

# **Malign Hematolojik Hastalıklarda İmmunfenotiplendirme**

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# **FCM güncel hematolojik uygulamalarda kullanımı giderek artmaktadır.**

Kısa sürede sonuç alınabilmesi

Kullanımının kolay olması

Anormal hücrelerin tanınması

FCM hematolojide en çok

- tanı
- lösemi-lenfoma-myelom sınıflaması
- MRD
- Prognoz
- Ve tedavinin takibi amacıyla kullanılmaktadır.

## **FCM nin tanıya katkıda bulunduğu hematolojik hastalıklar**

### **FCM ile tanı konulabilir**

- B ALL
- T-ALL
- B-CLL,SLL,MCL
- Hairy Cell Lösemi
- AML
- PNH

### **FCM tanı/ayırıcı tanıda yardımcıdır**

- Foliküler NHL
- Diğer low grade lenfoma
- Büyük B hücreli NHL
- Burkitt's Lenfoma
- Large garnüler lenfosit hast.
- Plazma hücre hast.
- MDS

# Klinik Kullanımı

Table 2. Clinical Applications of Flow Cytometry Immunophenotyping

Disease	Diagnosis	Classification	Prognosis and/or Staging	Disease Monitoring
Acute Leukemias	Yes	Yes	Controversial	Yes
Chronic Lymphoproliferative Disorder (CLPD)	Yes	Yes	Yes	Yes
Myelodysplasia (MDS)	To be established	No	To be established	No
Paroxysmal Nocturnal Hemoglobinuria (PNH)	Yes	Yes	No	Yes
Mastocytosis	Yes	Yes	No	Yes
Primary Thrombocytopathies	Yes	Controversial	Yes	No
Primary Immunodeficiencies	Yes	Yes	No	No
HIV Infection	No	Yes	Yes	Yes
Transplantation Outcome	Yes	Yes	Yes	Yes

# Akut Lösemi Tanısı

Sitolojik inceleme (PK ve/veya KI)

My Grunwald Giemsa Boyası/Histokimyasal Boyalar

Blast oranı, morfolojik displastik değişiklikler

İmmunfenotipleme ile tanı/köken aldığı hücre/sınıflama

Karyotipleme ile sitogenetik anomali

Moleküller gen analizleri

## **İmmunfenotipleme Hedefleri:**

Blastların köken aldığı hücre serisi(leri)nin belirlenmesi  
Bifenotipik akut lösemilerin tanınması  
Mevcut sınıflamalara göre (ör EGIL, FAB, GEIL, WHO) bir değerlendirme yapabilmek  
Takipte kullanılabilecek anormal antijen dağılımlarının belirlenmesi

Sitogenetik anomaliler için ipucu  
Prognostik değerlendirme

## **FCM verileri ile tanı koyabilmek için:**

- Normal hematopoietik matürasyon
  - Kazanılan/kaybedilen抗原
  - Antigen sitesindeki değişimler (sadece var/ yok değil)
- Sık görülen anormal immunfenotipik değişiklikler bilinmelidir
- Reaktiflerin performansı
- Örneklerin hazırlanması önem taşır

# 2008 WHO Classification of AML

- AML with recurrent genetic abnormalities
  - AML with t(8;21) (q22;q22) (*RUNX1-RUNX1T1*)
  - AML with inv(16)(p13.1q22) or t(16,16) (p13.1;q22) (*CBFB-MYH11*)
  - Acute promyelocytic leukemia with t(15;17)(q24.1;q21.1) (*PML-RARA*)
  - AML with t(9;11)(p22;q23) (*MLLT3-MLL*)
  - AML with t(6;9)(p23;q34) (*DEK-NUP214*)
  - AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) (*RPN1-EVI1*)
  - AML (megakaryoblastic) with t(1;22)(p13;q13) (*RBM15-MKL1*)
  - Provisional entity: AML with mutated *NPM1*
  - Provisional entity: AML with mutated *CEBPA*
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- AML, not otherwise specified
  - AML minimally differentiated
  - AML without maturation
  - AML with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic and monocytic leukemia
  - Acute erythroid leukemia
  - Acute megakaryocytic leukemia
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm

# New Acute Myeloid Leukemia subtypes 2016

- AML with *RUNX1* mutation (provisional)
  - Elderly male, poor prognosis
- AML with *BCR-ABL 1* (provisional)
  - Antigen receptor deletion (IGH)
- AML with **biallelic** *CEBPA* mutations (*CEBPA<sup>dm</sup>*)
- Familial AML/MDS (multiple types)
- Promoted to full entity (No longer provisional)
  - Acute Myeloid Leukemia with *NPM1* mutation
  - Acute Myeloid Leukemia with *CEBPA<sup>dm</sup>*

#### Mature B-cell neoplasms

Chronic lymphocytic leukemia/small lymphocytic lymphoma  
Monoclonal B-cell lymphocytosis\*  
B-cell prolymphocytic leukemia  
Splenic marginal zone lymphoma  
Hairy cell leukemia  
*Splenic B-cell lymphoma/leukemia, unclassifiable*  
*Splenic diffuse red pulp small B-cell lymphoma*  
*Hairy cell leukemia-variant*  
Lymphoplasmacytic lymphoma  
*Waldenström macroglobulinemia*  
Monoclonal gammopathy of undetermined significance (MGUS), IgM\*  
 $\mu$  heavy-chain disease  
 $\gamma$  heavy-chain disease  
 $\alpha$  heavy-chain disease  
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A\*  
Plasma cell myeloma  
Solitary plasmacytoma of bone  
Extraosseous plasmacytoma  
Monoclonal immunoglobulin deposition diseases\*  
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)  
Nodal marginal zone lymphoma  
*Pediatric nodal marginal zone lymphoma*  
Follicular lymphoma  
*In situ* follicular neoplasia\*  
Duodenal-type follicular lymphoma\*  
Pediatric-type follicular lymphoma\*  
*Large B-cell lymphoma with IRF4 rearrangement\**  
Primary cutaneous follicle center lymphoma  
Mantle cell lymphoma  
*In situ* mantle cell neoplasia\*  
Diffuse large B-cell lymphoma (DLBCL), NOS  
*Germinal center B-cell type\**  
*Activated B-cell type\**  
*T-cell/histiocyte-rich large B-cell lymphoma*  
Primary DLBCL of the central nervous system (CNS)  
Primary cutaneous DLBCL, leg type  
*EBV<sup>+</sup> DLBCL, NOS\**  
*EBV<sup>+</sup> mucocutaneous ulcer\**  
DLBCL associated with chronic inflammation

#### Lymphomatoid granulomatosis

Primary mediastinal (thymic) large B-cell lymphoma  
Intravascular large B-cell lymphoma  
ALK<sup>+</sup> large B-cell lymphoma  
Plasmablastic lymphoma  
Primary effusion lymphoma  
*HHV8<sup>+</sup> DLBCL, NOS\**  
Burkitt lymphoma  
*Burkitt-like lymphoma with 11q aberration\**  
High-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements\*  
High-grade B-cell lymphoma, NOS\*  
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

#### Mature T and NK neoplasms

T-cell prolymphocytic leukemia  
T-cell large granular lymphocytic leukemia  
*Chronic lymphoproliferative disorder of NK cells*  
Aggressive NK-cell leukemia  
Systemic EBV<sup>+</sup> T-cell lymphoma of childhood\*  
Hydroa vacciniforme-like lymphoproliferative disorder\*  
Adult T-cell leukemia/lymphoma  
Extranodal NK/T-cell lymphoma, nasal type  
Enteropathy-associated T-cell lymphoma

Provisional entities are listed in italics.

\*Changes from the 2008 classification.

Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and

**Table 1. (continued)**

Monomorphic epitheliotropic intestinal T-cell lymphoma*
<i>Indolent T-cell lymphoproliferative disorder of the GI tract*</i>
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30 <sup>+</sup> T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous γδ T-cell lymphoma
<i>Primary cutaneous CD8<sup>+</sup> aggressive epidermotropic cytotoxic T-cell lymphoma</i>
<i>Primary cutaneous acral CD8<sup>+</sup> T-cell lymphoma*</i>
<i>Primary cutaneous CD4<sup>+</sup> small/medium T-cell lymphoproliferative disorder*</i>
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
<i>Follicular T-cell lymphoma*</i>
<i>Nodal peripheral T-cell lymphoma with TFH phenotype*</i>
Anaplastic large-cell lymphoma, ALK <sup>+</sup>
Anaplastic large-cell lymphoma, ALK <sup>-</sup> *
<i>Breast implant-associated anaplastic large-cell lymphoma*</i>
<b>Hodgkin lymphoma</b>
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma

**Hodgkin lymphoma**

Nodular lymphocyte predominant Hodgkin lymphoma

Classical Hodgkin lymphoma

  Nodular sclerosis classical Hodgkin lymphoma

  Lymphocyte-rich classical Hodgkin lymphoma

  Mixed cellularity classical Hodgkin lymphoma

  Lymphocyte-depleted classical Hodgkin lymphoma

**Posttransplant lymphoproliferative disorders (PTLD)**

Plasmacytic hyperplasia PTLD

Infectious mononucleosis PTLD

Florid follicular hyperplasia PTLD\*

Polymorphic PTLD

Monomorphic PTLD (B- and T-/NK-cell types)

Classical Hodgkin lymphoma PTLD

**Histiocytic and dendritic cell neoplasms**

Histiocytic sarcoma

Langerhans cell histiocytosis

Langerhans cell sarcoma

Indeterminate dendritic cell tumor

Interdigitating dendritic cell sarcoma

Follicular dendritic cell sarcoma

Fibroblastic reticular cell tumor

Disseminated juvenile xanthogranuloma

Erdheim-Chester disease\*

Provisional entities are listed in italics.

\*Changes from the 2008 classification.

**Table 1. 2016 WHO classification of mature lymphoid, histiocytic,**

# İmmunfenotiplemede kritik noktalar

## Uygun panelin seçilmesi

Köken aldığı hücre?

Bifenotipi?

Sınıflama?

Aberan fenotip?

## Uygun teknigin seçilmesi

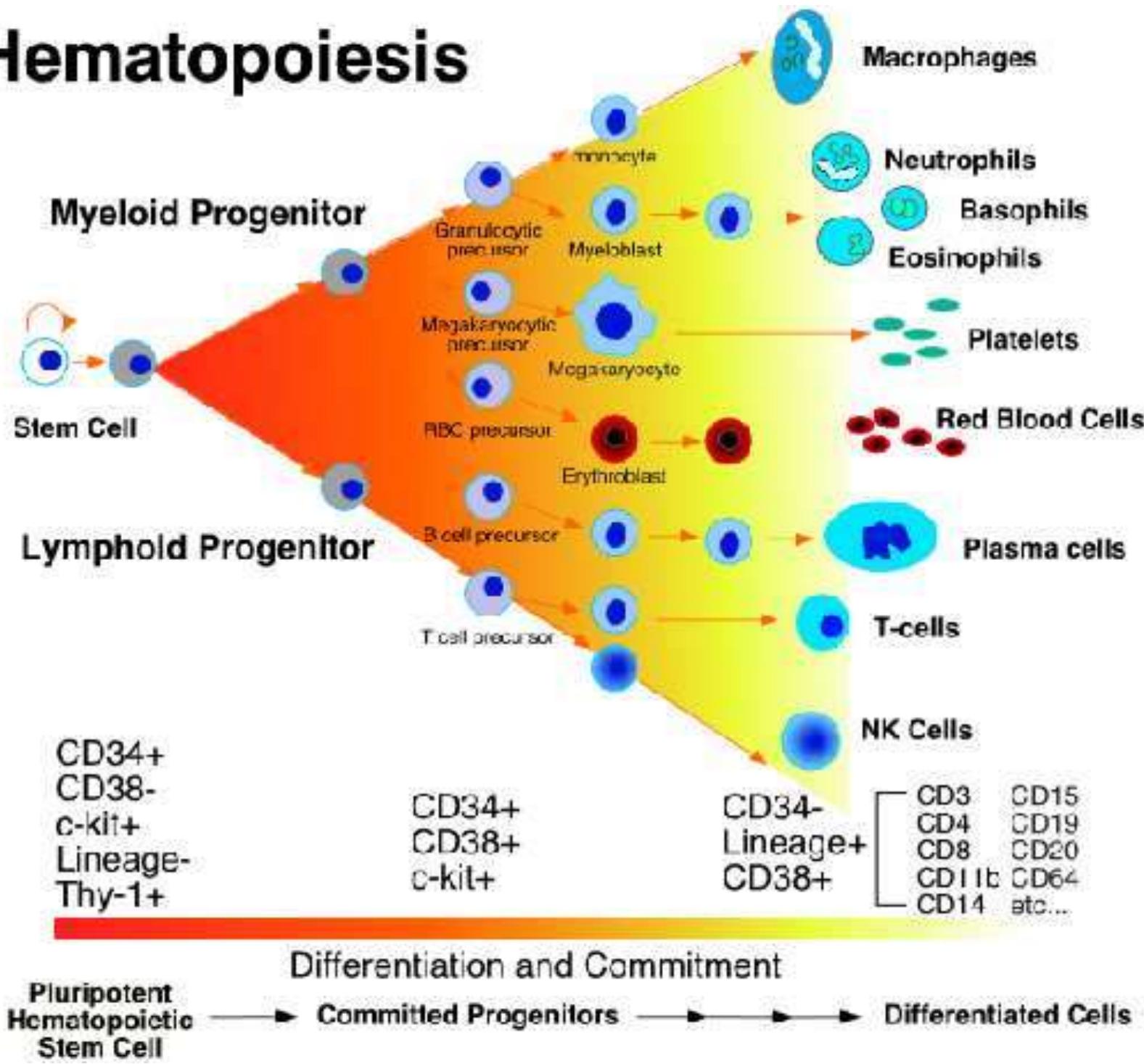
Hücre hazırlığı (dansite gradienti , eritrosit uzaklaştırma hangi lizis sol?, işlemden geçmemiş hücrelerle çalışmak )

Gate alma (CD45? FSC-SSC?)

Kullanılan antikor ( hangi klon? Seçilen florokrom?)

# **Hücre Serilerinin Belirlenmesi**

# Hematopoiesis



# Anormal hematopoez

Neoplastik hematopoetik hücrelerin kontroksuz  
çoğalması

Blast artışı (AML – ALL)

Bir veya daha fazla seride myelodisplazi

Periferik yükim artışına bağlı artmış hematopoetik hücre  
üretimi

Kemo/immünoterapi sonrası veya enfeksiyon yanıtına  
bağlı kemik iliği rejenerasyonu

Prekürsör B hücre artış

Mature B hücreleri var

Prekürsör B hücre diferansiasyonunda duraklama

Prekürsör B hücre rölatif artış

Mature B hücreleri az

**KEMİK İLİĞİ  
KOMPOZİSYONU**



**MAJOR DEĞİŞİKLİKLER  
(sayı, oran)**

# Normal Hematopoiez

*(Lochem, et al., 2004 Cytometry Part B 60B;1-13)*

## B hücre diferansiasyonu

Tdt / CD20 / CD19 / CD10  
CD45 / CD34 / CD19 / CD22

## Monositik diferansiasyon

CD34 / CD117 / CD45 / CD 13.33  
CD14 / CD33 / CD45 / CD34

## Granülositik diferansiasyon

CD34 / CD117 / CD45 / CD13+33  
CD16 / CD13 / CD45 / CD11b

## Eritroid diferansiasyon

CD71 / CD235a / CD45 / CD117

# Normal Hematopoez

(Lochem, et al., 2004 Cytometry Part B 60B;1-13)

## B hücre diferansiasyonu

TdT / CD20 / CD19 / CD10

CD45 / CD34 / CD19 / CD22

CD19 ----- pan B marker

CD10 ve CD20 ----- 4 farklı gelişim dönemi

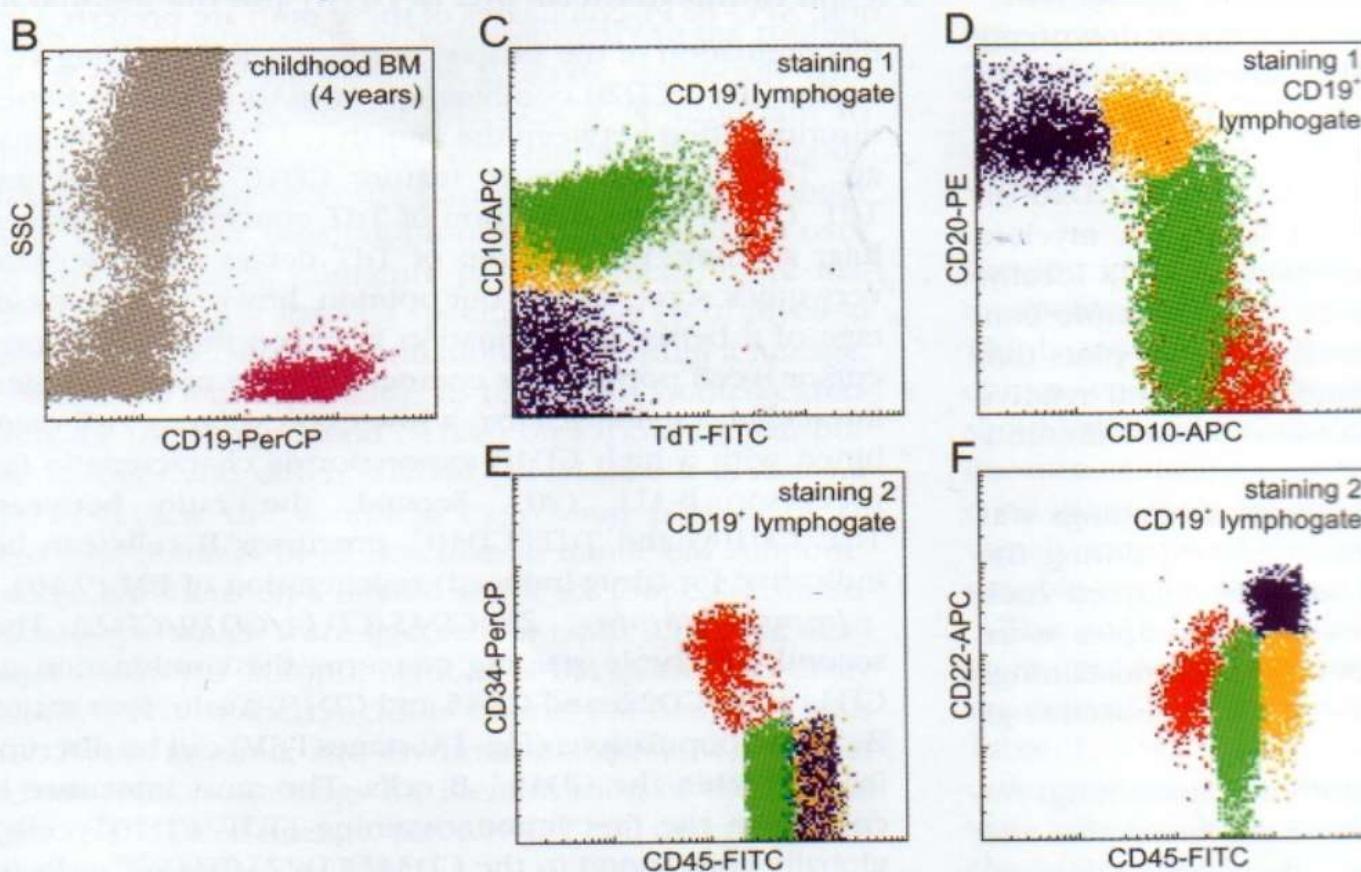
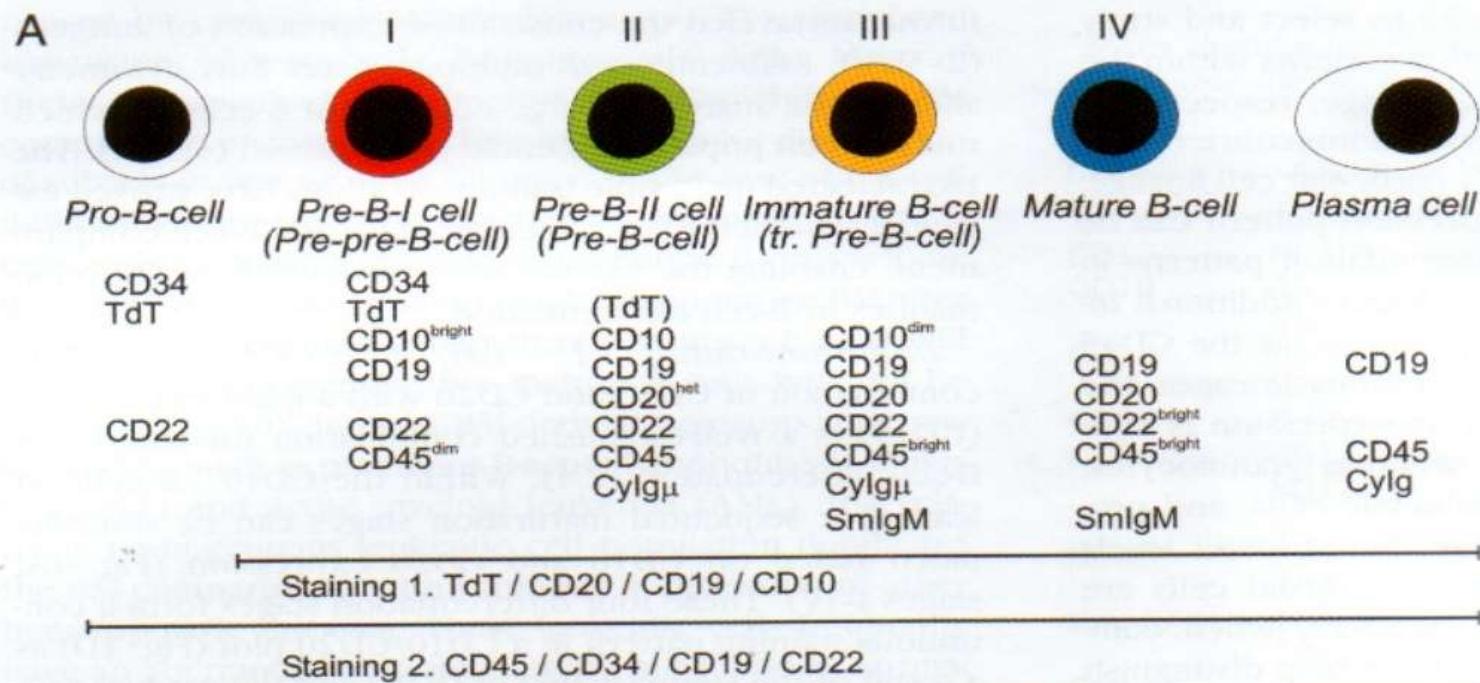
CD10 hücre olgunlaştıkça azalıyor

CD20 hücre olgunlaştıkça artıyor

CD10 (-) ve CD20 (-) hücreler plazma hücreleri

CD10 ve TdT ----- rejeneratif KI

TdT (+) CD10(+) / TdT (-) CD10 (+) prekürsör B hücre oranı



# Yaş-İmmunfenotip ilişkisi

IMMUNOPHENOTYPIC REFERENCE PATTERNS OF NORMAL BONE MARROW

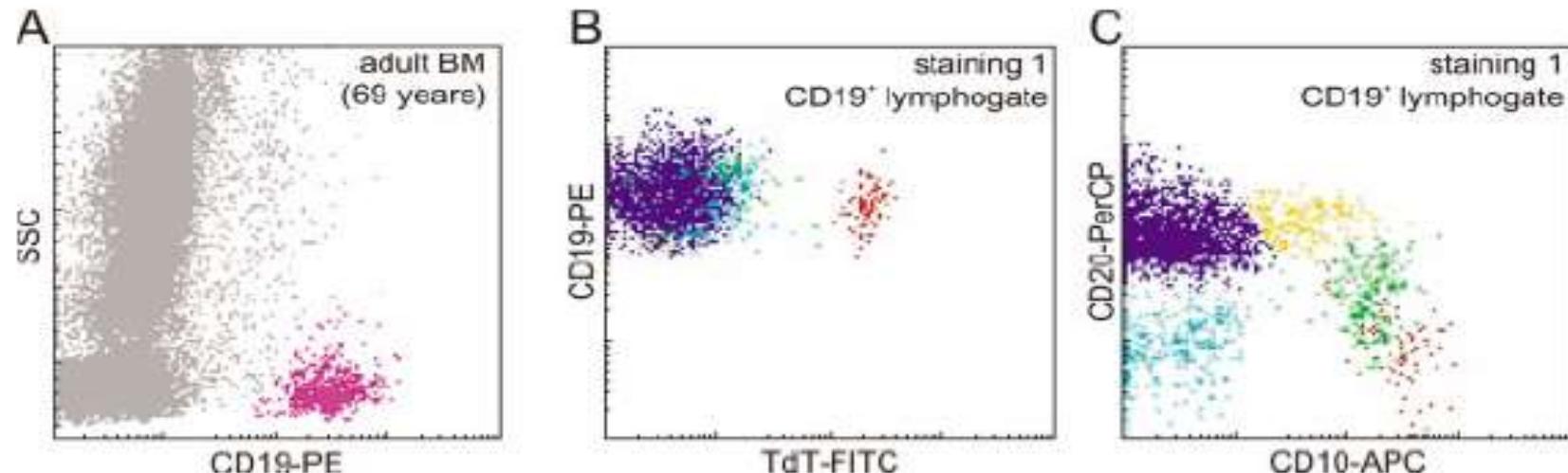


FIGURE 2

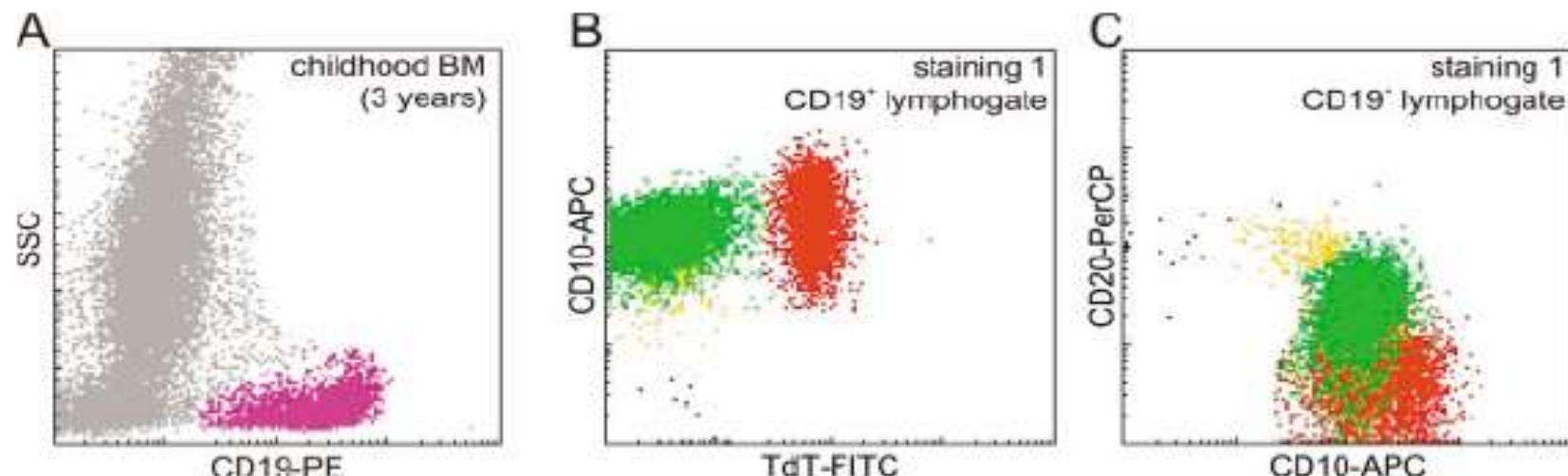


FIGURE 3

VAN LOCHM ET AL.

# Normal Hematopoez

(Lochem, et al., 2004 Cytometry Part B 60B;1-13)

## Monositik diferansiasyon

CD34 / CD117 / CD45 / CD13.33

AMAÇ: Monoblast ile myeloblastları diğer prekürsör hücrelerden immünenotipik olarak ayırmak

Monoblast ile myeloblast immünenotipik olarak ayrılamıyor

CD45 dim expresyon: tüm prekürsör hücreler (monoblast, myeloblast, prekürsör B hücreleri ve eritroblast)

CD34: granülositik, monositik, B hücre prekürsörlerinde pozitif

CD117: granülositik ve monositik prekürsörlerde pozitif

CD 13.33: granülositik ve monositik prekürsörlerde pozitif

CD45<sup>dim</sup> CD34<sup>+</sup> CD117<sup>+</sup> CD13.33<sup>+</sup> hücreler myelo/monoblast

CD45<sup>dim</sup> CD34<sup>+</sup> CD117<sup>-</sup> CD13.33<sup>-</sup> hücreler prekürsör B hücre

# Normal Hematopoez

*(Lochem, et al., 2004 Cytometry Part B 60B;1-13)*

## Monositik diferansiasyon

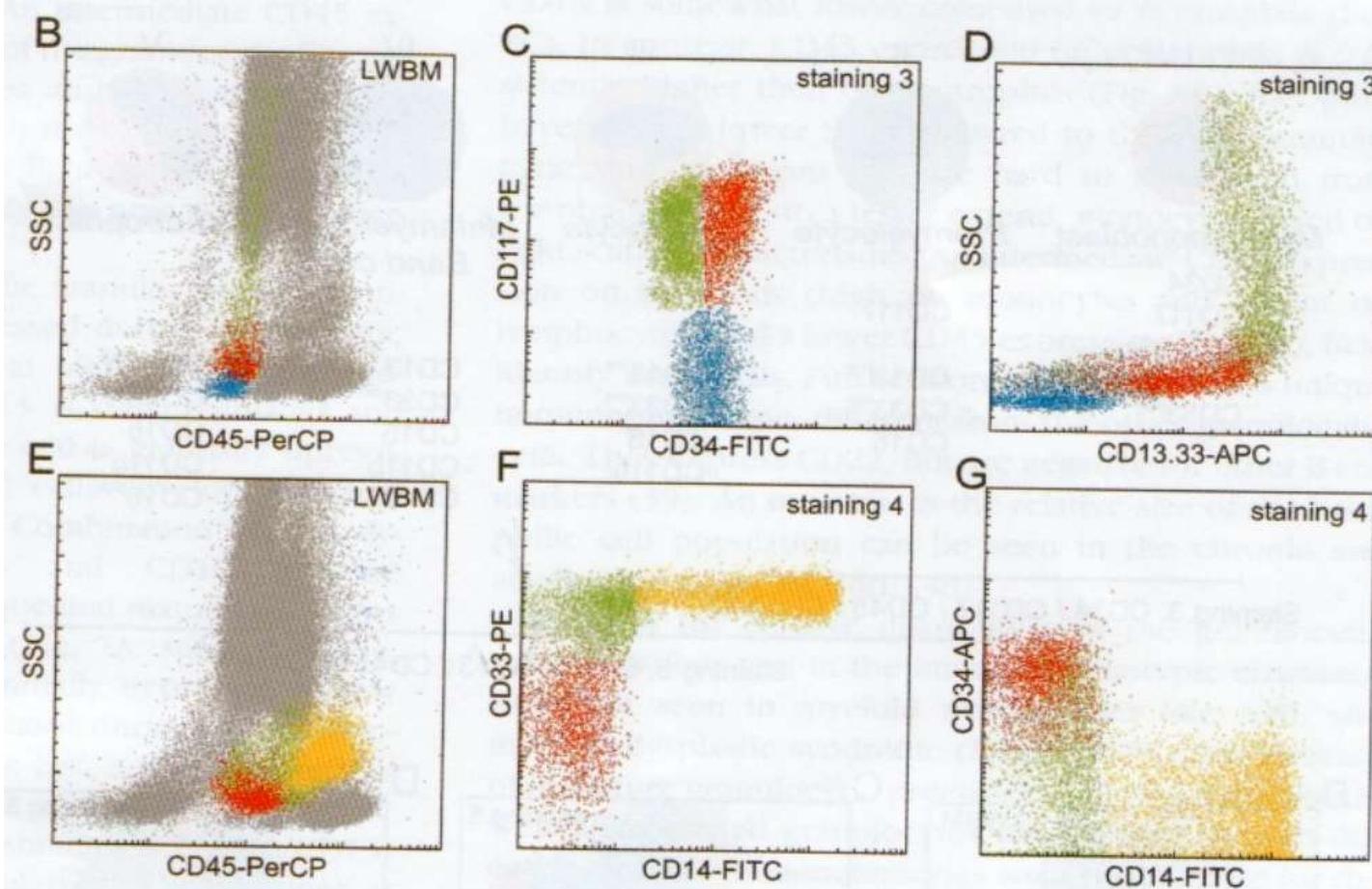
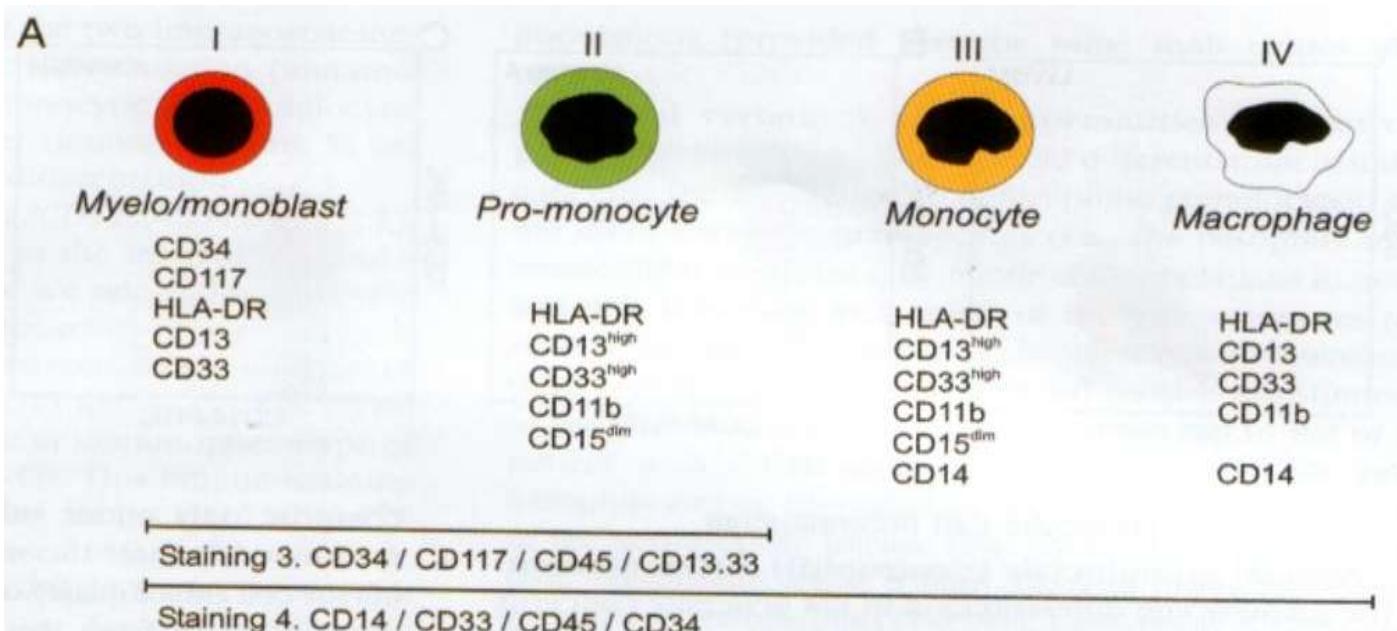
CD34 / CD117 / CD45 / CD13.33

CD14 / CD33 / CD45 / CD34

Promonosit: CD45<sup>orta</sup> CD34<sup>-</sup> CD33<sup>yüksek</sup>

Monoblast --- promonosit dönüşümü: CD33 artıyor ve CD34 kayboluyor (CD45 orta expresyon)

Monosit: CD14<sup>+</sup>



# Normal Hematopoez

*(Lochem, et al., 2004 Cytometry Part B 60B;1-13)*

## Granülositik diferansiasyon

**CD34/CD117/CD45/CD13+33**

AMAÇ: Monoblast ile myeloblastları diğer prekürsör hücrelerden immünfenotipik olarak ayırmak

**CD16/CD13/CD45/CD11b**

Ağırlıklı olarak nötrofil diferansiasyonu gözleniyor, eozinifil ve bazofil diferansiasyonu çok az

CD45 ekspresyonu

Granülositik seride orta

Lenfosit ve monositlerde yüksek

CD13 ekspresyonu

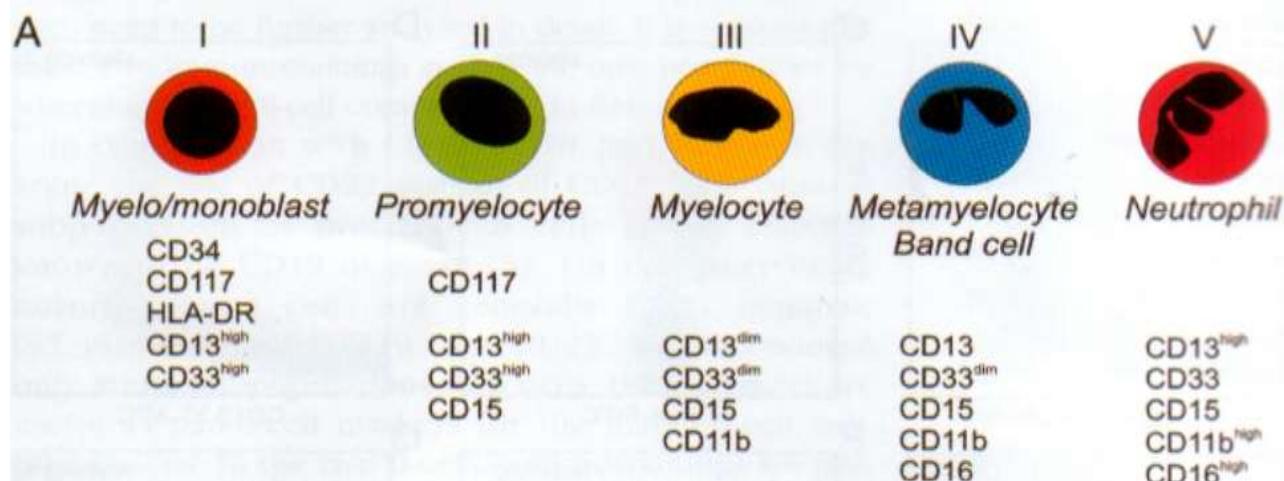
Myeloblast ve promyelositte yüksek

Myelositte dim

Segment nötrofilde yüksek

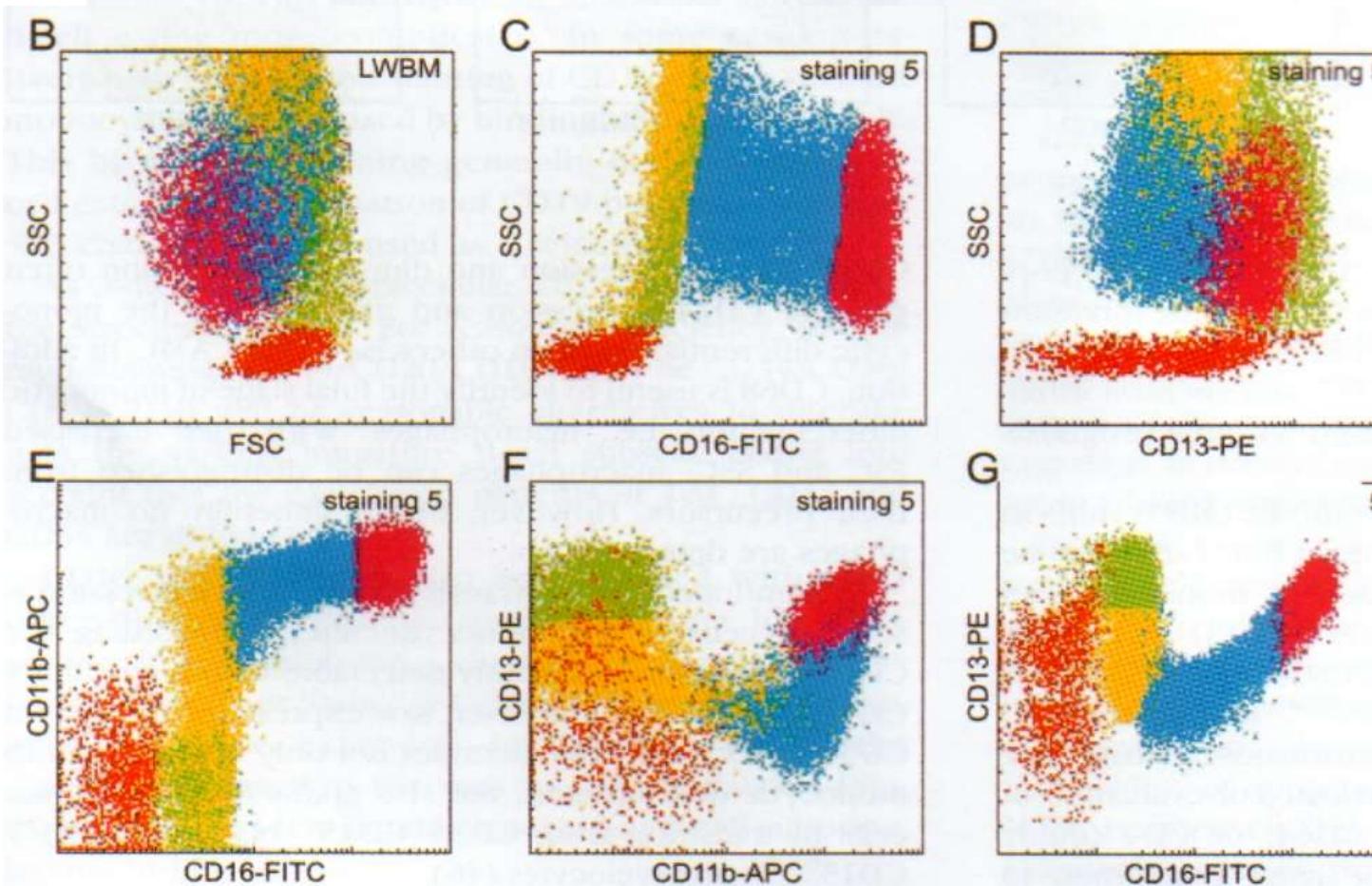
CD11b ve CD16 ekspresyonu

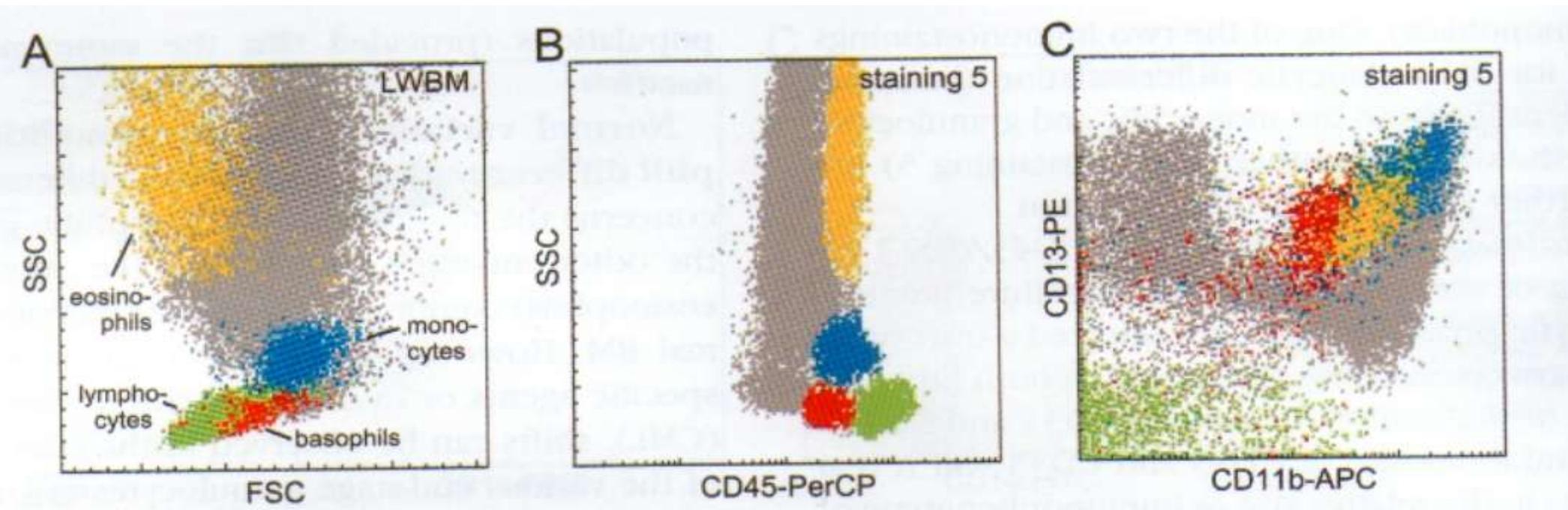
Başlangıçta düşük, hücre olgunlaşıkça artıyor



Staining 3. CD34 / CD117 / CD45 / CD13.33

Staining 5. CD16 / CD13 / CD45 / CD11b





# Normal Hematopoez

(Lochem, et al., 2004 Cytometry Part B 60B;1-13)

## Eritroid diferansiasyon

CD71 / CD235a / CD45 / CD117

Permabilizasyon / lize edici ajanlar ve NH4Cl çekirdekli eritroid hücreleri parçalamamaktadır.

CD45 ekspresyonu

CD117<sup>+</sup> ve CD71<sup>+</sup> olan eritroid prekürsör ve proeritroblastlarda dim pozitif,

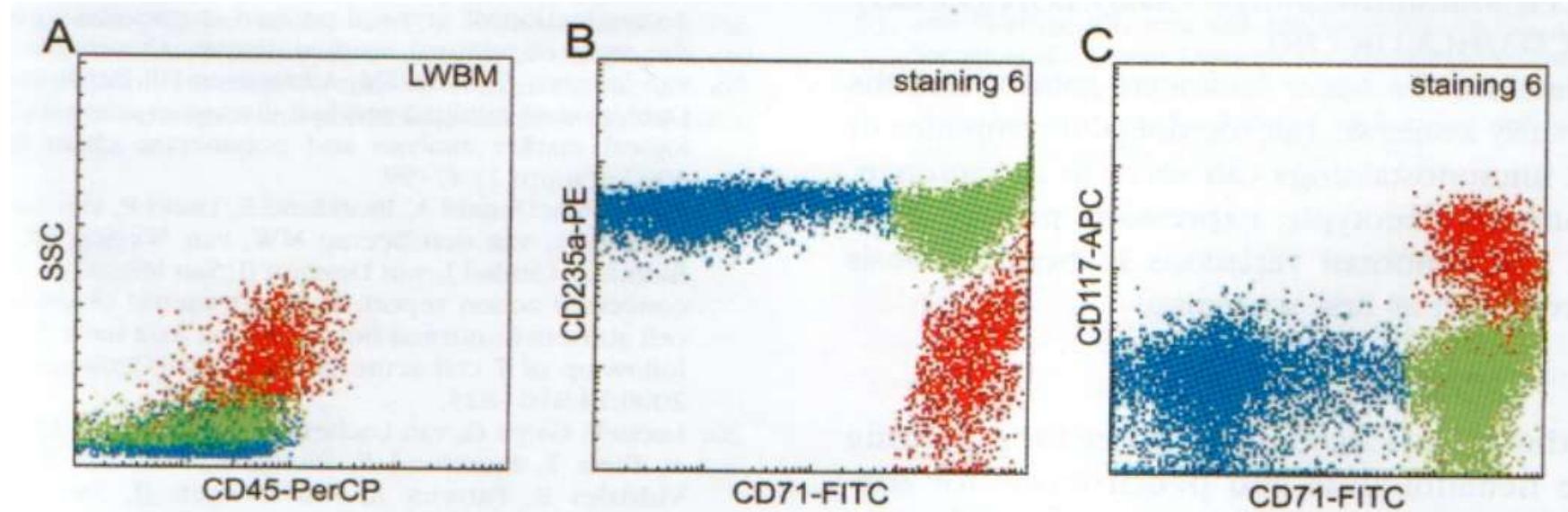
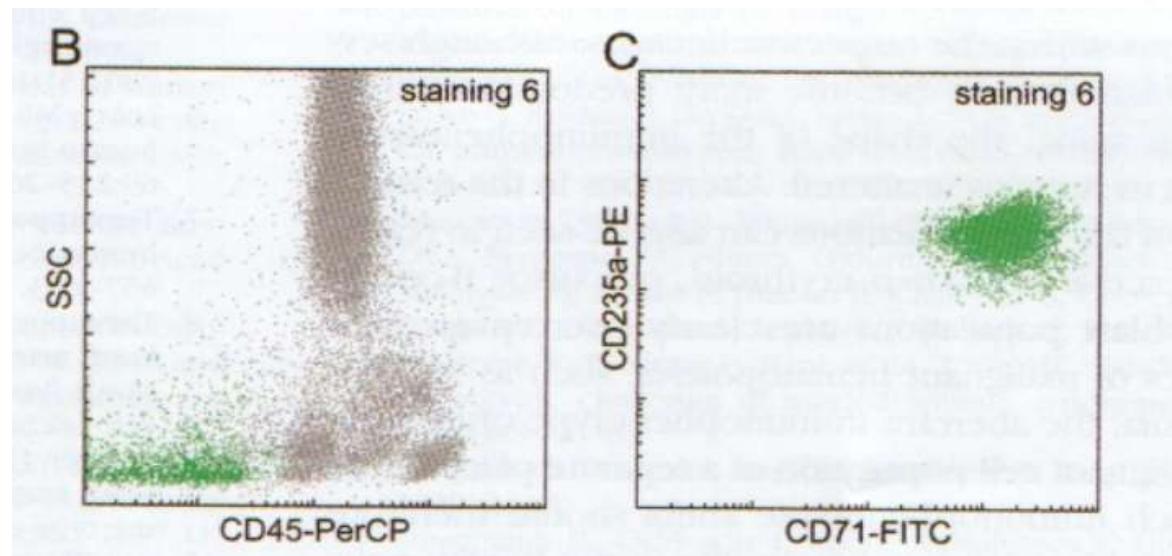
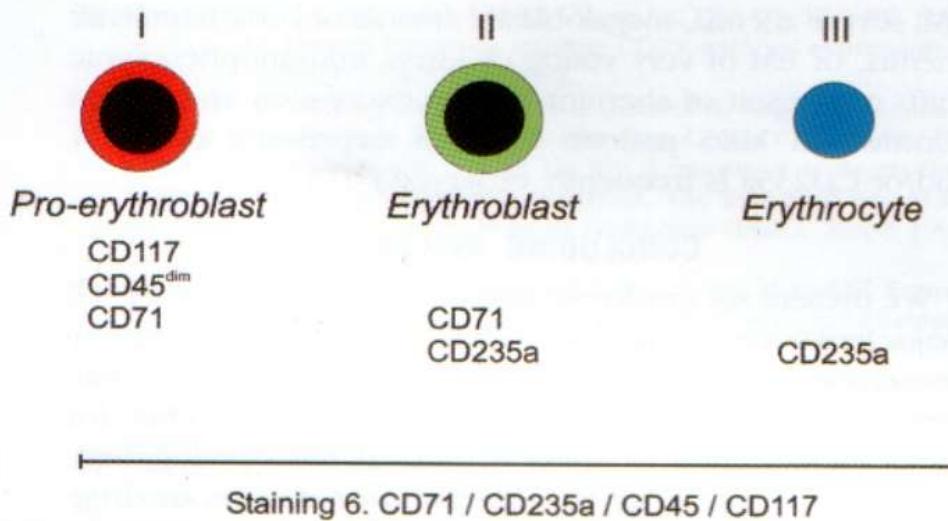
Daha olgun hücrelerde negatif

CD71 ekspresyonu

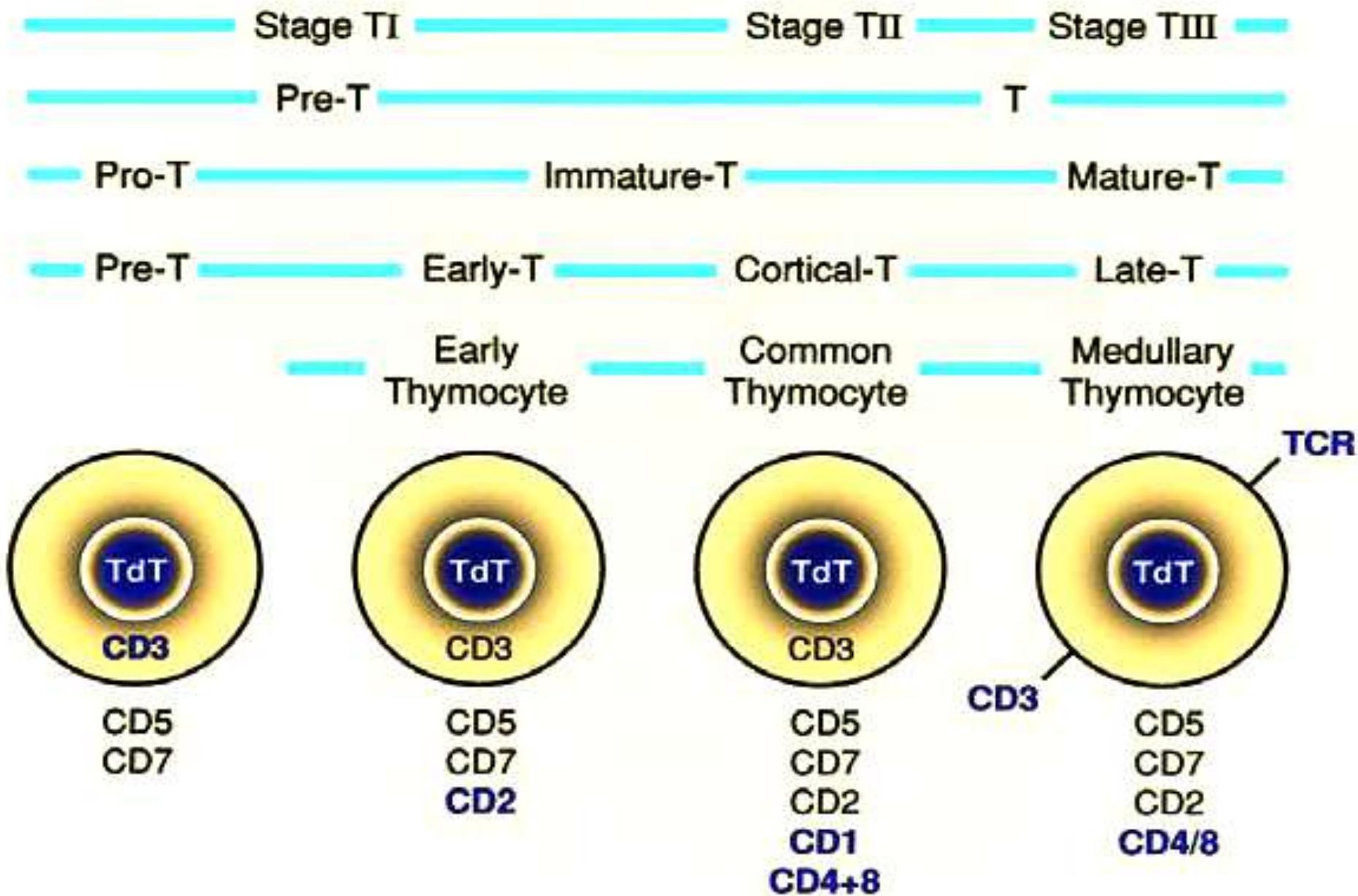
Olgunlaşma ile ekspresyonu artar, hücrenin çekirdeğini yitirmesi ile negatif olur

CD235a ekspresyonu

Eritroblast aşamasından itibaren eritrositler dahil pozitif



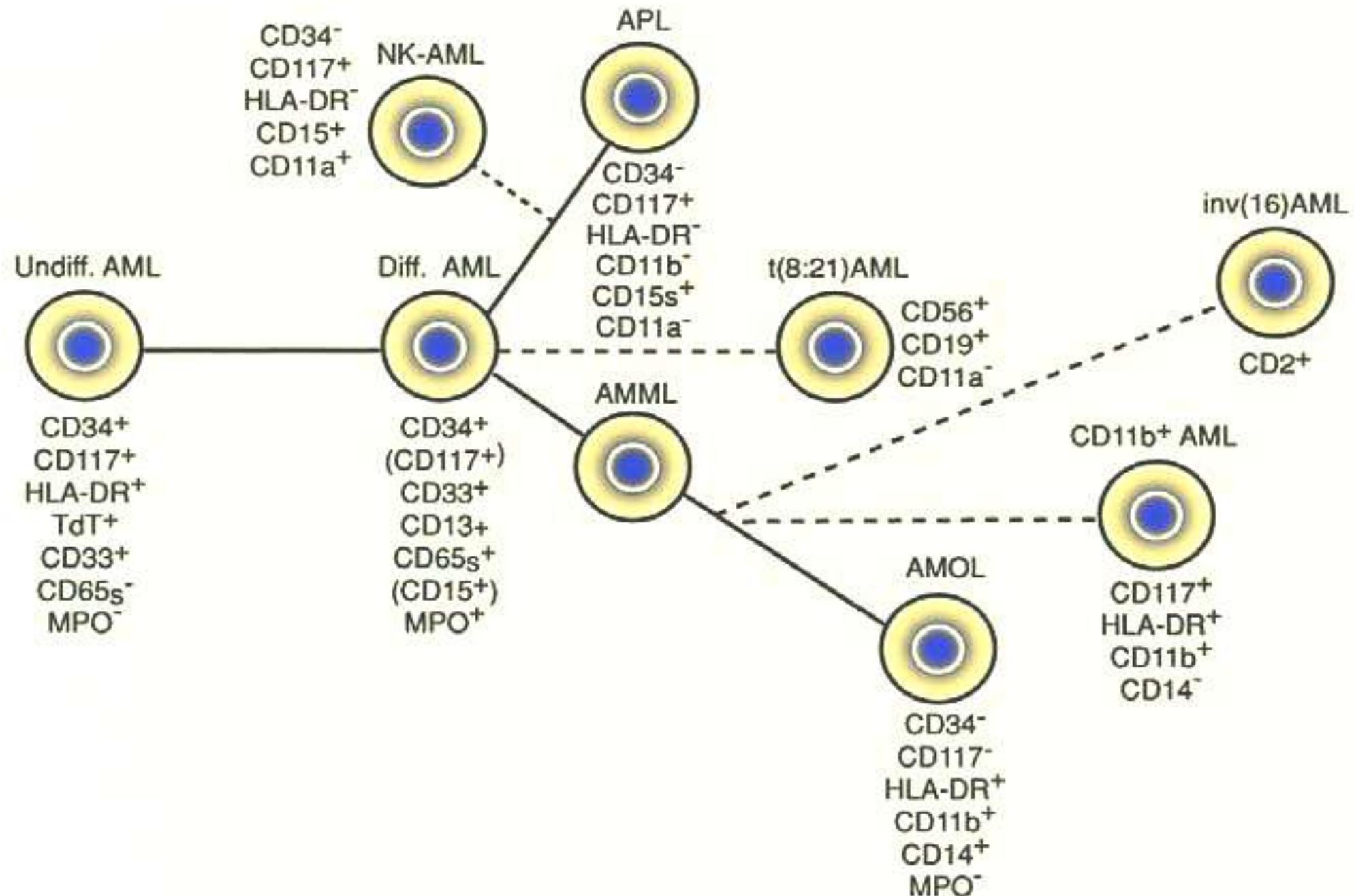
# T hücrelerin gelişimi



# Akut Lösemi Tanısında kullanılabilecek temel antikorlar

Hücre Serisi	B	T	M
Hücre serisi belirleyici	cCD79a cCD22 <b>CD19</b>	cCD3 <b>CD3</b> <b>CD7</b>	<b>cMPO</b> <b>CD13</b> <b>CD33</b>
Matürasyon belirleyici	<b>CD34</b> <b>TdT</b>	<b>CD34</b> <b>TdT</b>	<b>CD34, D117</b> CD15,CD11a CD11b,HLA DR
Diğer	<b>CD10, CD20</b> CD22,c IgM K, $\lambda$	CD1, CD2 CD3, CD5 CD4,CD8	<b>CD14, CD68</b> CD41, CD61 cCD235a(Gly-A)

# AML SINIFLAMASI



# ALL SINIFLAMASI

B ALL

-Prekürsor B ALL

- Pro-B ALL
- Common B ALL
- Pre-B ALL
- Transisionel B ALL
- Matür B ALL

T ALL

-İmmatür T ALL

Pro-T ALL

İmmatür T ALL

-Common (Kortikal) T ALL

Pretimik T ALL

Timik T ALL

- Matür T ALL

# BİFENOTİPİK LÖSEMI

## TANIM:

Birden fazla serİYE aİt CD işaretleri

Mixed lineage

Hibrid akut lösemi

acute leukemias of ambiguous lineage

Bilineal akut lösemi

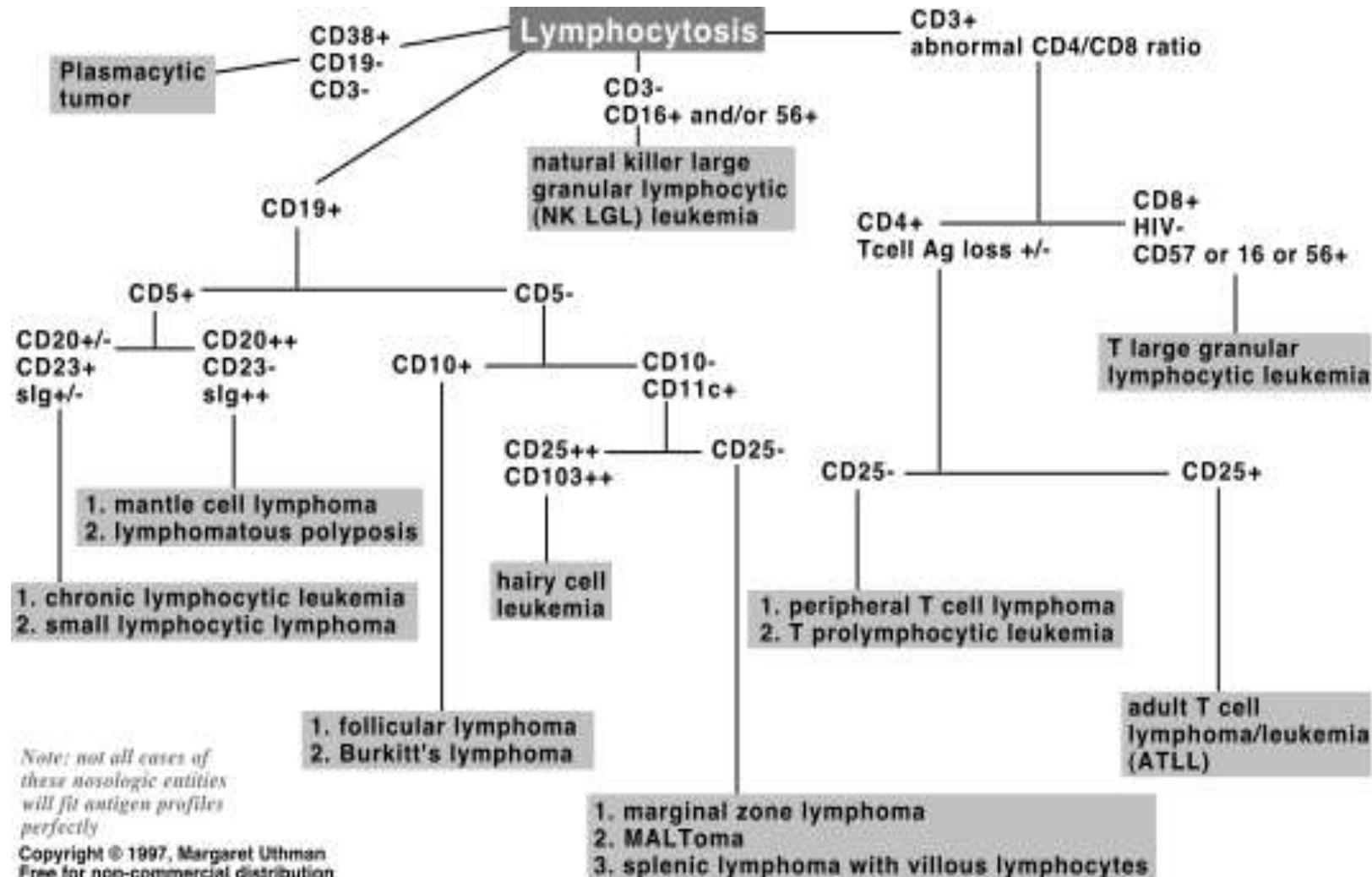
Table 4. Scoring system for the definition of biphenotypic acute leukaemias according to EGIL.

Points	B-lineage	T-lineage	Myeloid lineage
2	CD79a Cylgμ CD22	CD3 TCRαβ TCRγδ	MPO (lysozyme)
1	CD19 CD10 CD20	CD2 CD5 CD8 CD10	CD13 CD33 CD65 CD117
0.5	TdT CD24	TdT CD7 CD1a	CD14 CD15 CD64

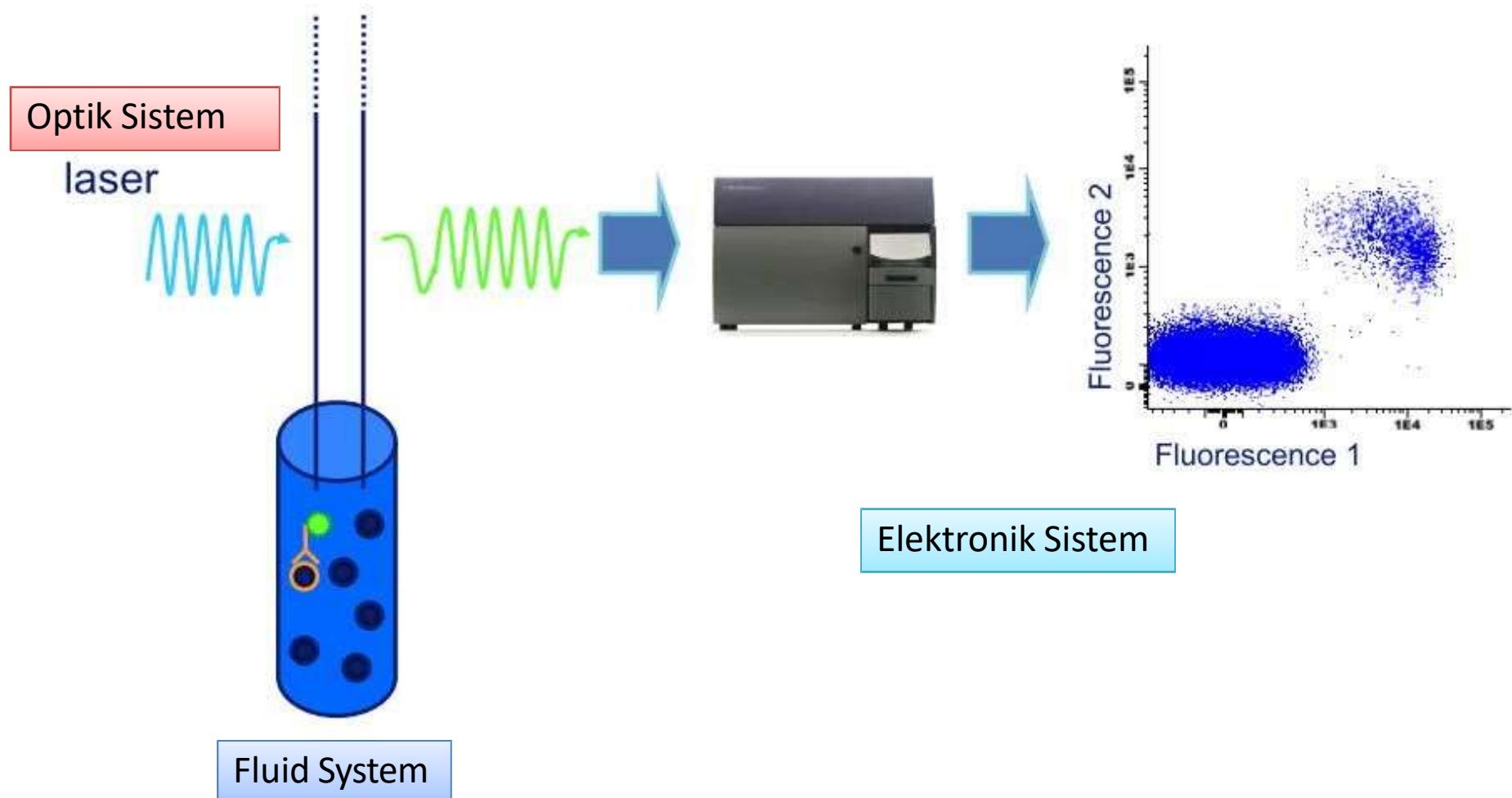
Biphenotypic acute leukaemia is defined when scores are over 2 for the myeloid markers and over 2 for one of the lymphoid lineages. For further details see refs. [19,51]. Unfortunately, this scoring system will erroneously define many obvious pro-B-ALL and some AML as biphenotypic leukaemias.

European Group for  
the Immunological  
Characterization of  
Leukemias  
(EGIL)

# LENFOMA İMMUNFENOTİPLENDİRME

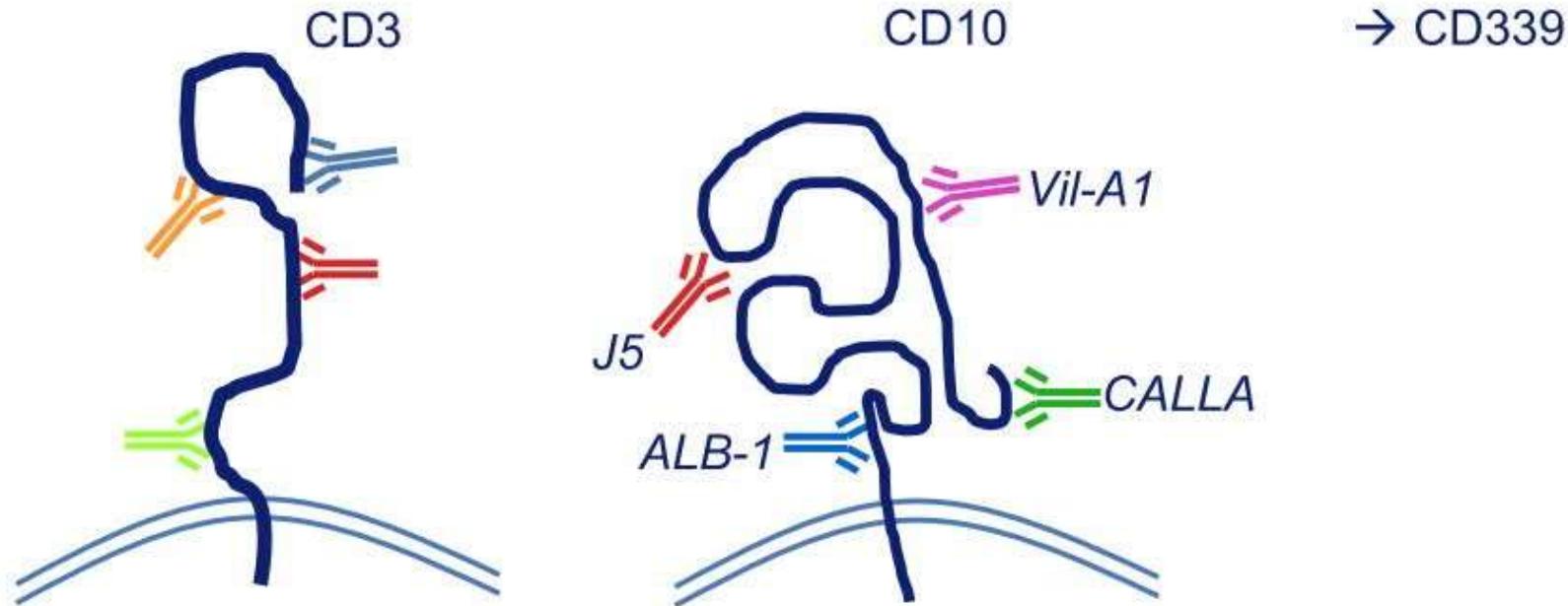


# Flow Cytometry



# Monoklonal Antikorlar

- CD=cluster of differentiation



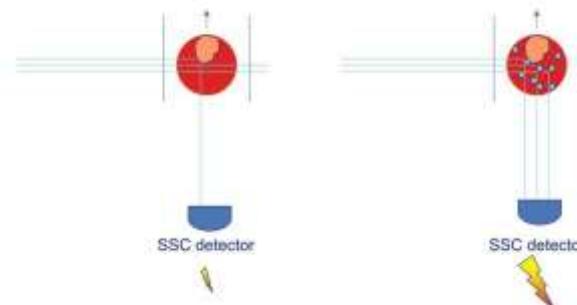
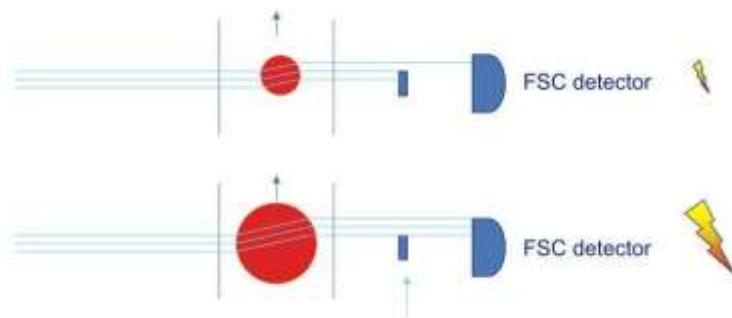
# Light Scatter

## Forward scatter (FSC)

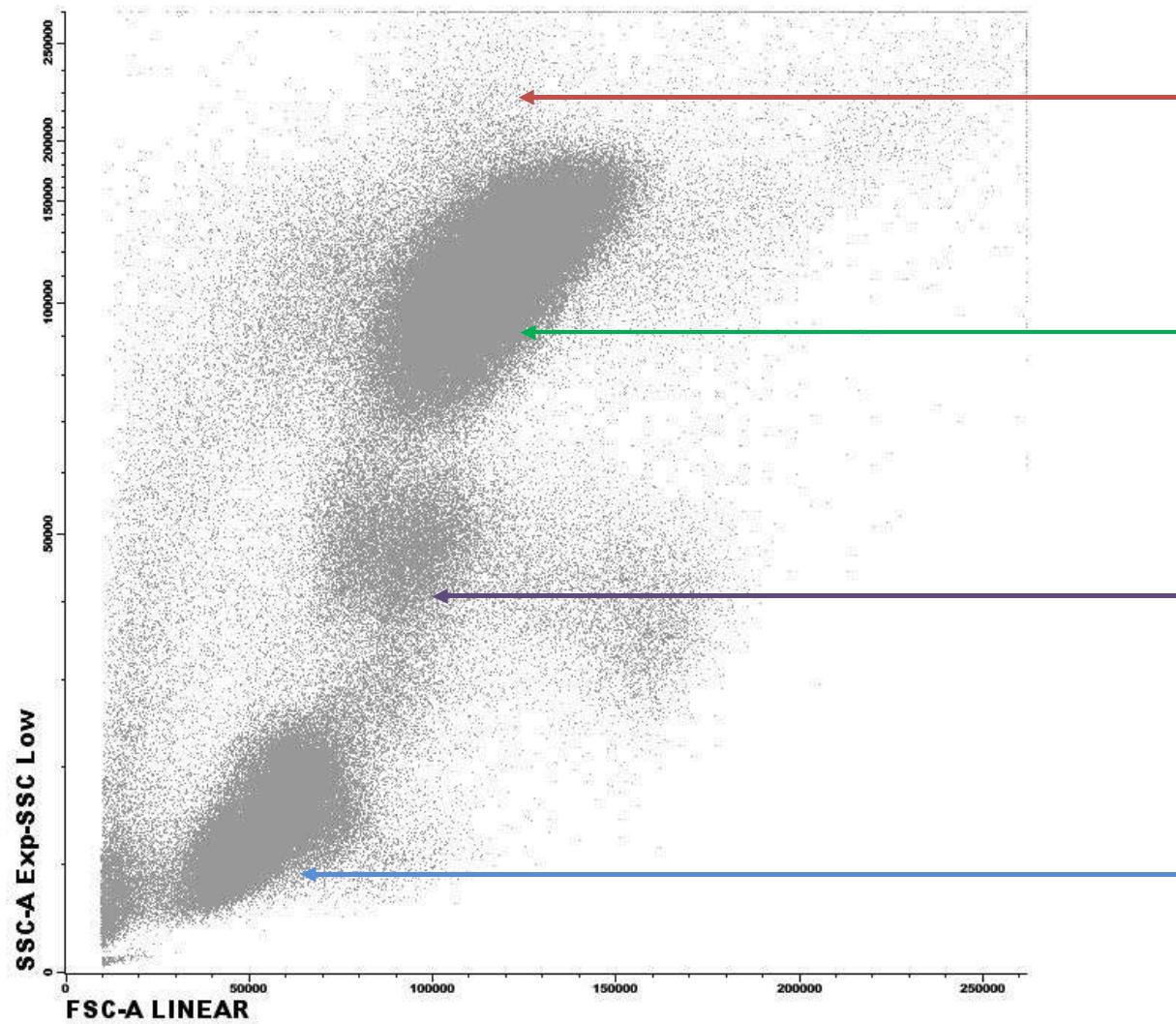
- Hücre büyüklüğü

## Side scatter (SSC)

- Hücre granularitesi



# Periferik Kan



# İmmüfenotiplendirme Medde Standardizasyon

- CLSI (Clinical Laboratory Standards Institute)
  - Hematolenfoid malignensilerde FC
- CCS (Clinical Cytometry Society)
  - 2006 Bethesda International Consensus' a göre hematolenfoid malignensilerde FC
- ESCCA (European Society for Clinical Cell Analysis)
  - [www.escca.eu](http://www.escca.eu)
- European Leukemia Net
  - [www.leukemia-net.org](http://www.leukemia-net.org)
- Latin American Consensus
  - [Cytometry B Clin Cytom. 2006 Jan;70\(1\):39-44.](http://Cytometry B Clin Cytom. 2006 Jan;70(1):39-44)

# **iki açıdan konsensuslar yetersiz!**

## **1. İmmunfenotiplendirme için önerdikleri markerların spesifik olmaması**

- MoAb klonu?
- Hangi florokrom?
- Tanı için uygun antikor kombinasyonu?

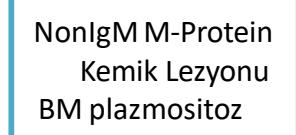
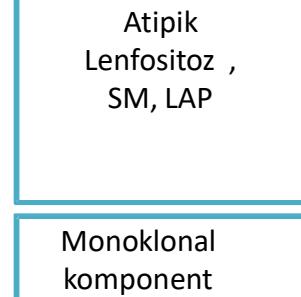
# **iki açıdan konsensuslar yetersiz!**

## **2. Net çalışma protokollerinin olmaması.**

- Panellerde hangi florokromlar bir arada olmalı?  
(kompenzasyon..)**
- Örnek hazırlama teknikleri**
- Uygun analiz nasıl yapılmalı?**



## Klinik endikasyon



BOS, vitreus biyopsisi vb şüpheli lenfoma tutulumu olabilecek hücre miktarının düşük olduğu örnekler

## Tarama

ALOT

LST

PCST

SST

Reaktif/Poliklonal

Klonal/aberant

Reaktif/Poliklonal

Klonal

Diger

BCP-ALL

T-ALL

AML/MDS

BCP-ALL' nin  
subtipleri

T-ALL' nin  
subtipleri

AML'nin  
subtipleri

BCR/ABL

B-CLPD

Limited

KLL

Non-KLL

B-CLPD

Broad

KLL

MCL

FCL

HCL

diger  
klonalB

T-CLPD

Aberrant γδ

Aberrant αβ

Reaktif

NK-CLPD

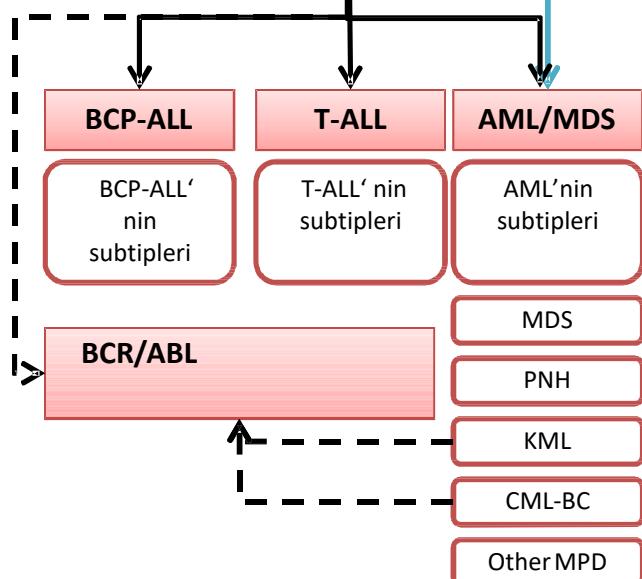
Aberrant NK cells

Reaktif

PCD

Atipik Plazma  
hücresi

## Tanımlama



BCR/ABL

# ALOT (Acute Leukemia Orientation Tube)

## Design for

- Akut Lösemiörneğinde ilk aşama blastik hücrenin hangi serİYE ait olduğunu değerlendirmek
- Daha sonra uygun ikinci bir panel B-ALL, T-ALL ,AML/MDS yapılır

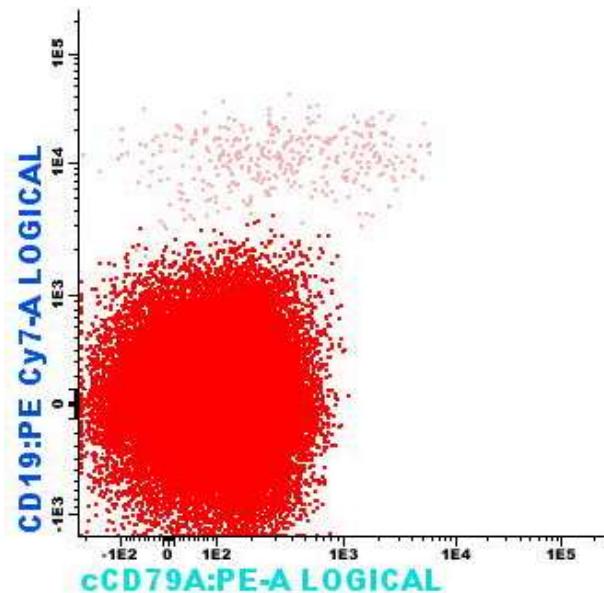
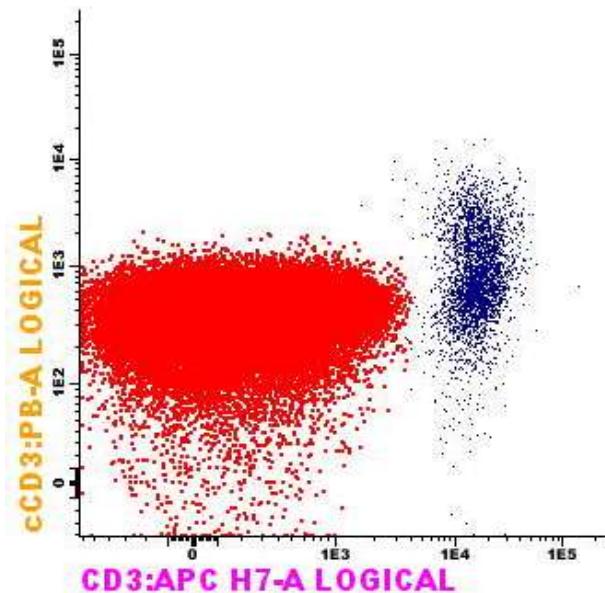
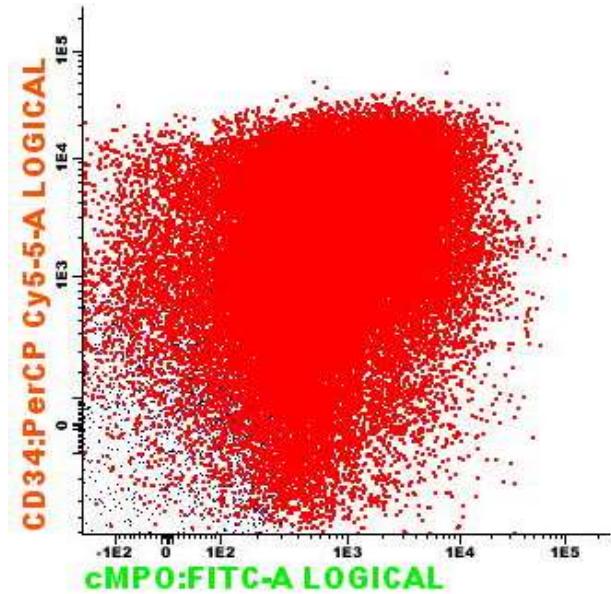
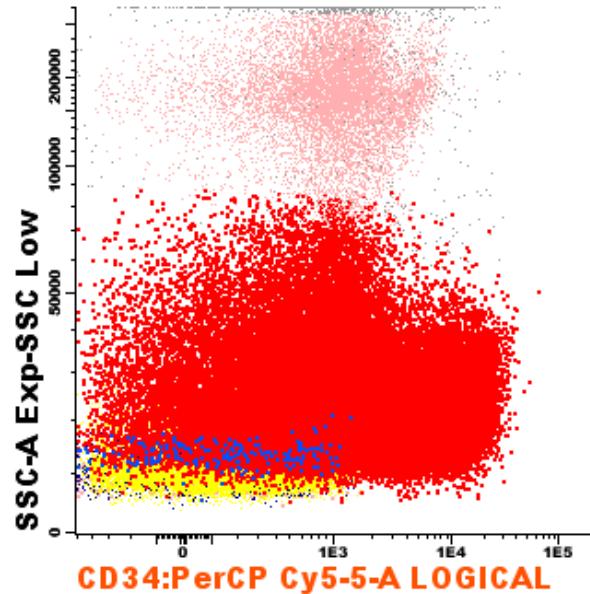
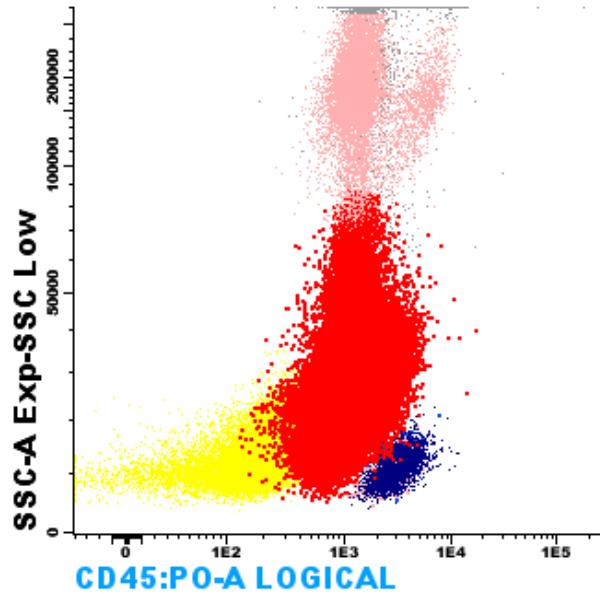
Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APCH7
cyCD3	CD45	cymPO	cyCD79a	CD34	CD19	CD7	smCD3

Target Antigen	Fluorochrome conjugate	Gating markers (first level)	Gating Markers (second level)	Immaturity markers	Lineage markers
cyMPO	FITC		X		My
cyCD79a	PE		X		B, T
CD34	PerCP Cy5.5	X		X	-
CD19	PECY7		X		B, My
CD7	APC		X	X	T, My
smCD3	APCH7		X		T
cyCD3	Pacific Blue		X		T
CD45	PO	X		X	-

## Panel 1. Composition of ALOT and technical information on reagents

	PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7
	CyCD3	CD45	CyMPO	CyCD79a	CD34	CD19	CD7	SmCD3
Marker	Fluorochrome	Clone	Source	Catalogue number	Application in EuroFlow panel			µl/test
CyCD3	PacB	UCHT1	BD Biosciences	558117	ALOT (also in T-ALL)			7
SmCD3	APCH7	SK7	BD Biosciences	641415	ALOT (also in T-ALL)			3
CD7	APC	124-1D1	eBioscience	17-0079-42	ALOT (also in T-ALL, AML)			2
CD19	PECy7	J3-119	Beckman Coulter	IM3628	ALOT (also in LST, SST, BCP-ALL, PCD)			5
CD34	PerCP Cy5.5	8G12	BD Biosciences	347222	ALOT (also in BCP-ALL, AML)			7
CD45	PacO	HI30	Invitrogen	MHCD4530	ALOT (also in LST, SST, BCP-ALL, T-ALL, AML, B-CLPD, T-CLPD, NK-CLPD)			5
CyCD79a	PE	HM57	Dako	R7159	ALOT			5
CyMPO	FITC	MPO-7	Dako	F0714	ALOT			3

# ALOT / AML



# LST (Lenfosit Tarama Tüpü)

## Design for

- İmmunfenotip olarak klonal ve aberant matür lenfositler değerlendirilir
- Atipik lenfositlerin normal ve reaktif lenfositlerden ayırmayı yapılır

Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20-CD4	CD45	CD8-Smlgλ	CD56-Smlgκ	CD5	CD19-TCRγδ	SmCD3	CD38

**12 COLORS IN ONE TUBE**  
**14 PARAMETERS**

# LST (Lenfosit tarama tüpü)

Marker	Hangi hücrenin belirteci	Positif tanı	Population subgrup	tanısal sınıflandırma	MRD marker	Prognostic
CD45	Mature lymphocytes and B-cell precursors	X	X			
CD19	B-cells, T- and NK-cells by exclusion	X		X	X	
CD20	B-cells, T- and NK-cells by exclusion	X	X	X	X	
Smlgk and λ	Smlg+ B-cells	X	X		X	
CD38	Plasma cells and B-cell precursors	X	X	X	X	X
SmCD3	T-cells, B- and NK-cells by exclusion	X		X	X	
CD4	CD4+ T-cells	X	X	X	X	
CD8	CD8hi T-cells and CD8lo NK-cells	X	X	X	X	
CD56	NK-cells	X	X		X	
TCRγδ	TCRγδ+ T-cells	X	X		X	
CD5	T-cells	X		X	X	

Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20-CD4	CD45	CD8-SmIgλ	CD56-SmIgκ	CD5	CD19-TCRγδ	SmCD3	CD38

## Identify

- Non-Hematopoetik hücreler (CD45, CD56, CD38, FSC, SSC)

Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20-CD4	CD45	CD8-SmIgλ	CD56-SmIgκ	CD5	CD19-TCRγδ	SmCD3	CD38

## Identify

- Non-Hematopoetik hücreler (CD45, CD38, CD56)
- **Hematopoetik hücreler**
  - **B-cell (CD19, CD20, CD45)**

Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20-CD4	CD45	CD8-SmIgλ	CD56-SmIgκ	CD5	CD19-TCRγδ	SmCD3	CD38

## Identify

- Non-Hematopoetik hücreler(CD45, CD38, CD56)
- Hematopoetik hücreler
  - B-cell (CD19, CD20, CD45)
    - Kappa (CD19, CD20, CD45, SmIgκ)
    - Lambda (CD19, CD20, CD45, SmIgλ)

Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20-CD4	CD45	CD8-SmIgλ	CD56-SmIgκ	CD5	CD19-TCRγδ	SmCD3	CD38

## Identify

- Non-Hematopoetik hücreler(CD45, CD38, CD56)
- Hematopoetik hücreler
  - B-cell (CD19, CD20)
    - Kappa (CD19, CD20, CD45, SmIgκ)
    - Lambda (CD19, CD20, CD45, SmIgλ)
  - T-cell (**SmCD3, CD45**)

Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20-CD4	CD45	CD8-SmIgλ	CD56-SmIgκ	CD5	CD19-TCRγδ	SmCD3	CD38

## Identify

- Non-Hematopoietic cells(CD45, CD38, CD56)
- Hematopoietic cells
  - B-cell (CD19, CD20)
    - Kappa (CD19, CD20, CD45, SmIgκ)
    - Lambda (CD19, CD20, CD45, SmIgλ)
  - T-cell (SmCD3, CD45)
    - Helper T (SmCD3, CD45, CD4)
    - Cytotoxic T (SmCD3, CD45, CD8)
    - γδ+ (SmCD3, CD45, TCRγδ)

Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20-CD4	CD45	CD8-SmIgλ	CD56-SmIgκ	CD5	CD19-TCRγδ	SmCD3	CD38

## Identify

- Non-Hematopoetik hücreler(CD45, CD38, CD56)
- **Hematopoetik hücreler**
  - B-cell (CD19, CD20)
    - Kappa (CD19, CD20, CD45, SmIgκ)
    - Lambda (CD19, CD20, CD45, SmIgλ)
  - T-cell (SmCD3, CD45)
    - Helper T (SmCD3, CD45, CD4)
    - Cytotoxic T (SmCD3, CD45, CD8)
    - γδ+ (SmCD3, CD45, TCRγδ)
  - **NK-cell (SmCD3, CD56, CD45, CD38)**

Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20-CD4	CD45	CD8-SmIgλ	CD56-SmIgκ	CD5	CD19-TCRγδ	SmCD3	CD38

## Identify

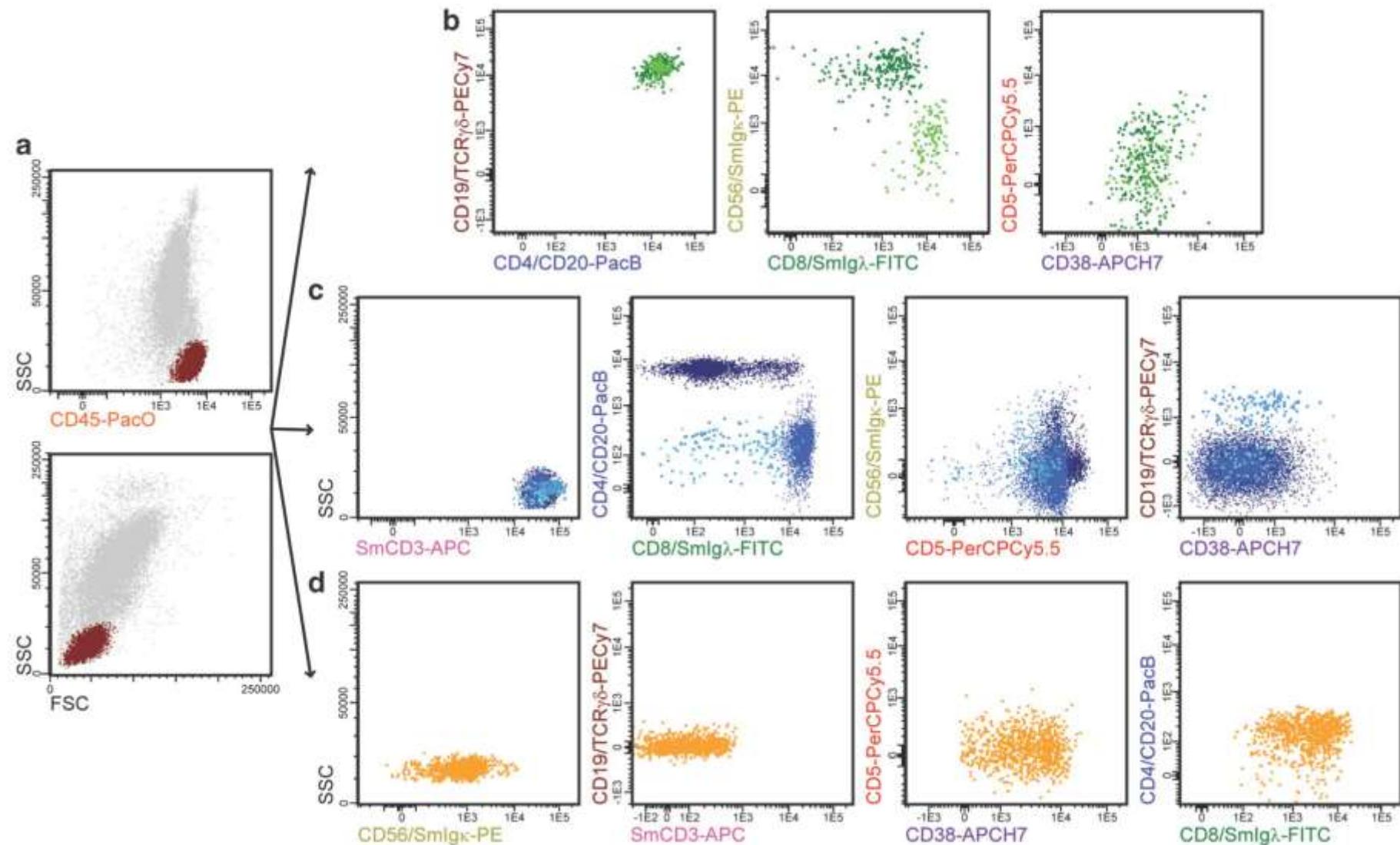
- Non-Hematopoetik hücreler(CD45, CD38, CD56)
- **Hematopoetik Hücreler**
  - B-cell (CD19, CD20)
    - Kappa (CD19, CD20, CD45, SmIgκ)
    - Lambda (CD19, CD20, CD45, SmIgλ)
  - T-cell (SmCD3, CD45)
    - Helper T (SmCD3, CD45, CD4)
    - Cytotoxic T (SmCD3, CD45, CD8)
    - γδ+ (SmCD3, CD45, TCRγδ)
  - NK-cell (SmCD3, CD56, CD45, CD38)
  - **Plasma cells (CD19, CD38, CD45, CD56)**

Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD20-CD4	CD45	CD8-SmIgλ	CD56-SmIgκ	CD5	CD19-TCRγδ	SmCD3	CD38

## Identify

- Non-Hematopoietik hücreler(CD45, CD38, CD56)
- **Hematopoietik hücreler**
  - B-cell (CD19, CD20)
    - Kappa (CD19, CD20, CD45, SmIgκ)
    - Lambda (CD19, CD20, CD45, SmIgλ)
  - T-cell (SmCD3, CD45)
    - Helper T (SmCD3, CD45, CD4)
    - Cytotoxic T (SmCD3, CD45, CD8)
    - γδ+ (SmCD3, CD45, TCRγδ)
  - NK-cell (SmCD3, CD56, CD45, CD38)
  - Plasma cells (CD19, CD38)
- **B-CLPD özgün markerlar (CD19, CD20, CD45)**

# Normal periferik kan / LST



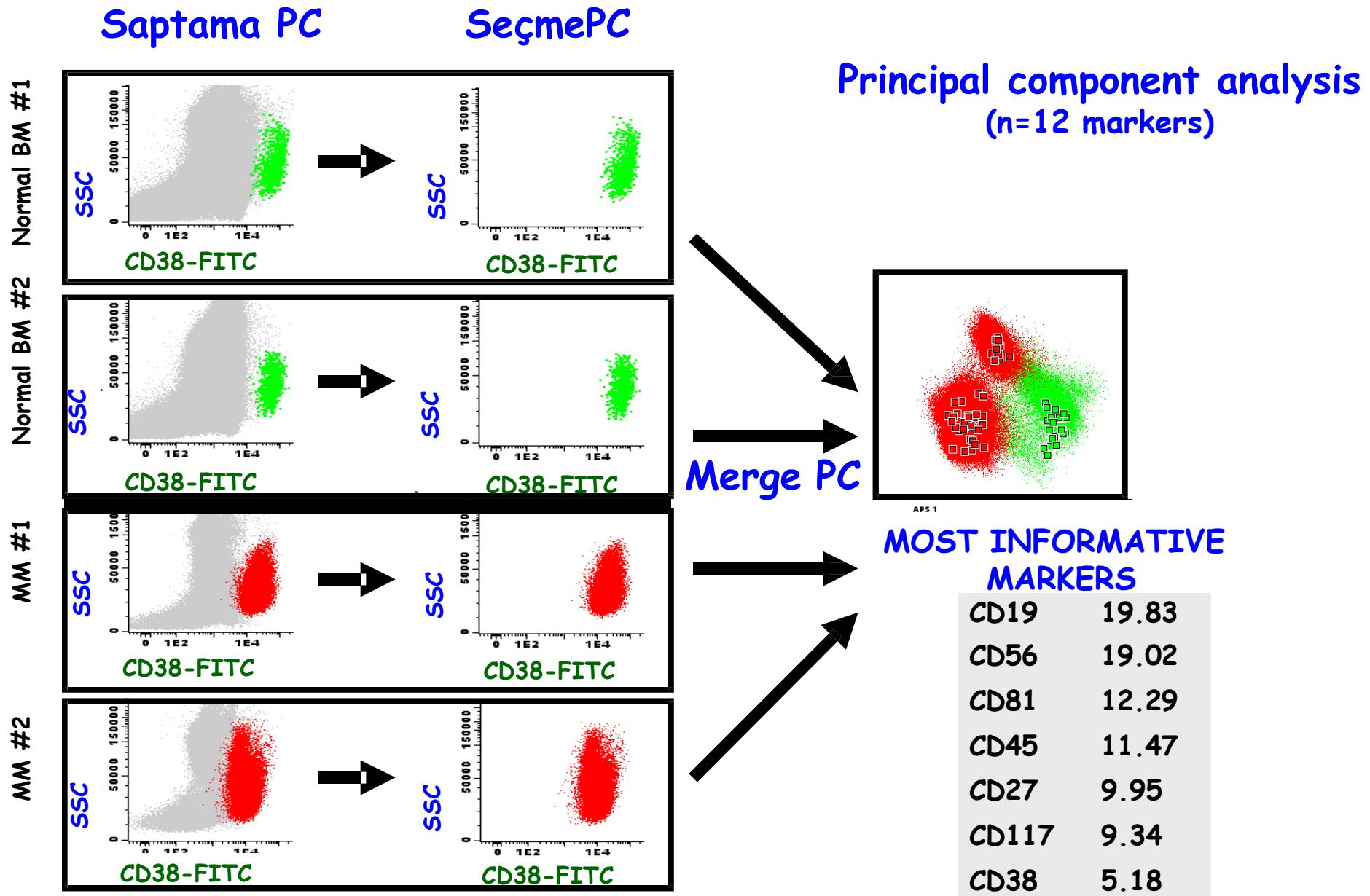
# PCST (Plasma hücresi tarama tüpü)

Pac Blue	Pac Orange	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD45	CD138	CD38	CD56	CD27	CD19	CyIgκ	CyIgλ

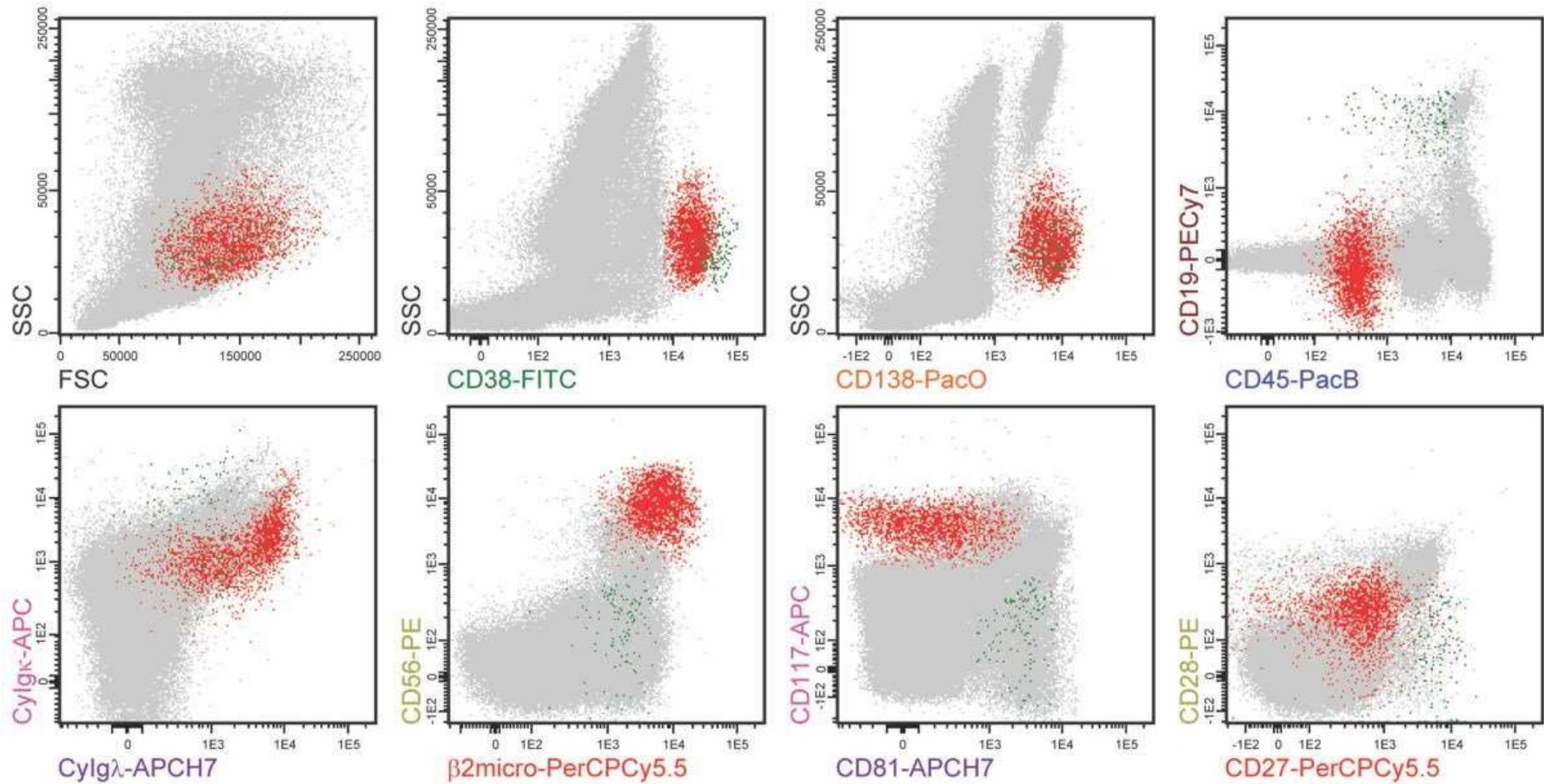
# PCD (Plasma Cell Dyscrasia)

Tube	Target Antigen	Identification of plasma cells	Aberrant markers	2 <sup>nd</sup> diagnostic level marker	Assessment of plasma cell clonality
BB markers	CD38	X	C		
	CD138	X			
	CD45	X	C		
	CD19	X	C		
Tube 1	Cylgκ				X
	Cylgλ				X
	CD56		C		
	β2 Micro			X	
Tube 2	CD27			X	
	CD28			X	
	CD117			X	
	CD81				

# EuroFlow MRD PANEL: MM



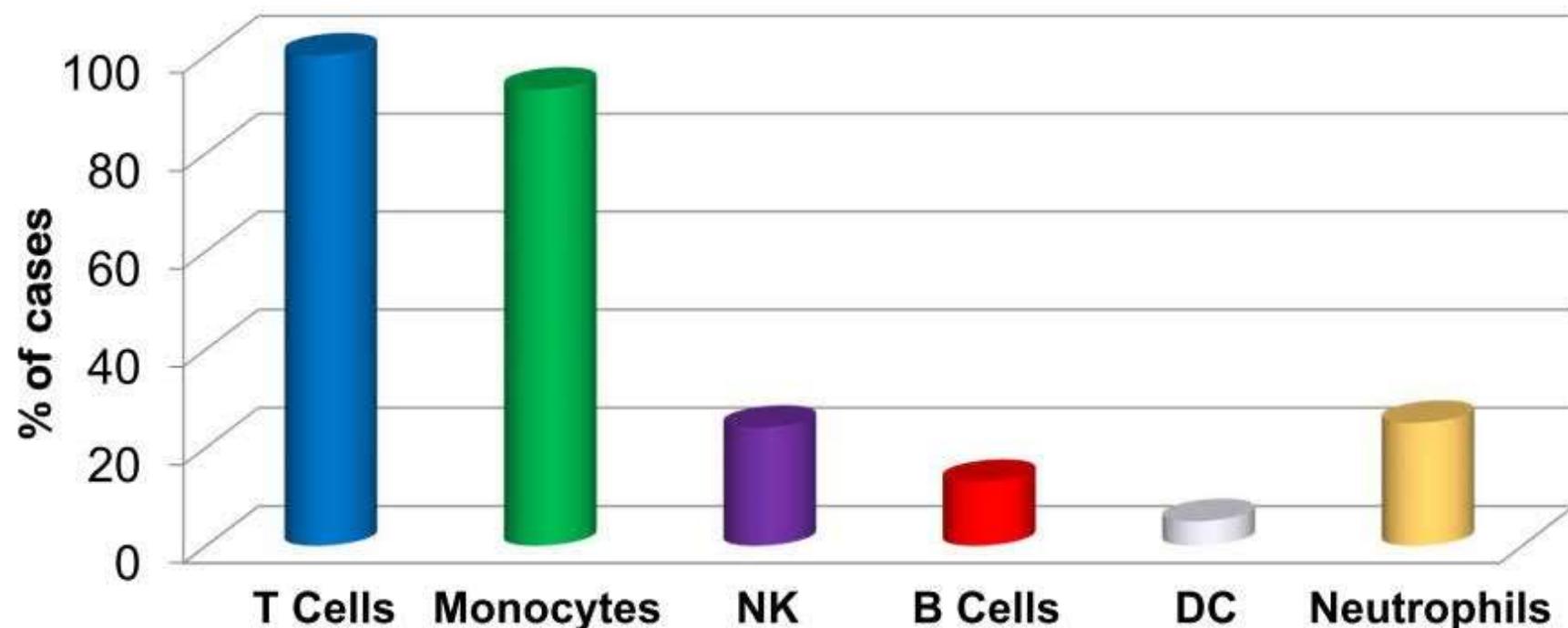
# MGUS



**SST  
(SMALL SCREENING TUBE)**

# Hücrelerin dağılımı

## Normal/Reaktif BOS Örneği



# SST (Small Screening Tube)

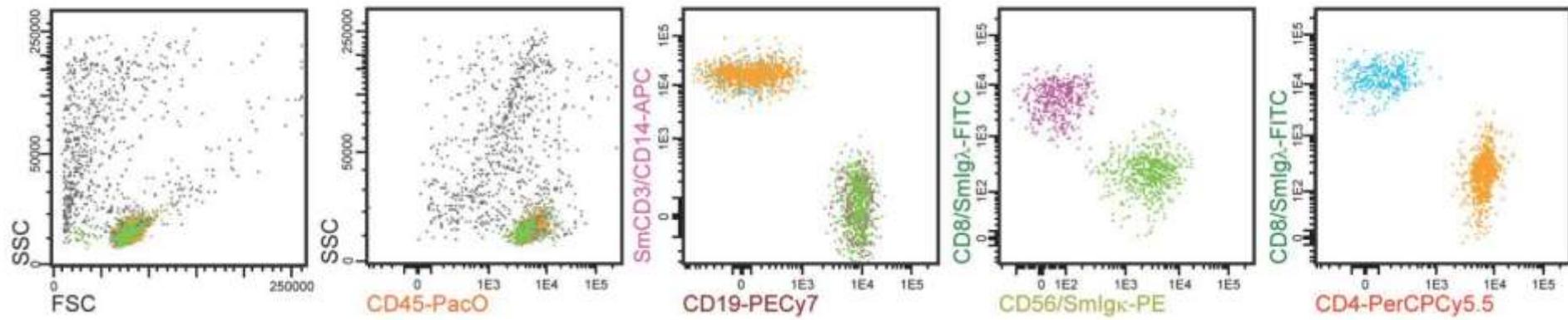
## Düzenlenmesi

- Çok az sayıdaki hücrelerden maksimum bilgi elde edilecek şekilde düzenlenmeli

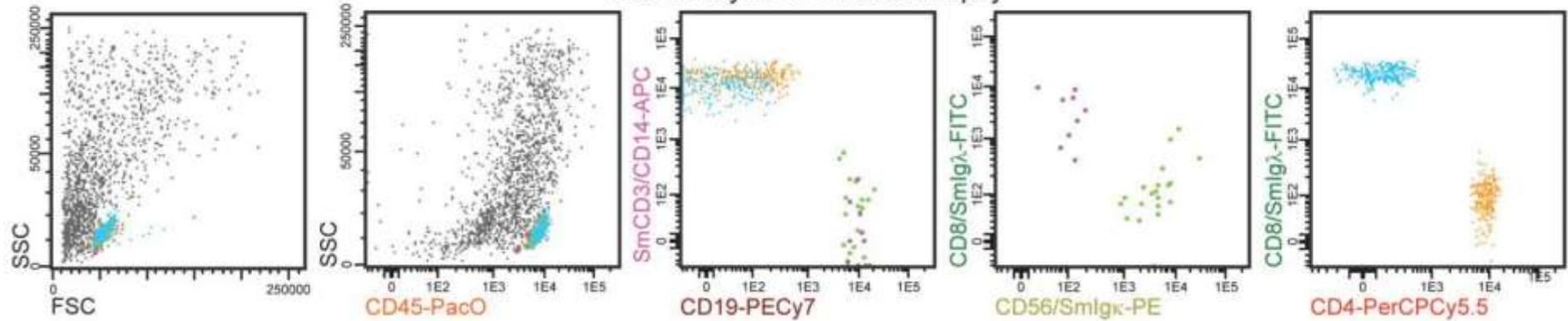
	PB	PO	FITC	PE	PerCP Cy5.5	PE-Cy7	APC	APC-H7
Screen	CD20	CD45	CD8-Smlgλ	CD56-Smlgκ	CD4	CD19	CD3-CD14	CD38

# SST Analizi

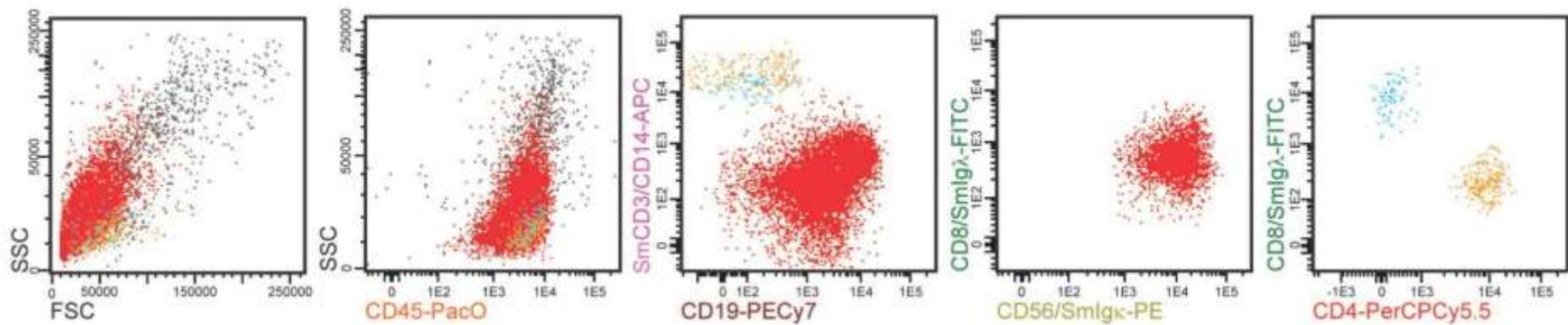
SST analysis of CSF sample



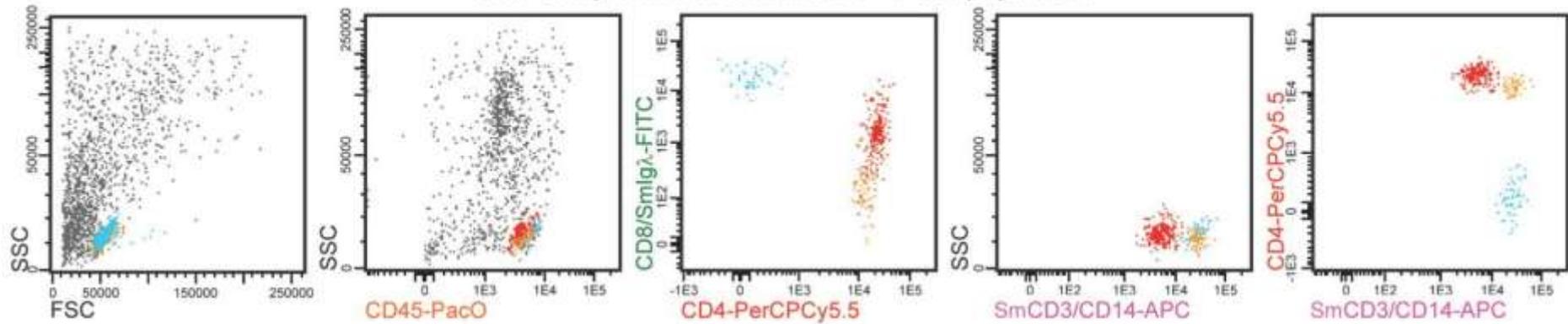
SST analysis of vitreous biopsy



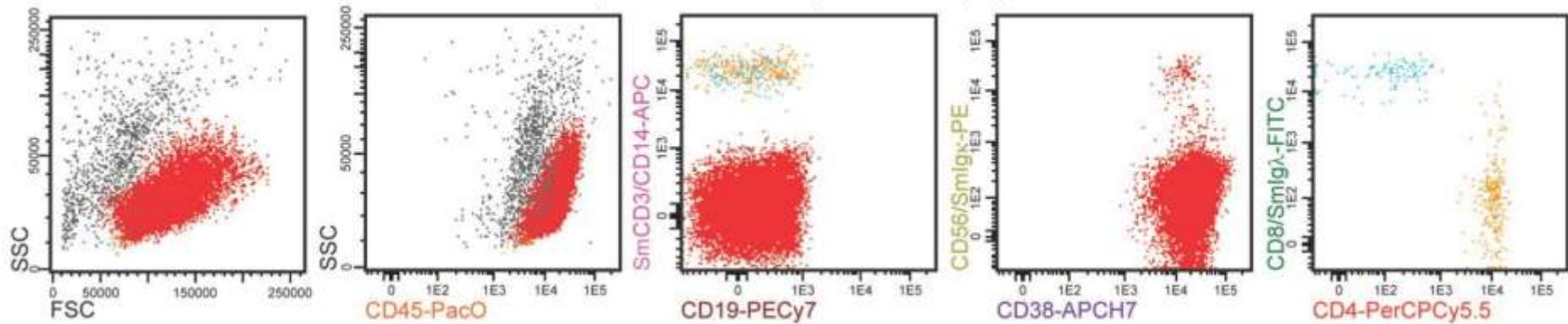
Vitreous biopsy with clonal Igκ<sup>+</sup> B-cell population



CSF sample with aberrant CD4<sup>+</sup> T-cell population



CSF sample with aberrant plasma cell population



# BOS tutulumunun FCM ve CC ile saptanması

## Aggressive B-NHL (n=123)

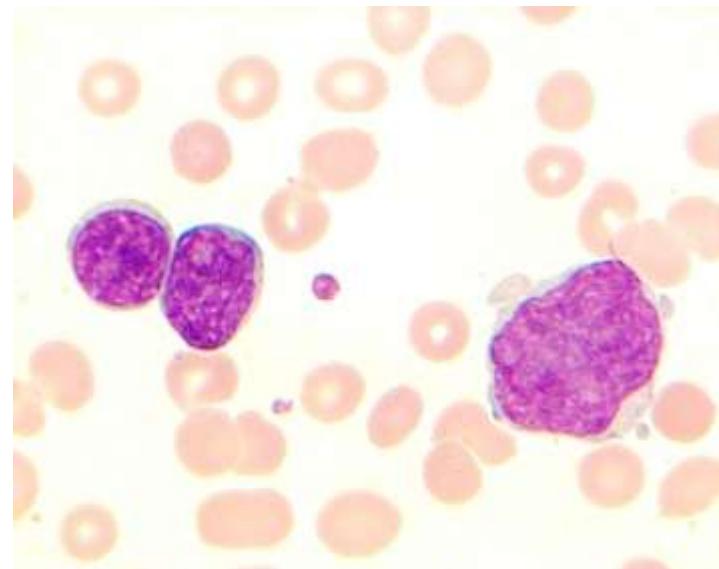
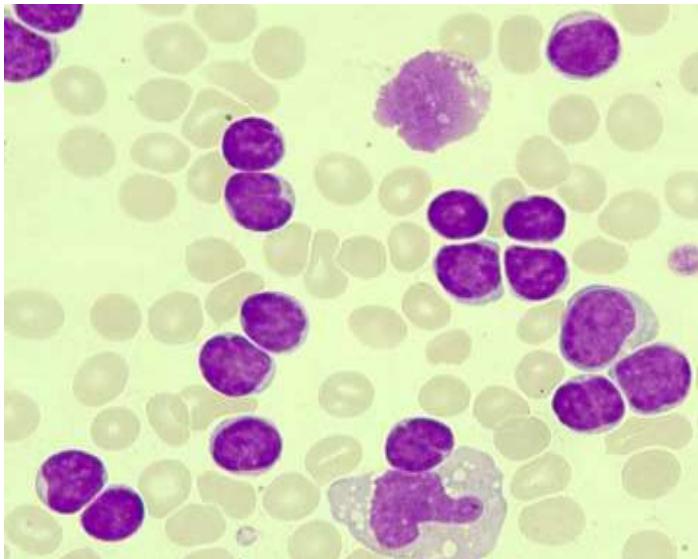
Conventional Cytology	Flow Cytometry			
	Negative		Positive	
	No.	%	No.	%
Negative	95/123	77	17/123	14
Positive	1*/123	1	7/123	6
Suspicious	--	--	3/123	2

\*The presence of neoplastic cells in this patient was ruled out by further immunocytochemical analyses (one cytospin slide was fixed in acetone and stained with CD20 monoclonal antibody L-26 [Dako, Glostrup, Denmark] using the ABC method).

	FCM	Cytology	P
CSF (+)	22% (27/123)	6% (6/123)	<0.0001

# Immuno-phenotype

	CD5	CD20	CD22	CD23	CD25	CD38	CD43	CD79b	CD81	FMC7	SIg
CLL	+	+(weak)	-/+	+	-	-/+	+(mod)	-/+	+(weak)	-	+(weak)
MCL	+	+(mod-strong)	+	-/+	-	-	-/+	+(strong)	+(mod)	+	+(mod-strong)



# FCM \* B-cell LPH

Percentile

20<sup>th</sup> 50<sup>th</sup> 80<sup>th</sup>

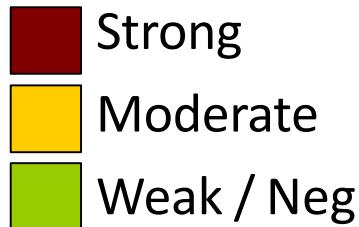
All weak

All strong

Heterogeneous

Category	20 <sup>th</sup> Percentile	50 <sup>th</sup> Percentile	80 <sup>th</sup> Percentile
All weak	Weak / Neg (Light Green)	Weak / Neg (Light Green)	Weak / Neg (Light Green)
All strong	Strong (Dark Red)	Strong (Dark Red)	Strong (Dark Red)
Heterogeneous	Weak / Neg (Light Green)	Moderate (Yellow)	Strong (Dark Red)

# FCM \* B-cell LPH



Percentile  
20<sup>th</sup> 50<sup>th</sup> 80<sup>th</sup>



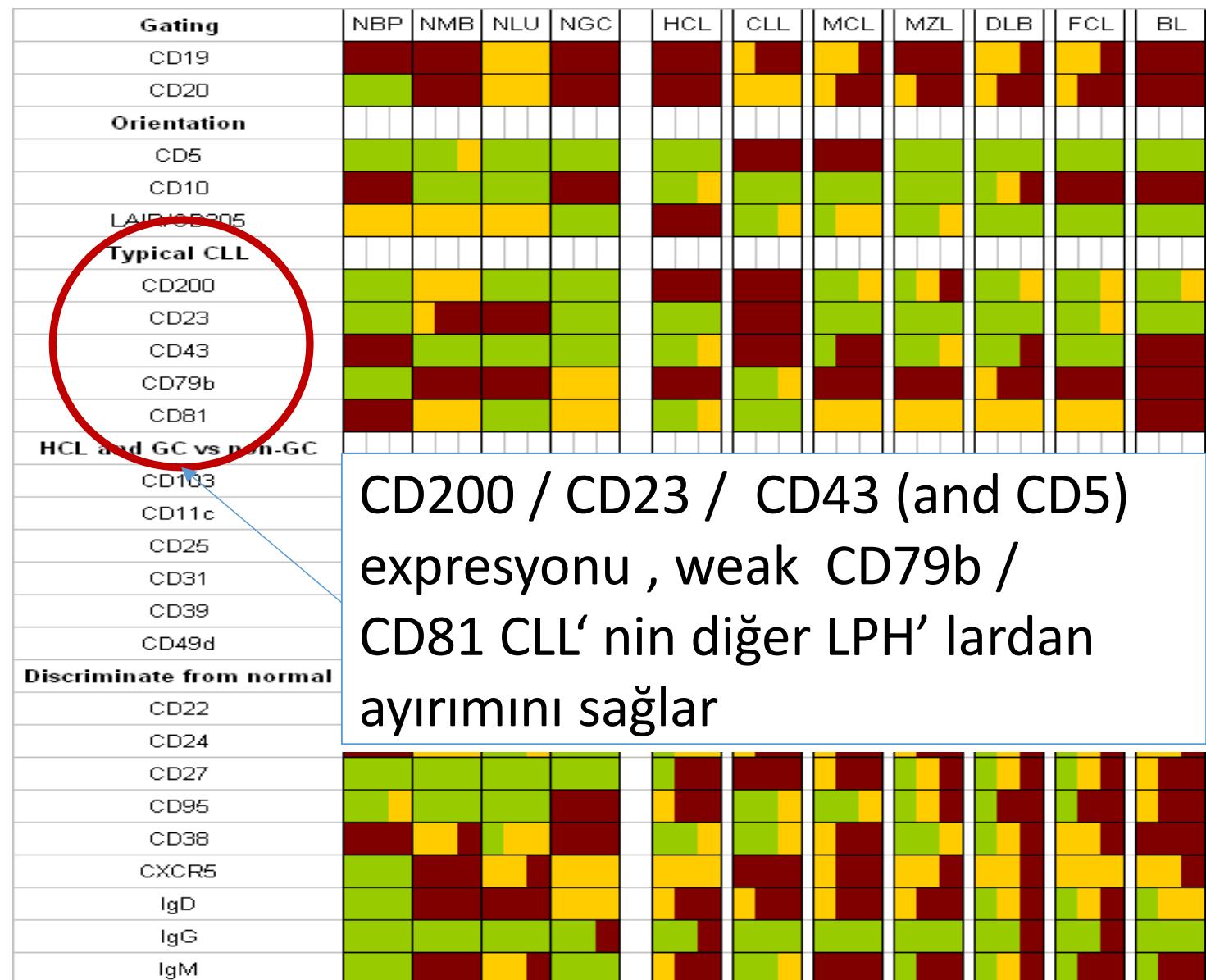
All weak



All strong



Hetero-  
geneous



## Panelde Hangi MoAb' lar olmalı

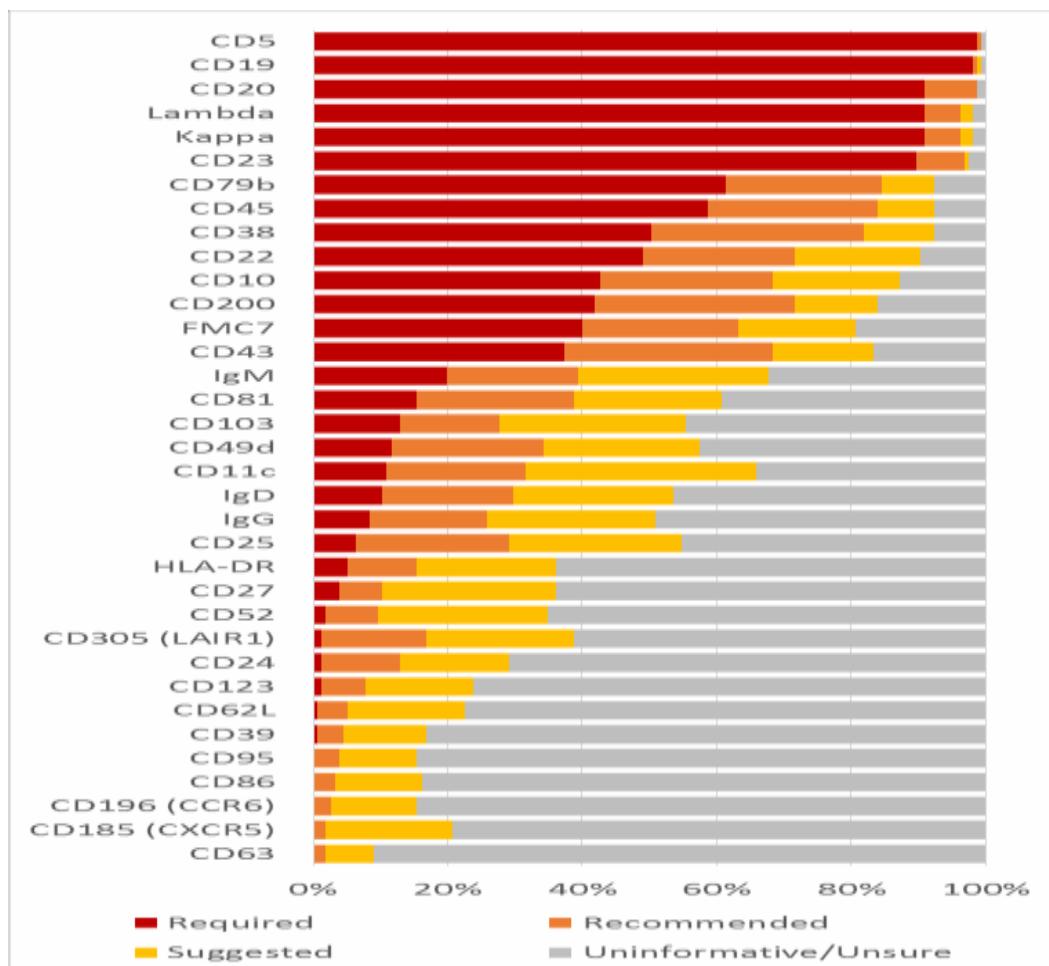
- CD5
- CD20
- Kappa/Lambda
- CD23
- CD200
- CD79b
- CD43
- ROR1

# Reproducible diagnosis of CLL by flow cytometry: an ERIC&ESCCA harmonisation project

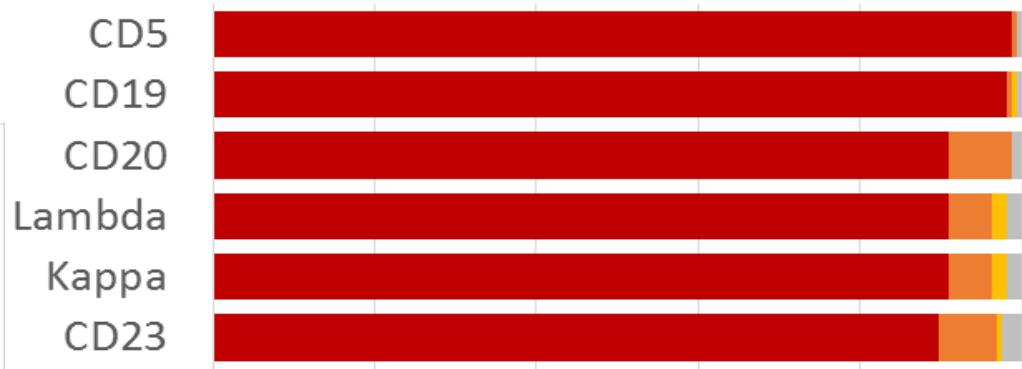
Andy C. Rawstron, Karl-Anton Kreuzer, Asha Soosapilla, Martin Spacek, Peter Gambell, Neil McIver-Brown, Katherina Psarra, Maria Arroz, Raffaella Milani, Javier de la Serna, M. Teresa Cedena, Ozren Jaksic, Josep Nomdedeu, Carol Moreno, Gian Matteo Rigolin, Antonio Cuneo, Preben Johansen, Hans Johnsen, Richard Rosenquist Brandell, Carston Utoft Niemann, David Westerman, Marek Trneny, Stephen Mulligan, Peter Hillmen, Mesude Falay, David Oscier, Michael Hallek, Paolo Ghia, Emili Montserrat.

On behalf of the European Research Initiated on CLL (ERIC) and the European Society for Clinical Cell Analysis (ESCCA)

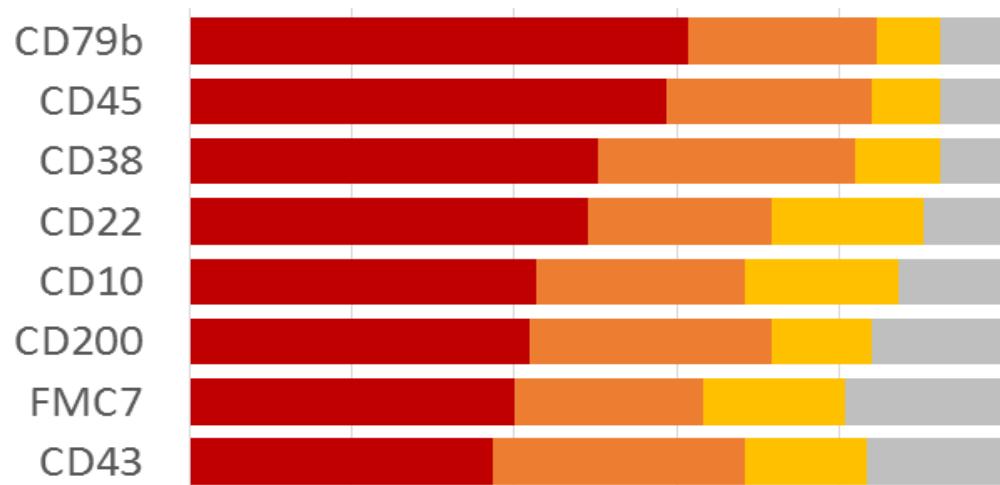
# 35 marker KLL tanısı için 154 ERIC/ESCCA üyesine önerildi



>75% 'nin cevabı → KLL tanısı için  
gereken moAb



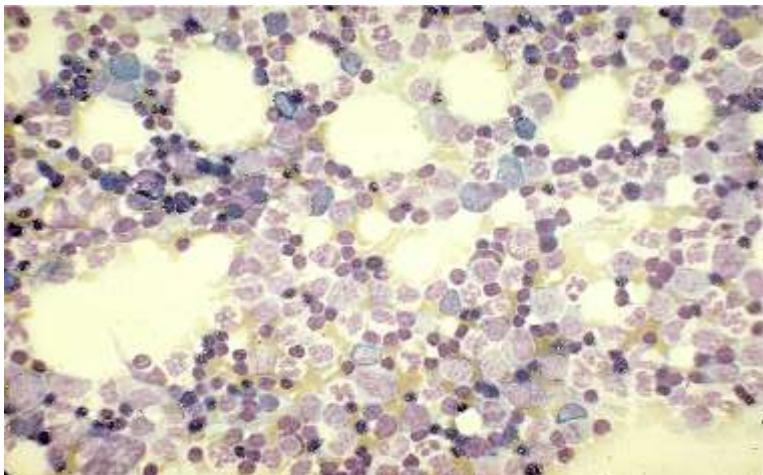
>50% 'nin cevabı → KLL tanısı için  
önerilen moAb



# Tanı için önerilen minimum panel

- Tanı İçin gereklisi:
  - CD19 / CD5 / CD23 / CD20 / Kappa / Lambda
- Tanı ve klinik çalışmalar için ilave edilebilir:
  - CD43 / CD79b / CD81\* / CD22 / CD10 / CD200\* / [ROR1]\*
- Sıklıkla Önerilen ancak tanı için gereklili olmayan:
  - CD45 / CD38 / FMC7
- Tanı için önerilen ama gerekmeyen
  - IgM/D and CD11c:

# FLOW SİTOMETRİ



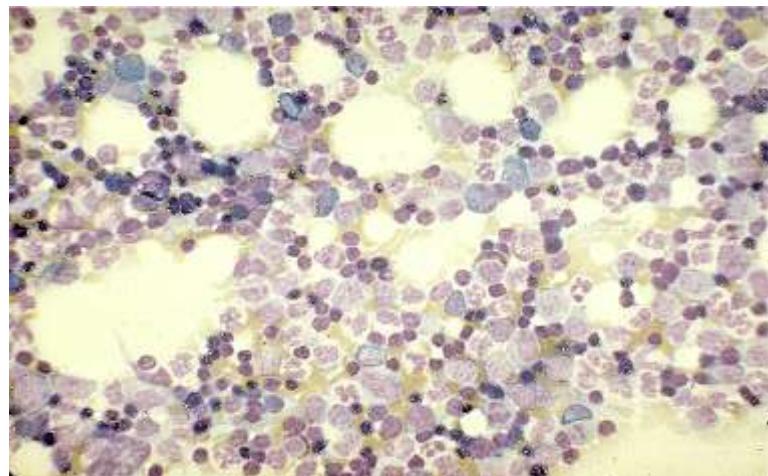
**!! ZOR İŞ**

**NE ARADIĞINI  
BİLMİYORSAN**

**?**

**NE BULDUĞUNU  
ANLAYAMAZSIN**

# FLOW SİTOMETRİ

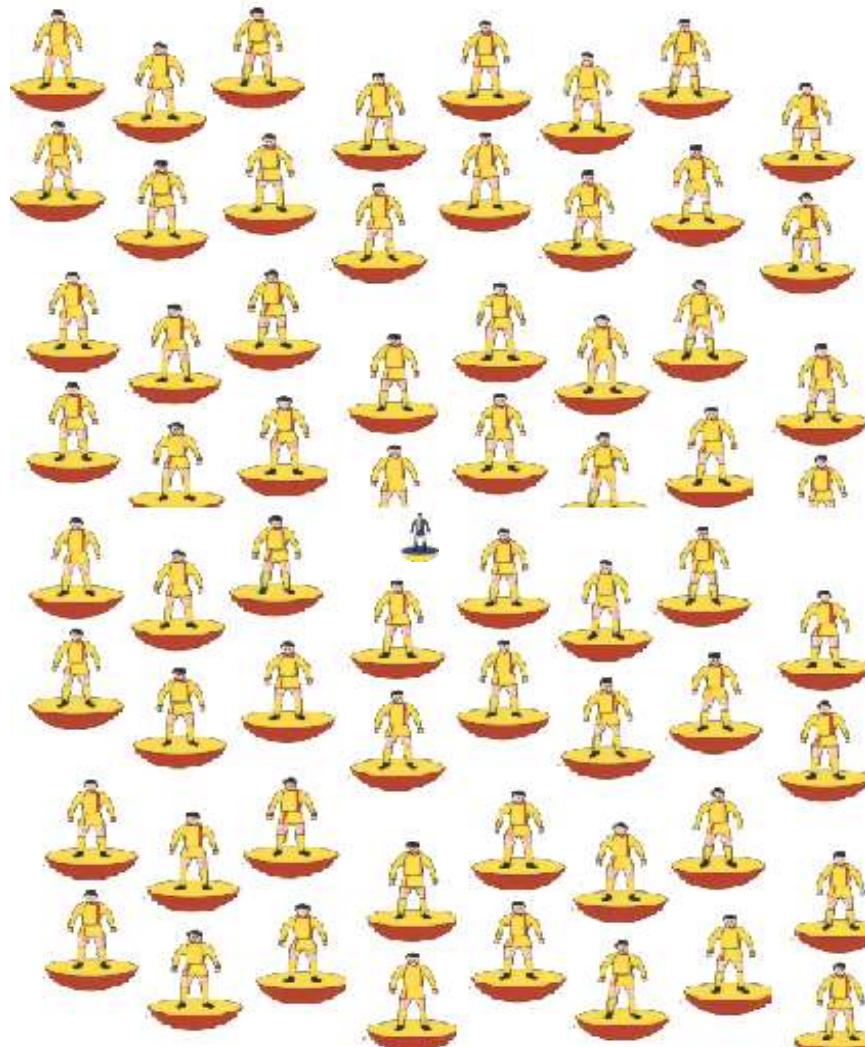


!! ZOR İŞ

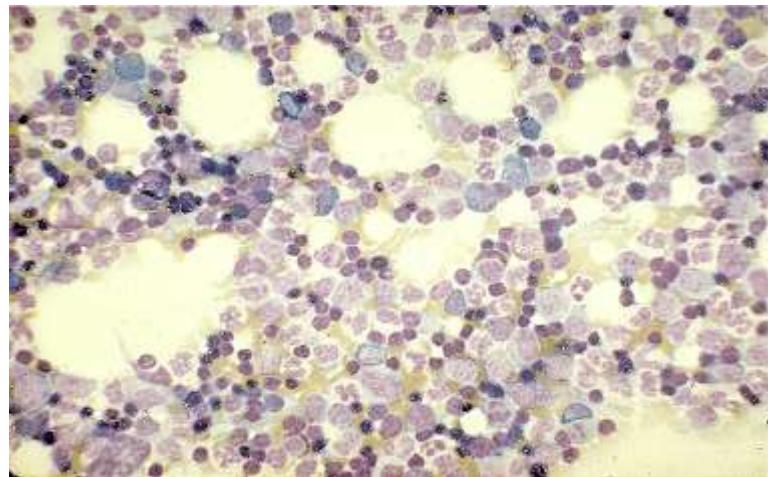
NE ARADIĞINI  
BİLMİYORSAN



NE BULDUĞUNU  
ANLAYAMAZSIN



# FLOW SİTOMETRİ



!! ZOR İŞ

NE ARADIĞINI  
BİLMİYORSAN



NE BULDUĞUNU  
ANLAYAMAZSIN

