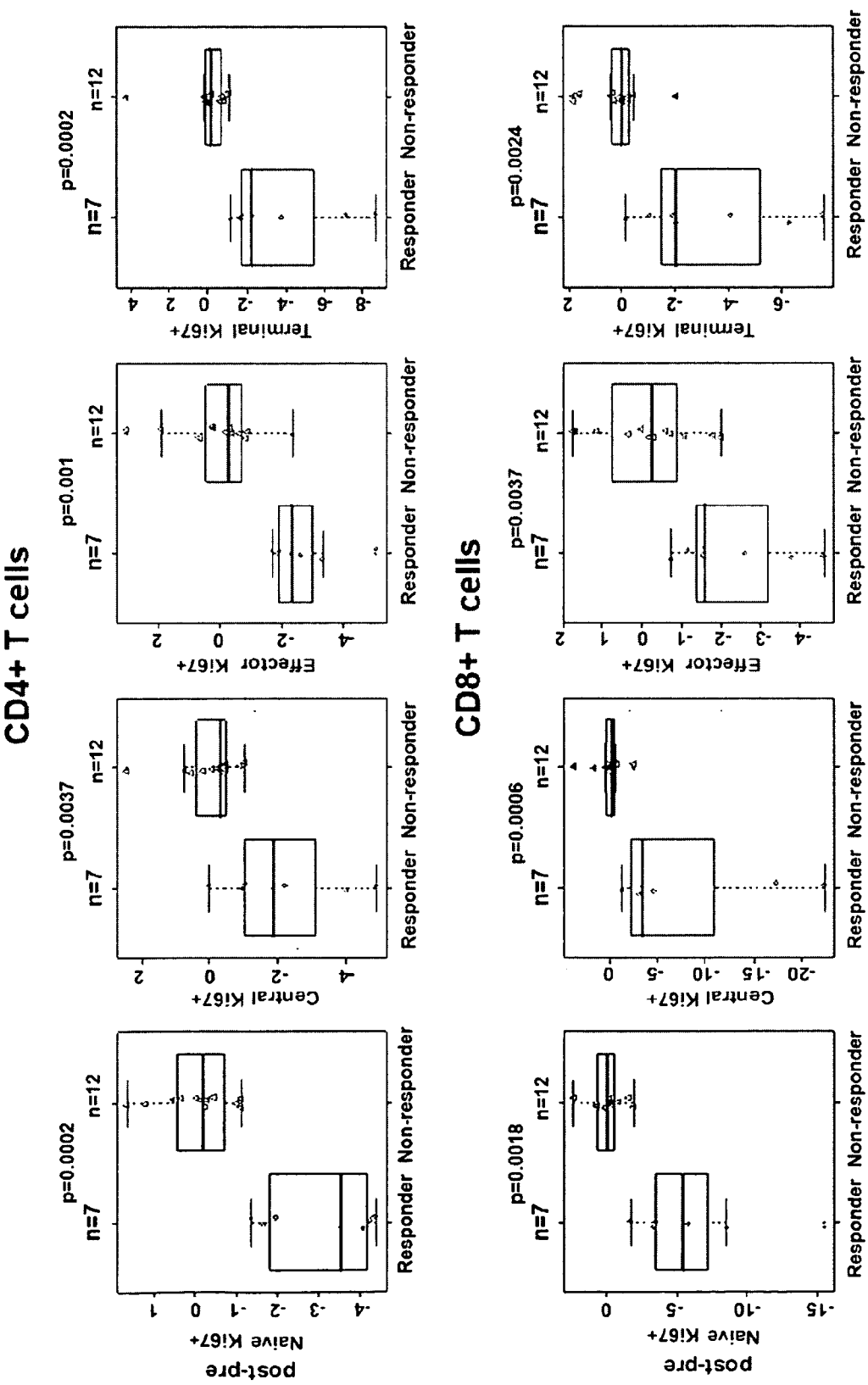


FIG. 5B

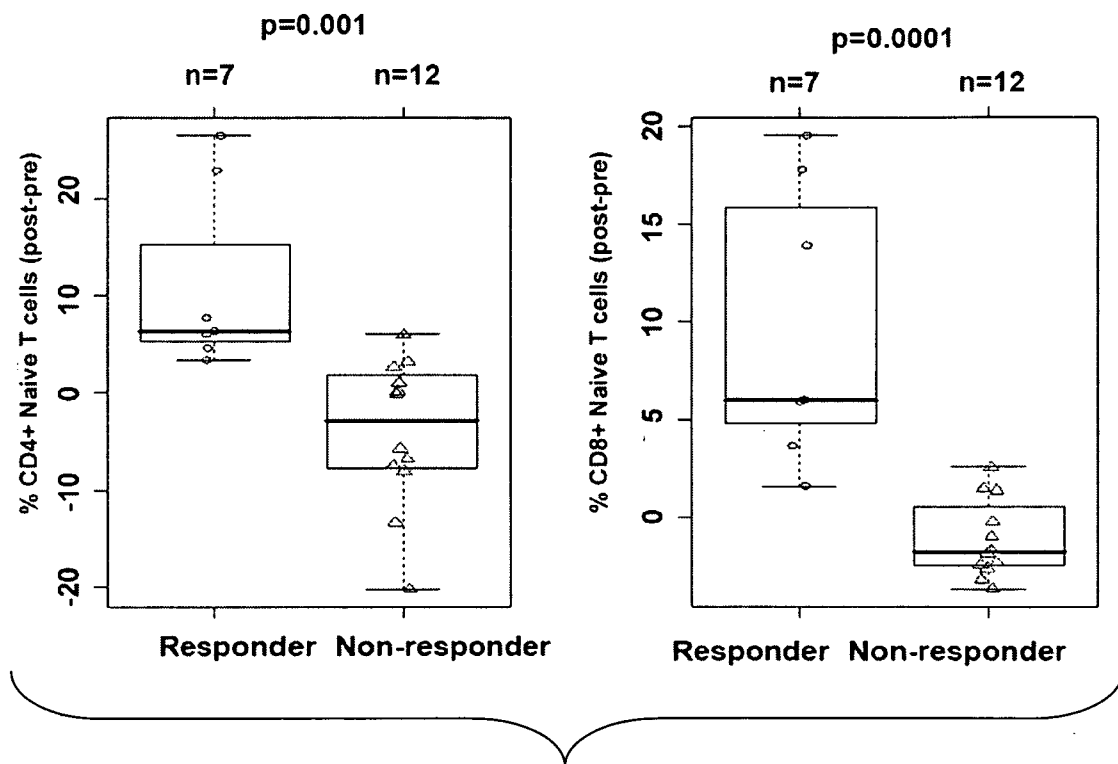


FIG. 5C

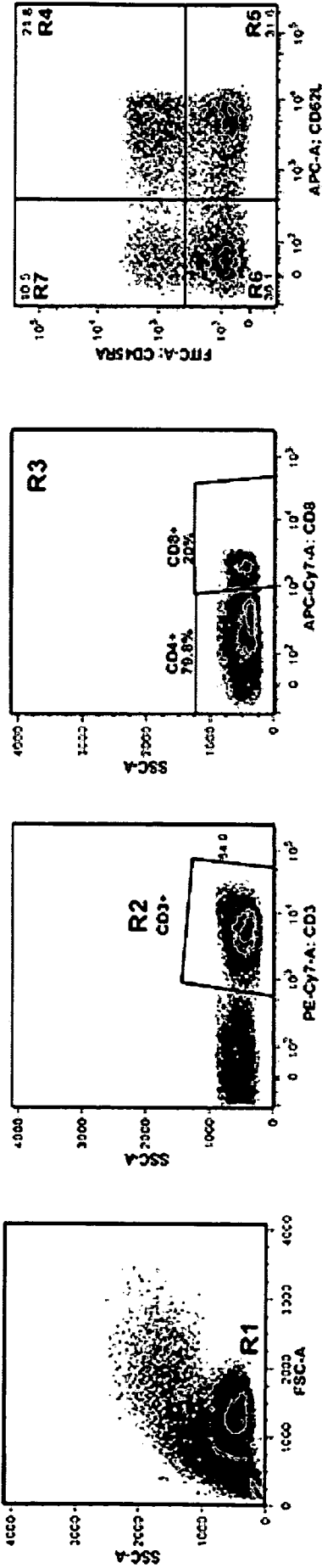


FIG. 6D

FIG. 6C

FIG. 6B

FIG. 6A

10/10

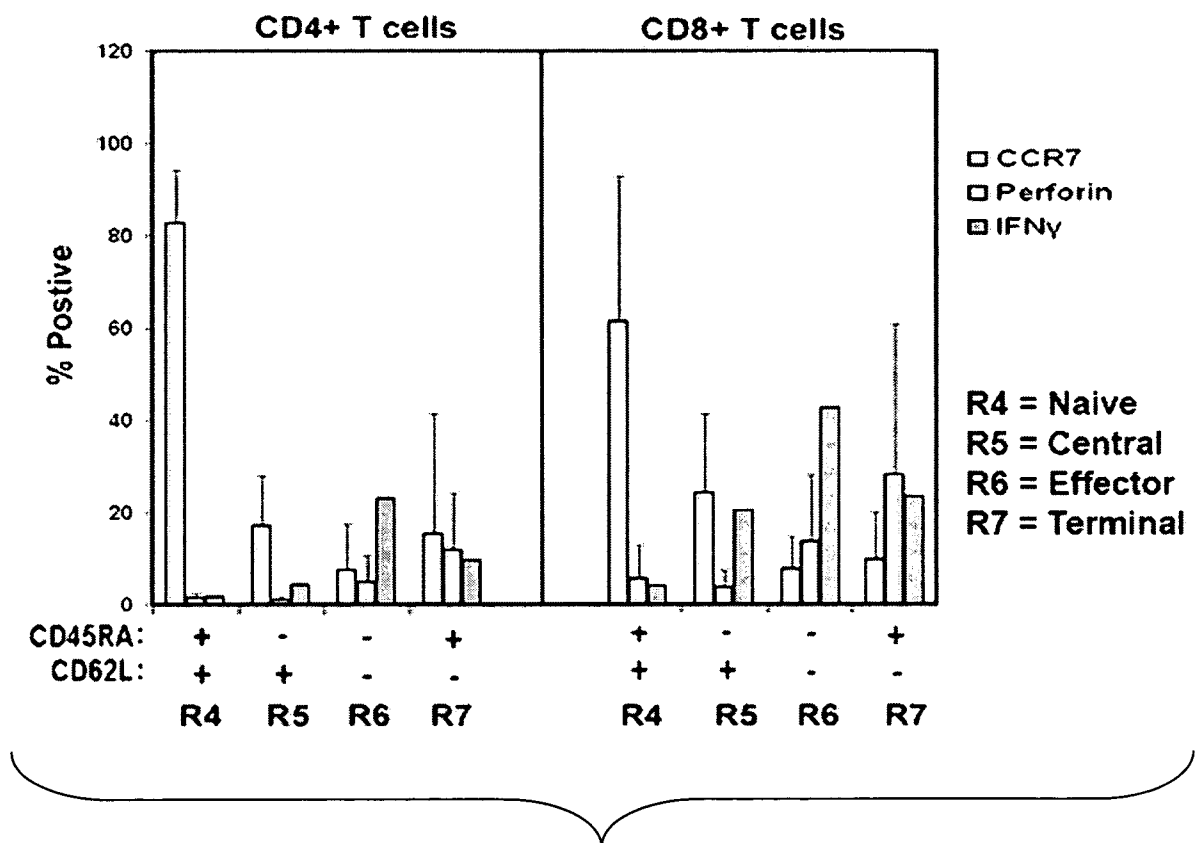


FIG. 6E