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(54) Titre : METHODE DE DETERMINATION DU PHENOTYPE DE CELLULES
(54) Title: A METHOD FOR DETERMINING THE PHENOTYPE OF CELLS

(57) Abrégé/Abstract:
The present invention provides a system, a device, and a kit for comparing the phenotypic state of a first unknown sample material with a sample material of a known phenotypic state. The present invention also provides a method of using the system, device and kit.

ABSTRACT

[0051] The present invention provides a system, a device, and a kit for comparing the phenotypic state of a first unknown sample material with a sample material of a known phenotypic state. The present invention also provides a method of using the system, device and kit.

A METHOD FOR DETERMINING THE PHENOTYPE OF CELLS

FIELD OF THE INVENTION

[0001] This invention is directed to methods for the rapid, efficient and accurate determination of the phenotype of cells. In particular, this invention is directed to methods employing antibodies.

BACKGROUND

[0002] The surface of every cell in the body is coated with proteins. These cell surface proteins have several functions. For example, the cell surface proteins may be receptors, which have the capability of selectively binding or adhering to other "signaling" molecules. Alternatively, these proteins may be functional or structural. For example, the protein may facilitate the adherence of the cell to a substrate, or the protein may facilitate the secretion of a molecule. Each cell type, for example a liver cell, has a certain combination of proteins on their surface that makes them distinguishable from other kinds of cells or cell systems.

[0003] The expression of cell surface proteins on a given cell type may vary, depending on a variety of factors. The may include, for example, changes as a result of cellular proliferation, differentiation, disease, isolation of the cell from the body and culture *in vitro*. For example, cell surface proteins have been reported to play vital roles in multiple steps of prostate cancer metastasis, such as detachment of tumor cells from the extracellular matrix, resistance of tumor cells to the detachment-induced apoptosis, adhesion of tumor cells to endothelial cells and angiogenesis to support tumor growth. There may be cell surface proteins either uniquely or differentially expressed in both epithelial and endothelial cells that are important for individual processes of prostate cancer metastasis.

[0004] Researchers have taken advantage of the biological uniqueness of cell surface proteins and chemical properties of certain compounds to tag or "mark" cells. Cells marked in this manner can readily be isolated and characterized. In many cases, a combination of multiple markers is used to identify a particular cell type.

[0005] Antibodies are used extensively as diagnostic tools in a wide array of different analyses. Monoclonal and recombinant antibodies may be used as probes to tag or mark cells. Antibody-based immunoassays are the most commonly used type of diagnostic assay and still one of the fastest growing technologies for the analysis of biomolecules. Another example with particular importance in diagnostics that has become a routine during the past decade is flow cytometric analysis. Flow cytometry is a method for quantifying components or structural features of cells primarily by optical means. Although it makes measurements on one cell at a time, it can process thousands of cells in a few seconds. Since different cell types can be distinguished by quantifying structural features, flow cytometry can be used to count cells of different types in a mixture. Flow cytometers may also be employed to select or "sort" cells based on their cell surface marker expression.

[0006] Intracellular components may also be reported by fluorescent probes, including total DNA/cell (allowing cell cycle analysis), newly synthesized DNA, specific nucleotide sequences in DNA or mRNA, filamentous actin, and any structure for which an antibody is available. Flow cytometry can also monitor rapid changes in intracellular free calcium, membrane potential, pH, or cell signaling pathways. For example, Krutzik *et al* (Journal of Immunology, 2005, 175: 2357-2365) state "phosphospecific flow cytometry has emerged as a powerful tool to analyze intracellular signaling events in complex populations of cells because of its ability to simultaneously discriminate cell types based on surface marker expression and measure levels of intracellular phosphoproteins. This has provided novel insights into the cell- and pathway-specific nature of immune signaling."

[0007] Flow cytometry analysis and sorting studies using monoclonal antibodies to define the surface markers on normal and neoplastic cell populations created the basis for routine clinical diagnostic assays that now range from leukemia classification to monitoring CD4 T-cell loss as HIV disease progresses.

[0008] Despite the versatility of flow cytometry, there are some drawbacks. For example, the antibodies chosen for flow cytometry reagents may not be optimal: They may not be specific, resulting in a high background staining. Furthermore, the efficiency of coupling the fluorochrome to the antibody may vary from batch to batch. Therefore, comparisons between populations of cells may be further complicated.

[0009] In addition, in order to assess multiple parameters on cell populations, a significant investment is required to purchase the required antibodies. For example, the analysis of 90 single parameters would require the purchase of 90 separate antibodies, which may amount to several tens of thousands of dollars and a large number of cells per analysis. This is problematic if the number of cells is finite and limited. Therefore, there is a significant need for cost effective, validated, reproducible reagents and antibodies for the phenotyping or analysis of cells.

SUMMARY

[0010] The present invention also provides a method for comparing the phenotypic state of a first unknown sample material with a sample material of a known phenotypic state. The method comprises the steps of:

a. Obtaining a first unknown sample material, and;
b. Contacting the first unknown sample material with reagents to detect the presence of phenotypic markers in the first unknown sample material, and;
c. Recording the presence of phenotypic markers in the first unknown sample material, and;
d. Obtaining a sample material of a known phenotypic state, and;
e. Contacting the sample material of a known phenotypic state with reagents to detect the presence of phenotypic markers in the sample material of a known phenotypic state, and;
f. Recording reagents the presence of phenotypic markers in the sample material of a known phenotypic state, and;
g. Reporting the differences between the unknown sample material and the sample material of a known phenotypic state.

[0011] The present invention provides a system for comparing the phenotypic state of a first unknown sample material with a sample material of a known phenotypic state. The system comprises:

a. A panel of phenotypic markers, and;

b. Reagents to detect the presence of phenotypic markers in the first sample material of unknown phenotypic state, and;
c. Apparatus to record the presence or absence of phenotypic markers in the first sample material of unknown phenotypic state, and;
d. Reagents to detect the presence of phenotypic markers in the sample material of a known phenotypic state, and;
e. Apparatus to record the presence or absence of phenotypic markers in the sample material of a known phenotypic state, and;
f. Apparatus to report differences between the first unknown sample material and the sample material of a known phenotypic state.

[0012] The present invention provides device for comparing the phenotypic state of a first unknown sample material with a sample material of a known phenotypic state. The device comprises:

a. A panel of phenotypic markers, and;
b. Reagents to detect the presence of phenotypic markers in the first sample material of unknown phenotypic state, and;
c. Apparatus to record the presence or absence of phenotypic markers in the first sample material of unknown phenotypic state, and;
d. Reagents to detect the presence of phenotypic markers in the sample material of a known phenotypic state, and;
e. Apparatus to record the presence or absence of phenotypic markers in the sample material of a known phenotypic state, and;
f. Apparatus to report differences between the first unknown sample material and the sample material of a known phenotypic state.

[0013] The present invention provides kit for comparing the phenotypic state of a first unknown sample material with a sample material of a known phenotypic state. The kit comprises:

a. A panel of phenotypic markers, and;
b. Reagents to detect the presence of phenotypic markers in a sample material.

[0014] These embodiments and more are described more fully in the following sections.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIGURE 1: Outline of the method of the present invention.

[0016] FIGURE 2: Analysis of the phenotypic state of a population of cells using the marker reagent array shown in Table 2.

[0017] FIGURE 3: Analysis on the effects of enzymatic treatment on the phenotypic state of different samples of populations of cell X, using the marker reagent array shown in Table 2.

[0018] FIGURE 4: Comparison of the phenotypic state of a sample of cell X with a sample of human dermal fibroblsts, using the marker reagent array shown in Table 2.

[0019] FIGURE 5: Phenotypic analysis of whole blood. Figure 5 A shows the level of expression of a selected epitope in heterogenous cell samples. Leukocyte, monocyte and granulocyte populations were selected based upon size and granularity. Percentage of positive events and Mean Fluorescence Intensity was assessed for all selected cell populations for each epitope tested. As an example the expression levels of CD63 are shown for all selected cell populations. Figure 5 B shows the percentage of positive events and expression levels of leukocytes, monocytes and granulocytes for the array of surface epitopes shown. Cell populations were selected and analyzed from three healthy donors in 11 separate tests (Mean ± SEM). Figure 5 C shows expression levels of the epitopes in the reagent array shown in table 1 for a leukocyte population taken from a healthy individual.

DETAILED DESCRIPTION

[0020] According to the present invention, assay components and methods for the characterization of the phenotype of cells are provided. As those of ordinary skill in the art will recognize, the invention has an enormous number of applications in diagnostic assay techniques. Reagents may be prepared, for example, so as to detect

or screen for any of a number of sample characteristics, pathological conditions, or reactants. The present invention provides panels of reagents that are selected to cover various applications.

[0021] Suitable panels of reagents may include, for example, a tumor marker panel including epitopes related to prostate cancer, breast cancer, hematological disorders, and other suitable tumor markers; an autoimmune disease panel comprising tests for rheumatoid factors and other markers associated with autoimmune disease; a therapeutic drug panel comprising tests for biomarkers related to the drug therapy of interest.

THE REAGENTS

[0022] Reagents suitable for use in the present invention may be antibodies that recognize phenotypic markers. The phenotypic markers may be proteins expressed on the surface of cells, or they may be proteins that are expressed intracellularly. Alternatively, they may be proteins that are secreted from the cell. Alternatively, the phenotypic markers may be nucleic acids, lipids, polysaccharides or any biomolecule.

[0023] Reagents suitable for use in the present invention may be antibodies that specifically the phenotypic markers. Alternatively, the reagents may be oligonucleotides or any molecule that specifically recognize the phenotypic marker. In one embodiment, the reagents are labeled with a fluorescent marker. Alternatively the reagent may be intrinsically fluorescent.

[0024] Antibodies suitable for use in the present invention may be obtained from commercial sources, or they may be generated for a specific application. In one embodiment, the antibodies are labeled with a fluorescent marker. Examples of antibodies and phenotypic markers suitable for use in the present invention are shown in **Table 1**.

[0025] Antibodies may be selected that represent phenotypic markers of a state of interest or can be selected randomly. For example, the phenotypic markers may be proteins that are typically expressed on stem cells. Alternatively, the phenotypic markers may be associated with a disease, such as, for example, cancer.

Alternatively, the phenotypic markers may be used to measure molecules in heterogeneous systems, such as, for example, blood.

THE REAGENT PANELS

[0026] The present invention provides cost effective, optimized reagents for the reproducible analysis of cells. In one embodiment, the reagent is in the form of a kit, comprising a multi-well plate containing antibodies that are lyophilized or otherwise configured allowing for convenient transport and storage. The multi-well plates may have different formats such as 6, or 8, or 12, or 24, or 96, or 384 wells and the like. The antibodies are selected according to the analysis that is to be performed. The antibodies may be selected to cover immune related epitopes. Alternatively, they may be selected to cover cancer epitopes, or stem cell epitopes. The antibodies may recognize extracellular molecules or they may recognize intracellular molecules.

[0027] The selected antibodies may be dispensed into multi-well plates according to the layouts shown in Tables 2 and 3. The layouts are unique to the specific phenotypic marker panel. Each well of the multiwell plate may contain a single antibody or multiple antibodies. The selected antibodies are provided in bulk quantities and dispensed into a large number of plates to reduce variation between samples. The antibodies may be stored in the plates in solution prior to analysis. Alternatively, the antibodies may be stored lyophilized and re-constituted prior to analysis.

THE METHOD

[0028] In one embodiment, the method of the present invention comprises a system that carries out the steps of collecting samples material of an unknown phenotypic state and collecting sample material of a known phenotypic state. The sample material may comprise, for example, cells, blood, or any biological sample. The system analyses the sample materials to identify patterns epitope markers in the sample material of known phenotypic state that is used as a comparator for the sample

material of unknown phenotypic state. The identity of the epitopes in the identified pattern may be known. Alternatively, more than one epitope in the identified pattern may be unknown. An outline of the method of the present invention is shown in **Figure 1**.

[0029] For example, the methods of the present invention may be used for quality control purposes. In this case, the sample material of known phenotypic state may be obtained from a control population of cells. This sample material may then be used as a comparator for the sample material of unknown phenotypic state that consists of populations of cell obtained from production runs. Changes from the patterns of epitope markers in the sample material of known phenotypic may be used to accept or reject production runs. An example of this is shown in **Example 1**.

[0030] Alternatively, the present invention may be used to report changes in the pattern of epitope markers in a population of cells following treatment with a factor or pharmaceutical agent. An example of this is shown in **Example 2**. Alternatively, the present invention may be used to report changes in the pattern of epitope markers between distinct populations of cells. An example of this is shown in **Example 3**.

[0031] In one embodiment, the present invention may also report changes in the pattern of epitope markers in distinct populations of cells in whole blood. The cell populations may be from a sample of blood from one patient. Alternatively, samples from more patient may be used.

[0032] The methods of the present invention may be employed to analyze one sample or, alternatively, more than one sample. In one embodiment, the high-throughput analysis of a large number of samples may be carried out. The steps of the method of the present invention may be carried manually. Alternatively, at least one step may be automated. Multiple copies of a phenotypic marker reagent plate may be made and used to analyze samples.

[0033] Analysis of the samples may be performed by any suitable platform known to those of ordinary skill in the art. Such platforms may include, for example, a flow cytometry apparatus, a mass spectrometry apparatus, or a fluorescent microscope, and the like.

[0034] Flow cytometry apparatuses rely upon flow of cells or other particles in a liquid flow stream in order to determine one or more characteristics of the cells under investigation. Further, the flow cytometry apparatus is useful for identifying the presence of certain cells or particles of interest, enumerating those cells or particles and, in some instances, providing a sorting capability so as to be able to collect those cells or particles of interest. In a typical flow cytometry apparatus, a fluid sample containing cells is directed through the flow cytometry apparatus in a rapidly moving liquid stream so that each cell passes serially, and substantially one at a time, through a sensing region. Cell volume may be determined by changes in electrical impedance as each cell passes through the sensing region. Similarly, if an incident beam of light is directed at the sensing region, the passing cells scatter such light as they pass there through. This scattered light serves as a function of cell shape and size, index of refraction, opacity, roughness and the like. Further, fluorescence emitted by labeled cells, or autofluorescent cells, which have been excited as a result of passing through the excitation energy of the incident light beam is detectable for identification of cells having fluorescent properties. After cell analysis is performed by the flow cytometry apparatus, those cells that have been identified as having the desired properties may be sorted if the apparatus has been designed with such capability.

[0035] Representative flow cytometry apparatuses are described in U.S. Pat. Nos. 3,826,364 and 4,284,412, and in the publication by Herzenberg *et al.*, "Fluorescence-activated Cell Sorting," Sci. Am. 234 (3): 108, 1976.

[0036] The present invention is further illustrated, but not limited by, the following examples.

EXAMPLES

EXAMPLE 1: QUALITY CONTROL; RELEASE CRITERIA

[0037] *Master plate preparation*: An antibody template was designed to monitor changes of expression across a wide array of epitopes in order to establish consistency of the cell product between production and expansion runs. Antibodies were selected and dispensed into a multiwell plate according to the layout shown in **Table 2**.

Antibodies were purchased from the sources shown in **Table 1**. Antibodies were diluted in PBS into a 96-well master plate and stored in a refrigerator prior to analysis. For each assay 10µl of master antibody solution was transferred from the master plate to a new 96 well plate as such that a copy was created. The array contained phycoerythrin fluorescein labeled antibodies against one specific marker per well. This eliminated the need for compensation of the machine, simplified the assay and reduced operator bias. In addition, the staining protocol was designed to minimize the number of cells needed for the assay and eliminate steps, such as washing, that can cause errors.

[0038] *Assay*: A vial of cells were thawed, washed and finally diluted to $2x10^5$ cells per ml in PBS and a 100µl sample was transferred to each well (20,000 cells/well) containing diluted antibodies. The plate was left to incubate for 30 minutes at 4°C. Next 100µl fixation buffer (Cytofix, BD catalog no. 554655) was added and left for 15 minutes. A FACSCalibur machine with a high through put system was setup for analysis and 10,000 events per well were acquired.

[0039] *Data analysis*: For this study the acquired data was exported to an analysis software program and the Mean Fluorescent Intensity (MFI) of each sample well was assessed. The MFI of the unstained sample was subtracted from each sample MFI and this delta MFI was plotted for each epitope. This was repeated eight times in independent experiments creating an average expression pattern for our cell product.

[0040] *Results*: Cells obtained from different production/expansion runs were routinely stained using the 96-well plate array and the MFI were plotted and compared against the pattern of epitope markers obtained from the sample material of known phenotypic state. The results are shown in **Figure 2**.

EXAMPLE 2: COMPARISON OF DISSOCIATION REAGENTS

[0041] *Method*: The methods and reagent panel used for this assay is the same as described in **Example 1**. Cells were grown in 3 separate culture flasks under identical conditions. These cultures were harvested each with a different dissociation enzyme mix. The cells were then stained using out 96-well method and the mean fluorescence

intensity was measured for each parameter and compared to the pattern of epitope markers obtained from the sample material of known phenotypic state.

[0042] *Results*: Each enzyme mix showed different results when compared to the pattern of epitope markers obtained from the sample material of known phenotypic state. Mixture C (Accutase) seems to affect the expression of certain phenotypic marker more than the standard mix A (Trypsin) and B (TriplE). For example the adhesion marker CD49f and the signaling molecule CD63 show lower expression levels after the harvesting procedure, which may affect the cell product's function. Results are shown in **Figure 3**.

[0043] *Conclusion*: This method allows for a rapid assessment of a wide array of proteins expressed on the cell surface. Changes to the phenotypic state that may occur following treatment with enzymes can readily be assessed by this method. The availability of a master plate allows for a quick assessment of phenotypic changes across a wide array of phenotypic markers due to treatment of the cells.

EXAMPLE 3: COMPARISON OF THE PHENOTYPIC STATE OF TWO DISTINCT CELL POPULATIONS

[0044] *Method*: The methods and reagent panel used for this assay were the same as described in **Example 1**. In this assay expression levels epitopes tested by the master plate on a different homogenous cell culture. The cell culture of interest was grown to confluency and harvested according our standard protocol. Both cell cultures were then stained in the 96-well master plate and analyzed according the methods described earlier. The phenotypic state of the cell populations were compared and key differences were identified.

[0045] *Results*: The two cell types have similar expression levels for most of the epitopes except for example CD11a and CD54. These epitopes were differentially expressed. We selected an arbitrary cut-off for significance at a delta of 25 MFI compared to the sample material of known phenotypic state. The results are shown in **Figure 4**.

[0046] *Conclusion*: We showed expression differences between two cell types in our panel. These differences can be further explored and can become important

starting points of further research especially if the phenotypic marker changes can be linked to in-vivo efficacy differences.

EXAMPLE 4: PHENOTYPIC ANALYSIS OF WHOLE BLOOD

[0047] *Method*: The methods and reagent panel used for this assay were the same as described in **Example 1**. Heparinized peripheral blood was obtained from healthy volunteers and white blood cells were isolated. The White blood cells were washed and suspended in PBS and transferred to the 96-well staining plate (50,000 cells per well) and data was acquired according to the standard protocol. Data analysis, however, is different since the sample contains multiple cell populations each with their specific expression pattern for each epitope. Analyzing the cells as a bulk may obscure these effects. Therefore three main populations were selected based upon size and granularity and each was analyzed for percentage positive events within the gate and expression levels of each epitope tested (**Figure 5 A,B and C**).

[0048] *Results*: Using the 96-well platform we able to quickly assess expression levels of our array of epitopes for three distinct populations in peripheral blood (**Figure 5**).

[0049] *Conclusion*: The 96 well plate can also be used for heterogeneous cell population. However the data processing and mining becomes significantly more complex. Novel analysis methods are in development to simplify the analysis, which is especially important when this method is expanded to multiple color flow cytometry. This method may be used to determine the pattern of epitope markers in easy accessible peripheral blood samples for healthy (non-disease phenotype) individuals and use this a comparator for cytomes obtained from individuals with a particular disease. Differences can be exploited for, but not limited to, development of diagnostics, measure drug efficacy and selection of treatment regiment.

TABLE 1: PHENOTYPIC MARKERS SUITABLE FOR USE IN THE PRESENT INVENTION

CD identification	MOLECULE	Gene Name	Fluorescence	company
CD3	CD3/T-cell antigen receptor (TCR)	CD3G/Z	PE	Caltag
CD4	OKT4, Leu 3a, T4	CD4	PE	BD Pharmingen
CD5	T1, Leu1	CD5	PE	BD PharMingen
CD7	Leu 9, 3A1, gp40, T cell leukemia antigen	CD7	PE	BD Pharmingen
CD8	MHC class I receptor	CD8	PE	BD PharMingen
CD9	type III transmembrane protein (platelet aggregation/activation)	CD9	PE	BD PharMingen
CD10	CALLA, membrane metallo-endopeptidase	MME	PE	BD Pharmingen
CD11a	alphaL; LFA-1, gp180/95	ITGAL	PE	BD Pharmingen
CD13	Aminopeptidase N, APN, gp150, EC 3.4.11.2	ANPEP	PE	BD Pharmingen
CD15	fucosyltransferase 4 (alpha (1,3) fucosyltransferase, myeloid-specific)	FUT4	PE	BD PharMingen
CD16	Fc gamma R III	FCGR3A	PE	Caltag
CD16a	Fc gamma R IIIa	FCGR3A	PE	BD Pharmingen
CD18	ß2 -Integrin chain, macrophage antigen 1	ITGB2	PE	BD Pharmingen

	(mac-1)			
CD19	B4, associated with CD21, CD81, CD225, Leu-13, Lyn, Fyn, Vav, PI3-kinase	CD19	PE	BD PharMingen
CD20	membrane-spanning 4-domains, subfamily A, member 1	MS4A1	PE	BD PharMingen
CD21	C3d receptor,CR2, gp140; EBV receptor	CR2	PE	BD Pharmingen
CD22	Bgp135; BL-CAM, Siglec2	CD22	FITC	BD Pharmingen
CD25	Interleukin-2 receptor (IL-2R, inflammatory response)	IL2RA	PE	BD PharMingen
CD29	Integrin ß1 chain; platelet GPIIa; VLA (CD49) beta-chain	ITGB1	PE	BD Pharmingen
CD30	tumor necrosis factor receptor superfamily, member 8. KI-1	TNFRSF8	PE	BD PharMingen
CD31	PECAM-1; platelet GPIIa'; endocam	PECAM1	PE	BD Pharmingen
CD32	Fcgamma receptor type II (FcgRII), gp40	FCGR2A	PE	BD Pharmingen
CD33	gp67	CD33	PE	BD PharMingen
CD34	My10, gp105-120	CD34	PE	BD PharMingen
CD35	C3b/C4b receptor; complement receptor type 1 (CR1)	CR1	PE	BD Pharmingen

CD36	platelet GPIV, GPIIIb, OKM-5 antigen	CD36	PE	BD Pharmingen
CD38	T10; gp45, ADP-ribosyl cyclase	CD38	PE	BD Pharmingen
CD40	Bp50, TNF Receptor 5	TNFRSF5	PE	BD PharMingen
CD44	Pgp-1; gp80-95, Hermes antigen, ECMR-III and HUTCH-I.	CD44	PE	BD PharMingen
CD45	LCA, B220, protein tyrosine phosphatase, receptor type, C	PTPRC	PE	BD Pharmingen
CD49a	Integrin a1 chain, very late antigen, VLA 1a	ITGA1	PE	BD Pharmingen
CD49b	Integrin a2 chain, VLA-2-alpha chain, platelet gpla	ITGA2	PE	BD Pharmingen
CD49c	Integrin a3 chain, VLA-3 alpha chain	ITGA3	PE	BD Pharmingen
CD49d	Integrin a4 chain,, VLA-4-alpha chain	ITGA4	PE	BD Pharmingen
CD49e	Integrin a5 chain,, VLA-5 alpha chain	ITGA5	PE	BD Pharmingen
CD49f	Integrin a6 chain,, VLA-6 alpha chain, platelet gplc	ITGA6	PE	BD Pharmingen
CD50	ICAM-3, intercellular adhesion molecule 3	ICAM3	fitc	BD Pharmingen
CD51	Integrin alpha chain, vitronectin receptor a	ITGAV	PE	BD Pharmingen

	chain			
CD54	ICAM-1, intercellular adhesion molecule 1	ICAM1	PE	BD Pharmingen
CD55	decay-accelerating factor (DAF, prevent cell damage)	DAF	PE	BD PharMingen
CD56	Neural cell adhesion molecule 1 (NCAM1), NKH-1 (NK cell marker)	NCAM1	PE	Caltag
CD58	Lymphocyte function-associated antigen-3 (LFA-3, interacts with CD2 during cell adhesion)	CD58	PE	BD Pharmingen
CD59	MACIF, MIRL, P-18, protectin	CD59	PE	BD Pharmingen
CD61	Glycoprotein IIIa, beta3 integrin	ITGB3	PE	BD Pharmingen
CD62E	E-selectin, LECAM-2, ELAM-1	SELE	PE	BD Pharmingen
CD62L	L-selectin, LAM-1, Mel-14	SELL	PE	Caltag
CD62P	P-selectin, granule membrane protein-140 (GMP-140)	SELP	PE	BD Pharmingen
CD63	LIMP, gp55, LAMP-3 neuroglandular antigen, granulophysin	CD63	PE	BD Pharmingen
CD64	FcgR1, FcgammaR1 (IgG-receptor)	FCGR1A	PE	Caltag

CD69	Early T-cell activation antigen	CD69	PE	BD PharMingen
CD71	Transferrin receptor	TFRC	PE	BD Pharmingen
CD73	Ecto-5'-nucleotidase	NT5E	PE	BD Pharmingen
CD79a	CD79A antigen (immunoglobulin-associated alpha), MB1	CD79A	PE	BD PharMingen
CD79b	CD79B antigen (immunoglobulin-associated beta), B29	CD79B	PE	BD PharMingen
CD80	B7-1; BB1	CD80	PE	BD Pharmingen
CD81	Target of an antiproliferative antibody (TAPA-1); M38	CD81	PE	BD Pharmingen
CD83	HB15	CD83	PE	BD Pharmingen
CD86	B7-2; B70	CD86	PE	Caltag Laboratories
CD87	plasminogen activator, urokinase receptor (PLAUR), uPAR	PLAUR	PE	BD PharMingen
CD88	C5a-receptor	C5R1	PE	BD Pharmingen
CD90	Thy-1	THY1	PE	BD PharMingen
CD95	APO-1, Fas, TNFRSF6	TNFRSF6	PE	BD Pharmingen
CD100	SEMA4D	SEMA4D	PE	Serotec
CD103	integrin, alpha E (human mucosal lymphocyte antigen 1;	ITGAE	PE	BD PharMingen

	alpha polypeptide)			
CD104	Integrin beta 4 subunit, TSP-1180	ITGB4	PE	BD Pharmingen
CD105	Endoglin	ENG	PE	Caltag
CD106	VCAM-1 (vascular cell adhesion molecule-1), INCAM-110	VCAM1	PE	BD Pharmingen
CD114	G-CSFR, HG-CSFR, CSFR3	CSF3R	PE	BD Pharmingen
CD117	SCFR, c-kit, stem cell factor receptor	KIT	PE	BD Biosciences
CD119	IFN gamma receptor alpha chain (GIR-208)	IFNGR1	PE	BD Pharmingen
CD120b	TNFRI; TNFRp55	TNFRSF1B	PE	BD Pharmingen
CD126	IL-6 receptor alpha chain	IL6R	PE	BD Pharmingen
CD134	Tumor Necrosis Factor receptor 4, OX40 (promotes expression of BCL2/BCL-xL)	TNFRSF4	PE	Caltag
CD135	fms-related tyrosine kinase 3	FLT3	PE	BD PharMingen
CD140b	b-platelet derived growth factor (PDGF) receptor	PDGFRB	PE	BD Pharmingen
CD141	Thrombomodulin (TM), fetomodulin	THBD	PE	BD Pharmingen
CD142	Tissue factor, thromboplastin,	F3	PE	BD Pharmingen

	coagulation factor III			
CD146	Muc 18, MCAM, Mel-CAM, s-endo	MCAM	PE	BD Pharmingen
CD147	Basigin, M6, extracellular metalloproteinase inducer (EMMPRIN)	BSG	PE	Serotec
CD151	Platelet-endothelial tetra-span antigen (PETA)-3	CD151	PE	BD Pharmingen
CD152	Cytolytic T lymphocyte-associated antigen (CTLA-4)	CTLA4	PE	BD PharMingen
CD163	M130, GHI/61, RM3/1	CD163	PE	BD PharMingen
CD164	MUC-24, MGC 24, multi-glycosylated core protein 24	CD164	PE	BD Pharmingen
CD165	AD2, gp 37		PE	BD Pharmingen
CD178	FAS ligand, CD95 ligand	TNFSF6	PE	Caltag Laboratories
CD181	CXCR1 CDw128a: IL-8 receptor alpha,	IL8RA	PE	BD Pharmingen
CD182	CXCR2	IL8RB	PE	BD Pharmingen
CD183	CXCR3 chemokine receptor, G protein-coupled receptor 9	CXCR3	PE	BD Pharmingen
CD184	CXCR4 chemokine receptor, Fusin	CXCR4	PE	BD Pharmingen
CD200	OX2 (regulates Mf	CD200	PE	BD PharMingen

	activity)			
CD273	B7DC, PDL2	PDCD1LG2	PE	BD Pharmingen
CD274	B7H1, PDL1	PDCD1LG1	PE	BD Pharmingen
CD275	B7H2, ICOSL	ICOSL		
CD276	B7H3	N/A		
CD277	BT3.1	BTN3A1		
CD278	ICOS	ICOS		
CD279	PD1	PDCD1		
CD280	ENDO180	MRC2		
CD281	TLR1	TLR1		
CD282	TLR2	TLR2		
CD283	TLR3	TLR3		
CD284	TLR4	TLR4		
CD289	TLR9	TLR9		
CD292	BMPR1A	BMPR1A		
CDw293	BMPR1B	BMPR1B		
CD294	CRTH2	GPR44		
CD295	LeptinR	LEPR		
CD296	ART1	ART1		
CD297	ART4	DO		
CD298	Na+/K+ -ATPase b3	ATP1B3		
CD299	DCSIGN-related	CD209L		
CD300a	CMRF35H			

CD300c	CMRF35A			
CD300e	CMRF35L1			
CD301	MGL, CLECSF14	CLECSF14		
CD302	DCL1	N/A		
CD303	BDCA2	CLECSF7		
CD304	BDCA4, Neuropilin 1	NRP1		
CD305	LAIR1	LAIR1		
CD306	LAIR2	LAIR2		
CD307	IRTA2	N/A		
CD309	VEGFR2, KDR	KDR		
CD312	EMR2	EMR2		
CD314	NKG2D	KLRK1		
CD315	CD9P1	PTGFRN		
CD316	EWI2	IGSF8		
CD317	BST2	BST2		
CD318	CDCP1	N/A		
CD319	CRACC	SLAMF7		
CD320	8D6A	N/A		
CD321	JAM1	F11R		
CD322	JAM2	JAM2		
CD324	E-Cadherin	CDH1		
CDw325	N-Cadherin	CDH2		

CD326	Ep-CAM	TACSTD1		
CDw327	siglec6	SIGLEC6		
CDw328	siglec7	SIGLEC7		
CDw329	siglec9	SIGLEC9		
CD331	FGFR1	FGFR1		
CD332	FGFR2	FGFR2		
CD333	FGFR3	FGFR3		
CD334	FGFR4	FGFR4		
CD335	NKp46	NCR1		
CD336	NKp44	NCR2		
CD337	NKp30	NCR3		
CDw338	ABCG2, BCRP	ABCG2		
CD339	Jagged-1	JAG1		
ABCG2				
alkaline phosphatase				
BCL-xL	BCL2 like-1	BCL2L1	PE	Chemicon
bCL-2	B-cell CLL/lymphoma-2	BCL2	PE	BD Pharmingen
CK 5	Cytokeratin 5			
CK 6	Cytokeratin 6			
CK 7	Cytokeratin 7			
CK 8	Cytokeratin 8			
CK 10	Cytokeratin 10			

CK 13	Cytokeratin 13			
CK 14	Cytokeratin 14			
CK 15	Cytokeratin 15			
CK 16	Cytokeratin 16			
CK 18	Cytokeratin 18			
CK 19	Cytokeratin 19			
EGFr	Epidermal growth factor receptor (growth and differentiation)	EGFR	PE	BD PharMingen
HGF-R	Hepatocyte growth factor receptor	HGFR		
NGFr	NGFR (p75)	NGFR	PE	BD Pharmingen
RANTES	CCL5 (chemokine (C-C motif) ligand 5	CCL5	PE	Caltag
b2-microglobulin	Associates with HLA class I antigen complex	B2M	PE	BD Pharmingen
HLA-ABC	MHC Class I ABC		PE	BD Pharmingen
HLA-DP	MHC II HLA-DP beta-1	HLA-DPB1	PE	Serotec
HLA DR			PE	BD Pharmingen
SSEA-1	Embryonic Stem Cell Antigen 1	SSEA1		
SSEA-3	Embryonic Stem Cell Antigen 3	SSEA3		
SSEA-4	Embryonic Stem Cell Antigen 4	SSEA4		

TABLE 2: AN EXAMPLE OF A STEM CELL PHENOTYPIC MARKER ARRAY PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	Unstain	Unstain	Unstain	Unstain	Unstain	Unstain	CD9	CD10	CD11a	CD13	CD14	CD16
B	CD18	CD25	CD29	CD31	CD32	CD34	CD35	CD36	CD40	CD44	CD45	CD49a
C	CD49b	CD49c	CD49d	CD49e	CD49f	CD51	CD54	CD55	CD58	CD59	CD61	CD62E
D	CD62L	CD62P	CD63	CD64	CD71	CD73	CD80	CD81	CD86	CD90	CD95	CD100
E	CD104	CD105	CD106	CD117	CD119	CD120a	CD126	CD134	CD140a	CD140b	CD141	CD142
F	CD146	CD147	CD178	CD181	CD182	CD183	CD184	CD200	CD273	CD274	β2-microglobulin	HLA-ABC
G	HLA-DP	EGFR	NGFR	RANTES	CD3	CD4	CD7	CD15	CD19	CD20	CD21	CD22
H	CD38	CD50	CD62L	CD83	CD88	CD112	CD114	CD151	CD164	CD165	CD172	HLA-DR

TABLE 3: AN EXAMPLE OF AN ONCOLOGY PHENOTYPIC MARKER ARRAY PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	Unstain	Unstain	Unstain	Unstain	Unstain	Unstain	CD3	CD4	CD5	CD7	CD8	CD9
B	CD10	CD11a	CD13	CD14	CD15	CD16	CD18	CD19	CD20	CD22	CD25	CD29
C	CD30	CD31	CD32	CD33	CD34	CD35	CD36	CD38	CD40	CD44	CD45	CD49a
D	CD49b	CD49c	CD49d	CD49e	CD49f	CD51	CD54	CD55	CD56	CD58	CD59	CD61
E	CD62E	CD62L	CD62P	CD63	CD64	CD69	CD71	CD73	CD79a	CD79b	CD80	CD81
F	CD86	CD87	CD90	CD95	CD100	CD103	CD104	CD105	CD106	CD117	CD119	CD120a
G	CD126	CD134	CD135	CD140a	CD140b	CD141	CD142	CD146	CD147	CD152	CD163	CD178
H	CD181	CD182	CD183	CD184	CD200	CD273	CD274	b2-micorglobulin	EGFR	HLA-ABC	HLA-1 (DP)	RANTES

[0050] Publications cited throughout this document are hereby incorporated by reference in their entirety. Although the various aspects of the invention have been illustrated above by reference to examples and embodiments, it will be appreciated that the scope of the invention is defined not by the foregoing description, but by the following claims properly construed under principles of patent law.

What is claimed is:

1) A system for comparing the phenotypic state of a first unknown sample material with a sample material of a known phenotypic state.

2) The system of claim 1 comprising:

a. A panel of phenotypic markers, and;

b. Reagents to detect the presence of phenotypic markers in the first sample material of unknown phenotypic state, and;

c. Apparatus to record the presence or absence of phenotypic markers in the first sample material of unknown phenotypic state, and;

d. Reagents to detect the presence of phenotypic markers in the sample material of a known phenotypic state, and;

e. Apparatus to record the presence or absence of phenotypic markers in the sample material of a known phenotypic state, and;

f. Apparatus to report differences between the first unknown sample material and the sample material of a known phenotypic state.

3) The system of claim 1, wherein the panel of phenotypic markers is in a multiwell plate.

4) The system of claim 1, wherein the apparatus to record the presence of phenotypic markers is selected from the group consisting of a microscope, a flow cytometer, a plate reader, and a gel electrophoresis apparatus.

5) A device for comparing the phenotypic state of a first unknown sample material with a sample material of a known phenotypic state.

6) The device of claim 5 comprising:

a. A panel of phenotypic markers, and;

b. Reagents to detect the presence of phenotypic markers in the first sample material of unknown phenotypic state, and;

c. Apparatus to record the presence or absence of phenotypic markers in the first sample material of unknown phenotypic state, and;

d. Reagents to detect the presence of phenotypic markers in the sample material of a known phenotypic state, and;

e. Apparatus to record the presence or absence of phenotypic markers in the sample material of a known phenotypic state, and;

f. Apparatus to report differences between the first unknown sample material and the sample material of a known phenotypic state.

7) The device of claim 5, wherein the panel of phenotypic markers is in a multiwell plate.

8) The device of claim 5, wherein the apparatus to record the presence of phenotypic markers is selected from the group consisting of a microscope, a flow cytometer, a plate reader, and a gel electrophoresis apparatus.

9) A kit for comparing the phenotypic state of a first unknown sample material with a sample material of a known phenotypic state.

10) The kit of claim 9 comprising:

a. A panel of phenotypic markers, and;

b. Reagents to detect the presence of phenotypic markers in a sample material.

11) The kit of claim 10, wherein the reagents are in a multiwell plate.

12) A method for using the system of claim 1, comprising the steps of:

a. Obtaining a first unknown sample material, and;

b. Contacting the first unknown sample material with reagents to detect the presence of phenotypic markers in the first unknown sample material, and;

c. Recording the presence of phenotypic markers in the first unknown sample material, and;

d. Obtaining a sample material of a known phenotypic state, and;

e. Contacting the sample material of a known phenotypic state with reagents to detect the presence of phenotypic markers in the sample material of a known phenotypic state, and;

f. Recording reagents the presence of phenotypic markers in the sample material of a known phenotypic state, and;

g. Reporting the differences between the unknown sample material and the sample material of a known phenotypic state.

13) A method for using the kit of claim 9, comprising the steps of:

a. Obtaining a first unknown sample material, and;

b. Contacting the first unknown sample material with a kit containing reagents to detect the presence of phenotypic markers in the first unknown sample material, and;

c. Recording the presence of phenotypic markers in the first unknown sample material, and;

d. Obtaining a sample material of a known phenotypic state, and;

e. Contacting the sample material of a known phenotypic state with a kit containing reagents to detect the presence of phenotypic markers in the sample material of a known phenotypic state, and;

f. Recording reagents the presence of phenotypic markers in the sample material of a known phenotypic state, and;

g. Reporting the differences between the unknown sample material and the sample material of a known phenotypic state.

Application number / numéro de demande: 2572530

Figures:

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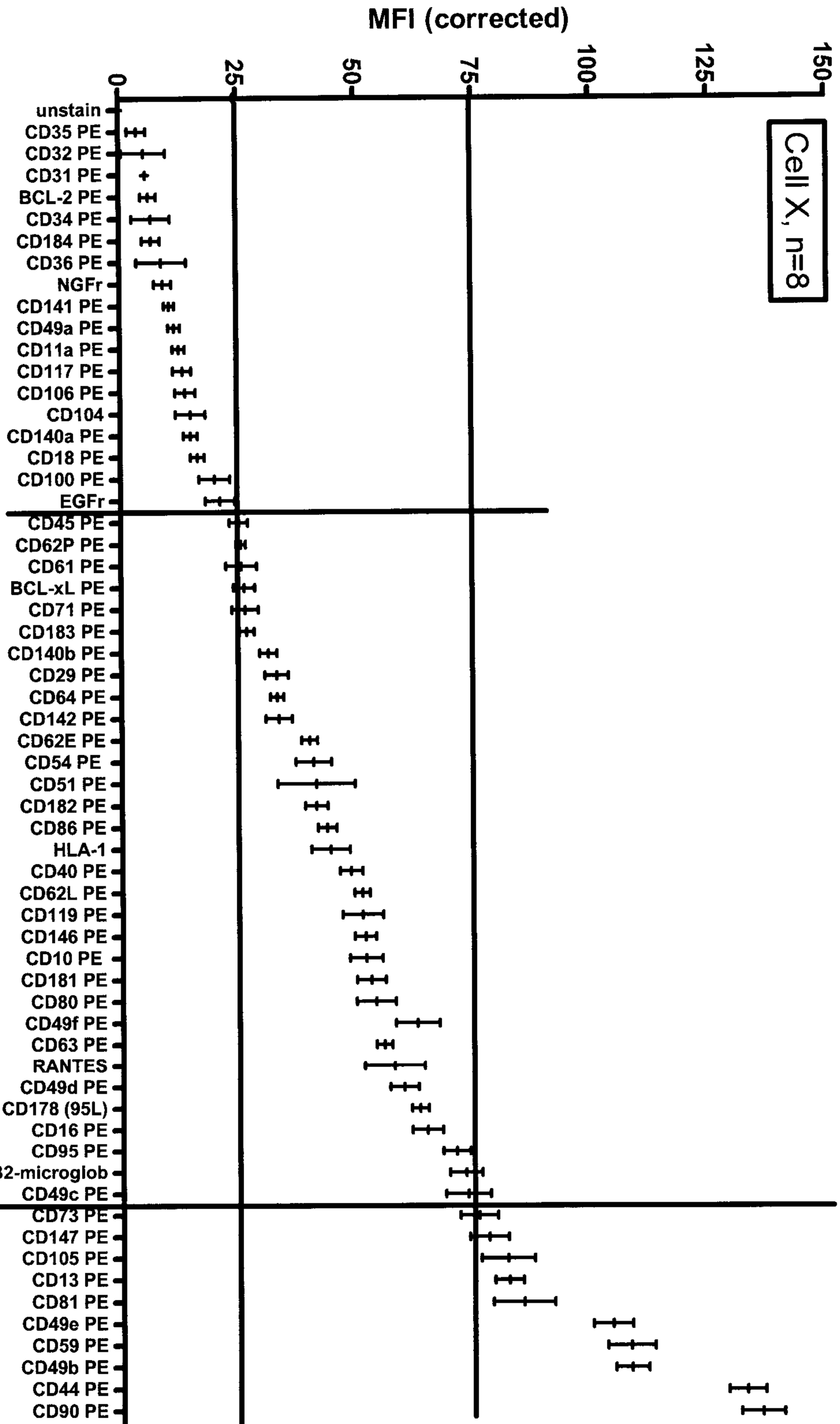
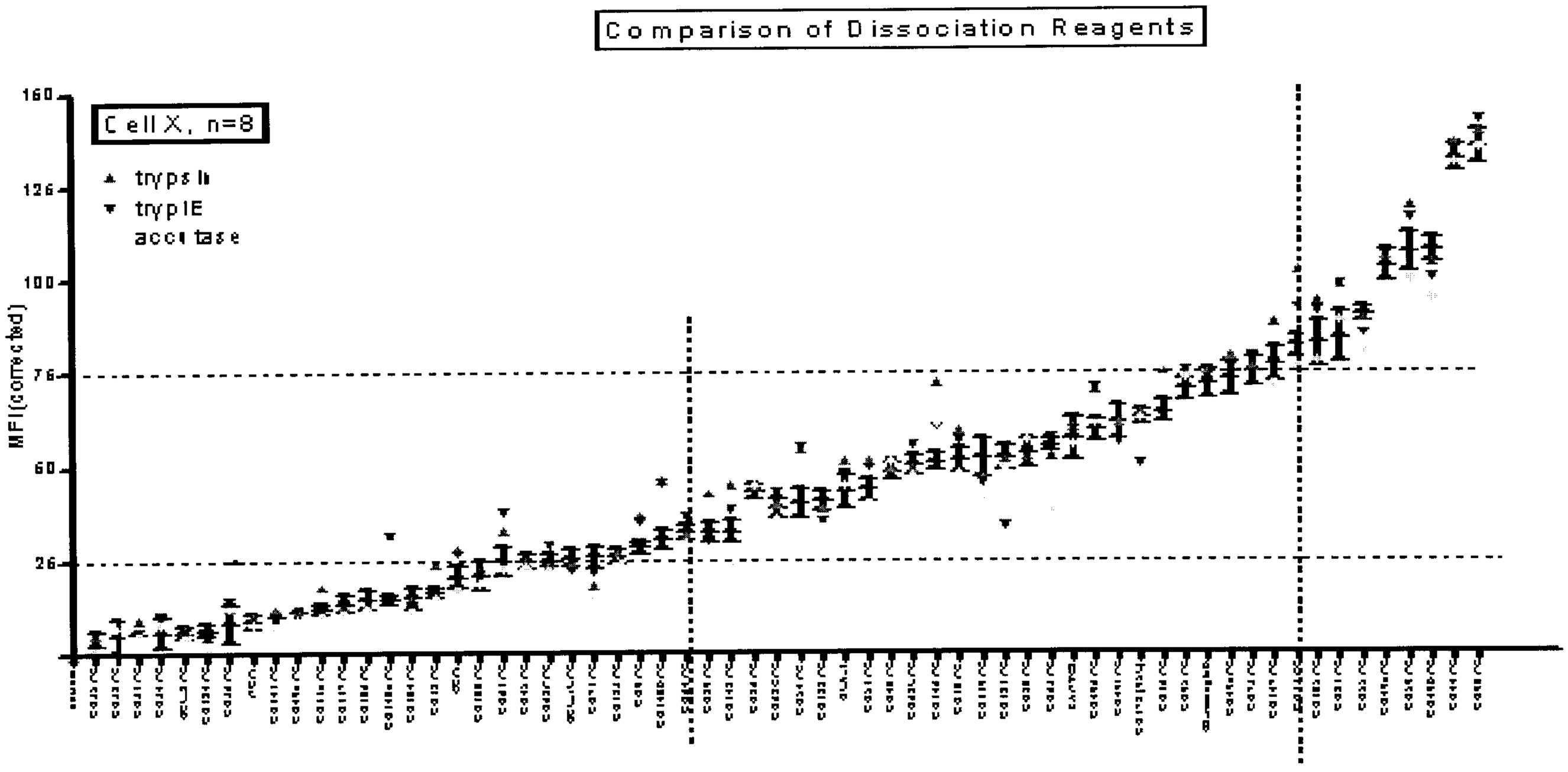

MFI (corrected)
0
25
50
75
100
125
150
Cell X, n=8
unstain
CD35 PE
CD32 PE
CD31 PE
BCL-2 PE
CD34 PE
CD184 PE
CD36 PE
NGFr
CD141 PE
CD49a PE
CD11a PE
CD117 PE
CD106 PE
CD104
CD140a PE
CD18 PE
CD100 PE
EGFr
CD45 PE
CD62P PE
CD61 PE
BCL-xL PE
CD71 PE
CD183 PE
CD140b PE
CD29 PE
CD64 PE
CD142 PE
CD62E PE
CD54 PE
CD51 PE
CD182 PE
CD86 PE
HLA-1
CD40 PE
CD62L PE
CD119 PE
CD146 PE
CD10 PE
CD181 PE
CD80 PE
CD49f PE
CD63 PE
RANTES
CD49d PE
CD178 (95L)
CD16 PE
CD95 PE
B2-microglob
CD49c PE
CD73 PE
CD147 PE
CD105 PE
CD13 PE
CD81 PE
CD49e PE
CD59 PE
CD49b PE
CD44 PE
CD90 PE

Figure 2:

CA 02572530 2006-12-28

Figure 3:



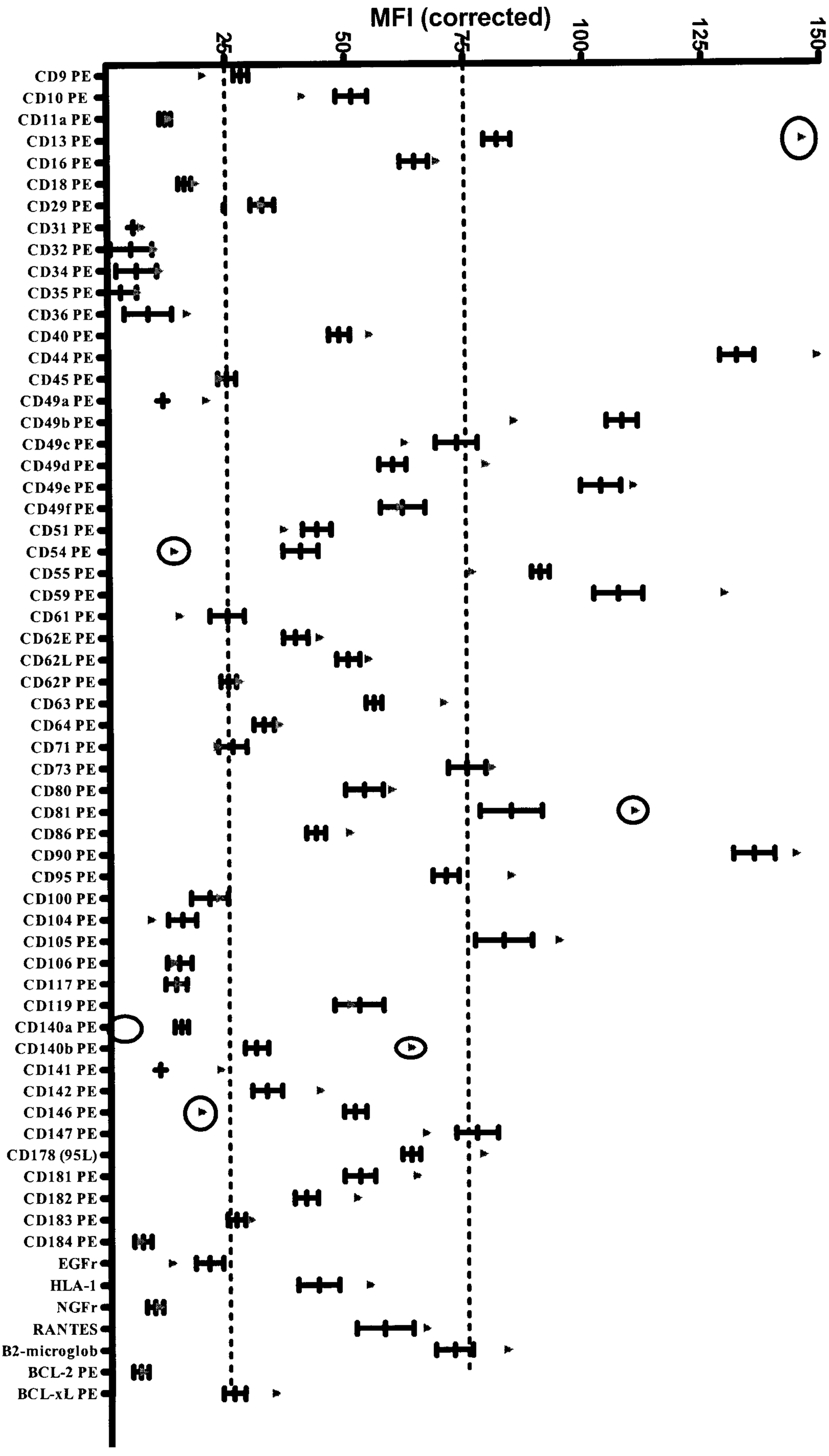

MFI (corrected)
25
50
75
100
125
150
CD9 PE
CD10 PE
CD11a PE
CD13 PE
CD16 PE
CD18 PE
CD29 PE
CD31 PE
CD32 PE
CD34 PE
CD35 PE
CD36 PE
CD40 PE
CD44 PE
CD45 PE
CD49a PE
CD49b PE
CD49c PE
CD49d PE
CD49e PE
CD49f PE
CD51 PE
CD54 PE
CD55 PE
CD59 PE
CD61 PE
CD62E PE
CD62L PE
CD62P PE
CD63 PE
CD64 PE
CD71 PE
CD73 PE
CD80 PE
CD81 PE
CD86 PE
CD90 PE
CD95 PE
CD100 PE
CD104 PE
CD105 PE
CD106 PE
CD117 PE
CD119 PE
CD140a PE
CD140b PE
CD141 PE
CD142 PE
CD146 PE
CD147 PE
CD178 (95L)
CD181 PE
CD182 PE
CD183 PE
CD184 PE
EGFr
HLA-1
NGFr
RANTES
B2-microglob
BCL-2 PE
BCL-xL PE

Figure 4:

Figure 5A:

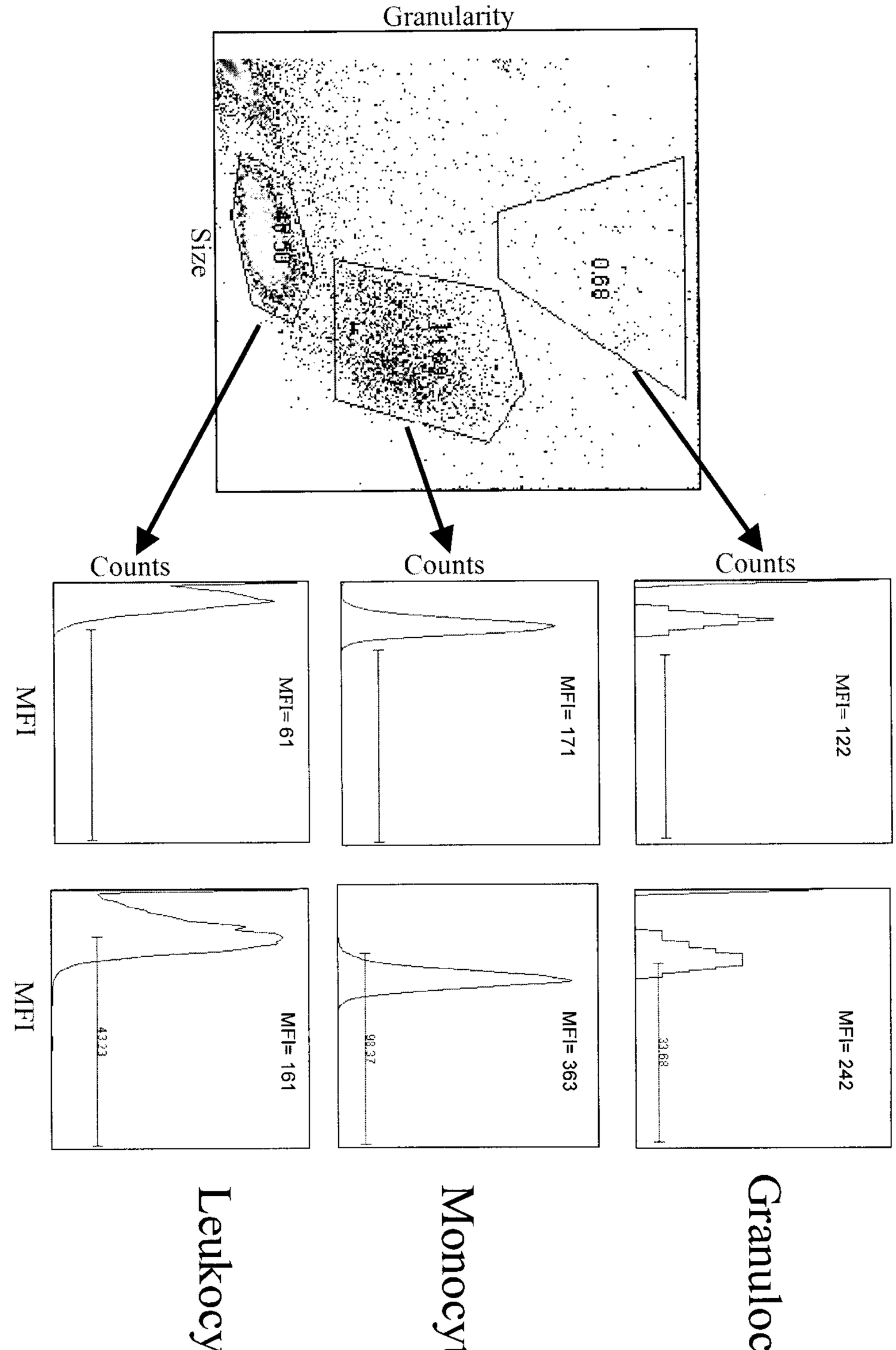

Granularity
Size
0.68
Counts
MFI= 61
MFI= 171
MFI= 122
Unstained
CD63
MFI= 242
33.68
MFI= 363
98.37
MFI= 161
43.23
MFI
Granulocytes
Monocytes
Leukocytes

Figure 5B:

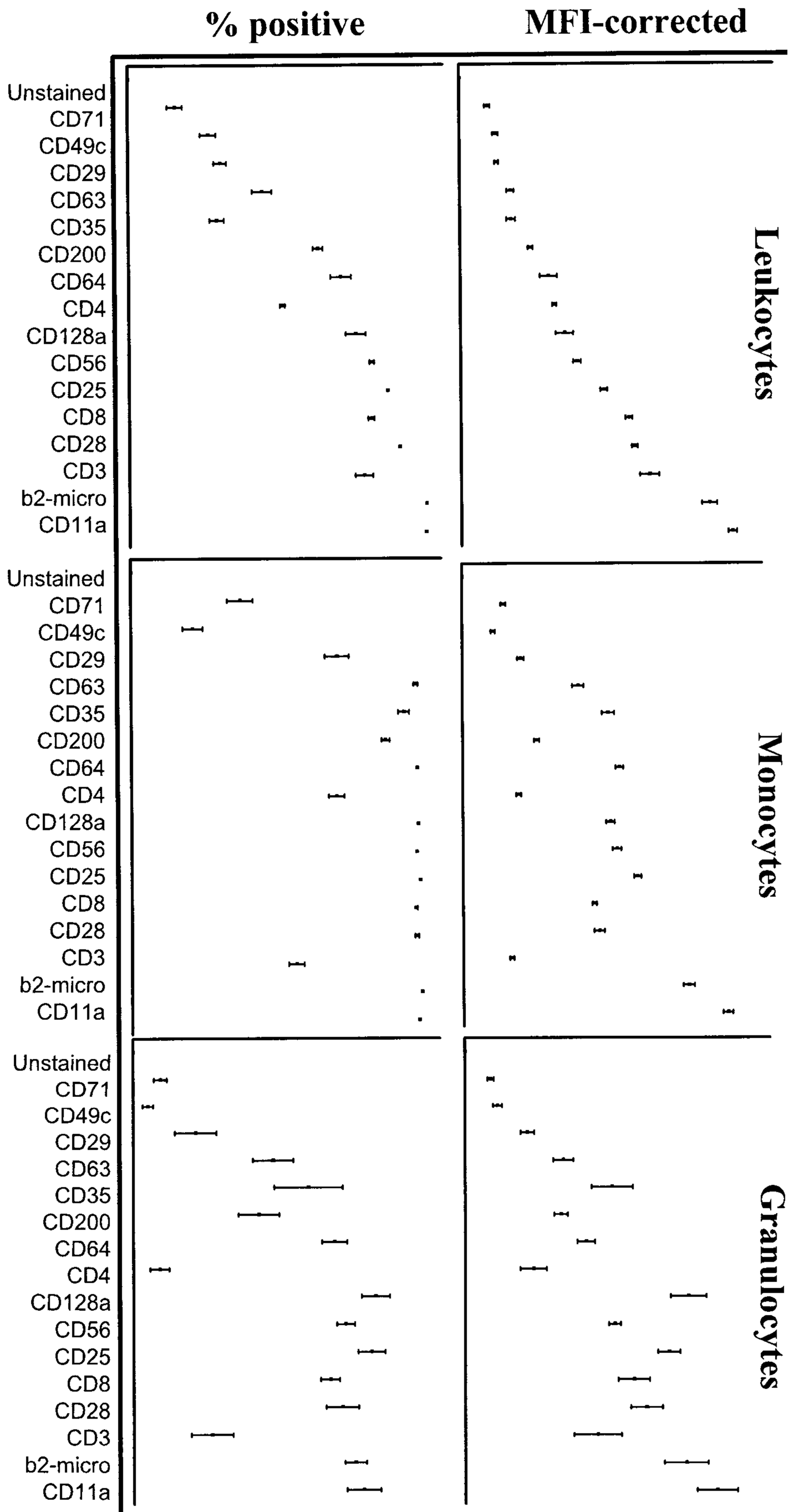
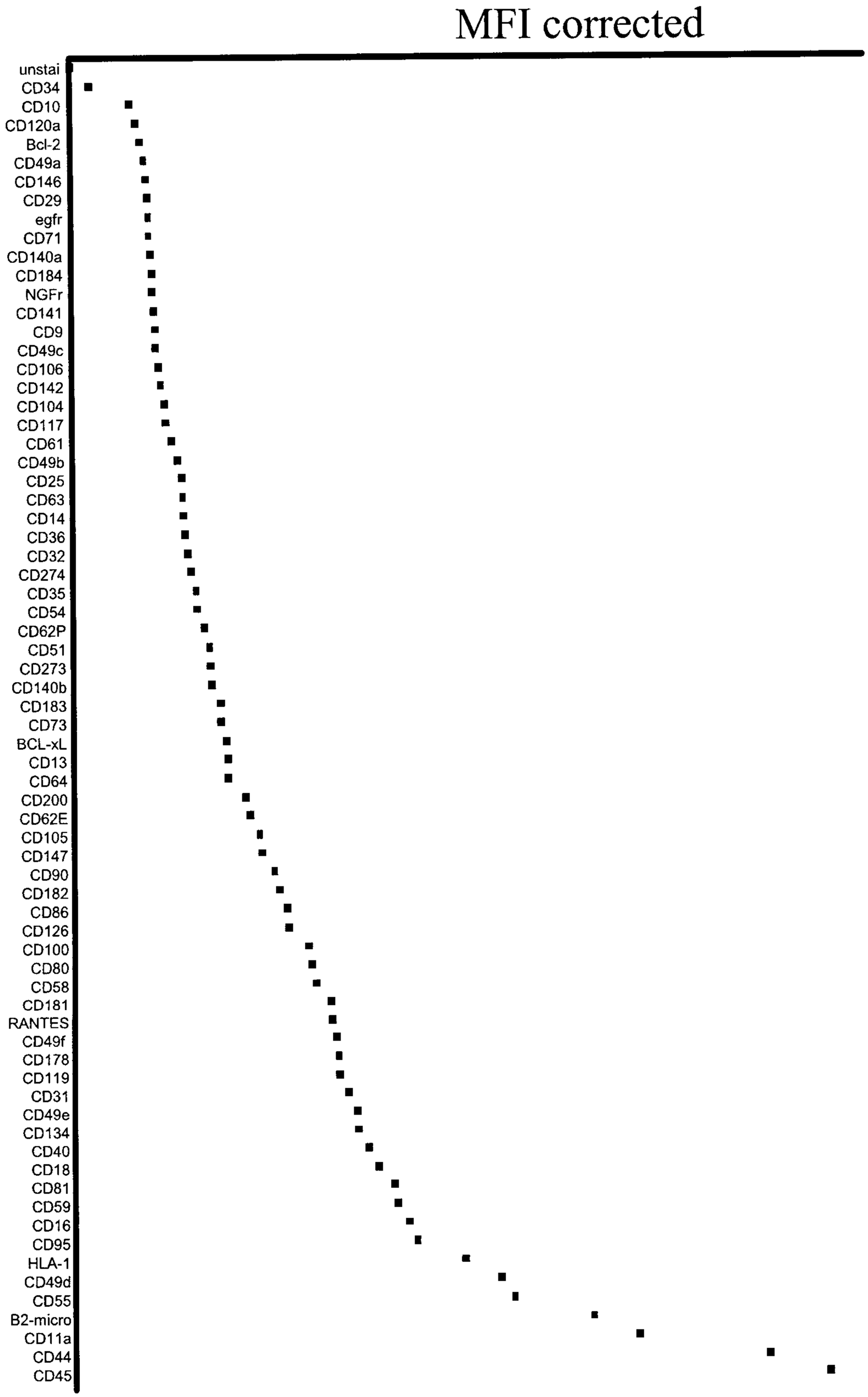

% positive
MFI-corrected
Leukocytes
Monocytes
Granulocytes
Unstained
CD71
CD49c
CD29
CD63
CD35
CD200
CD64
CD4
CD128a
CD56
CD25
CD8
CD28
CD3
b2-micro
CD11a

Figure 5C:


MFI corrected
unstai
CD34
CD10
CD120a
Bcl-2
CD49a
CD146
CD29
egfr
CD71
CD140a
CD184
NGFr
CD141
CD9
CD49c
CD106
CD142
CD104
CD117
CD61
CD49b
CD25
CD63
CD14
CD36
CD32
CD274
CD35
CD54
CD62P
CD51
CD273
CD140b
CD183
CD73
BCL-xL
CD13
CD64
CD200
CD62E
CD105
CD147
CD90
CD182
CD86
CD126
CD100
CD80
CD58
CD181
RANTES
CD49f
CD178
CD119
CD31
CD49e
CD134
CD40
CD18
CD81
CD59
CD16
CD95
HLA-1
CD49d
CD55
B2-micro
CD11a
CD44
CD45