Integrazione emocitometria – citofluorimetria nei Pazienti non ospedalizzati

MONZA 22 NOVEMBRE **2015**

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AVVERTENZA

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È normale scambiare la mononucleosi per leucemia?

(archivio consulti: per vedere la pagina aggiornata clicca QUI)

lunedì 9 settembre 2013 #1

Utente 308xxx

Utente uomo

Iscritto dal 2013

È normale scambiare la mononucleosi per leucemia?

Salve dottori.
Nello scorso mese di giugno ho subito una disavventura medica sulla quale vorrei un vostro parere. Ho contratto la mononucleosi infettiva da EBV (questo l'ho saputo a luglio, come capirete) e l'8 sono comparsi i classici sintomi: febbre, astenia, cefalea, placche sulle tonsille, splenomegalia (19 cm).
Il medico mi prescrive il Mono-Test che risulta negativo; nell'emocromo appaiono "linfociti atipici" e ci indirizziamo verso il CMV (citomegalovirus). Il 19 altre analisi mostrano che anche il CMV è negativo e inoltre appaiono "16% blasti" (15.000 WBC/mmc).

10.09.2013

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A questo punto vengo mandato d'urgenza in un ospedale oncologico con sospetto di leucemia. Gli ematologi lì fanno uno striscio di sangue periferico e confermano che è in corso "una malattia proliferativa" (dice il primario), che ci sono blasti nel sangue periferico, tutto ci fa pensare ad una leucemia. Agoaspirato midollare. La sera, ci viene chiaramente detto che è leucemia e si tratta solo di capire se è linfoide o mieloide, parlano di trapianto di midollo, chemioterapia... questo accade il 20 giugno. Mi ricovero in oncoematologia.
Il 21 mattina (vi lascio immaginare che nottata orrenda per me e per la mia famiglia) però arriva una clamorosa smentita.

L'emolinfopatologo ha esaminato il midollo e ha escluso assolutamente la leucemia: è un'infezione virale. Tante scuse dai medici. Qualche test più approfondito ed emerge subito l'EBV. La febbre se ne va il 3 luglio e ad ora sto benissimo.

La mia domanda è: è possibile che uno striscio di sangue periferico di mononucleosi possa essere confuso da oncoematologi con uno leucemico??

E’ possibile che uno striscio di sangue periferico di mononucleosi possa essere confuso con uno leucemico?

Dovrebbe esssere impossibile, perché un buon morfologo ha tutti gli elementi per operare questa diagnosi differenziale!

Leukocytosis

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D. S. CHABOT-RICHARDS, T. I. GEORGE

Lymphocytosis may be morphologically divided into polymorphic and monomorphic populations.

Leukocytosis

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D. S. CHABOT-RICHARDS, T. I. GEORGE

Polymorphic lymphocytosis is most consistent with a reactive process, while monomorphic populations are concerning for lymphoproliferative neoplasm.

POLYMORPHIC LYMPHOCYTOSIS
THE «ATYPICAL LYMPHOCYTE»

- **Atypical lymphocytes contain mature coarse red-purple nuclear chromatin** in contrast to immature forms which contain pale-staining immature nuclear chromatin with large amounts of parachromatin
- **Atypical lymphocytes possess abundant cyto**plasm, not scanty as in immature forms

O'Connor B H
A Color Atlas and Instruction Manual of Peripheral Blood Cell Morphology
1984 Lippincott William Wilkins

CLASSIFICAZIONE DELLE CELLULE DI DOWNEY

	TIPO I	TIPO II	TIPO III
dimensioni	piccole	da medie a grandi	da medie a grandi
nucleo	indentato o lobulato	rotondo o ovale	rotondo o ovale
cromatina	a zolle	moderatamente a zolle	dispersa
nucleoli	assenti o piccoli	assenti o piccoli	prominenti
citoplasma	scarso, lievemente basofilo	abbondante, pallido, grigio-azzurro	abbondante, basofilo
granuli	rari o assenti	assenti	assenti

VOI

SIETE CONVINTI?

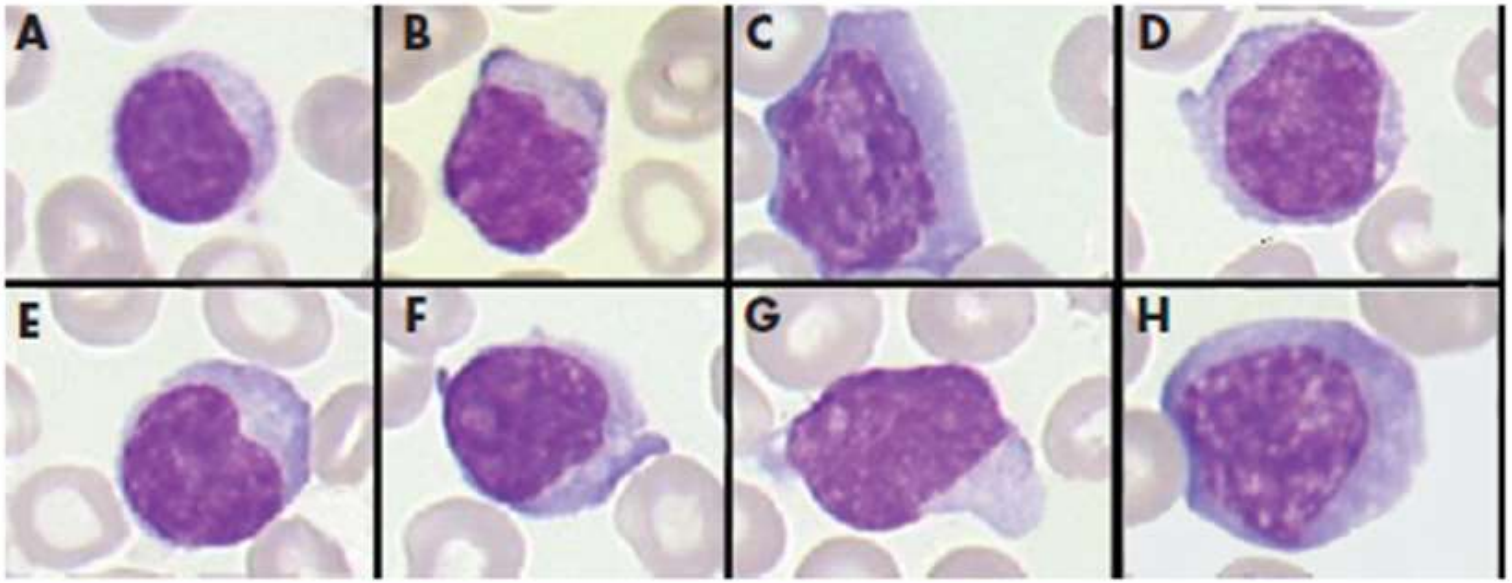
IO NON

COMPLETAMENTE

Van der Meer et al, J Clin Pathol 2007

100 white blood cells were randomly selected, microphotographated and processed in a PowerPoint presentation, which was sent to 157 different hospital laboratories in The Netherlands. A leucocyte differential was requested.

Van der Meer et al, J Clin Pathol 2007



All the participants were asked to differentiate the lymphocytes into normal lymphocytes, atypical lymphocytes, plasmacells, prolymphocytes or lymphoblasts.

VAN DER MEER 2007

RESULT OF THE SURVEY

- For 49 lymphocytes there was no concordance in subtyping.
- One cell was classified as normal lymphocyte or a plasma cell
- Nine cells were classified as normal or atypical or prolymphocyte
- Nine cells were classified as normal or atypical or plasma cell

VAN DER MEER 2007

RESULT OF THE SURVEY

- For seven cells, four different subtypes were mentioned
- To one cell, five different assessment were attributed
- One cell was shown twice, and the second time was classified as another subtype by the same observer

VAN DER MEER 2007

Van der Meer et al, J Clin Pathol 2007

All the cells were from a blood smear of an orthopaedic patient (female, age 3 years) with a lymphocytosis which was normal for a child of that age.

NORMAL SUBJECT AGE 3 YEARS

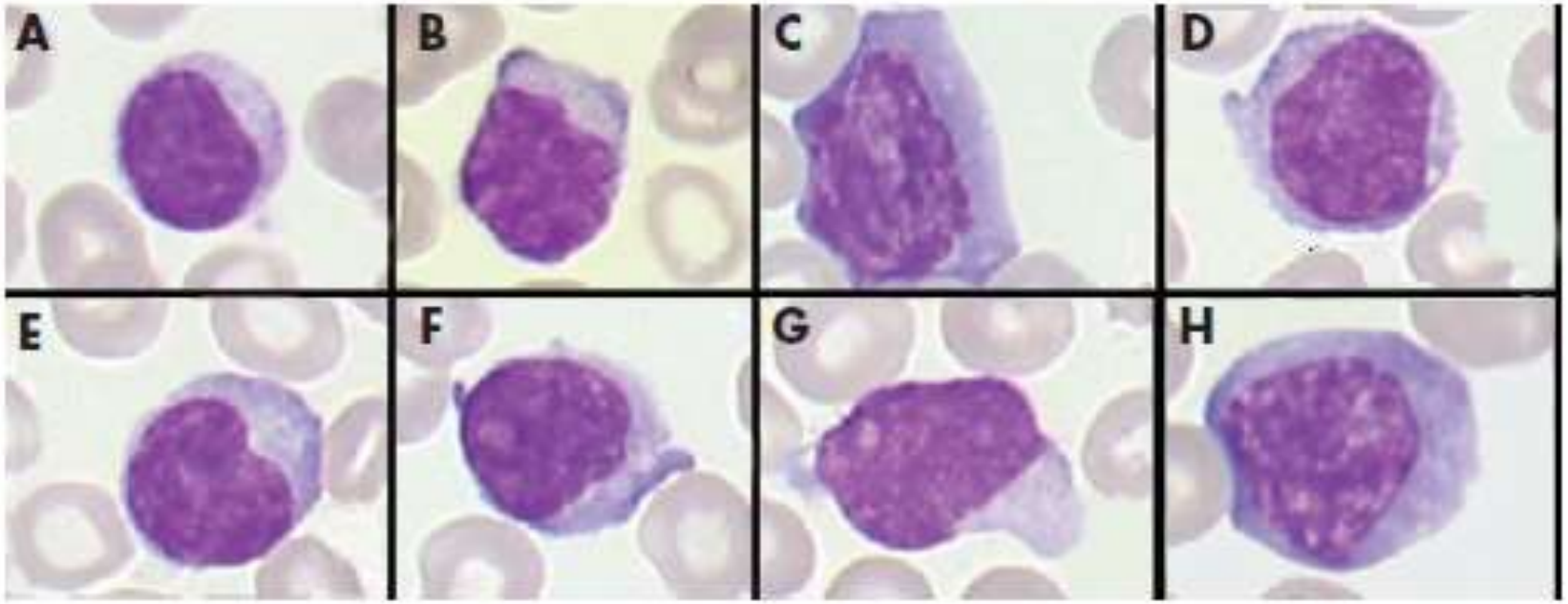


Figure 1 Examples of the different lymphocytes. Lymphocytes classified as: (A) lymphocyte with >90% accordance; (B) normal or atypical; (C) normal or plasma cell; (D) normal, atypical or prolymphocyte; (E) normal, atypical or plasma cell; (F) normal, atypical, prolymphocyte or plasma cell; (G) normal, atypical, prolymphocyte or blast; (H) normal, atypical, prolymphocyte, plasma cell or blast.

VAN DER MEER 2007

Integrazione emocitometria – citofluorimetria

- 1) DISTINZIONE DEL LINFOCITA ATIPICO DAL BLASTO
- 2) DISTINZIONE DEL LINFOCITA REATTIVO DALL'ELEMENTO DI UNA CLPD (CHRONIC LYMPHOPROLIFERATIVE DISEASE)

DISTINZIONE TRA LINFOCITA ATIPICO E BLASTO

VALUTAZIONE SEMI-QUANTITATIVA DELL'ESPRESSIONE DI CD45 DA PARTE DEGLI ELEMENTI SOSPETTI

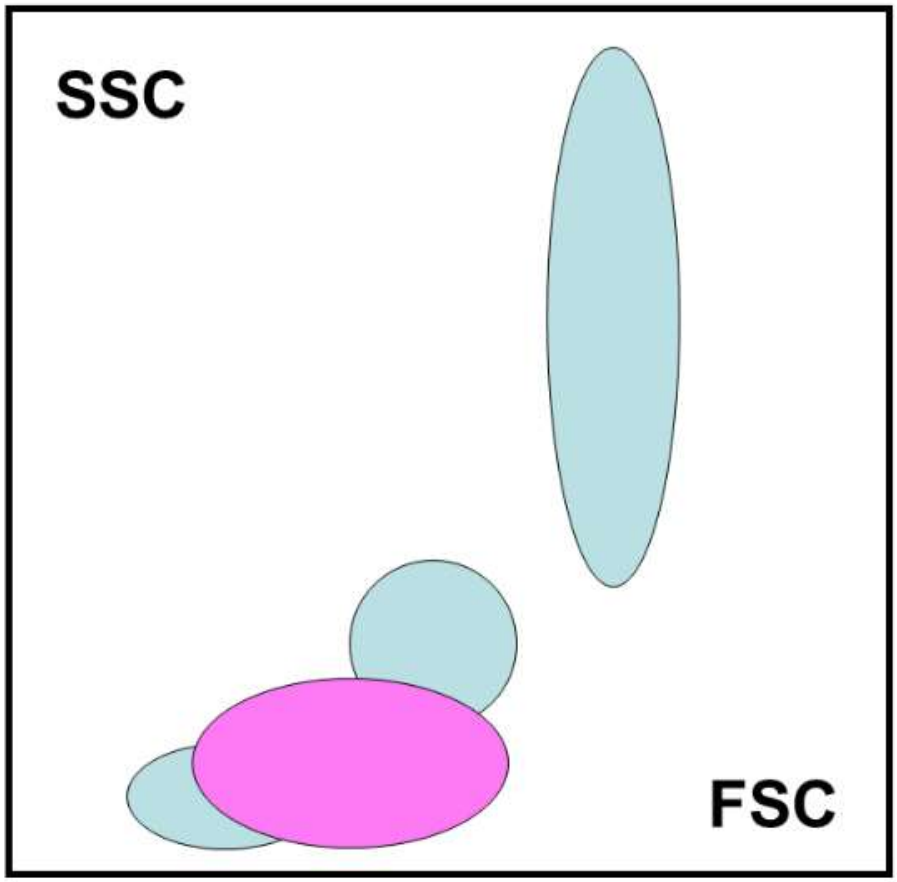
CYTOMETRICALLY SPEAKING ….

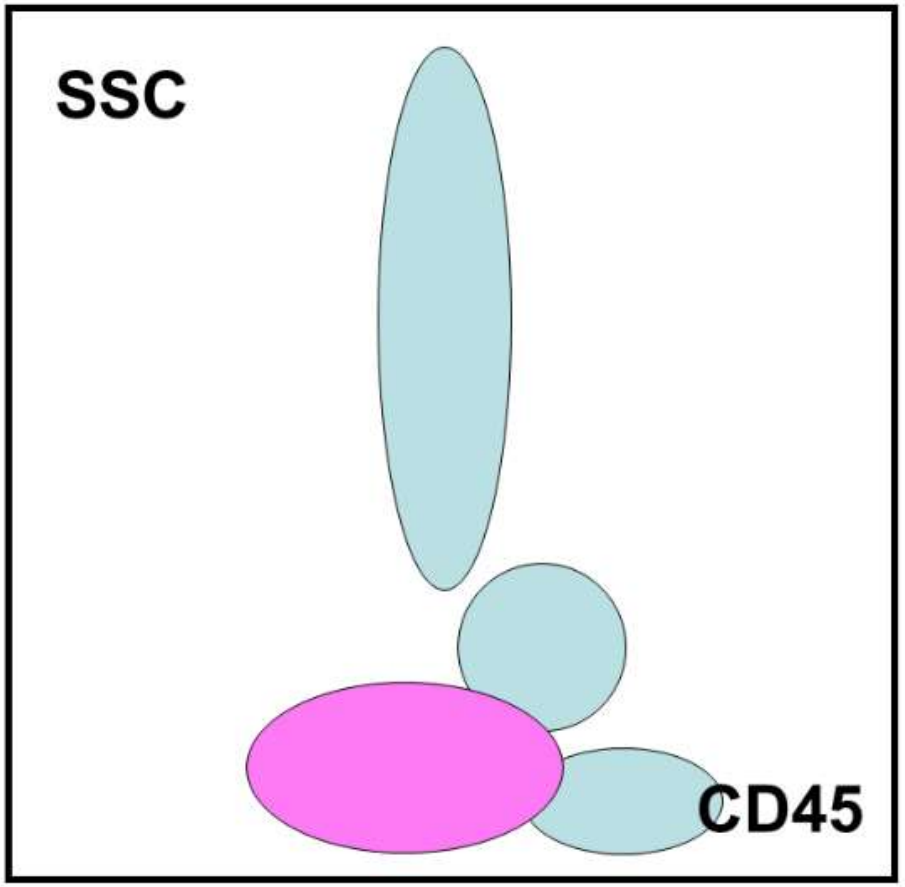
THERE IS NO ACCEPTED AND/OR SHARED DEFINITION OF BLASTIC CELL

NEVERTHELESS, FROM AN EMPIRICAL CYTOMETRIC APPROACH

- A BLAST IS CHARACTERIZED BY:
  - **LOW VALUES OF CD45 EXPRESSION**
  - VARIABLE VALUES OF FSC EXPRESSION (≥ LYMPH) DEPENDING ON CELL VOLUME
  - LOW VALUES OF SSC EXPRESSION (≥ LYMPH) DEPENDING ON GRANULARITY
  - EXPRESSION OF DIFFERENT ANTIGENS DEPENDING ON
    - HEMATOPOIETIC LINEAGE
    - MATURATIONAL STAGE
    - GENETIC FEATURES RELATED TO NEOPLASTIC TRANSFORMATION

ABNORMAL CELL DETECTION
FSC/SSC & CD45 EXPRESSION





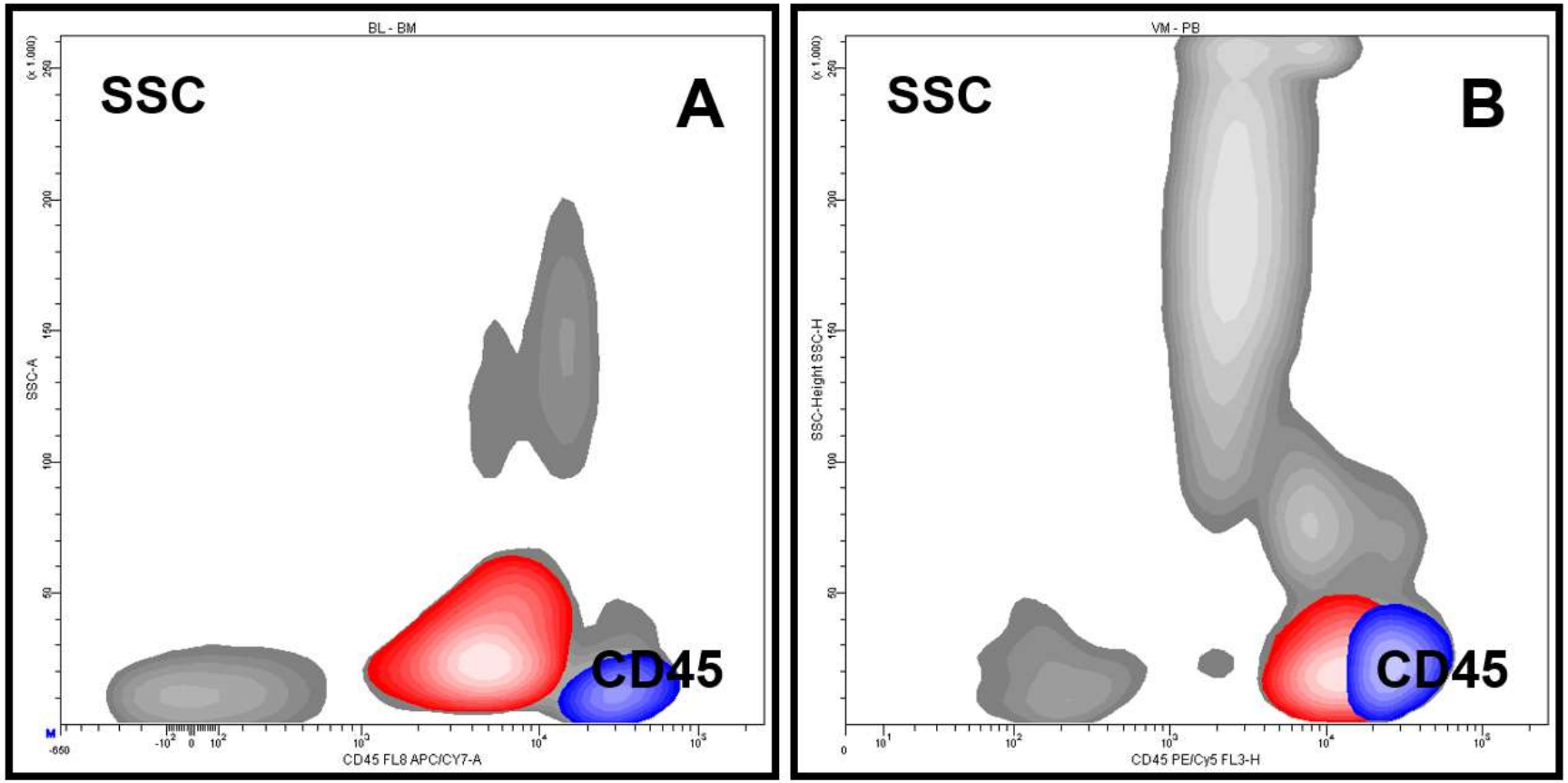
CAVEAT!

NOT ALL THE BLASTIC CELLS EXPRESS CD45 IN THE SAME WAY

CD45 EXPRESSION

- B-ALL WITH HYPERDIPLOID KARYOTYPE
  - VERY LOW, ALMOST ABSENT
- OTHER B-ALLs, AMLs
  - FROM LOW TO INTERMEDIATE
- T-ALLs
  - CLOSE TO MATURE LYMPHOCYTE EXPRESSION

B-ALL (sx), T-ALL (dx)
DIFFERENTIAL EXPRESSION OF CD45



ORTOLANI, FLOW CYTOMETRY OF HEMATOLOGICAL MALIGNANCIES, WILEY-BLACKWELL 2011

CAVEAT!

NOT ALL THE CD45 DIMLY POSITIVE CELLS ARE BLASTIC CELLS

BEWARE OF UFOs!

UNIDENTIFIED

FEEBLY CD45 POSITIVE

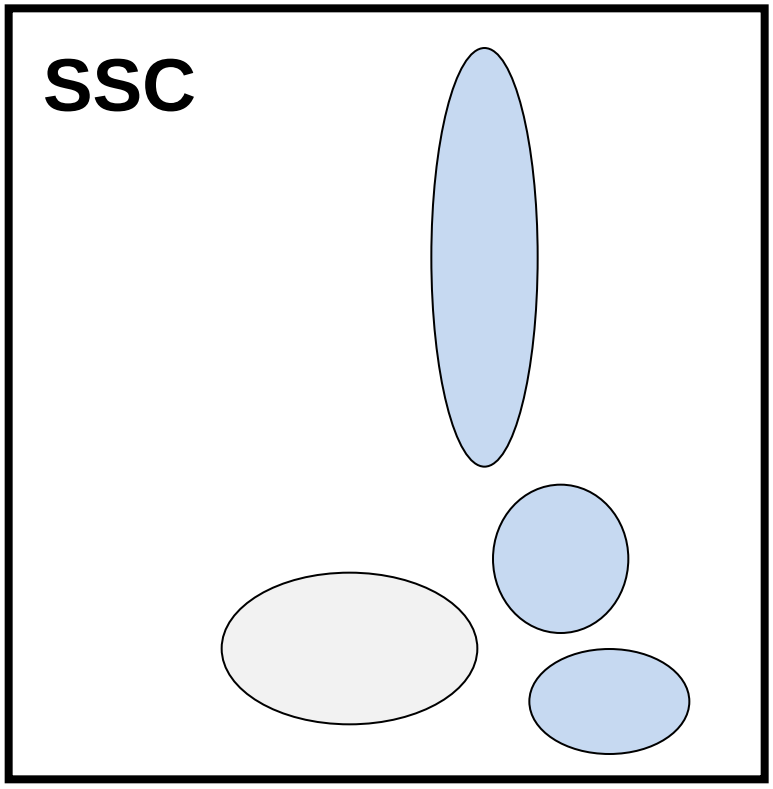
OBJECTS

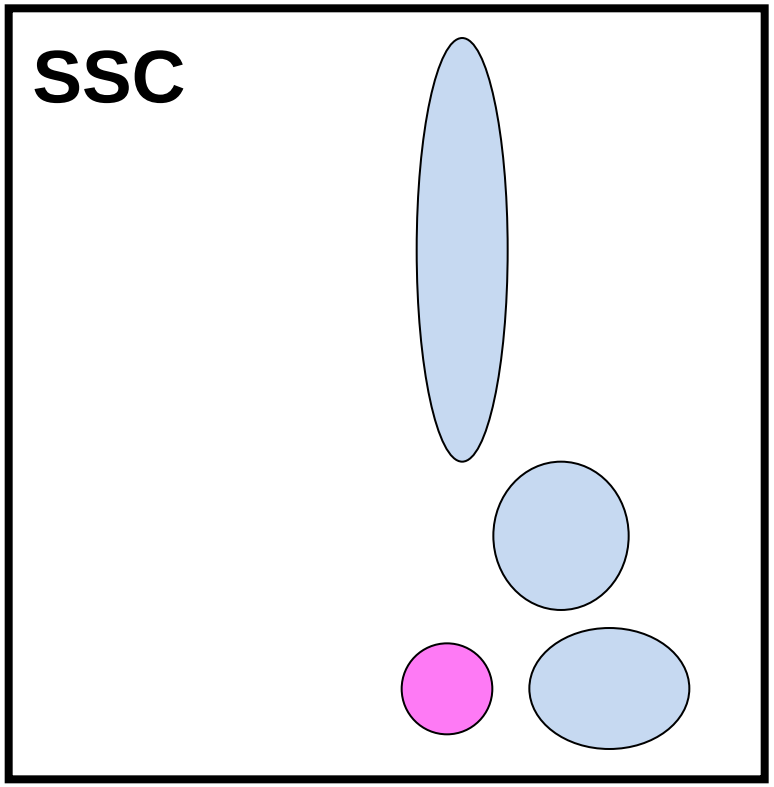
CD45 IS DIMLY EXPRESSED ON

- 1) BASOPHILS
- 2) HEMATOGONES
- 3) PLASMA CELLS
- 4) CELLS OF SOME PARTICULAR TYPE OF B-NHL
  - BURKITT LYMPHOMA IN PLASMACYTOID DIFF. (*)
  - PLASMABLASTIC LYMPHOMA
  - ISOLATED CASES OF DLBCL
  - OTHERS
- 5) THYMOCYTES

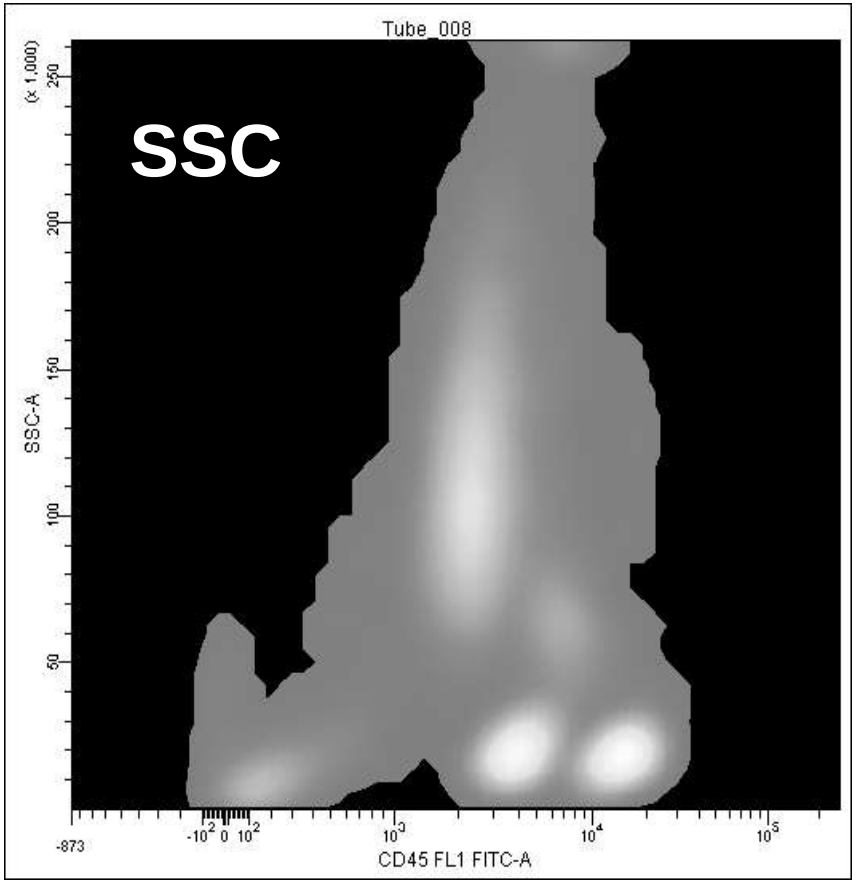
*4142/7465

BASOPHILS' CYTOMETRIC APPEARANCE

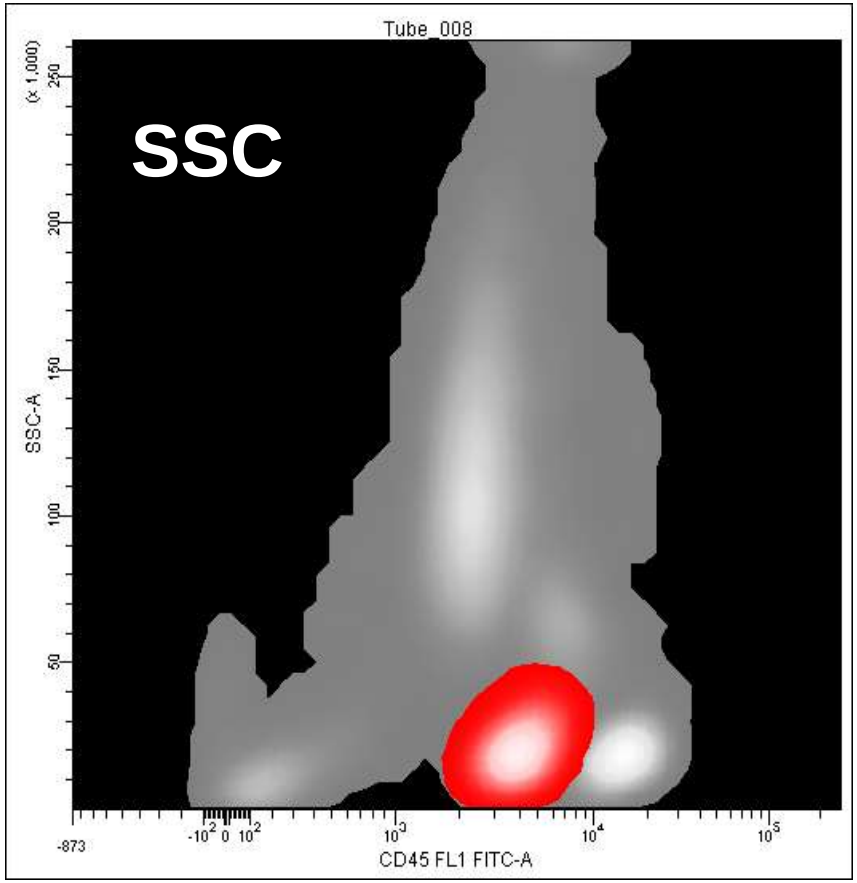




194/9274 (ESCCABase NAMEFILE 75805)
CML – ACCELERATED PHASE

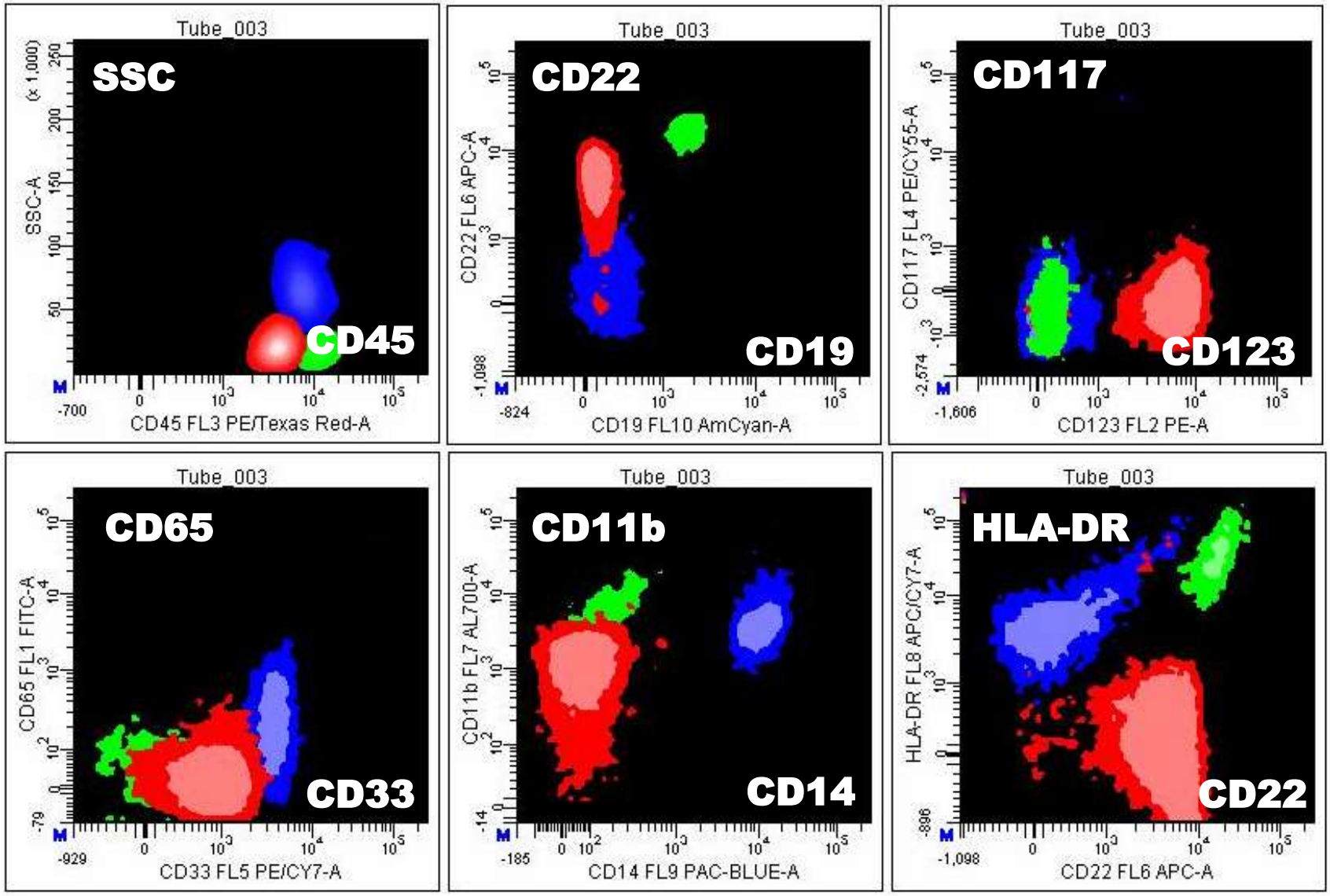


CD45

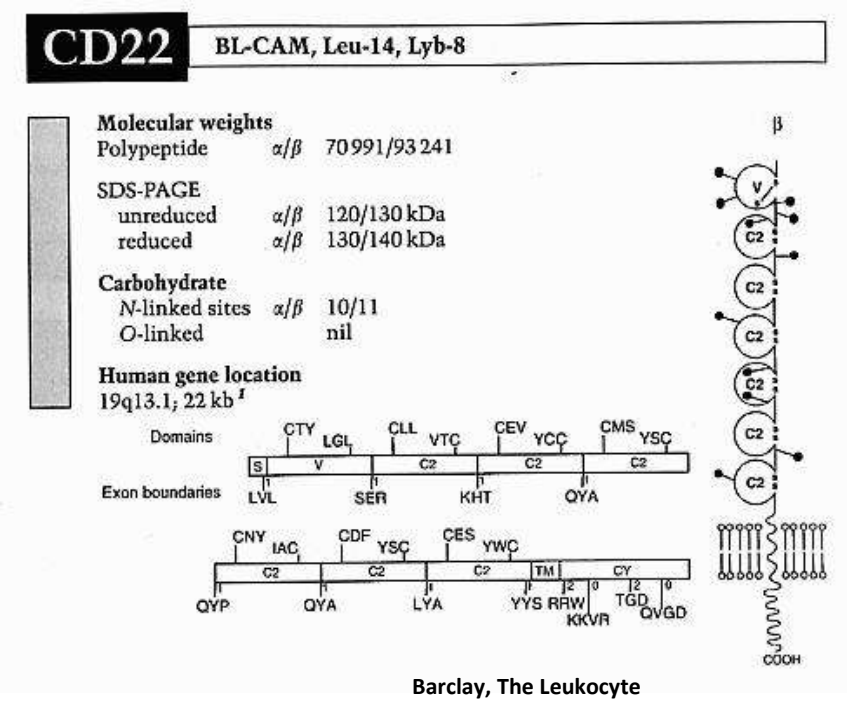


CD45

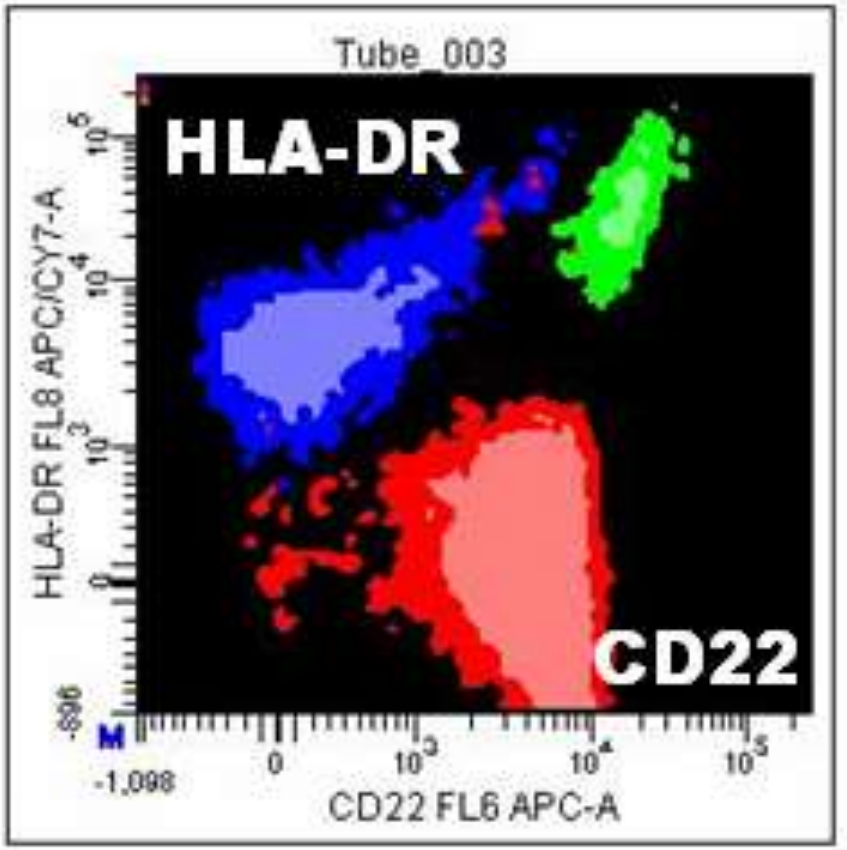
TEN COLOR ANALYSIS OF CD45± EVENTS IN A CASE OF CML IN ACCELERATED PHASE



CD22 – A CYTOMETRIC TIP
SOME CLONES STAIN BASOPHILS AND PLASMACYTOID DENDRITIC CELLS

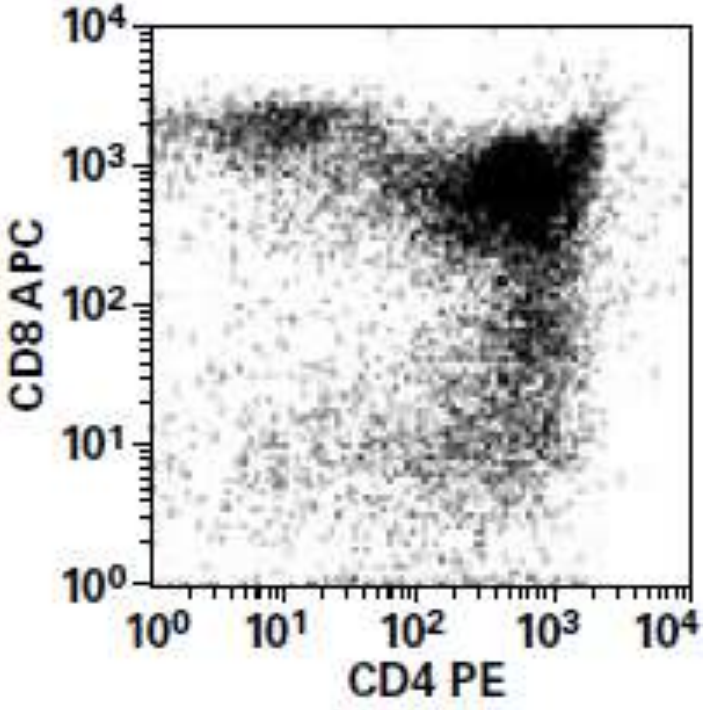


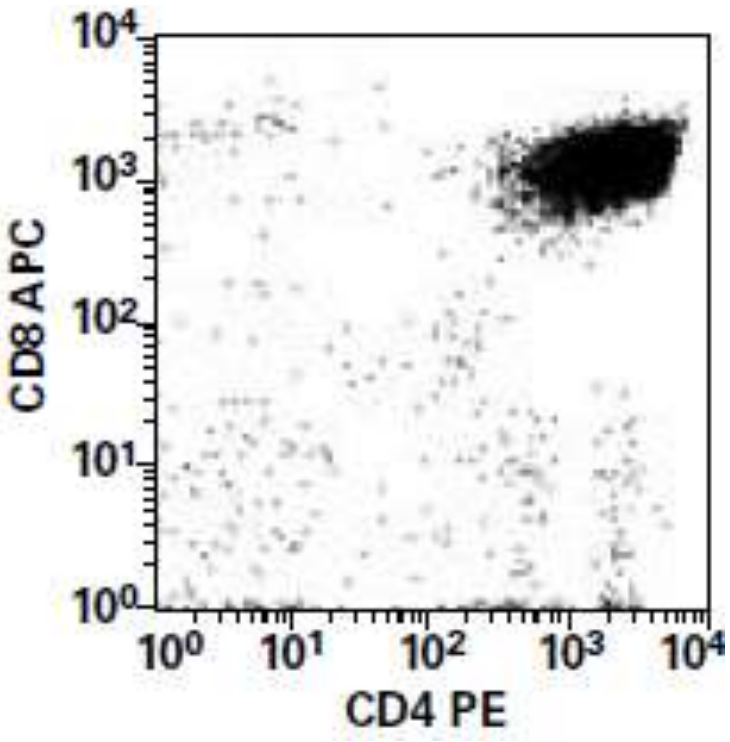
Barclay, The Leukocyte Antigen FactsBook, AP



Flow Cytometry in the Differential Diagnosis of Lymphocyte-Rich Thymoma From Precursor T-Cell Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma

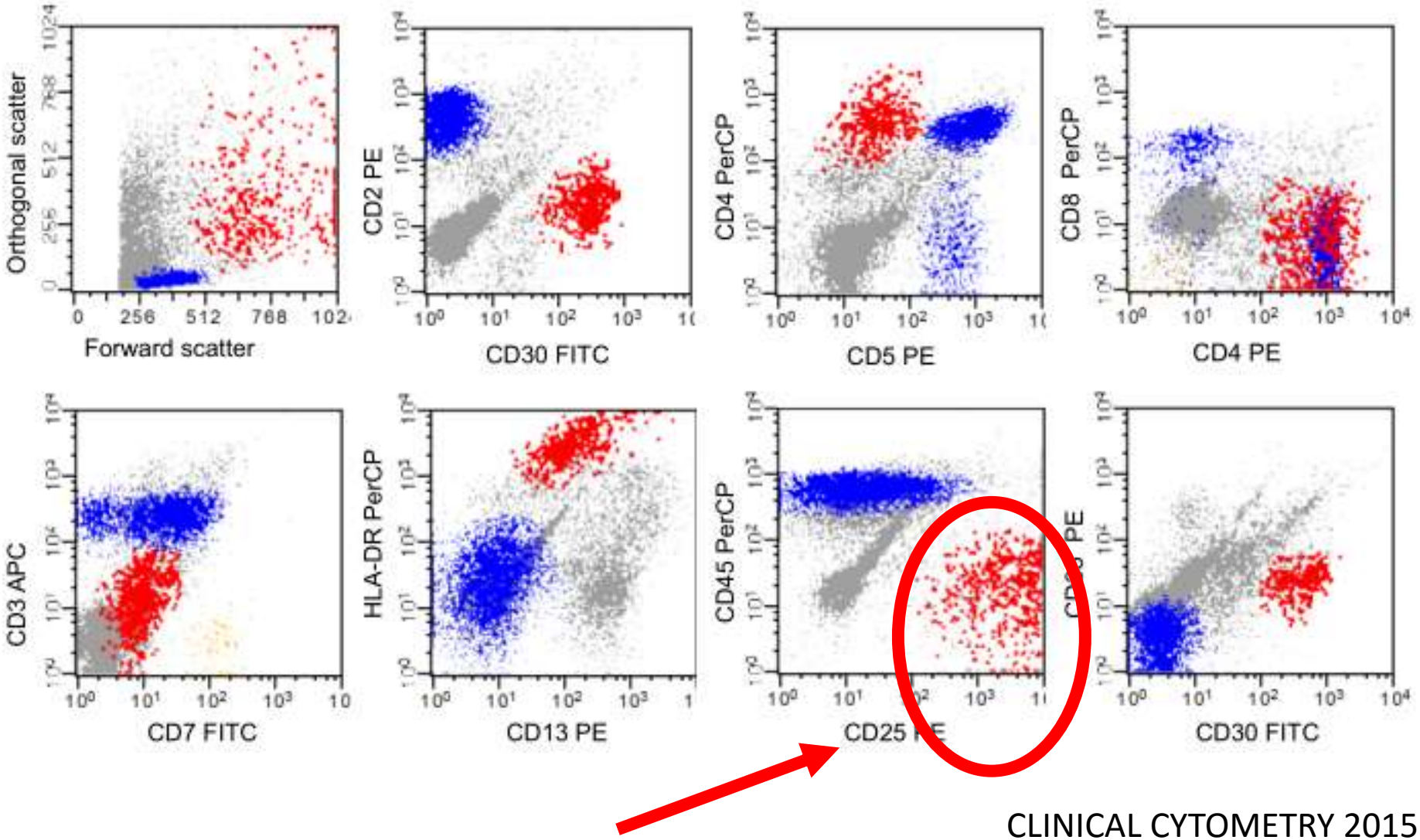
Shiyong Li, MD, PhD, Jonathan Juco, MD, Karen P. Mann, MD, PhD, and Jeannine T. Holden, MD





Immunophenotypic characteristics of breast implant-associated anaplastic large-cell lymphoma by flow cytometry

Crystal Montgomery-Goecker[1], Franklin Fuda[1], Jo Ellen Krueger[2], Weina Chen[1]



CLINICAL CYTOMETRY 2015

Leukocytosis

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D. S. CHABOT-RICHARDS, T. I. GEORGE

Polymorphic lymphocytosis is most consistent with a reactive process, while monomorphic populations are concerning for lymphoproliferative neoplasm.

Leukocytosis

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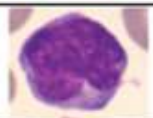
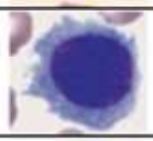
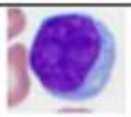
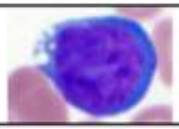
The types of cells present and morphology can help to guide additional work-up.

MONOMORPHIC LYMPHOCYTOSIS

SMALL, ROUND NUCLEI		**CLL** **MBL** PBL Burkitt MCL T-PLL	Flow cytometry FISH
FOLDED OR CLEAVED NUCLEI		**FL** **MCL** **Atypical CLL** T-cell Pertussis*	Flow cytometry FISH *CCND1, BCL2* Tissue biopsy
CONVOLUTED NUCLEI		**Sezary syndrome** **Adult T-cell leukemia**	Flow cytometry T-cell clonality
VILLOUS CYTOPLASM		**HCL** **SMZL** **HCLV** T-PLL LPL	Flow cytometry
PLASMACYTOID		**LPL** Plasma cell myeloma Plasma cell leukemia	Flow cytometry SPEP/UPEP
GRANULES		**T-LGL** NK cell leukemia	Flow cytometry T-cell clonality KIR profile
PROMINENT NUCLEOLI		**T-PLL** B-PLL HCLV MCL	Flow cytometry Cytogenetics
LARGE CELLS		**Burkitt Leukemia** **DLBCL** MCL ALCL	Flow cytometry FISH *MYC*

CHABOT-RICHARDS, 2014

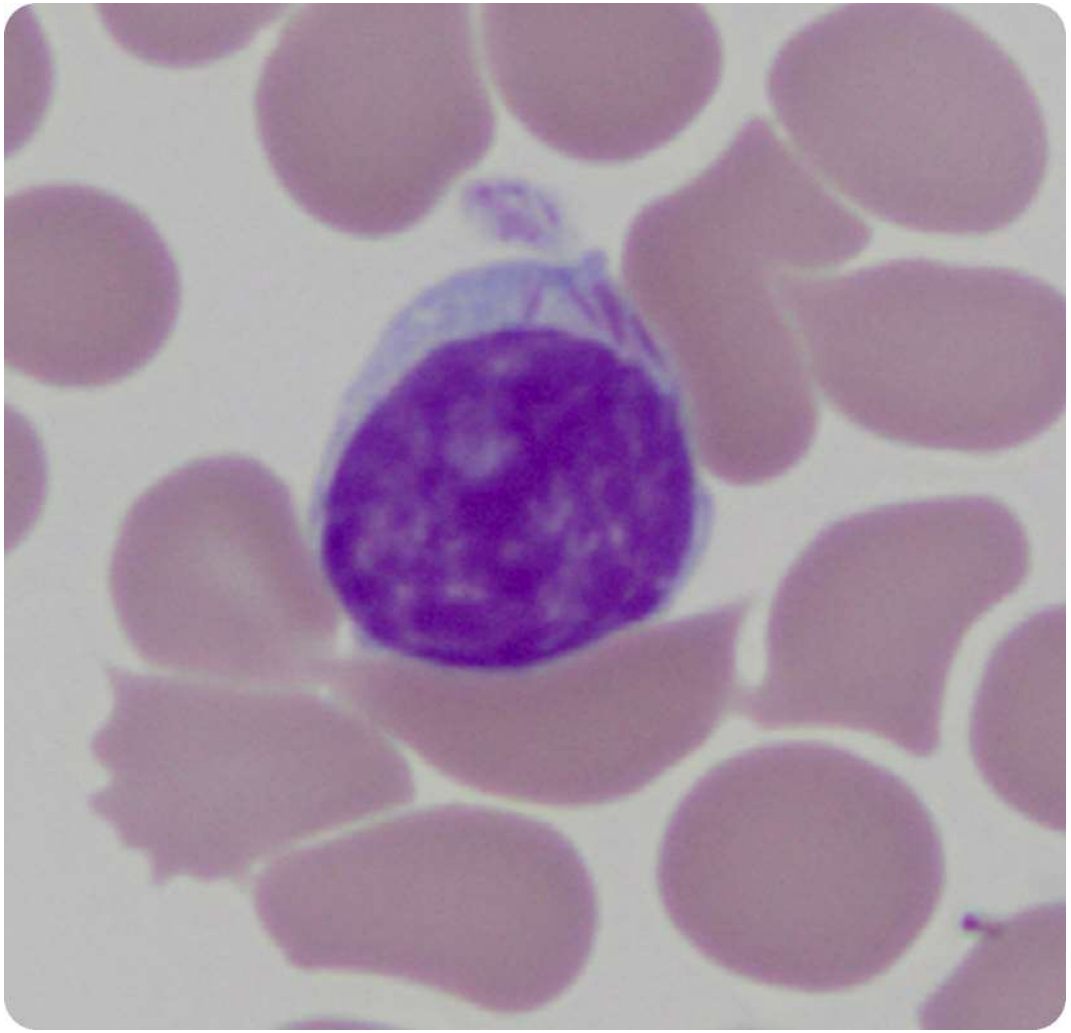
VOI

SIETE CONVINTI?

IO NON

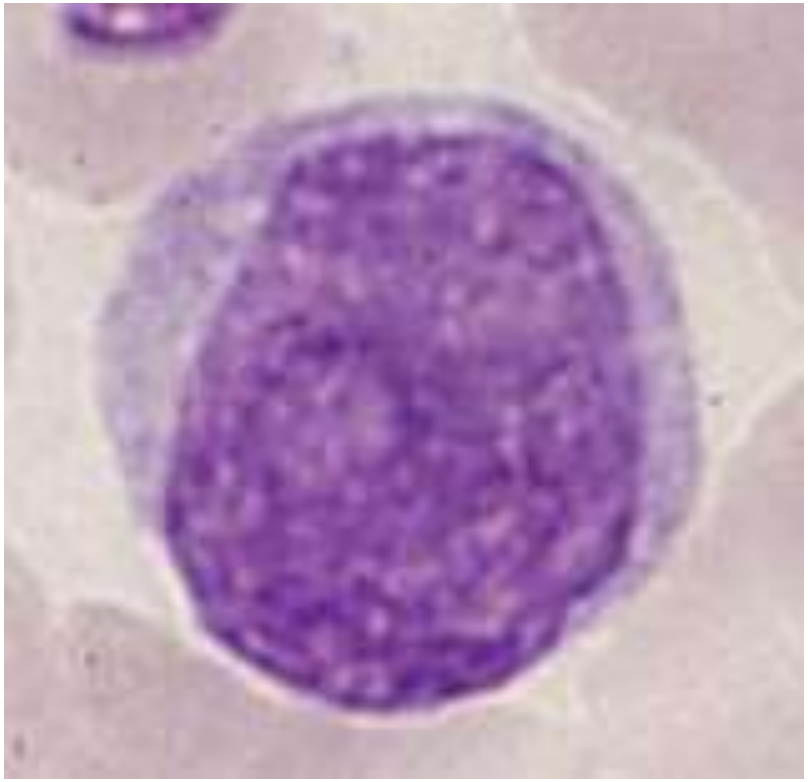
COMPLETAMENTE

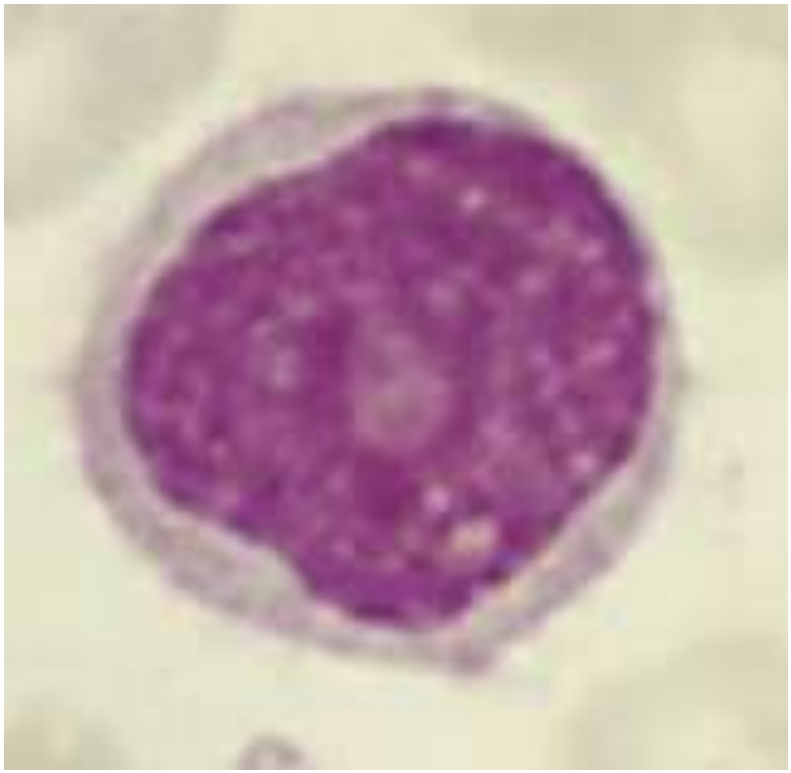
BACK TO CYTOMORPHOLOGY



Blood
Volume 124(16):2607-2607
October 16, 2014

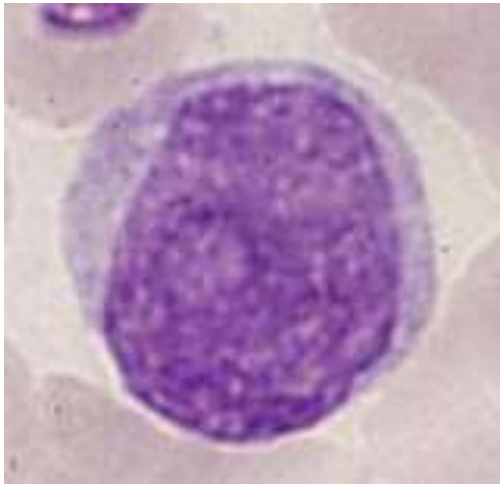
BACK TO CYTOMORPHOLOGY

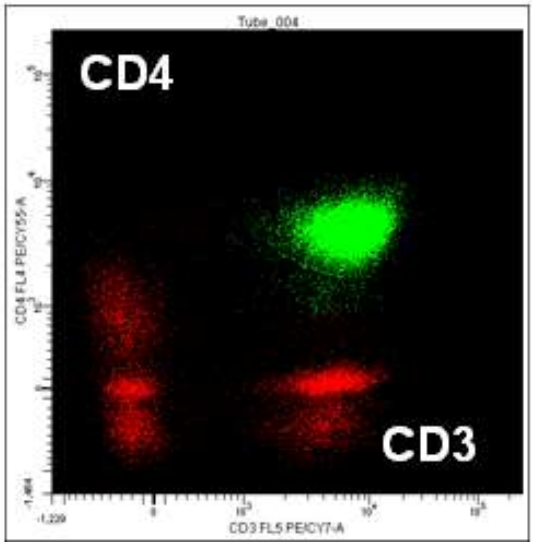


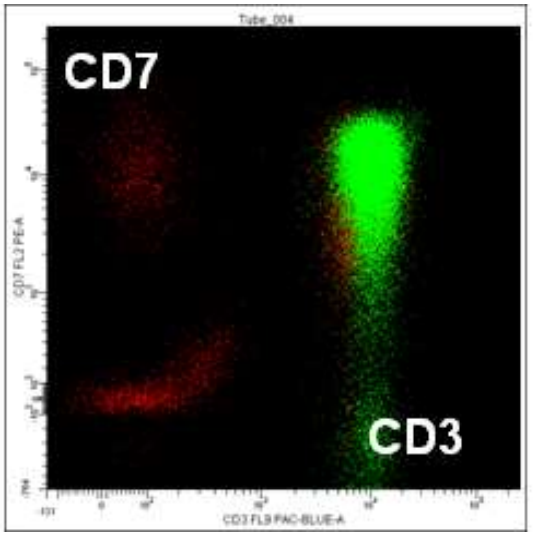


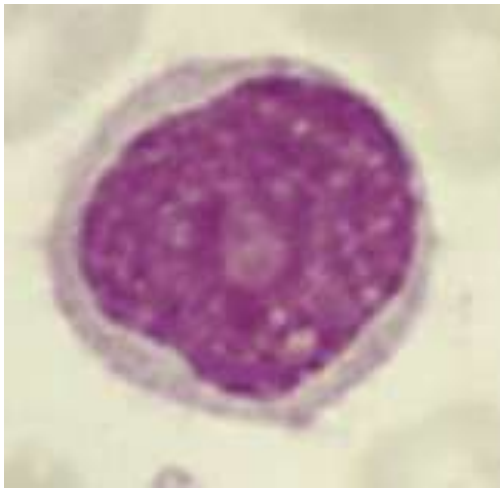
Personal archive

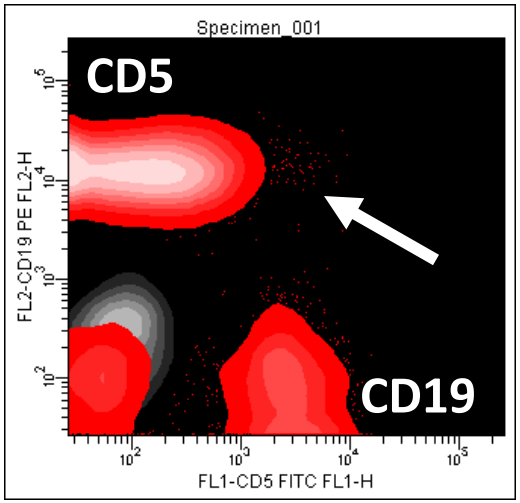
BACK TO CYTOMORPHOLOGY

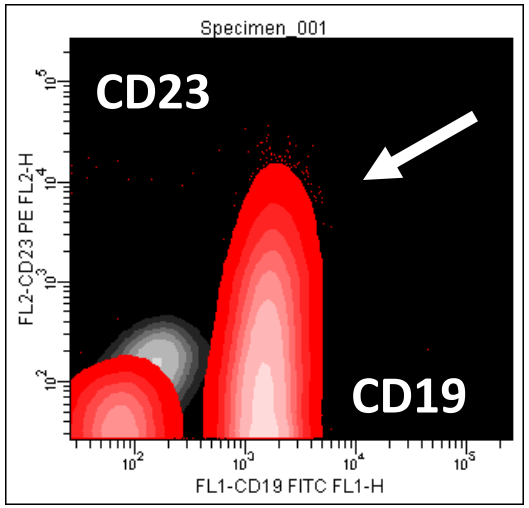












Sohn SK, et al. Hepatosplenic T-cell lymphoma: prolymphocytic transformation 18 months after splenectomy. Int J Hematol 1997;66:227

We report a young woman with pancytopenia and huge splenomegaly who was also found to have peripheral T-cell lymphoma with massive infiltration of T-cell evident in the liver and spleen. A liver biopsy showed predominant sinusoidal infiltration of pan-T cell antibody-stained T-lymphoid cells. Histologic examination of the spleen revealed numerous tumor cells predominantly infiltrated in the cords and sinuses of the red pulp, which were identical to those described in the liver. Several clusters of small round abnormal cells were observed in marrow cytology. Although the patient felt well during 18 months after the splenectomy was done, the patient eventually manifested a huge hepatomegaly, showed increasing white blood cell count to 42 x 10(9)/l, and numerous prolymphocytes (66.9%) in the bone marrow. **This change represented a prolymphocytic transformation of the patient's original hepatosplenic T-cell lymphoma.**

Prolymphocytoid transformation of follicular lymphoma with coexpression of CD5 and CD10

SEAN K. LAU, LAWRENCE M. WEISS, YIBIN ZHANG, & QIN HUANG

Histologic transformation of follicular lymphoma is usually to a diffuse large B-cell lymphoma.

We present a rare example of a histologic transformation of follicular lymphoma manifested by prolymphocytoid morphology

and an unusual immunophenotype characterized by coexpression of CD5 and CD10. The transformed prolymphocytoid lymphoma was positive for CD5 and CD10 antigens by both flow cytometry and immunohistochemistry. The case also expressed bcl-2 and bcl-6 proteins, and exhibited t(14;18), consistent with derivation from a pre-existing follicular lymphoma. Polymerase chain reaction analysis of the immunoglobulin kappa light chain genes derived from the follicular lymphoma and prolymphocytoid lymphoma showed identical rearranged bands, suggesting clonal identity of the two neoplasms. The basis for coexpression of CD5 and CD10 remains unclear. Because the preceding low-grade follicular lymphoma was positive only for CD10 and did not express CD5, CD5 expression appears to be an acquired phenomenon accompanying the process of histologic transformation in this particular case. Prolymphocytoid transformation, similar to other histologic forms of transformation of follicular lymphoma, appears to accompany clinical progression of disease.

Leukemia & Lymphoma, March 2006; 47(3): 541–547

Fridrik MA et al, Morphological and immunological changes of hairy cell leukemia during alpha-2-interferon therapy. Blut 1989;58;261.

We describe a patient who presented with the clinical picture of hairy cell leukemia (HCL). Bone marrow and peripheral blood lymphoma cells showed morphological and immunological features of HCL.

Under recombinant alpha-2-interferon (alpha-2-IF) therapy the characteristic morphology changed from HCL to prolymphocytic leukemia (PLL).

At diagnosis the lymphoma cells expressed CD24 and FMC7 surface antigen, but stained negative for surface immunoglobulins, light chains and anti-CD5. During alpha-2-IF treatment surface antigen expression changed to CD24, CD5 and FMC7. Surface IgD and lambda light chains became strongly positive. Southern Blot analysis of peripheral blood mononuclear cells showed two rearranged immunoglobulin bands at diagnosis but only one upon alpha-2-IF therapy. These data suggest, that this patient suffered from a biclonal lymphoma, HCL and PLL. While undergoing alpha-2-IF treatment the HCL came into remission, whereas the PLL clone proved to be poorly sensitive to alpha-2-IF therapy.

Kawada E et al. Primary nonsecretory plasma cell leukemia: a rare variant of multiple myeloma. Ann Hematol 1999;78;25

We report a patient with primary nonsecretory plasma cell leukemia. These cells resembled prolymphocytes and expressed only CD38 and CD56. Due to the atypical morphology and lack of monoclonal immunoglobulins in both serum and urine, it was difficult to make a correct diagnosis. Electron-microscopic and immunocytochemical cytoplasmic studies were useful.

Mature B-Cell Leukemias With More Than 55% Prolymphocytes

A Heterogeneous Group That Includes an Unusual Variant of Mantle Cell Lymphoma

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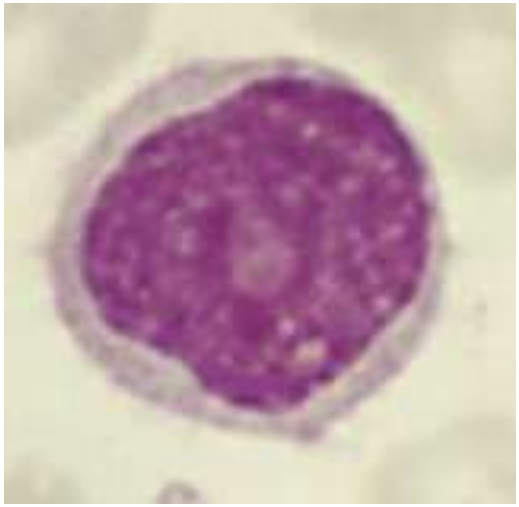
We studied 20 cases of mature B-cell leukemia with more than 55% prolymphocytes in peripheral blood or bone marrow, fulfilling the French-American-British criteria for B-cell prolymphocytic leukemia (PLL). Cases segregated into 3 groups: de novo PLL, 6; PLL occurring in patients with a previous well-established diagnosis of chronic lymphocytic leukemia (PLL-HxCLL), 10; and t(11;14)(q13;q32)-positive neoplasms, 4. All cases expressed monotypic immunoglobulin light chain, and most were positive for CD5. All t(11;14)-positive neoplasms were CD23- and uniquely positive for cyclin D1. Cytogenetic abnormalities were present in 19; in all 19, the karyotype was complex, indicating clonal evolution and genomic instability. The most frequent cytogenetic abnormality in de novo PLL involved chromosome 7 in 4 cases. Trisomy 12 or add(12p) was present in 4 cases of PLL-HxCLL. We conclude that mature B-cell leukemias with more than 55% prolymphocytes are a heterogeneous group that includes t(11;14)-positive neoplasms, which we suggest are best classified as mantle cell lymphoma.

We suggest that prolymphocytic morphologic features are a common end-stage of transformation for several B-cell neoplasms.

Am J Clin Pathol 2001;115:571-581

LE INSIDIE DELLA CITOMORFOLOGIA



B-PLL

T-PLL

MCL

FL

HCL

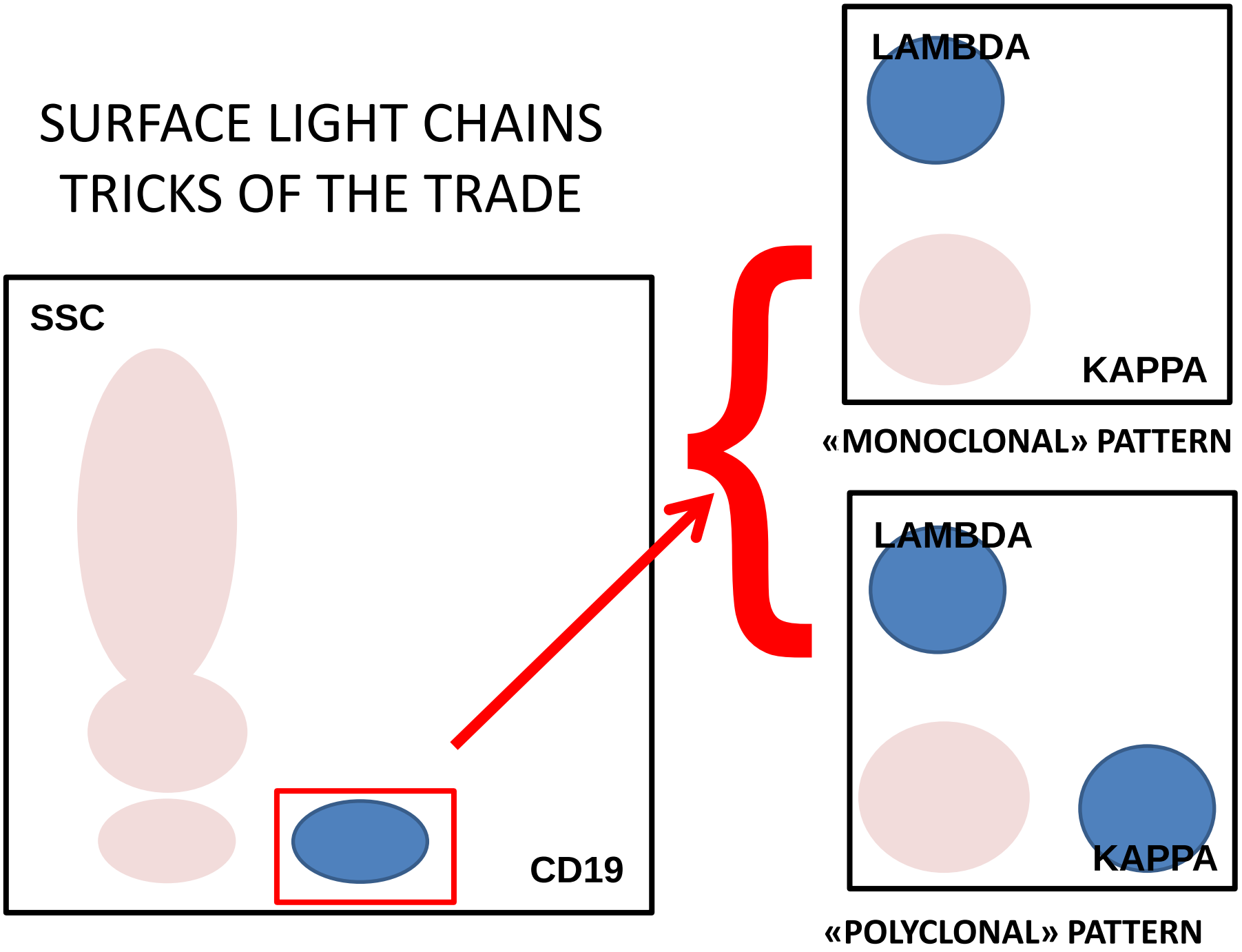
HSTCL

PCL

Ulteriore esempio di integrazione emocitometria – citofluorimetria

DISTINZIONE DEL LINFOCITA REATTIVO DALL'ELEMENTO DI UNA CLPD

SURFACE LIGHT CHAINS
TRICKS OF THE TRADE

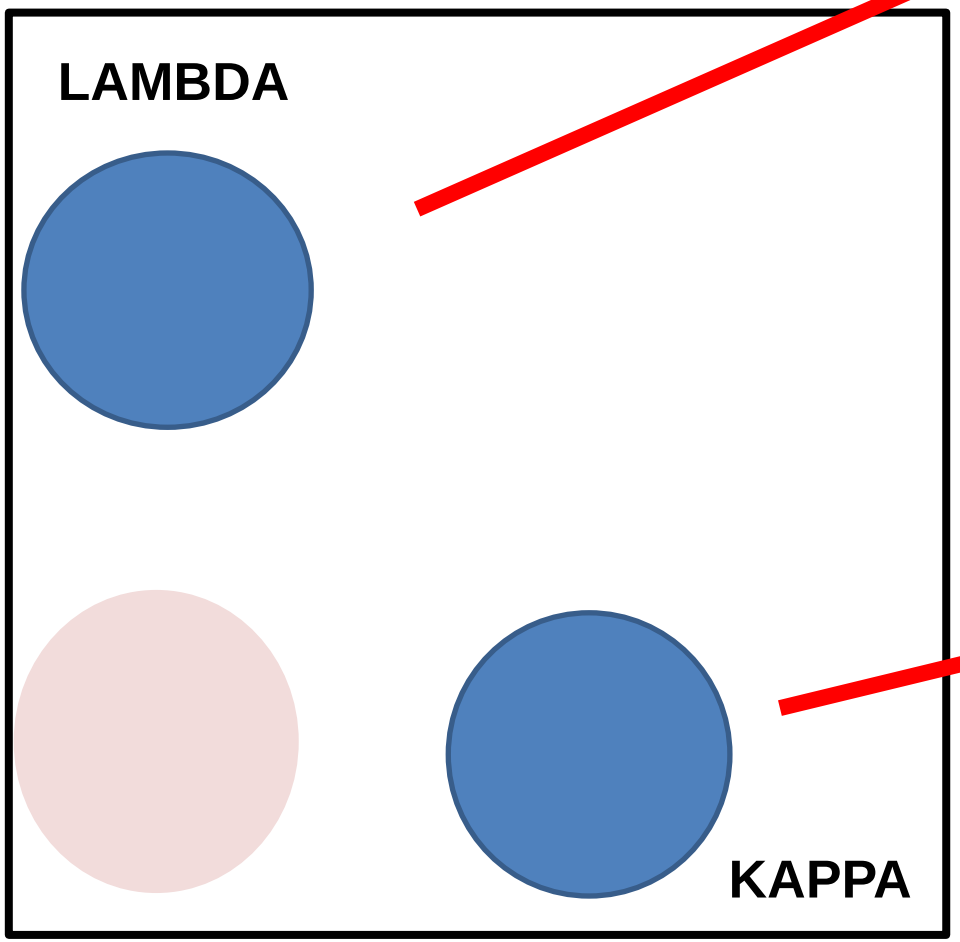


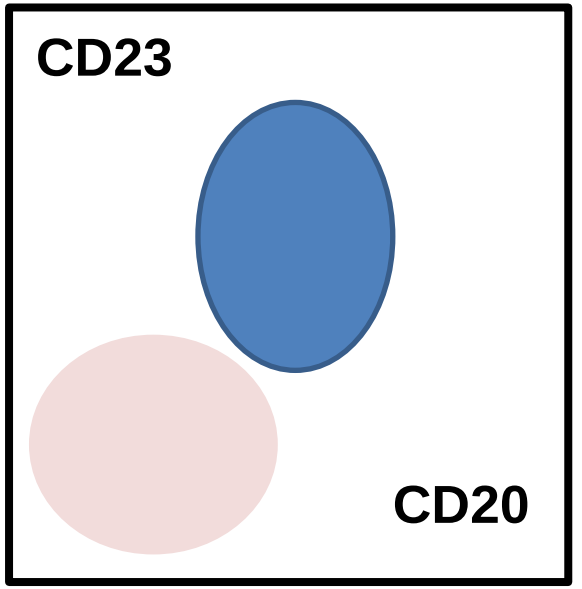
SURFACE LIGHT CHAINS
TRICKS OF THE TRADE

<u>AVOIDING MISTAKES</u>

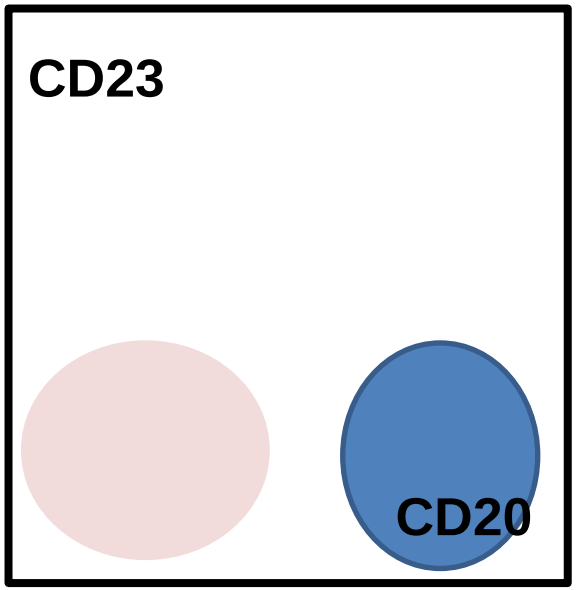
IS A «POLYCLONAL» PATTERN **ALWAYS** REALLY POLYCLONAL ?

SURFACE LIGHT CHAINS
TRICKS OF THE TRADE





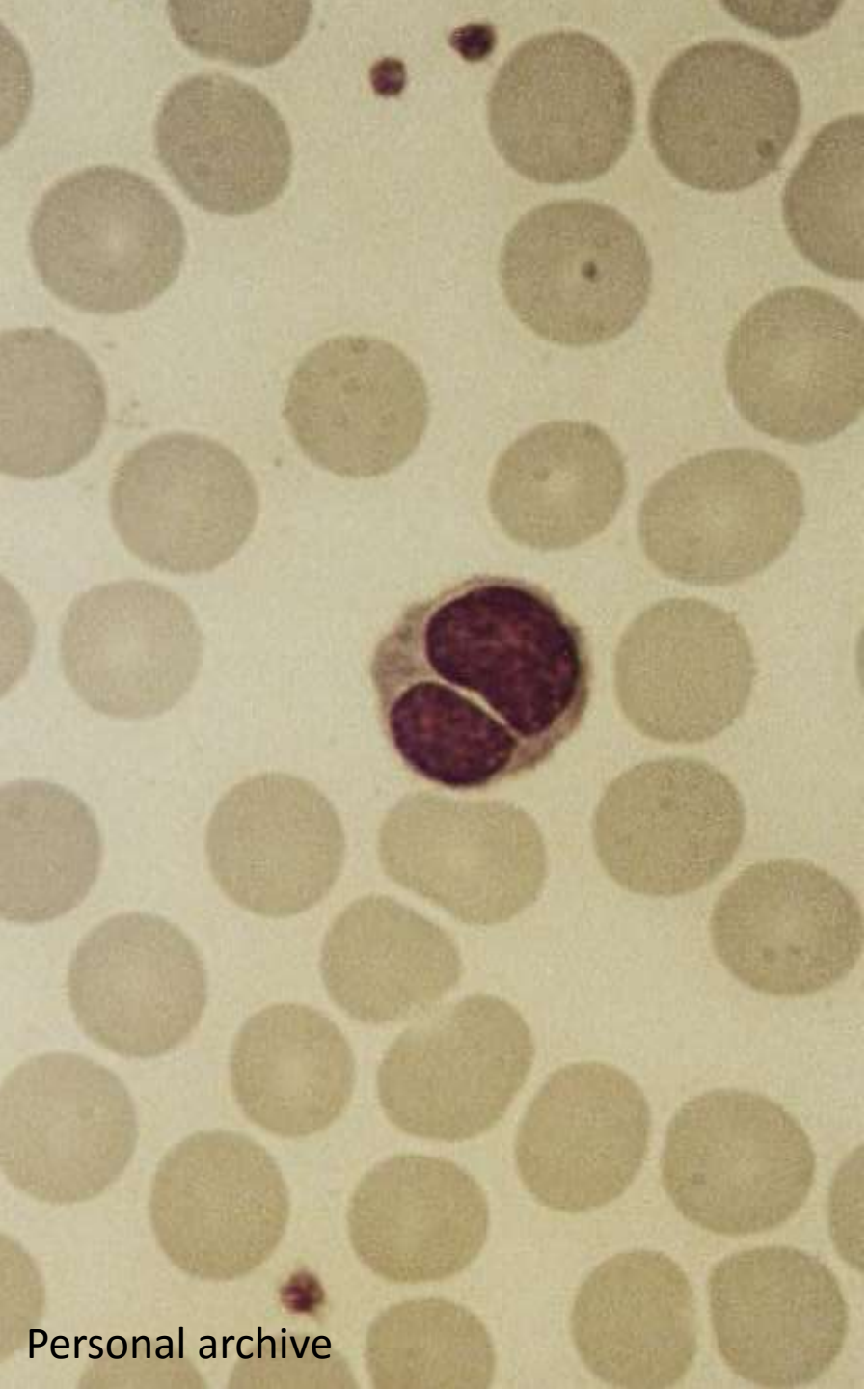
PATHOLOGICAL POPULATION



SURFACE LIGHT CHAINS
TRICKS OF THE TRADE

AVOIDING MISTAKES

IS A **REALLY POLYCLONAL** PATTERN
ALWAYS NORMAL ?



Personal archive

POLICLONALITY PROVEN WITH MOLECULAR BIOLOGY ASSAYS

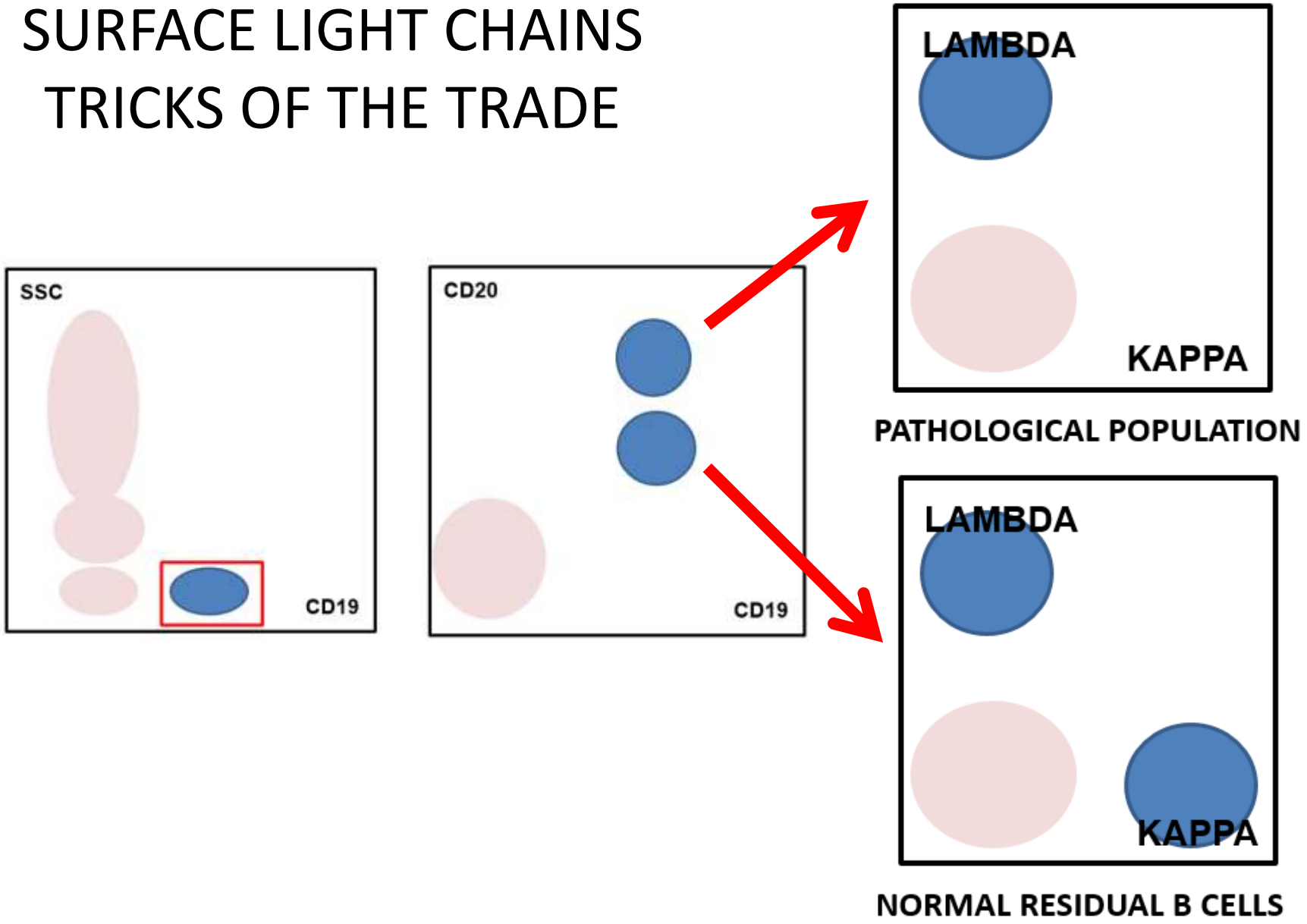
- Female gender
- Young age
- Smoking habit
- Polyclonal IgM hypergammaglobulinemia
- HLA-DR7 haplotype
- Binucleate lymphocytes in peripheral blood
- Phenotype: CD5-, CD10-, CD11c-, CD19+, CD20++, CD22±, CD23-, CD25-, CD27+, CD37+, CD43-, CD103-

SURFACE LIGHT CHAINS
TRICKS OF THE TRADE

AVOIDING MISTAKES

IS A «HOMOGENEOUS» POPULATION
ALWAYS HOMOGENEOUS ?

SURFACE LIGHT CHAINS
TRICKS OF THE TRADE

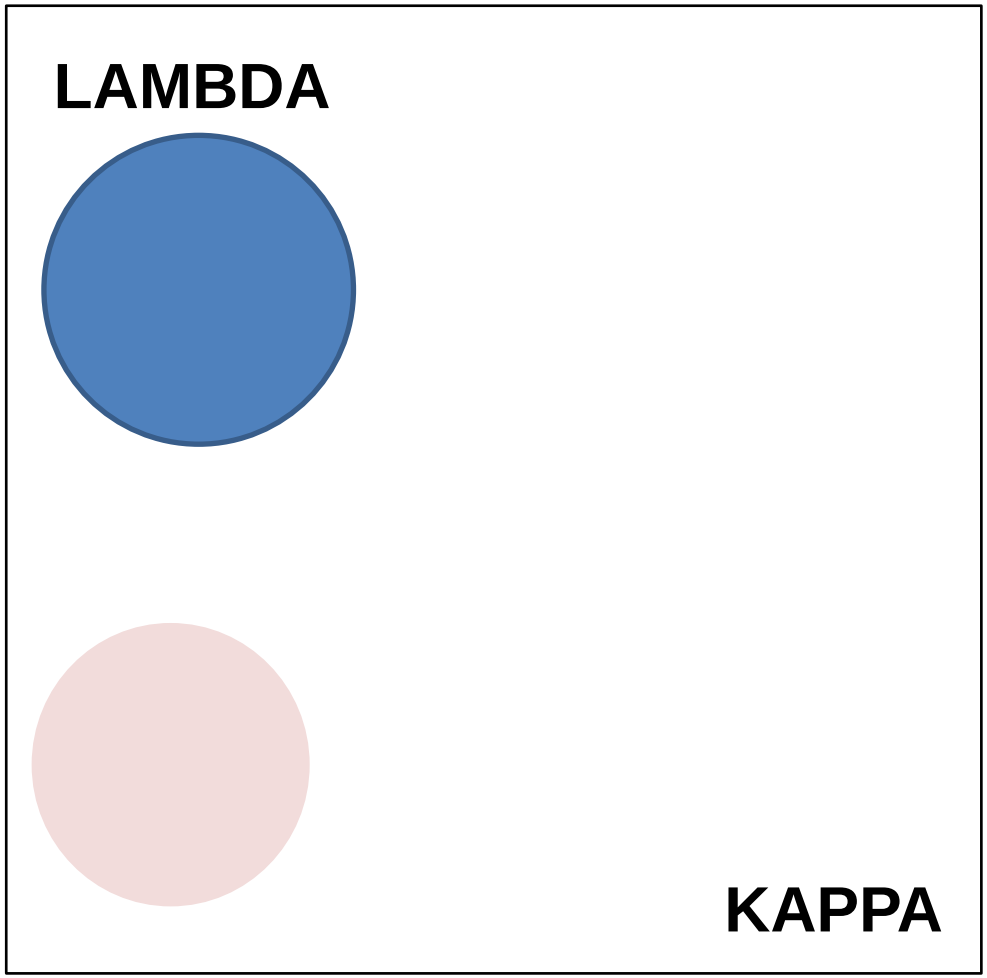


SURFACE LIGHT CHAINS
TRICKS OF THE TRADE

AVOIDING MISTAKES

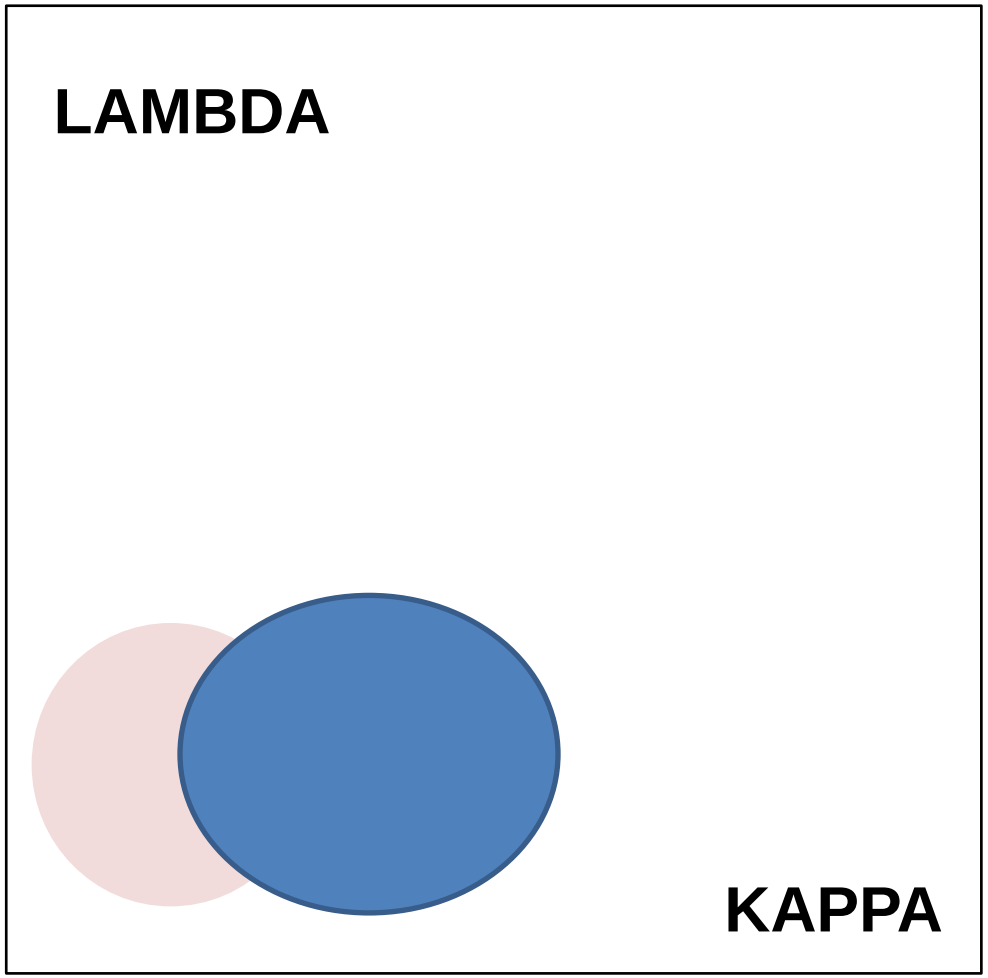
MAY I OBTAIN ADDITIONAL INFORMATION FROM A PATHOLOGICAL PATTERN?

MOST PROBABLE INTERPRETATION



1) HIGH INTENSITY
2) SUGGESTION: BL, MCL, OTHER THAN B-CLL

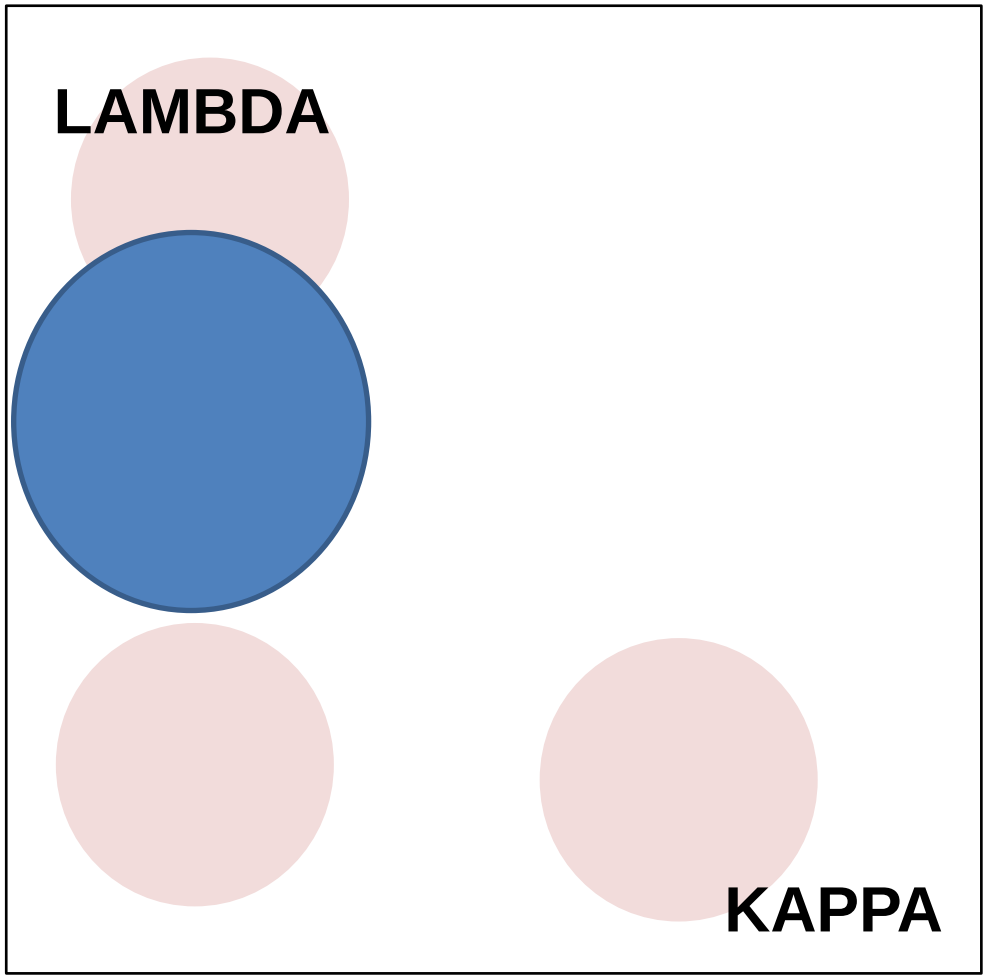
MOST PROBABLE INTERPRETATION



1) LOW INTENSITY
2) SUGGESTION:

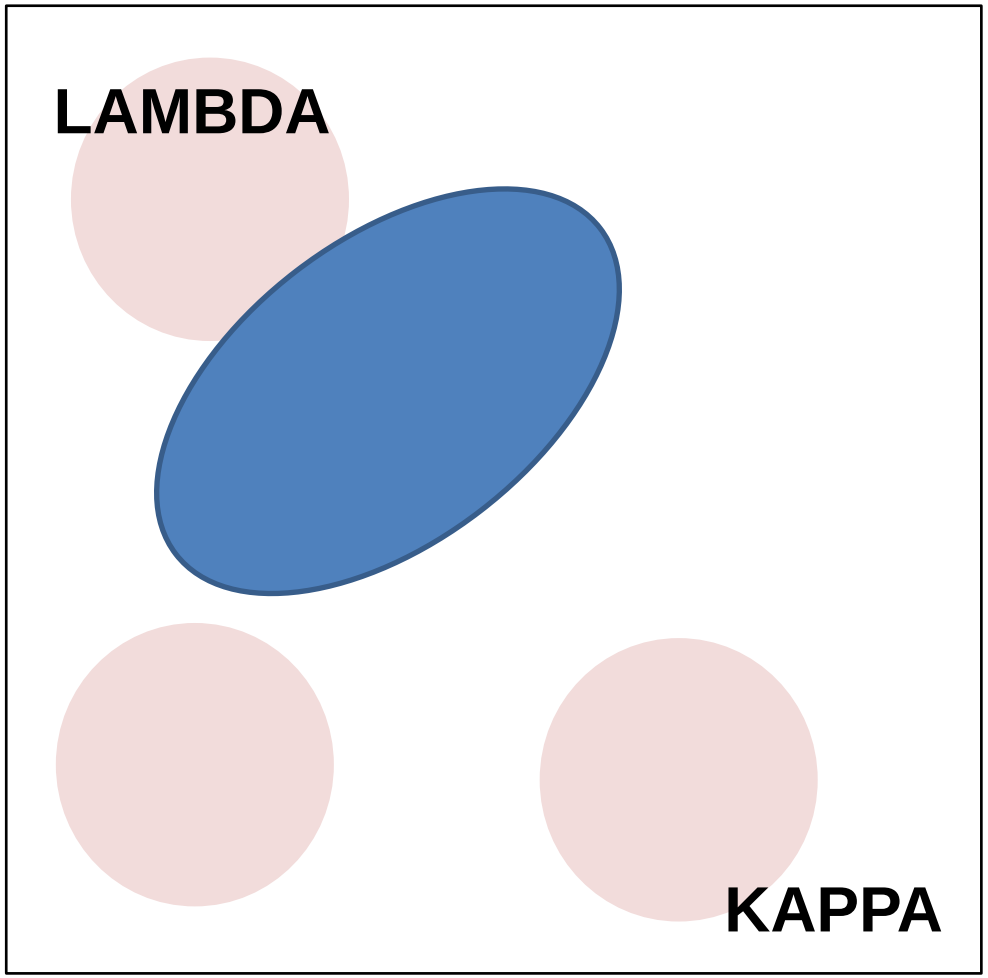
B-CLL

MOST PROBABLE INTERPRETATION



1) NORMAL RESIDUAL B CELL POPULATION (BLUE) + LOW INTENSITY λ RESTRICTED B SUBSET (RED)
2) SUGGESTION:
   - INITIAL DISEASE?
   - RELAPSE?

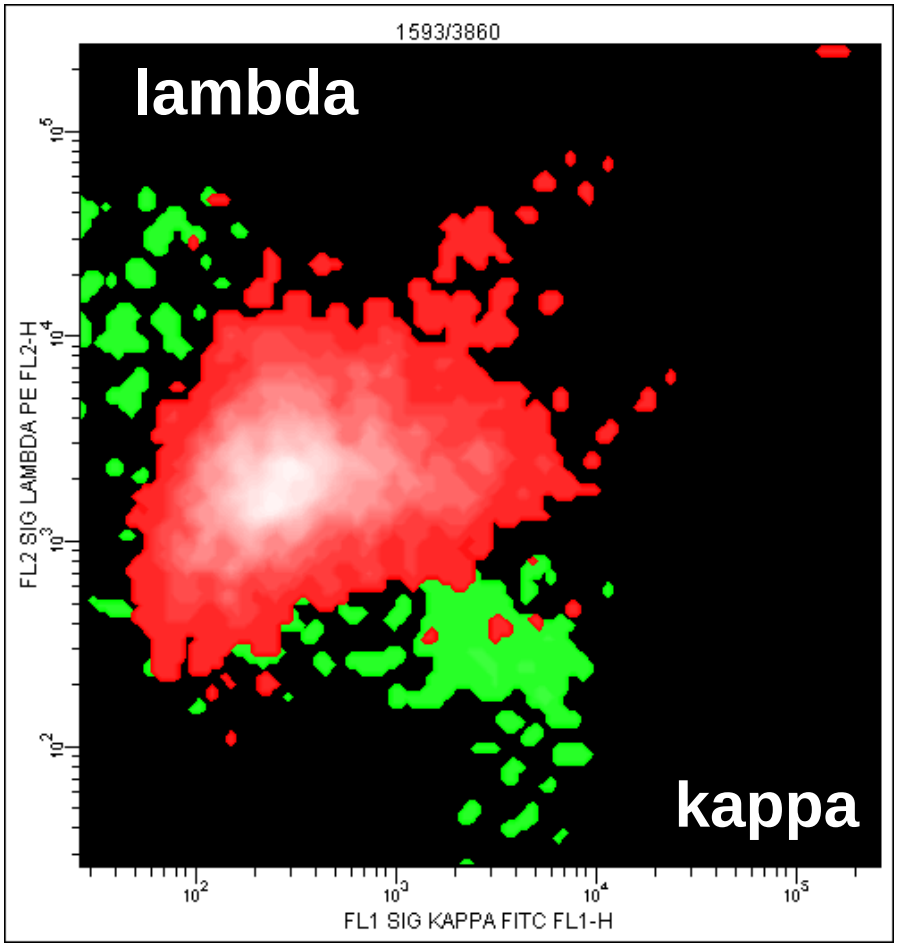
MOST PROBABLE INTERPRETATION

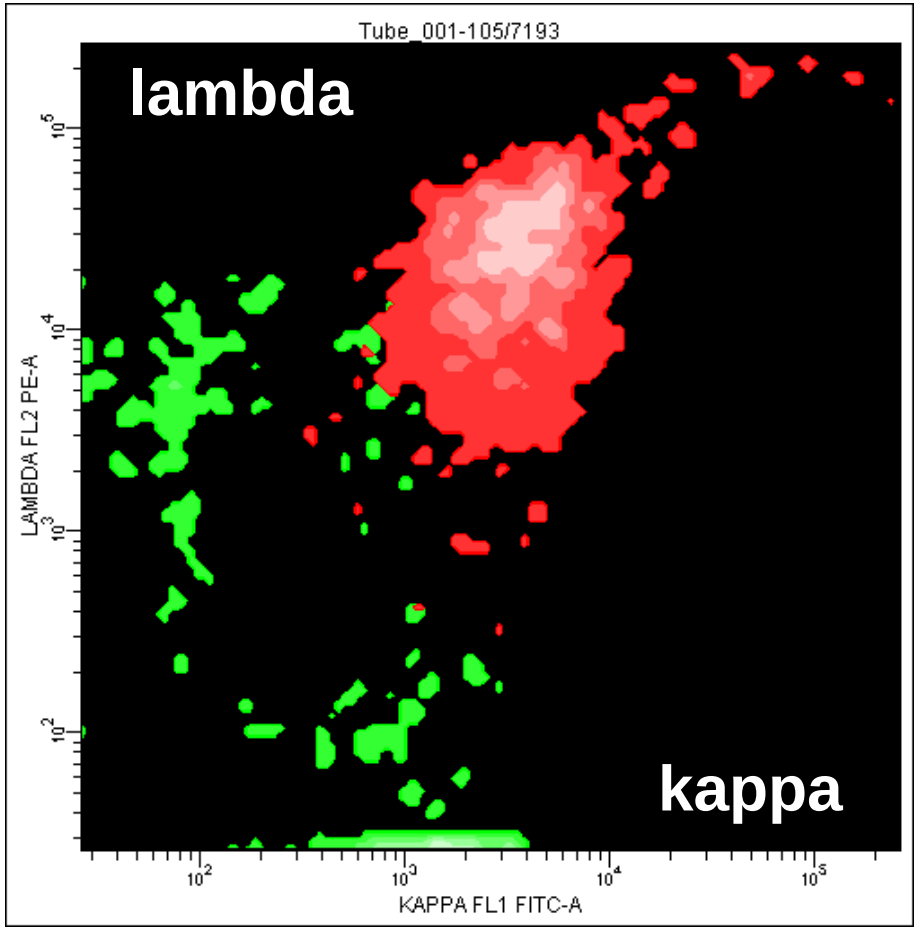


1) NORMAL RESIDUAL B CELL POPULATION (BLUE) + λ RESTRICTED B SUBSET (RED) WITH A SLIGHTLY «DOUBLE POSITIVE» APPEARANCE (BLUE)
2) SUGGESTION:

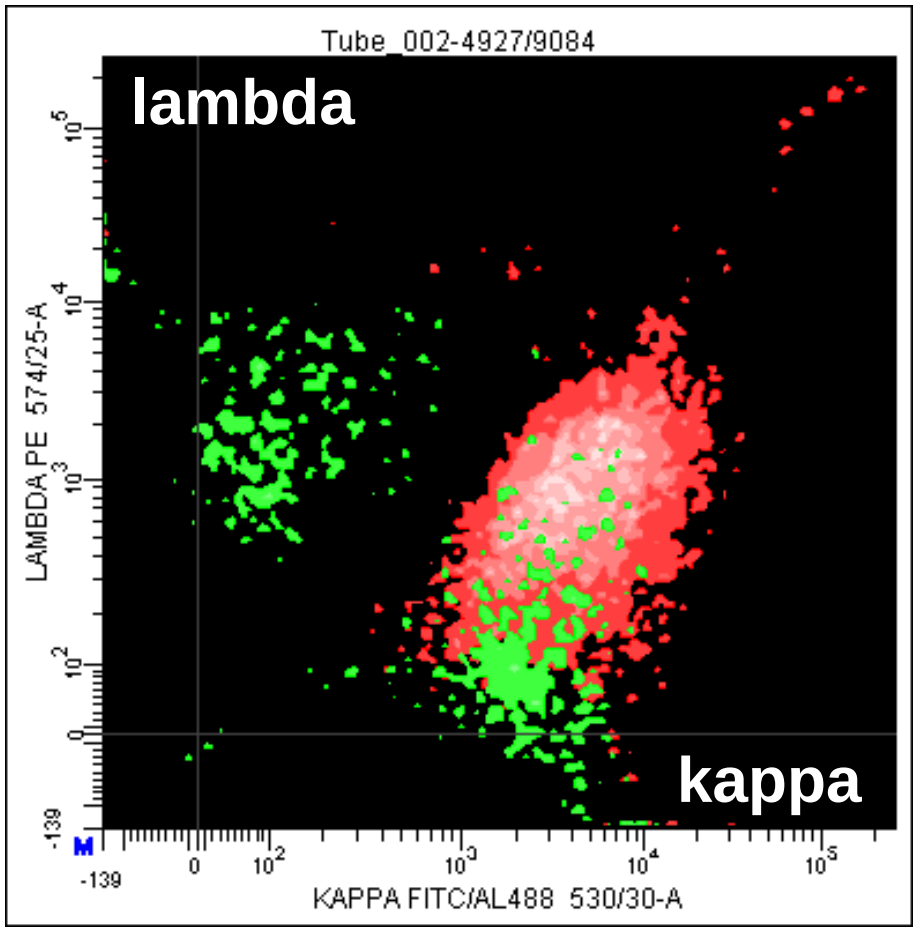
   HCL?

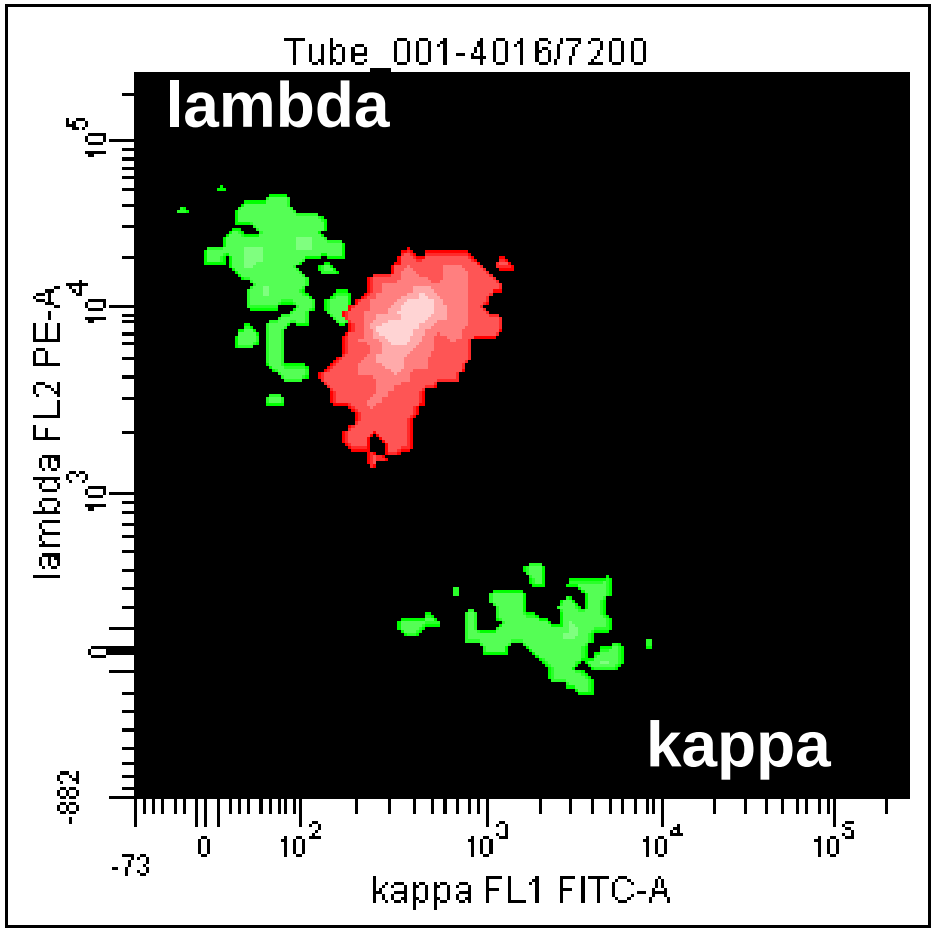
TYPICAL (AND UNEXPLAINED) LIGHT CHAIN BEHAVIOR IN HCL CELLS (RED)





TYPICAL (AND UNEXPLAINED) LIGHT CHAIN BEHAVIOR IN HCL CELLS (RED)





THERE ARE MORE THINGS IN FLOW CYTOMETRY, HORATIO, THAN ARE DREAMT OF IN YOUR PHILOSOPHY ...

Shakespeare, Hamlet, Act 1, Scene V