Flow cytometry: rules and application
medical biotechnology

antibodies

flow cytometry

laser analysis
Production of antibodies

Polyclonal antibodies

Monoclonal antibodies

Monospecific → monovalent affinity bind to the same epitope
ELISA

direct ELISA

Antibody immobilised on the surface of the test well

A test sample is added and a corresponding antigen attaches to the immobilised antibodies

When an enzyme-labelled antibody is added, it links with the antigen

It causes a colour change that is proportional to the amount of antigen present

The enzyme substrate reacts with the enzyme

An enzyme substrate is added

enzyme substrate
test sample
antibody
enzyme-labelled antibody

indirect ELISA

Enzyme’s substrate (●) is added, and reaction produces a product that causes a visible color change (○).
Monoclonal antibodies in diagnostics

Monoclonal antibodies are important tool for diagnosing diseases
- pregnancy testing
- cancer screening
- diagnosis of hepatitis B, cystic fibrosis or HIV

Advantages of monoclonal antibody as diagnostic tool
- Rapid testing
- High sensitivity
- Less material needed to be taken from patients

http://classes.midlandstech.edu/carterp/Courses/bio225/chap18/lecture5.htm
Cloning of mouse variable genes into human constant-region genes generates chimeric antibodies.

Humanized antibodies are generated by the insertion of mouse complementarity-determining regions (CDRs) onto human constant and variable domain frameworks.

Fully human antibodies can be generated by the selection of human antibody fragments from in vitro libraries, by transgenic mice and through selection from human hybridomas.
<table>
<thead>
<tr>
<th>International non-proprietary name</th>
<th>Trade name</th>
<th>Type</th>
<th>Indication first approved</th>
<th>First EU (US) approval year</th>
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<tbody>
<tr>
<td>Abciximab</td>
<td>Reopro</td>
<td>Anti- GPIb/IIa; Chimeric IgG1 Fab</td>
<td>Prevention of blood clots in angioplasty</td>
<td>1995* (1994)</td>
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<tr>
<td>Daclizumab</td>
<td>Zenapax</td>
<td>Anti-IL2R, Humanized IgG1</td>
<td>Prevention of kidney transplant rejection</td>
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<td>Infliximab</td>
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<td>Anti-TNF, Chimeric IgG1</td>
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<td>Trastuzumab</td>
<td>Herceptin</td>
<td>Anti-HER2, Humanized IgG1</td>
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<td>Gentuzumab ozogamicin</td>
<td>Mylotarg</td>
<td>Anti-CD33, Humanized IgG4</td>
<td>Acute myeloid leukemia</td>
<td>NA (2000#)</td>
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<td>Alectumab</td>
<td>MabCampath, Campath-1H</td>
<td>Anti-CD52, Humanized IgG1</td>
<td>Chronic myeloid leukemia</td>
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<td>Adalimumab</td>
<td>Humira</td>
<td>Anti-TNF; Human IgG1</td>
<td>Rheumatoid arthritis</td>
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<td>Eflizumab</td>
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<td>Psoriasis</td>
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<td>Cetuximab</td>
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<td>Anti-EGFR; Chimeric IgG1</td>
<td>Colorectal cancer</td>
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<td>Omalizumab</td>
<td>Xolar</td>
<td>Anti-IgE; Humanized IgG1</td>
<td>Asthma</td>
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<tr>
<td>Ranibizumab</td>
<td>Lucentis</td>
<td>Anti-VEGF, Humanized IgG1 Fab</td>
<td>Macular degeneration</td>
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<td>Certolizumab pegol</td>
<td>Cinqia</td>
<td>Anti-TNF; Humanized Fab pegylated</td>
<td>Cronn disease</td>
<td>2009 (2005)</td>
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<td>Golimumab</td>
<td>Simponi</td>
<td>Anti-TNF, Human IgG1</td>
<td>Rheumatoid and psoriatic arthritis, ankylosing spondylitis</td>
<td>2009 (2009)</td>
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<td>Canakinumab</td>
<td>Ilaris</td>
<td>Anti-IL1b, Human IgG1</td>
<td>Muckle-Wells syndrome</td>
<td>2009 (2009)</td>
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<tr>
<td>Catumaxomab</td>
<td>Removab</td>
<td>Anti-EPAC1/CD43, Rat mouse biopspecific mAb</td>
<td>Malignant ascites</td>
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<td>Ustekinumab</td>
<td>Stelara</td>
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<td>Tolizumab</td>
<td>RoActienda, Actemra</td>
<td>Anti-HLAG, Humanized IgG1</td>
<td>Rheumatoid arthritis</td>
<td>2010 (2010)</td>
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<td>Cetuximab</td>
<td>Arzerra</td>
<td>Anti-CD20; Human IgG1</td>
<td>Chronic lymphocytic leukemia</td>
<td>2010 (2009)</td>
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<tr>
<td>Boelimunab</td>
<td>Benlysta</td>
<td>Anti- ELYS; Human IgG1</td>
<td>Systemic lupus erythematosus</td>
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<td>Iplimunab</td>
<td>Yervoy</td>
<td>Anti-CTLA-4; Human IgG1</td>
<td>Metastatic melanoma</td>
<td>2011 (2011)</td>
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<td>Brentuximab vedotin</td>
<td>Adocstra</td>
<td>Anti-CD30; Chimeric IgG1; immunoconjugate</td>
<td>Hodgkin lymphoma</td>
<td>2012 (2011)</td>
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<tr>
<td>Pertuzumab</td>
<td>Perjeta</td>
<td>Anti-HER2; Humanized IgG1</td>
<td>Breast Cancer</td>
<td>2013 (2012)</td>
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<tr>
<td>Ado-Trastuzumab ontanone</td>
<td>Kadoyla</td>
<td>Anti-HER2; Humanized IgG1; immunoconjugate</td>
<td>Breast cancer</td>
<td>2013 (2013)</td>
</tr>
<tr>
<td>Vadiluzumab (Pending)</td>
<td></td>
<td>Anti-alpha4beta7 integrin; Humanized IgG1</td>
<td>Ulcerative colitis, Crohn disease</td>
<td>In review (In review)</td>
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<tr>
<td>Ramucirumab (Pending)</td>
<td></td>
<td>Anti-VEGFR2; Human IgG1</td>
<td>Gastric cancer</td>
<td>In review (In review)</td>
</tr>
<tr>
<td>Ogivtitumab</td>
<td>Gasveya</td>
<td>Anti-CD20; Human IgG1; Glycoengineered</td>
<td>Chronic lymphocytic leukemia</td>
<td>In review (2013)</td>
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<tr>
<td>Siltuximab (Pending)</td>
<td></td>
<td>Anti-IL-6; Chimeric IgG1</td>
<td>Castleman disease</td>
<td>In review (In review)</td>
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<tr>
<td>Saculimunab (Pending)</td>
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<td>Anti-IL-17a; Human IgG1</td>
<td>Immunesuppression</td>
<td>In review (NA)</td>
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<tr>
<td>Nivolumab (Pending)</td>
<td></td>
<td>Anti-PD1; Human IgG4</td>
<td>Melanomasuppression</td>
<td>NA (NA) [In review in Japan]</td>
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<td>Lambrolizumab (Pending)</td>
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<td>Anti-PD1; Humanized IgG4</td>
<td>Melanoma</td>
<td>NA (In review)</td>
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<tr>
<td>Dinutuximab (Pending)</td>
<td></td>
<td>Anti-G2; Chimeric IgG1</td>
<td>Neuroblastoma</td>
<td>In review (NA)</td>
</tr>
</tbody>
</table>
Monoclonal antibodies in flow cytometric analysis

- basic research, monitoring of disease,
- medical diagnostics (diagnosis of blood cancers),
- evaluation of tumor response to therapy
Flow cytometry - definition

First fluorescence-based flow cytometry device was developed in 1968 by Wolfgang Göhde from the University of Münster, Germany and commercialized in 1968/69 by German developer and manufacturer Partec through Phywe AG in Göttingen.

Originally named – „pulse cytophotometry”; renamed for „flow cytometry” in 1978.

- Method of analysis of the properties of individual cells in a heterogeneous population.
- Cells analyzed are suspended in a stream of fluid and passed by an electronic detection apparatus.
- The ability to test objects of sizes ranging from bacteria to mature human blood cells.
The rule of working

- A beam of light (laser) of a single wavelength is directed onto a hydrodynamically-focused stream of fluid
- The cells in the stream cross the laser beam individually
- A number of detectors are aimed at the point where the stream passes through the light beam: one in line with the light beam (Forward Scatter or FSC) and several perpendicular to it (Side Scatter (SSC) and one or more fluorescent detectors).

Evaluation of cells:
- size
- structure
- phenotype
- functionality
- viability

Collecting, processing and signal analysis
Hydrodynamic focusing

Sample – cell suspension

Sheath fluid (płyn otaczający)

Laminar flow

cells cross the laser beam individually
Point of lighting of the stream

Hydrodynamic focusing

Side scatter, SSC (rozproszenie boczne)

Forward scatter, FSC (rozproszenie wdłużne)
Forward scatter characteristics (FSC)

Signal proportional to the size of the cell

Small

Medium

Large

Threshold

Small cells and debris
Scattering of light at larger angles is a measure of granularity of cells and complexity of its structure.

- Analyzed light is perpendicular to the laser beam.
Size and granularity of blood cells

Forward (FSC) and side (SSC) scatter allow to distinguish major types of blood cells.

"Dot plot"
Another parameter: fluorescence of the cell

Light of different wavelengths is directed through the filters and dichroic mirrors to different photomultipliers.

FACS - fluorescence-activated cell sorting

Detectors: photomultipliers (fotopowielacze)
Fluorescent labeling of cells

- Use of antibodies which recognize specific antigens on the cell surface – **Immunophenotyping**
- The antibodies are conjugated to fluorescent dyes

**Fluorochrome excitation**

![Fluorochrome excitation diagram]

**Mixture of cells is labeled with fluorescent antibody**

![Mixture of cells diagram]
Types of fluorescent dyes

- **organic**
  - Ethidium bromide
  - Phicoerythrin (PE)
  - Fluorescein
  - Alexa fluor 350

- **fluorescent proteins**
  - Green Fluorescent Protein - GFP

- **quantum dots**
  - The energy of the photon depends on the size of the core: smaller core → shorter wave

- **tandem dyes** - achieve greater Stokes’s shifts
  - More of the possible emission wavelength with a single excitation wavelength
Lasers / fluorochromes
An example of double staining with the use of dyes: Alexa Fluor 488 and R-PE excited with 488 nm wavelength.

Partial overlap of emission spectra may result in a false detection of the signal in the two channels, while cells are stained only in one channel.
Multiparameter analysis

- Possibility of the use of several different fluorescence channels (in practice up to 8-10)

- Possibility of gating and distinguishing target cell populations
Flow cytometry applications

- Use of antibodies which recognize **specific antigens on the cell surface** – proteins, glycoproteins, phospholipids

- Ability of determination of **intracellular antigens** - the need for cell fixation and permeabilization of the cell membranekomórkowej
Bone marrow stem cells:

mesenchymal stem cells (MSCs)  hematopoietic stem cells (HSCs)

In the postnatal bone marrow, MSCs reside around sinusoids, maintain a niche for HSCs, support hematopoiesis and replenish the differentiated compartment of osteoblasts and adipocytes during tissue growth and turnover.

Hematopoietic stem cells / hematopoiesis

HSC

multipotent hematopoietic stem cell

CMP

common myeloid progenitor

megakaryocyte

proerythroblast

basophilic erythroblast

promegakaryocyte

polychromatic erythroblast

thrombocytes

erthrocyte

thrombopoiesis

erythropoiesis

thrombocytes

erthrocyte

erythrocyte

mast cell

HSC

myeloblast

monoblast

promonocyte

promonocytes

b. band

e. band

n. band

basophils

eosinophils

neutrophils

granulopoiesis

macrophage

dendritic cell

monocyte

dendritic cell

CLP

common lymphoid progenitor

lymphoblast

prolymphocyte

small lymphocyte

B lymphocyte

T lymphocyte

NK cell

lymphocytes

plasm cell

lymphoid dendritic cell

mature cells

committed progenitors

stem cells
Identification of hematopoietic cells based on surface or functional markers

LT-HSC, long-term repopulating HSC; ST-HSC, short-term repopulating HSC; MPP, multipotent progenitor; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; MEP, megakaryocyte/erythroid progenitor; GMP, granulocyte–macrophage progenitor. The encircled pluripotent population, LT-HSC, ST-HSC and MPP are Lin-, Sca-1+, c-kit+ as shown

FACS analysis of mouse hematopoietic stem cell subsets – gating strategy

Larsson & Karlsson, Oncogene (2005) 24, 5676–5692


http://stemcellassays.com
Physiological balance of hematopoiesis

Hematopoietic cells may be missing or do not function properly

Hematopoietic cells may undergo malignant transformation into an abnormally proliferating leukemic cells

Its regulation depends on: cytokines, cellular interactions, transcription and metabolic factors

Leukemias (acute or chronic) and lymphomas can be regarded as malignant counterparts of normal hematopoietic cells in different maturation stages.
Flow cytometric immunophenotyping and functional studies of blood/ bone marrow/ other material

Flow cytometry in primary immunodeficiency

Targeted analysis of immune cells:

1. Are all cells present in normal frequencies?
2. Do they have all relevant proteins expressed?
3. Is their function normal?
Primary immunodeficiencies are disorders in which part of the immune system is missing or does not function normally.

Mostly genetic disorders.

The majority are diagnosed in children under the age of one, although milder forms may not be recognized until adulthood.
Both T lymphocytes and often B lymphocytes, regulators of adaptive immunity, are dysfunctional or decreased in number.

The main members are various types of severe combined immunodeficiency (SCID):

**T-/B+ SCID (T cells predominantly absent):**
- Common γ chain deficiency
- JAK3 deficiency
- Interleukin 7 receptor chain α deficiency
- CD45 deficiency
- CD3δ/CD3ε deficiency

**T-/B- SCID (both T and B cells absent):** RAG 1/2 deficiency, DCLRE1C deficiency, **adenosine deaminase (ADA) deficiency**, reticular dysgenesis.
ADA deficiency - first controlled trial of gene therapy - 1990

ADA - an enzyme involved in purine metabolism
- breakdown of adenosine from food and for the turnover of nucleic acids in tissues

ADA deficiency
- autosomal recessive metabolic disorder
- accounts for about 15% of all cases of SCID
- treatments include: transplant, gene therapy

Ashanti de Silva
X-linked SCID – common γ chain deficiency (most cases of SCID)

- mutations in the gene encoding the common γ chain (on X-chromosome), shared by the receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21

--> widespread defects in interleukin signaling and near complete failure of the immune system to develop and function, with low or absent T cells and NK cells and non-functional B cells

- treatments include: transplant, gene therapy

ex vivo infection of CD34+ cells with retroviral vector with a correct gene of γ chain of cytokine receptor

David Vetter – “bubble boy”
Primary immunodeficiencies
- enumeration of lymphocytes subsets in PB

Peripheral blood
lymphocyte subpopulations

Healthy patient (control)
Primary immunodeficiencies
- enumeration of lymphocytes subsets in PB

**SCID T-B-NK- (ADA deficiency)**

![Flow cytometry plots showing lymphocyte subsets](image)

<table>
<thead>
<tr>
<th>Population</th>
<th>%Parent</th>
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</thead>
<tbody>
<tr>
<td>limfocyty</td>
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<tr>
<td>limf B</td>
<td>11.6</td>
</tr>
<tr>
<td>limf T</td>
<td>5.8</td>
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<tr>
<td>NK</td>
<td>7.2</td>
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<td>NKT</td>
<td>1.4</td>
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<tr>
<td>klasyczne T</td>
<td>7.2</td>
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</table>
Primary immunodeficiencies  
- enumeration of lymphocytes subsets in PB

SCID T-B+NK- (common γ chain mutation)

Blood from infant – 4h after childbirth
DNA replication

The use of dyes which stoichiometrically bind to DNA allows to determine the amount of DNA in the cells and in turn the phase of cell cycle.
Cell proliferation - checking function of immune cells & others

- staining of cell membranes with the lipid fluorescent dye
- with each cell division, the amount of dye per cell is two times lower
- the possibility to track the amount of divisions
- CFSE dye enables the visualization of eight or more generations of proliferating cells before the signal is overwhelmed by intrinsic cellular auto-fluorescence
Primary immunodeficiencies – checking expression of relevant protein

Chronic granulomatous disease → NADPH oxidase mutation

Immune cells have impaired ability to form reactive oxygen compounds (most importantly, the superoxide radical)
-> formation of granulomata in many organs
Primary immunodeficiencies

Chronic granulomatous disease

anti-gp91 phox monoclonal antibody

healthy

X-linked CGD

Monoclonal antibody – Information only about the epitop not the whole protein!!
Detection of reactive oxygen species – checking function of immune cells & others

- Dye undergoing activation by cellular enzymes and converted to fluorescent product by reactive oxygen species

Unstimulated cells

Stimulated cells

H₂DCFDA-AM

H₂DCF
(Non-fluorescent)

DCF
(Fluorescent)

ROS

Cell Membrane

Cellular Esterase Activity
Flow-cytometric immunophenotyping is the sole technique that fulfils the requirements of high speed, broad applicability at diagnosis and during follow-up of immunological and hematological disorders with accurate focusing on the cell population of interest.
Leukemias and lymphomas can be regarded as malignant counterparts of normal hematopoietic cells in different maturation stages.

**Acute leukemia** - rapid increase in the number of immature blood cells

**Chronic leukemia** - excessive build up of relatively mature, but still abnormal, white blood cells

### Four major kinds of leukemia

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Acute</th>
<th>Chronic</th>
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</thead>
<tbody>
<tr>
<td>Lymphocytic leukemia</td>
<td>Acute lymphoblastic leukemia (ALL)</td>
<td>Chronic lymphocytic leukemia (CLL)</td>
</tr>
<tr>
<td>(or &quot;lymphoblastic&quot;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myelogenous leukemia</td>
<td>Acute myelogenous leukemia (AML)</td>
<td>Chronic myelogenous leukemia (CML)</td>
</tr>
<tr>
<td>(also &quot;myeloid&quot; or &quot;nonlymphocytic&quot;)</td>
<td>(or myeloblastic)</td>
<td></td>
</tr>
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</table>

**ALL** - Acute lymphoblastic leukemia
B-ALL from B lineage
T-ALL from T lineage

**AML** - Acute myeloid leukemia
- M0  minimally differentiated acute myeloblastic leukemia
- M1  acute myeloblastic leukemia, without maturation
- M2  acute myeloblastic leukemia, with granulocytic maturation
- M3  promyelocytic, or acute promyelocytic leukemia (APL)
- M4  acute myelomonocytic leukemia
  - M4eo myelomonocytic together with bone marrow eosinophilia
- M5  (M5a) acute monoblastic leukemia
  (M5b) acute monocytic leukemia
- M6  (M6a) acute erythroid leukemias, including erythro leukemia
  (M6b) very rare pure erythroid leukemia
- M7  acute megakaryoblastic leukemia
Surface marker characteristics of different stage of B cell differentiation

Hematopoietic malignancies usually display aberrant or unusual antigen expression -> Multiparameter flow cytometry diagnostics

Physiological shift in B-cell compartment

Normal B-cell development

Age-related shifts in B-cell compartment of elderly individuals

Regenerating B cell precursor in bone marrow after cytotoxic treatment
Chronic myeloid leukemia (CML)

15-20% all leukemias in adults
marked leucocytosis – basophils and neutrophils are increased

Mutated chromosome Philadelphia -> formation of hybrid gene Bcr/Abl and fusion protein of constitutive, unregulated kinase activity

TREATMENT:
Gleevec – targets the cause of CML – a specific inhibitor of a small family of tyrosine kinases, including Bcr-Abl, Kit and PDGF receptor

Diagnostics of chronic myeloid leukemia (CML)

- morphologic evaluation of the peripheral smear and bone marrow aspirate

- identification of the t(9;22) Philadelphia chromosome translocation by FISH technique, or BCR-ABL1 fusion gene by RT-PCR

FISH – fluorescence in situ hybridization

Antibody recognizing BCR attached to a capture bead

ABL-directed phycoerythrin (PE)–conjugated detection antibody

Sandwich Complex

http://www.slh.wisc.edu/clinical/cytogenetics/fish/
Other applications of flow cytometry - cell viability, apoptosis & necrosis

**Apoptosis** – programmed cell death (nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation)

**Necrosis** - cell death following mechanical damage

- **The use of different DNA dyes**
  - **7-ADD** – penetrates only damaged cell membranes
  - **HOECHST** – penetrates through intact cell membranes, but apoptotic cells show higher permeability
  - **ANNEXIN V** for studying the externalization of phosphatidylserine

**The possibility of assessment of changes in asymmetric structure of cell membrane** – **ANNEXIN V** for studying the externalization of phosphatidylserine
Cell sorter

- Heterogenous population
- Cell analysis, decision of sorting of selected cell subpopulation
- The separation of the stream into individual droplets (a piezoelectric element). At the time of detachment of the droplet with the cell of interest, it is charging positively or negatively
- Droplets that contain the desired cell are charged and are deflected in an electric field
- Possibility of sorting of 6 different populations at the same time

MoFlo (Beckman Coulter)
Magnetic cell sorting

Heterogeneous population of lymphocytes is mixed with antibodies coupled to paramagnetic particles or beads and poured over an iron wool mesh.

When a magnetic field is applied the coupled cells stick to the iron wool; unlabeled cells are washed out.

The magnetic field is removed releasing the coupled cells.

COBE® Spectra Apheresis System – sorting for transplantation.

Peripheral blood stem cell harvested by apheresis.

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Figure A-27 Immunobiology, 7ed. (© Garland Science 2008)
Analysis of hematopoietic potential of cells

Single sorting of HSC → colonies containing different types of blood cells
GFP+ HSC for bone marrow transplantation

Tracking bone marrow progeny

an example - Adult hematopoietic stem cells contribute to lymphatic endothelium

between 200 to 500 sorted GFP⁺ c-kit⁺, Sca-1⁺, lineage⁻ (KSL) cells, hereafter referred to as HSCs, were transplanted into lethally irradiated (1200 cGy) recipient mice

-> GFP⁺ cells comprising between 80–90% of nucleated hematopoietic cells (assessed by flow cytometry)

donor-derived lymphatic endothelium:
GFP⁺, Lyve-1⁺ and/or VEGFR-3⁺, CD45⁻ and F4/80⁻

Magnetic sorting (apheresis) for transplantation in patients

**Blood Stem Cell Transplantation**

- **Mobilization of progenitors by G-CSF** - donor receives subcutaneous injections of filgrastim (rG-CSF) twice daily for three or four days in preparation for apheresis

- Peripheral blood stem cell harvested by **apheresis (leukapheresis)**
  - "adequate number of hematopoietic progenitor cells for full hematopoietic recovery in an allogeneic recipient without the disadvantages of a marrow harvest under general anesthesia for the donor"

- During **apheresis**, blood is removed from a vein in one arm, fractionated continuously on an automated cell separator to collect the stem cells, and the remaining cells are returned through a vein in the opposite arm

- For **autologous** and **allogeneic** (the cells come from a matched related or unrelated donor)

- Bone pain in response to the filgrastim injections which cause marrow expansion

Shlomchik, Nature Reviews Immunology 7, 340-352 (May 2007)
Innovative agents for peripheral blood progenitor cells mobilization

Pro-inflammatory cytokines such as G-CSF can indirectly affect HSCs by altering the bone marrow microenvironment, disrupting the stem cell niche, and leading to HSC mobilization into the blood.

**AMD-3100** – reversible inhibitor of CXCR4/SDF-1 binding which mobilizes CD34+ cells into peripheral blood. Administration of AMD-3100 after 4-5 days of G-CSF results in 3 fold increase of CD34+ cells mobilization.

Schuettpelz and Link, Front. Immunol., 19 July 2013
Imaging flow cytometry (ImageStream system)

flow cytometry + digital microscopy

- The system combines a precise method of electronically tracking moving cells with a high resolution multispectral imaging system to acquire multiple images of each cell in different imaging modes.

- Simultaneous acquiring of several images of each cell:
  - Side-scatter (darkfield) image
  - Several fluorescence images

CCD camera (Time Delay Integration)

- An image of each channel is biased at a different angle
Image stream applications

Zymosan internalization

Cell Signaling
DNA Damage and Repair
Cell Death
Co-localization
Cell Cycle and Mitosis
Parasitology
Cell-Cell Interactions
Autophagy
Microbiology
Morphology
Targeted Immunotherapy
Oncology
Internalization
Stem Cell Differentiation
Oceanography

Not Internalized

Internalized

Zymosan internalization
Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope. Broad spectrum of applications in diagnostics - therapeutic monoclonal antibodies

Flow cytometry (with the use of monoclonal antibodies) is of particular importance in basic research, monitoring of disease, medical diagnostics (diagnosis of blood cancers), evaluation of tumor response to therapy

Flow cytometric immunophenotyping with (monoclonal) antibodies allows the recognition of leukocyte subsets

Usage of multiparameter analyses allow the dissection of differentiation and maturation pathways as well as the detection of specific proteins

Hematopoietic cells may be missing or do not function properly (primary immunodeficiencies). On the other hand hematopoietic cells may undergo malignant transformation into an abnormally proliferating leukemic cells.

Flow-cytometric immunophenotyping is the sole technique that fulfils the requirements of high speed, broad applicability at diagnosis and during follow-up of immunological and hematological disorders with accurate focusing on the cell population of interest
### Classical leukocytes markers

<table>
<thead>
<tr>
<th>Immature markers</th>
<th>T-cell markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34: precursor marker</td>
<td>CD1: common thymocyte marker</td>
</tr>
<tr>
<td>TdT: terminal deoxynucleotidyl transferase</td>
<td>CD2: pan-T-cell marker</td>
</tr>
<tr>
<td>CD117: myeloid precursor marker</td>
<td>CD4: helper T-cell marker</td>
</tr>
<tr>
<td>HLA-DR: precursor cells (and APC cell)</td>
<td>CD8: cytotoxic T-cell marker</td>
</tr>
<tr>
<td></td>
<td>CD3: mature T-cell marker</td>
</tr>
<tr>
<td>B-cell markers</td>
<td>TCR: T-cell receptor</td>
</tr>
<tr>
<td>CD10: immature B-cell marker</td>
<td>Myeloid markers</td>
</tr>
<tr>
<td>CD19: pan-B-cell marker</td>
<td>CD13 and CD33: pan-myeloid</td>
</tr>
<tr>
<td>CD20: mature B-cell marker</td>
<td>CD14: monocytic marker</td>
</tr>
<tr>
<td>Smlg: membrane bound Ig</td>
<td>CD15: granulocytic marker</td>
</tr>
<tr>
<td>Cylg: cytoplasmic Ig</td>
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