



**CORSO TEORICO-PRACTICO PER LA GESTIONE OTTIMALE DEI PAZIENTI
AFFETTI DA LINFOMA MANTELLARE, LINFOMA FOLLICOLARE
E LEUCEMIA LINFATICA CRONICA**

*Aula CERM5, AOU Città della Salute e della Scienza di Torino – Prendio Molinette
Via Cherasco 15, Torino*

21-23 Maggio 2018

**LA CITOFUORIMETRIA NELLA DIAGNOSI E
NELLA DEFINIZIONE DELLA RISPOSTA**

Paola Omedè



Laboratorio Citofluorimetria
Divisione Universitaria di Ematologia

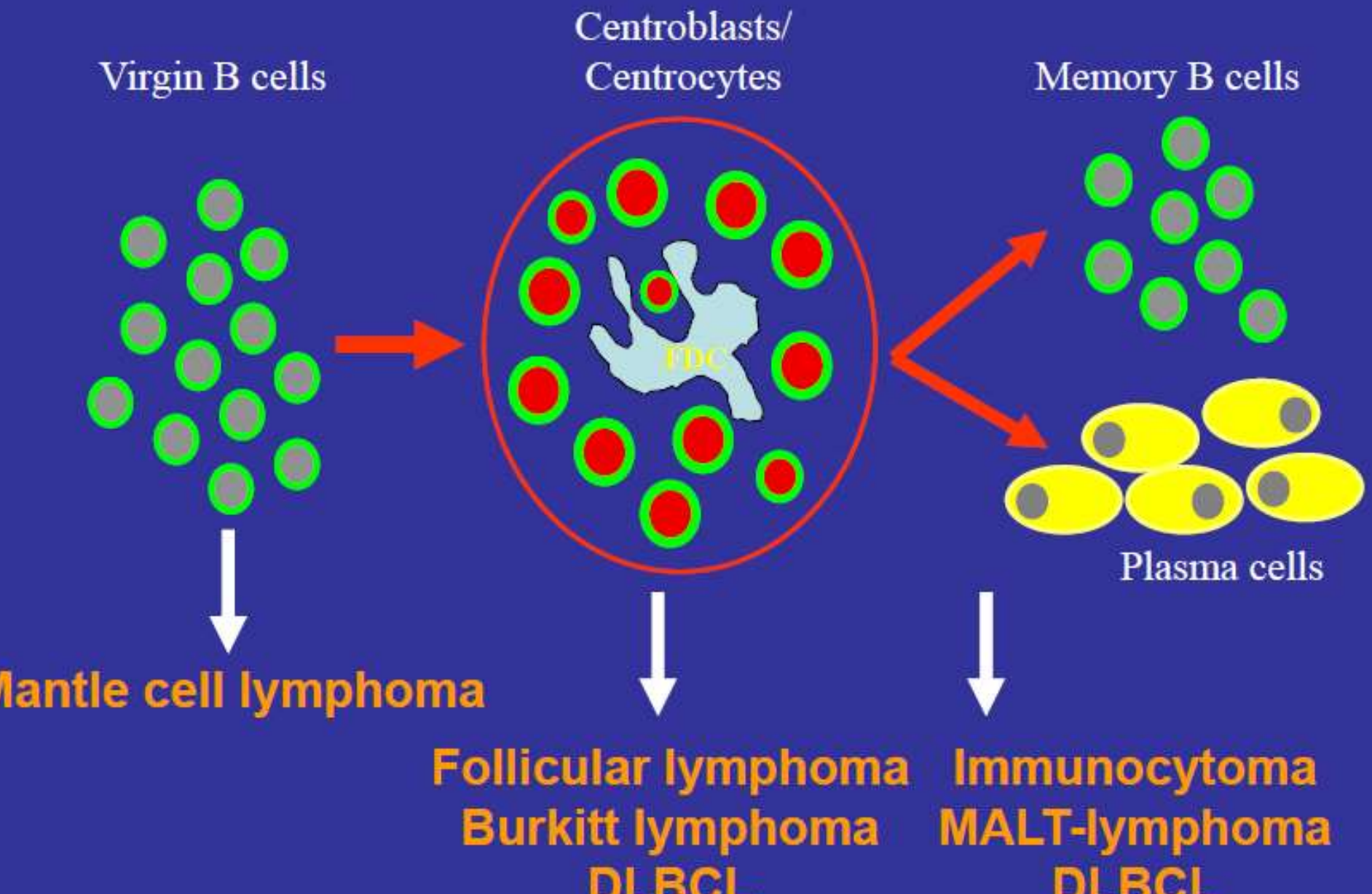
DISORDINI LINFOPROLIFERATIVI CRONICI

Leucemia Linfatica Cronica

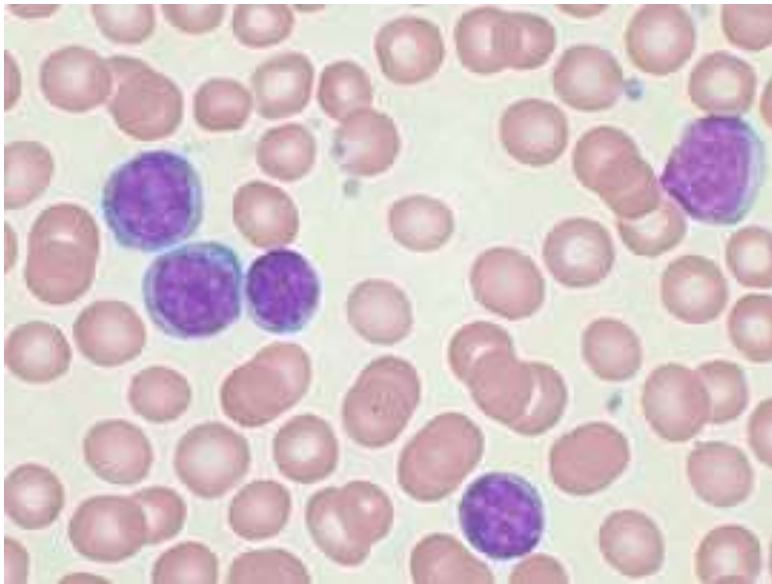
Linfoma Mantellare

Linfoma Follicolare

HISTOGENESIS of B-cell Neoplasia

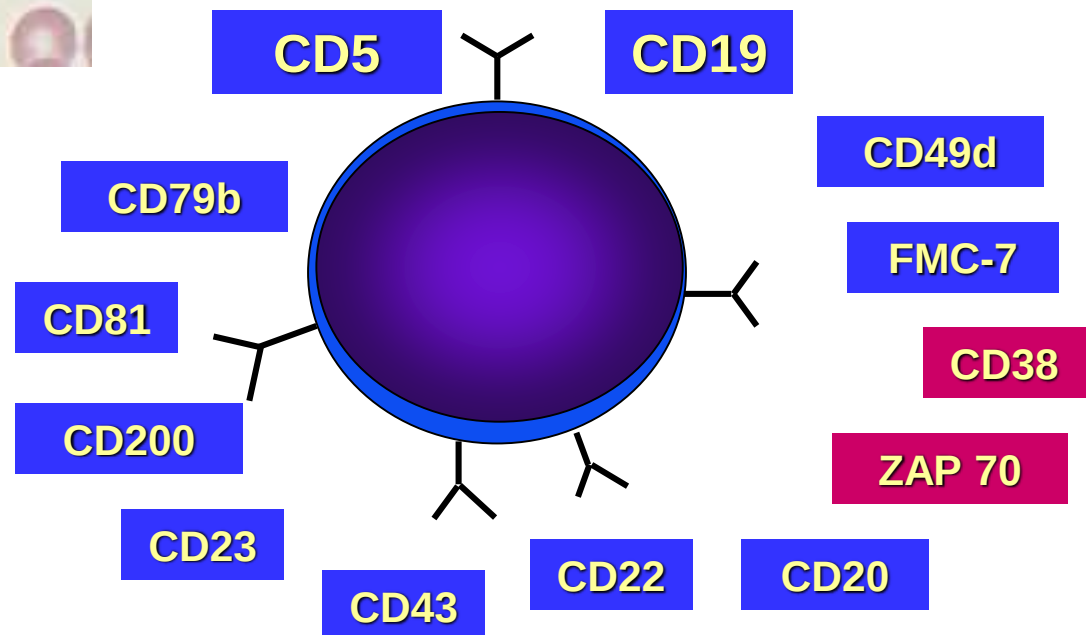


MORFOLOGIA



CLL

CITOFUORIMETRIA



CLL: DIAGNOSI

- 5000 B linfociti monoclonali/ μ L nel PB
- clonalità confermata in citofluorimetria
- presenza di prolinfociti
- presenza di ombre di Gumprecht

CITOFLUORIMETRIA

DIAGNOSI

FATTORI PROGNOSTICI

MALATTIA MINIMA RESIDUA

blood

2008 111: 5446-5456

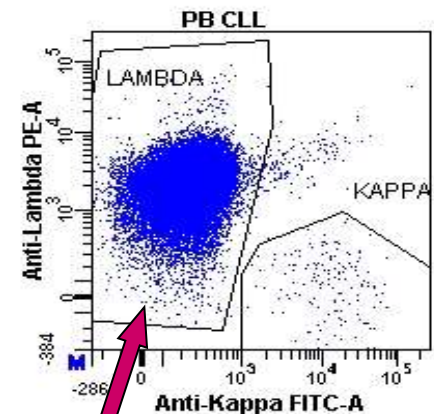
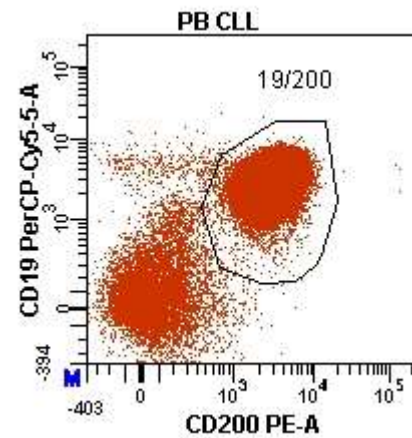
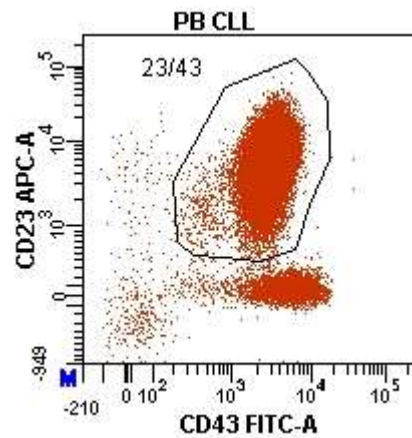
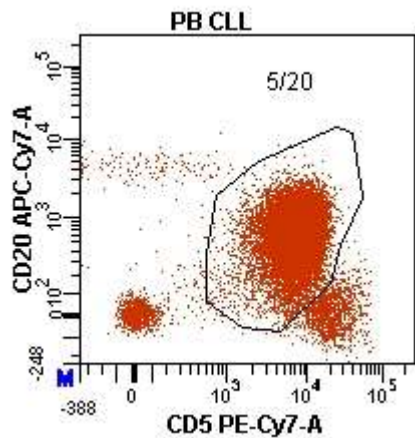
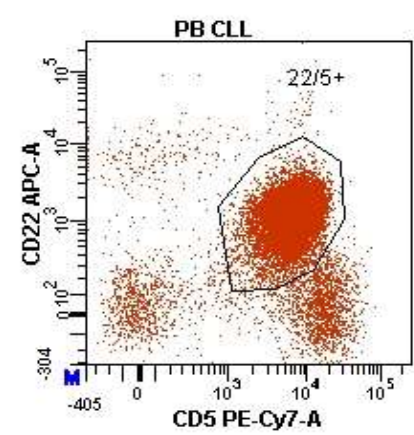
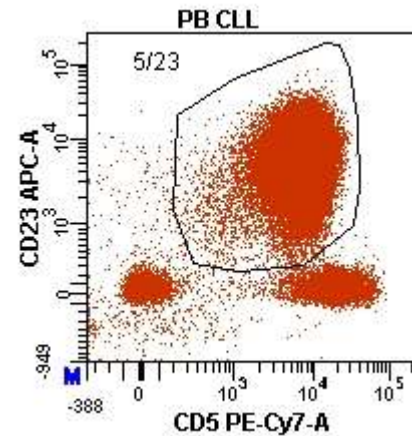
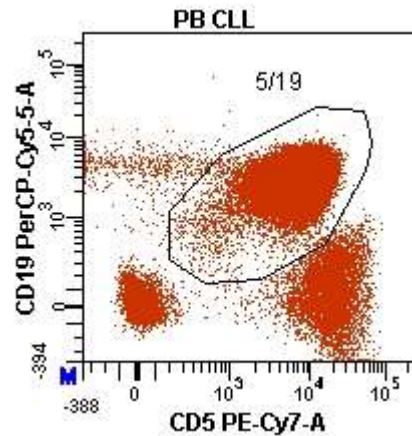
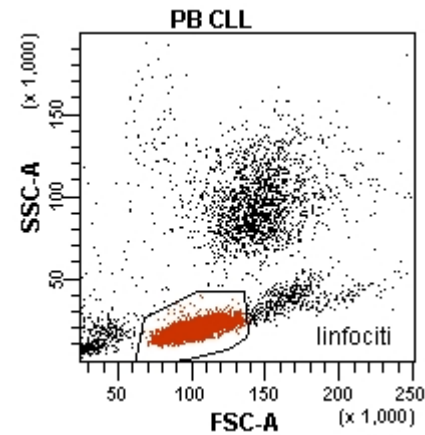
Prepublished online Jan 23, 2008;

doi:10.1182/blood-2007-06-093906

Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute Working Group 1996 guidelines

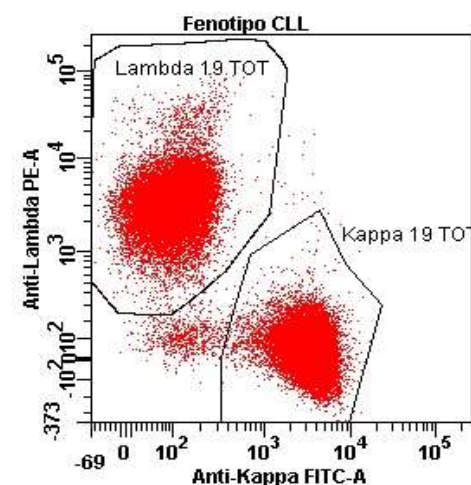
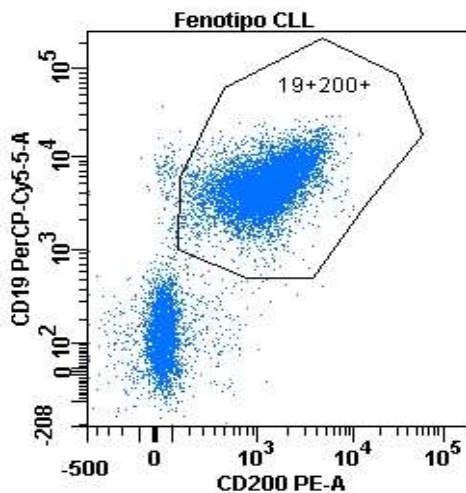
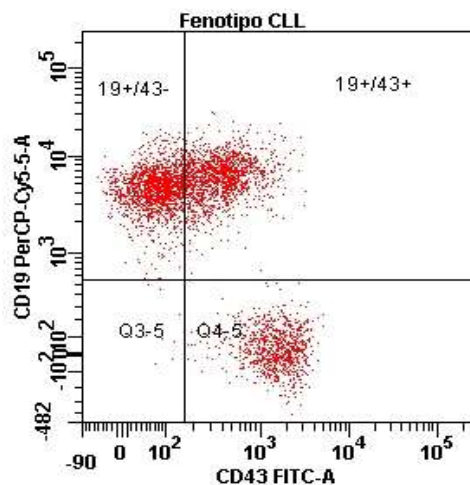
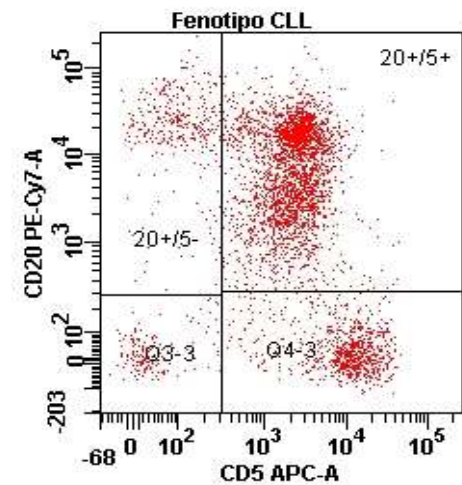
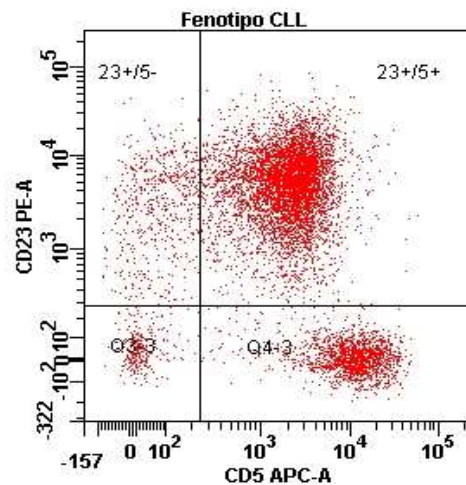
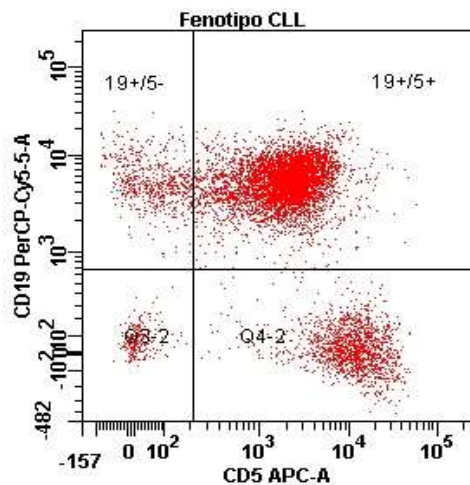
Michael Hallek, Bruce D. Cheson, Daniel Catovsky, Federico Caligaris-Cappio, Guillaume Dighiero, Hartmut Döhner, Peter Hillmen, Michael J. Keating, Emili Montserrat, Kanti R. Rai and Thomas J. Kipps

CLL: DIAGNOSI

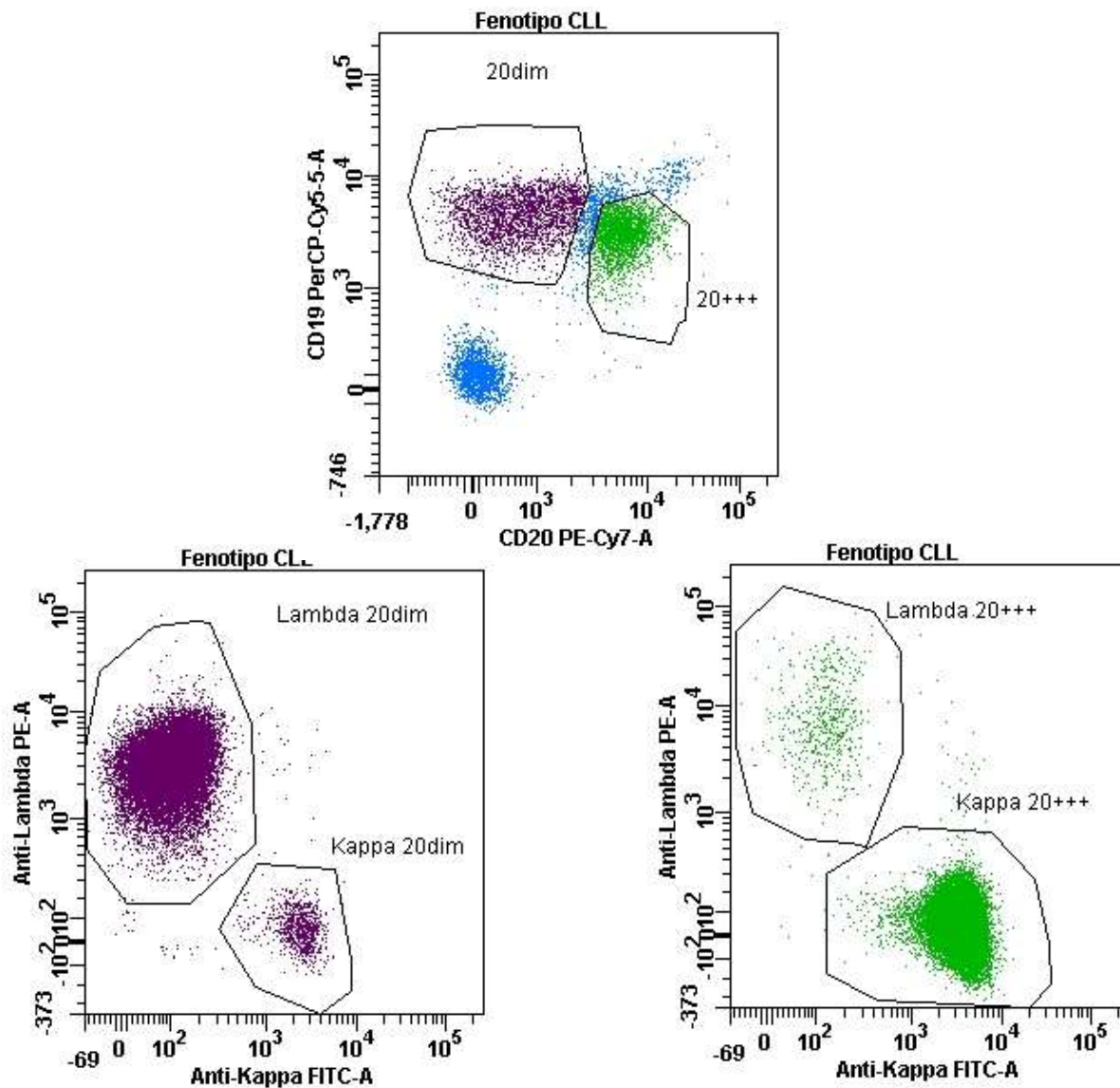


catene leggere delle Ig monoclonali

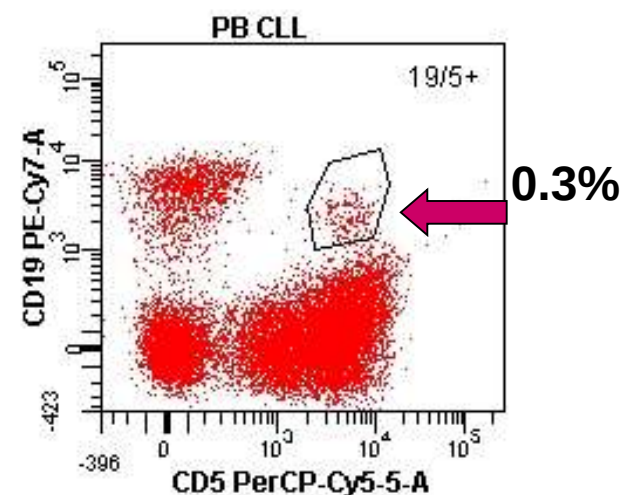
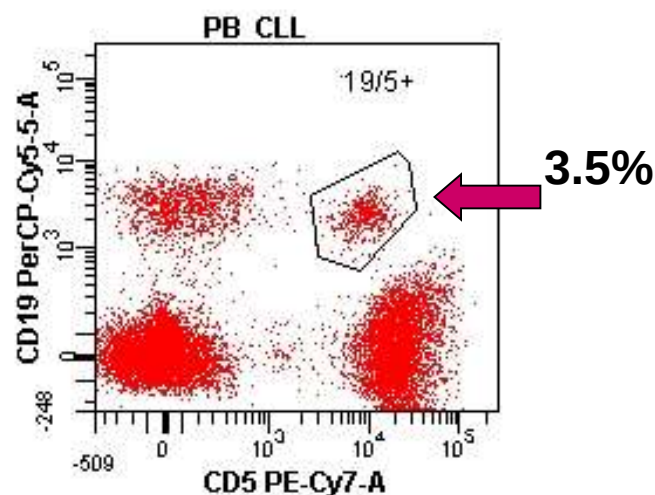
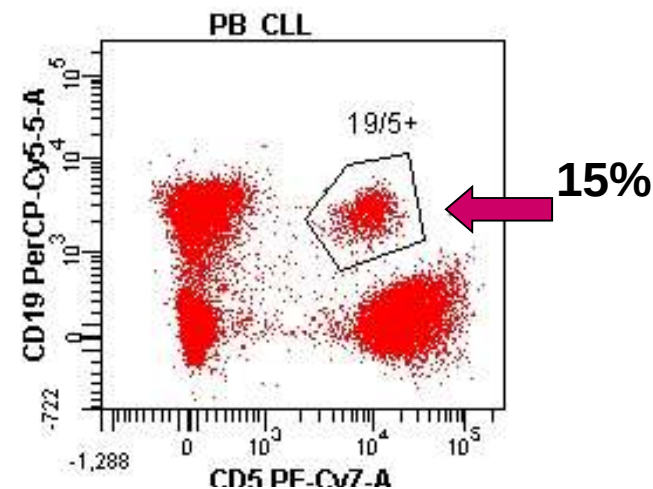
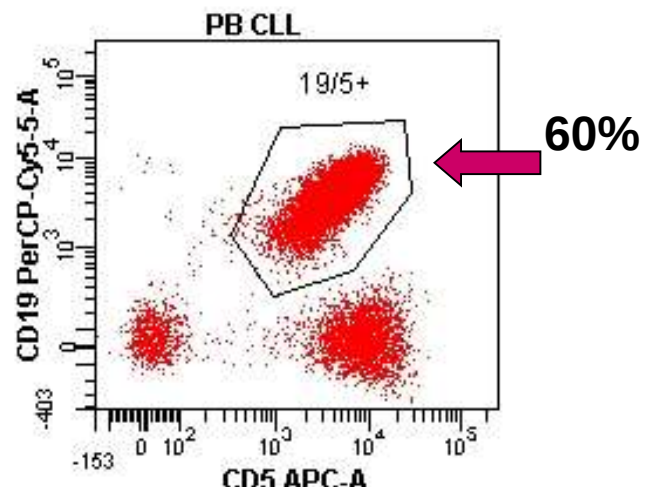
CLL ATÍPICA



CLL ATIPICA



MONITORAGGIO della RISPOSTA alla TERAPIA



VALUTAZIONE della MMR

Metodi QUALITATIVI

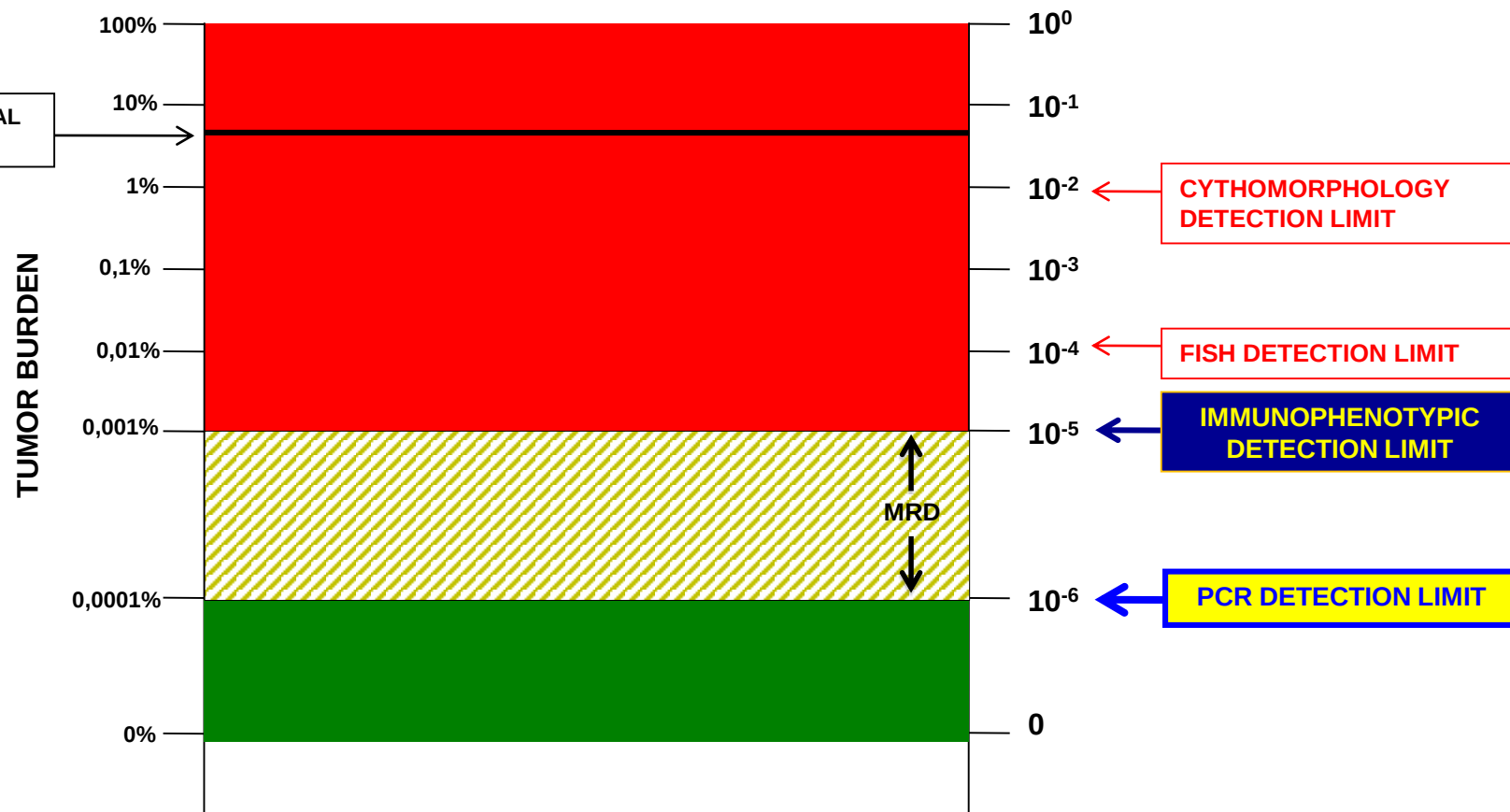
- IgH PCR
- Citofluorimetria

Metodi QUANTITATIVI

- ASO IgH PCR
- Citofluorimetria multiparametrica
- NGS
- NGF

Malattia Minima Residua (MMR)

Identificazione di cellule tumorali residue mediante tecniche molto sensibili





ORIGINAL ARTICLE

International standardized approach for flow cytometric residual disease monitoring in chronic lymphocytic leukaemia

AC Rawstron¹, N Villamor^{2,3}, M Ritgen⁴, S Böttcher⁴, P Ghia⁵, JL Zehnder⁶, G Lozanski⁷, D Colomer^{2,3}, C Moreno^{2,3}, M Geuna⁸, PAS Evans¹, Y Natkunam⁶, SE Coutre⁶, ED Avery⁹, LZ Rassenti⁹, TJ Kipps⁹, F Caligaris-Cappio⁵, M Kneba⁴, JC Byrd⁷, MJ Hallek¹⁰, E Montserrat^{2,3} and P Hillmen¹

slgλ	Slgκ	CD19	CD5
CD45	CD14	CD19	CD3
CD20	CD38	CD19	CD5
CD81	CD22	CD19	CD5
CD43	CD79b	CD19	CD5

“Either 4-color flow cytometry (MRD flow) or allele-specific oligonucleotide PCR is reliably sensitive down to a level of approximately one CLL cell in 10000 leukocytes.”

“...patients will be defined as having a clinical remission in the absence of MRD when they have blood or marrow with less than one CLL cell per 10000 leucocytes.”

MMR-: <0.01% cellule di CLL

MMR+: >0.01% cellule di CLL

ORIGINAL ARTICLE

Improving efficiency and sensitivity: European Research Initiative in CLL (ERIC) update on the international harmonised approach for flow cytometric residual disease monitoring in CLL

AC Rawstron^{1,2}, S Böttcher³, R Letestu^{4,5}, N Villamor⁶, C Fazi⁷, H Kartsios¹, RM de Tute¹, J Shingles¹, M Ritgen³, C Moreno⁸, K Lin⁹, AR Pettitt⁹, M Kneba³, E Montserrat⁶, F Cymbalista^{4,5}, M Hallek¹⁰, P Hillmen¹¹ and P Ghia⁷ on behalf of the European Research Initiative in CLL (ERIC)

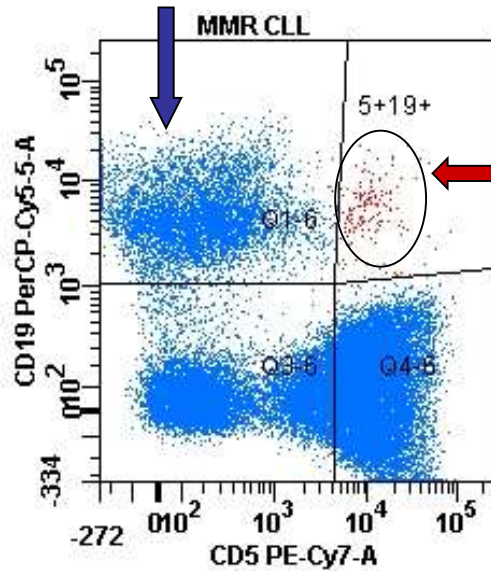
Table 1. Antibodies used for four-CLR and six-CLR analysis

Test	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
4-CLR 1	λ (1-155-2)	κ (TB28-2)	CD19 (SJ25C1)	—	CD5 (L17F12)	—
4-CLR 2	CD45 (2D1)	CD14 (MOP9)	CD19 (SJ25C1)	—	CD3 (SK7)	—
4-CLR 3	CD20 (L27)	CD38 (HB7)	CD19 (SJ25C1)	—	CD5 (L17F12)	—
4-CLR 4	CD81 (JS-81)	CD22 (S-HCL-1)	CD19 (SJ25C1)	—	CD5 (L17F12)	—
4-CLR 5	CD43 (1G10)	CD79b (SN8)	CD19 (SJ25C1)	—	CD5 (L17F12)	—
6-CLR 1	CD3 (SK7)	CD38 (HB7)	CD5 (L17F12)	CD19 (SJ25C1)	CD79b (SN8)	CD20 (L27)
6-CLR 2	CD81 (JS-81)	CD22 (S-HCL-1)	CD5 (L17F12)	CD19 (SJ25C1)	CD43 (1G10)	CD20 (L27)

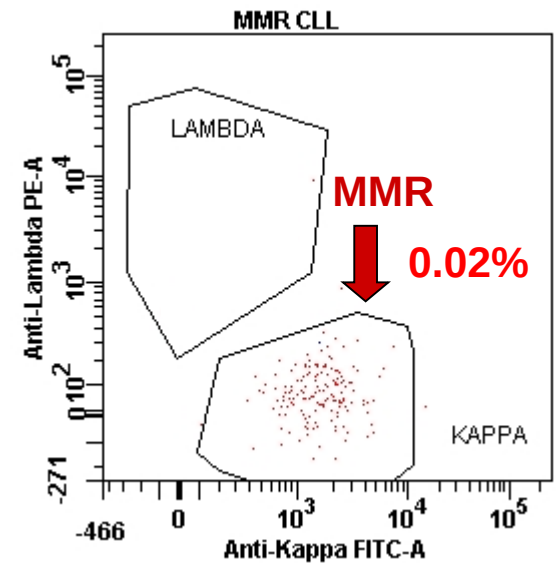
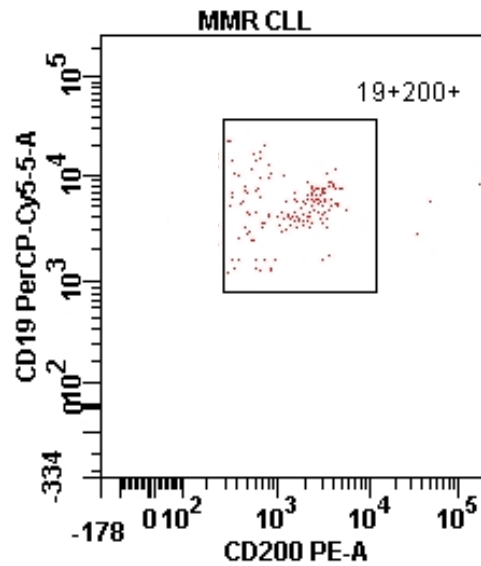
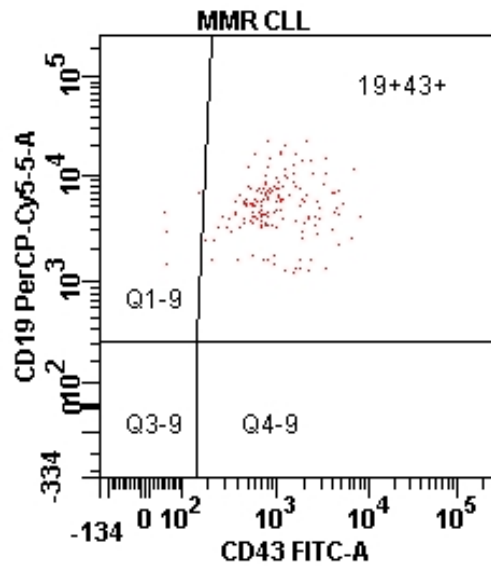
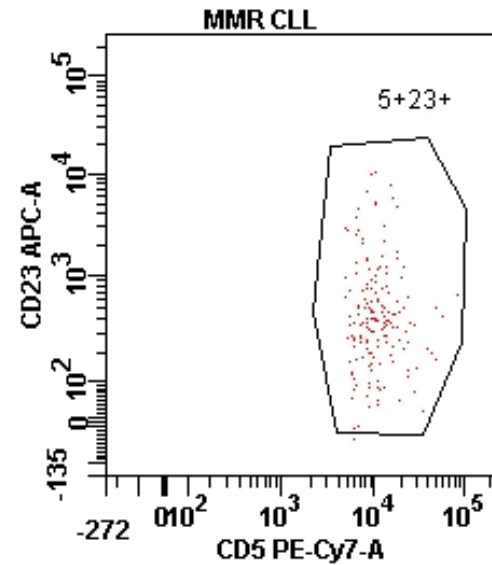
Abbreviation: CLR, colour.

MMR in CLL

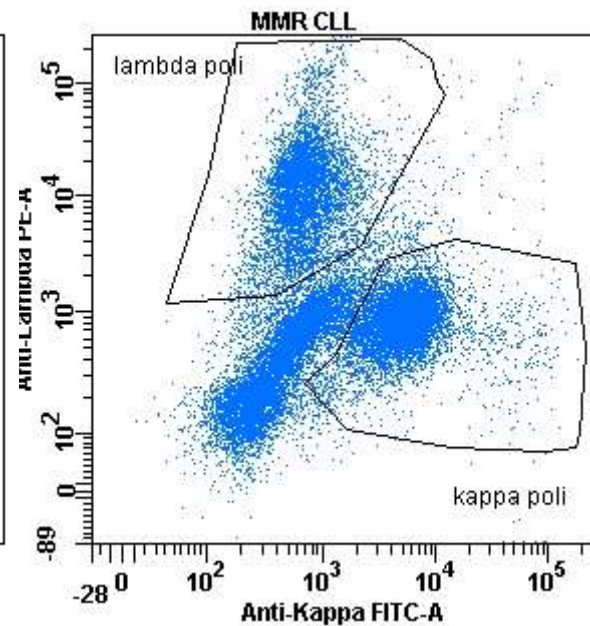
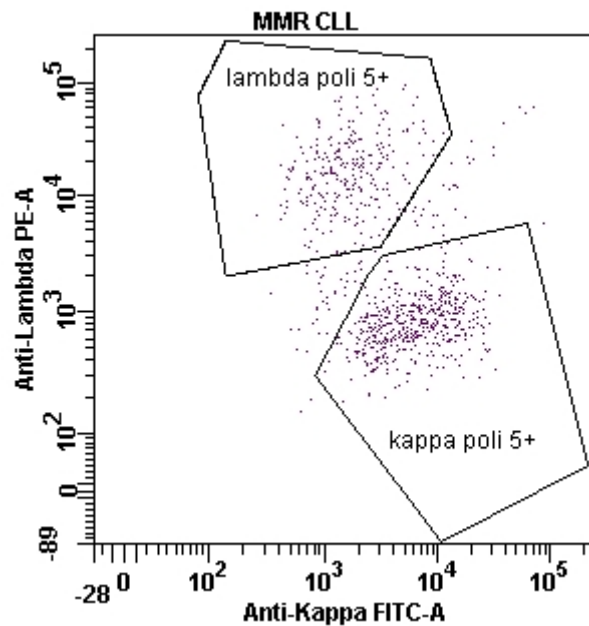
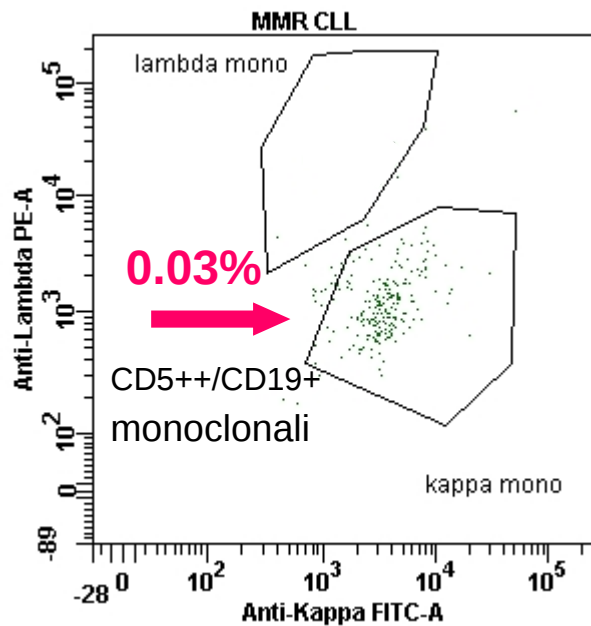
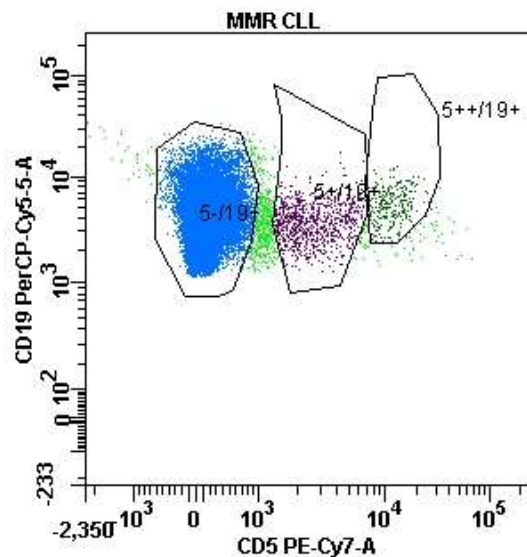
B policlonali 2.95%



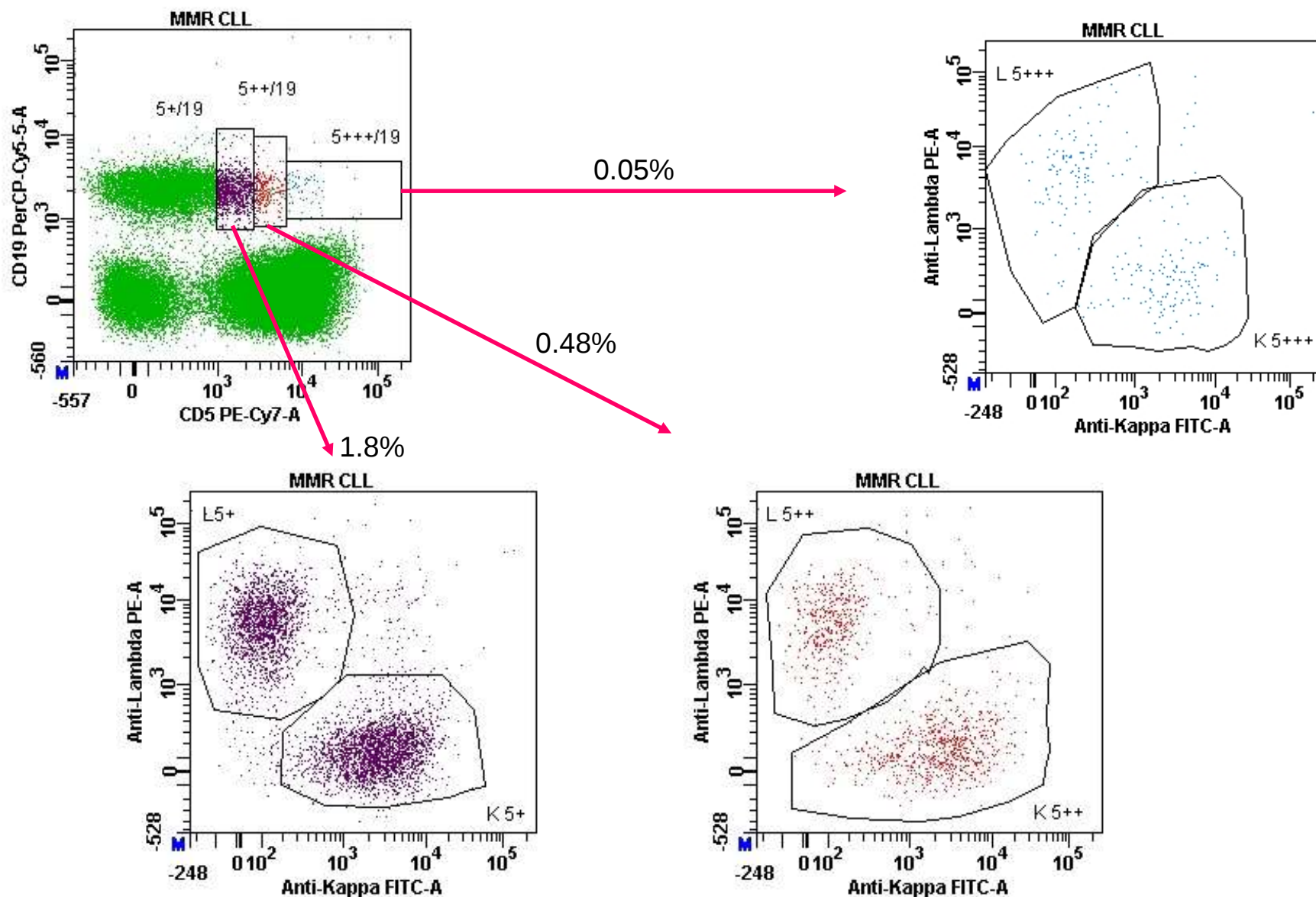
B monoclonali 0.02%



MMR in CLL



Remissione Fenotipica in CLL



blood

Prepublished online December 17, 2013;
doi:10.1182/blood-2013-08-523704

Monoclonal B-cell lymphocytosis in healthy blood donors: an unexpectedly common finding

Youn K. Shim, Jane M. Rachel, Paolo Ghia, Jeff Boren, Fatima Abbasi, Antonis Dagklis, Geri Venable, Jiyeon Kang, Heba Degheidy, Fred V. Plapp, Robert F. Vogt, Jay E. Menitove and Gerald E. Marti

.....low-count MBL: low risk to progress to CLL

.....CLL-like MBL: 1-2% per year progress to symptomatic CLL

Monoclonal B-cell lymphocytosis: recommendations from the Dutch Working Group on CLL for daily practice.

[te Raa GD](#)¹, [van Oers MH](#), [Kater AP](#); [HOVON CLL working party](#).

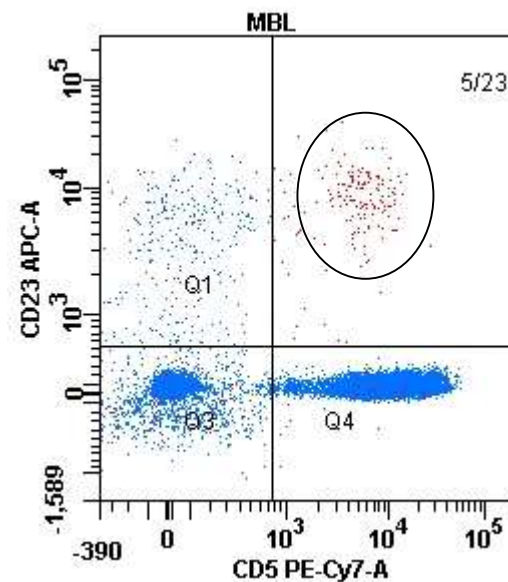
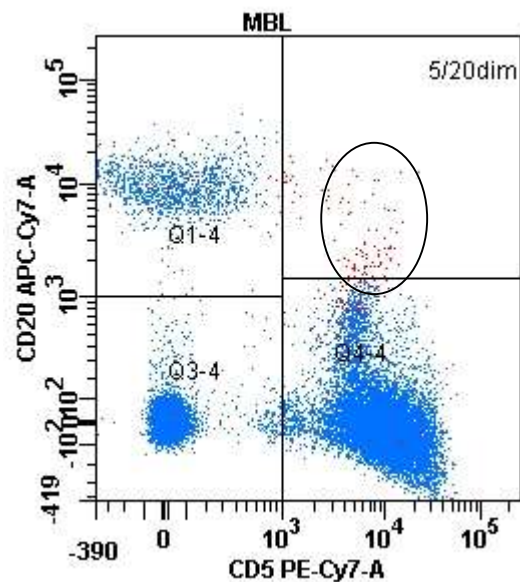
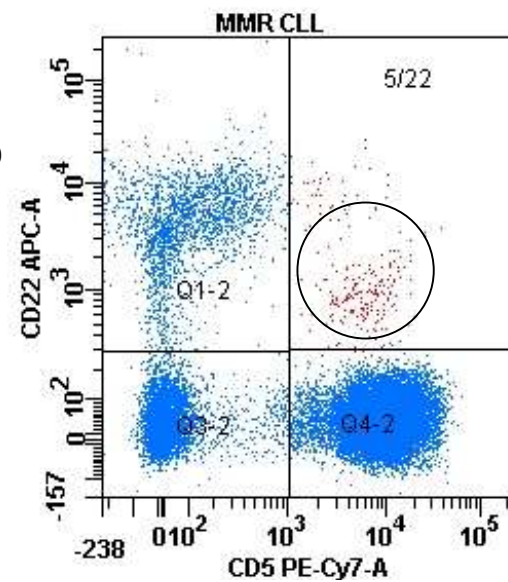
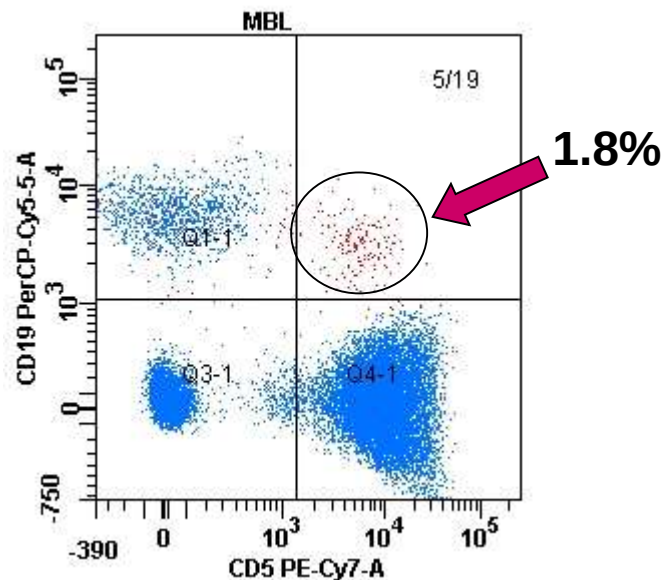
The prevalence of MBL in the general population with an age over 40 years is 3 to 5%.

Subjects with MBL develop CLL requiring treatment at a rate of 1 to 2% per year.

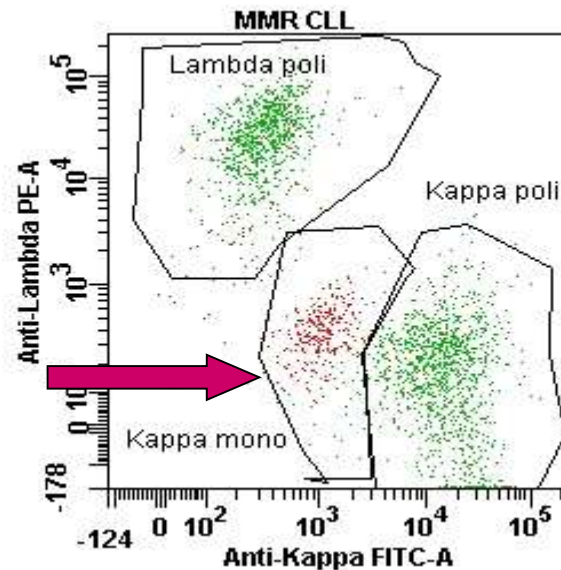
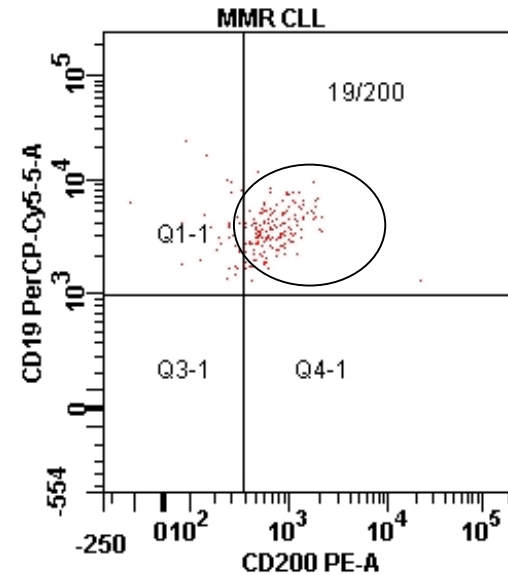
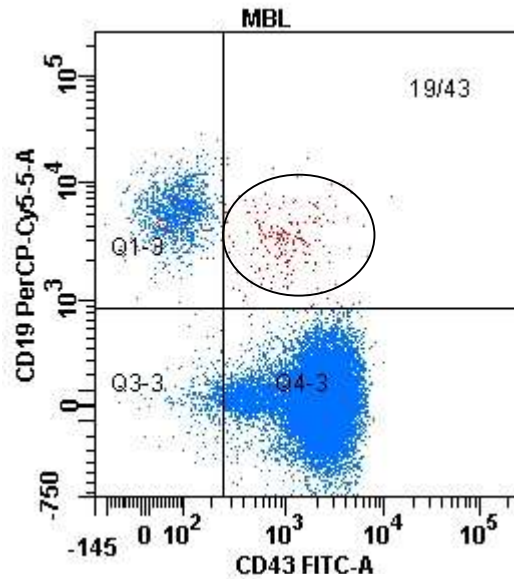
Monoclonal B Lymphocytosis

- <5000 B linfociti monoclonali/mL
- 1-2% /anno progrediscono a CLL

MBL: Monoclonal B Lymphocytosis



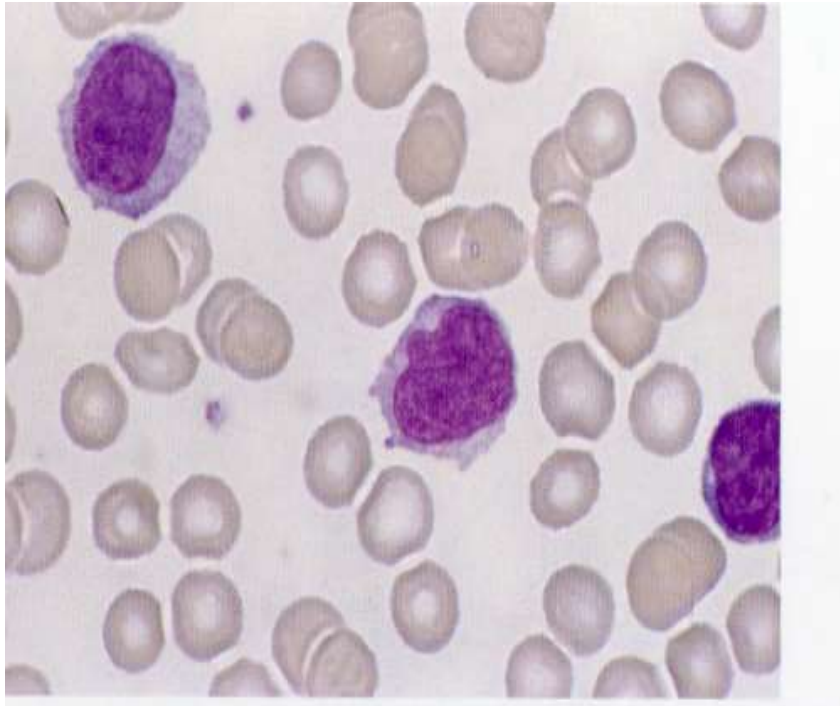
MBL: Monoclonal B Lymphocytosis



B policlonali: 5.8%

B monoclonali: 1.8%

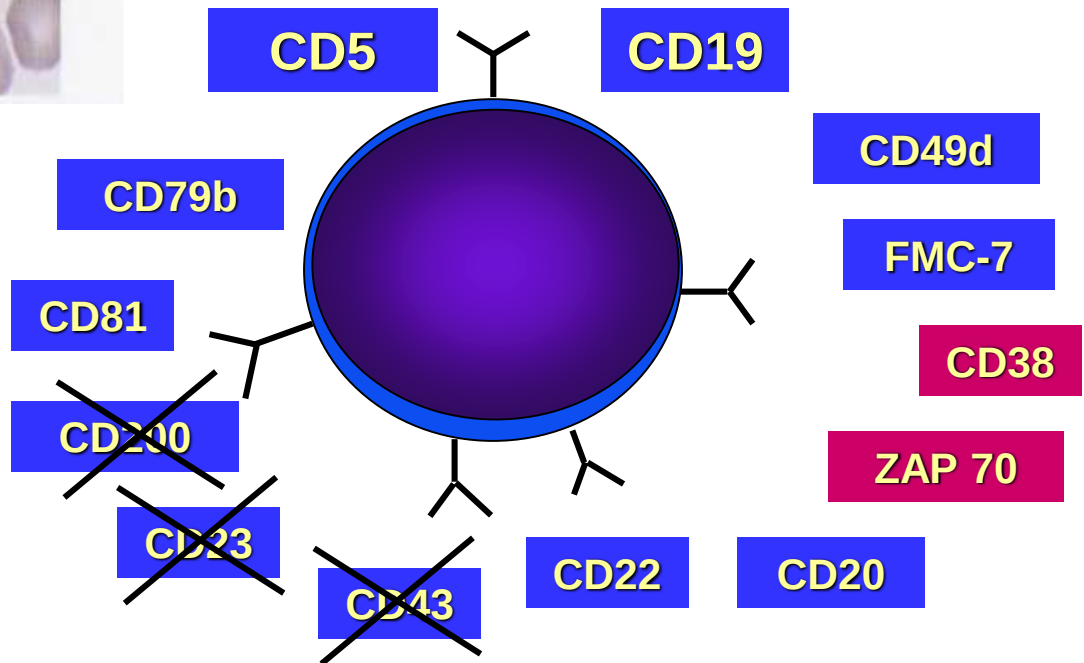
MORFOLOGIA



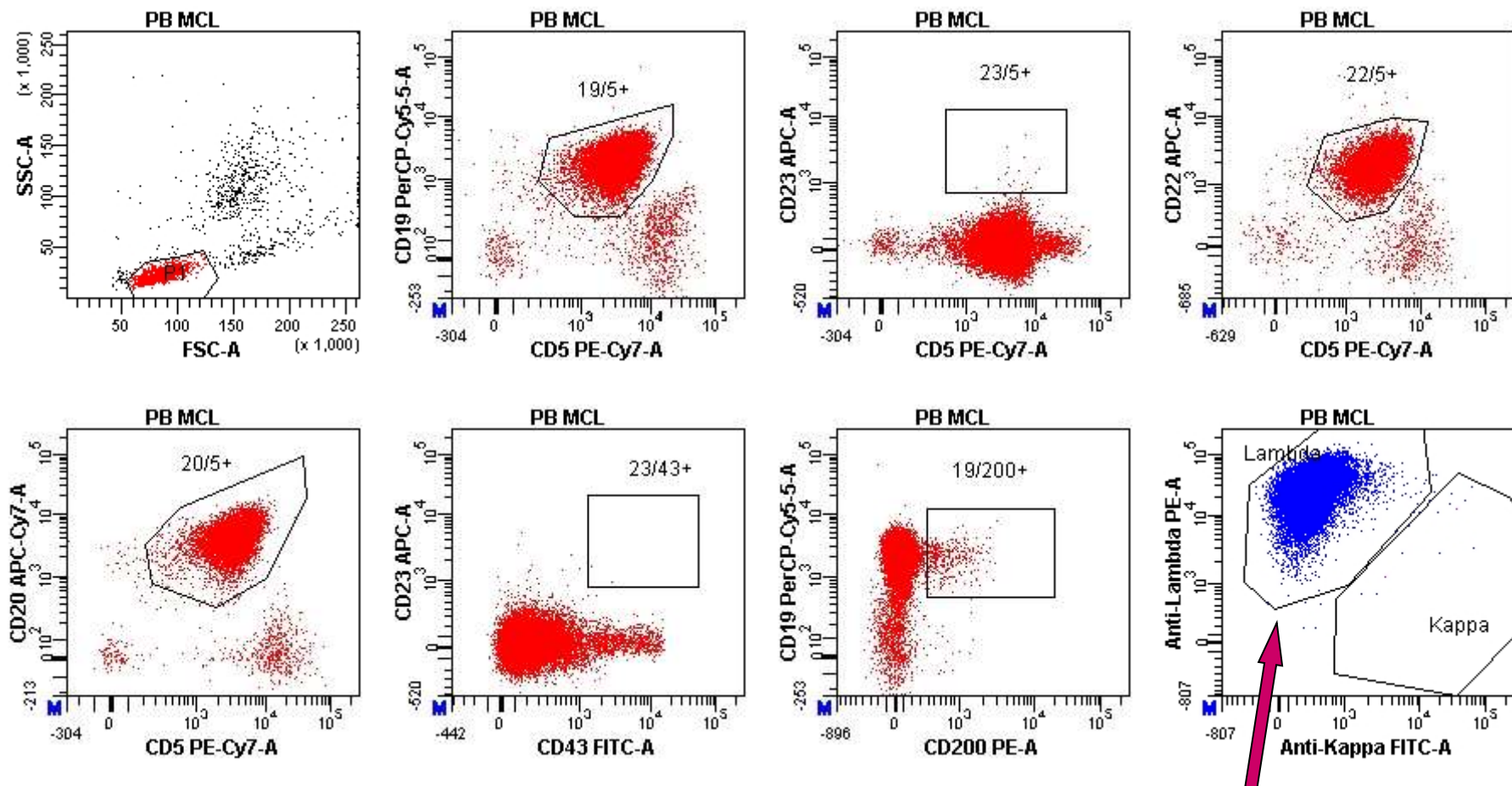
MCL

CITOFUORIMETRIA

t (11;14) → positiva



MCL: IMMUNOFENOTIPO



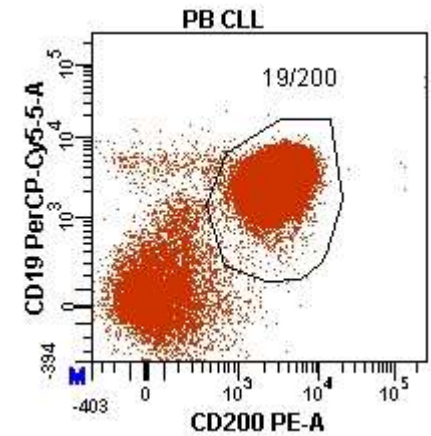
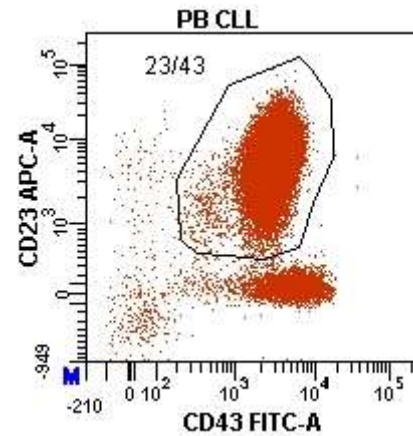
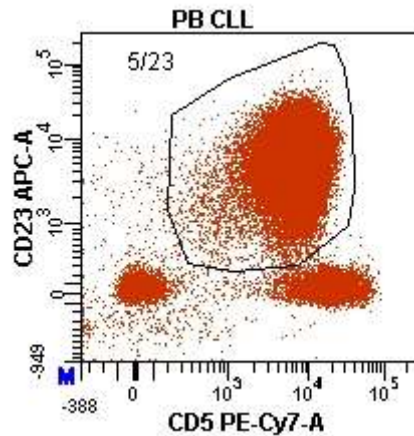
catene leggere delle Ig monoclonali

CLL e MCL: differenze immunofenotipiche

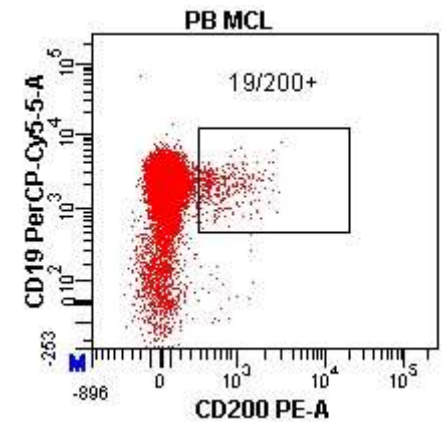
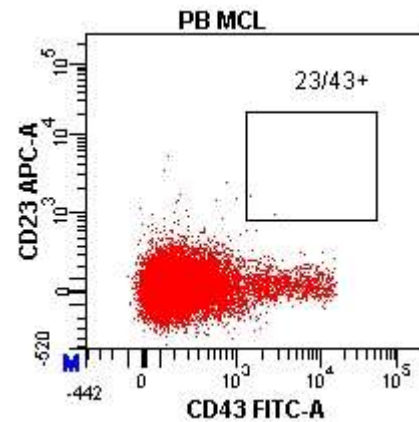
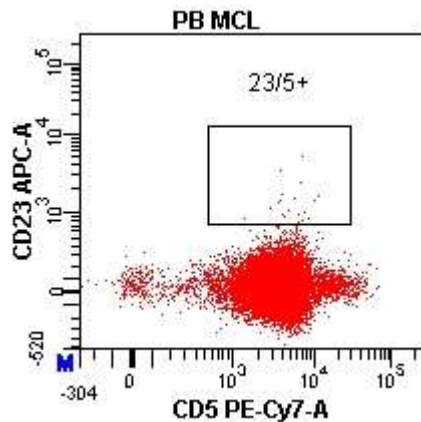
- Espressione di CD23
- Espressione di CD200
- Espressione di CD43
- Intensità di espressione di CD20
- Intensità di espressione di catene leggere delle Ig

CLL e MCL: differenze immunofenotipiche

CLL

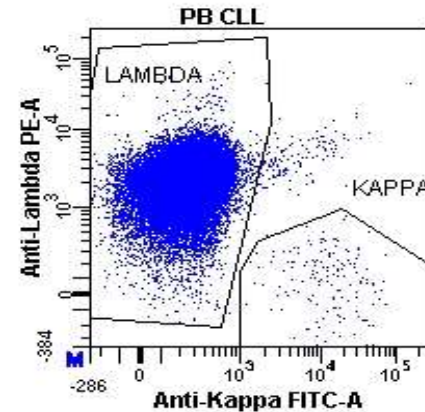
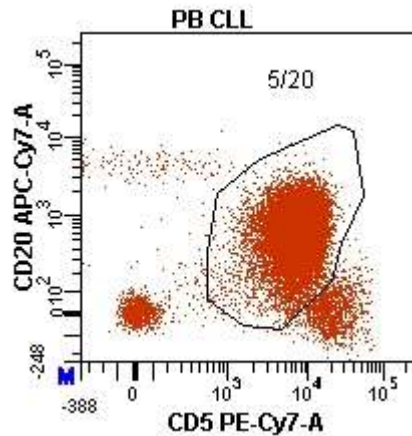


MCL

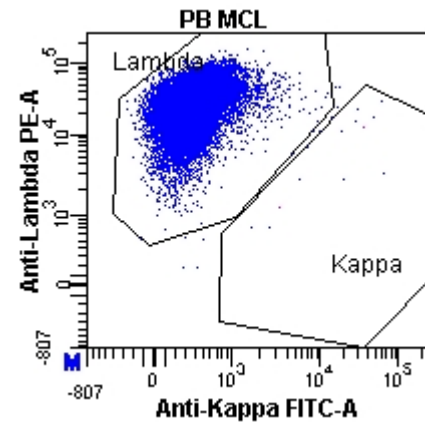
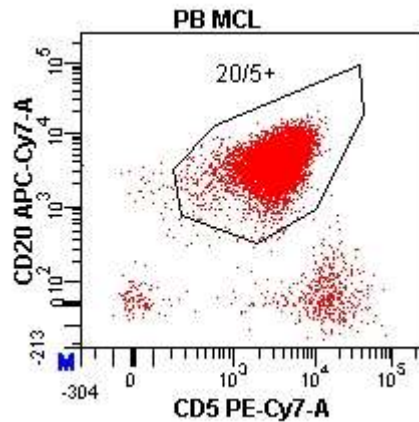


CLL e MCL: differenze immunofenotipiche

CLL



MCL



Challagundla P, L. Medeiros LJ, Kanagal-Shamanna R, Miranda RN, and Jorgensen JL

Differential expression of CD200 in B-cell neoplasms by flow cytometry can assist in diagnosis, subclassification, and bone marrow staging.

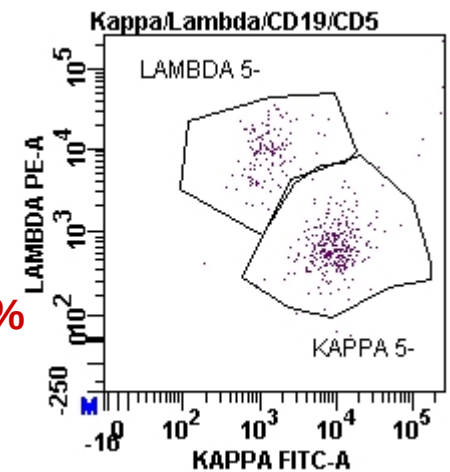
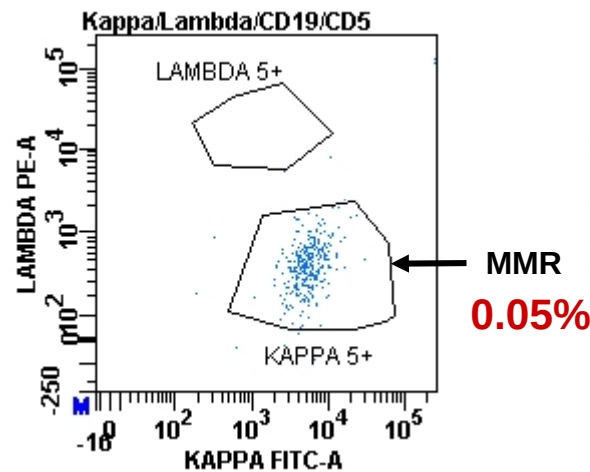
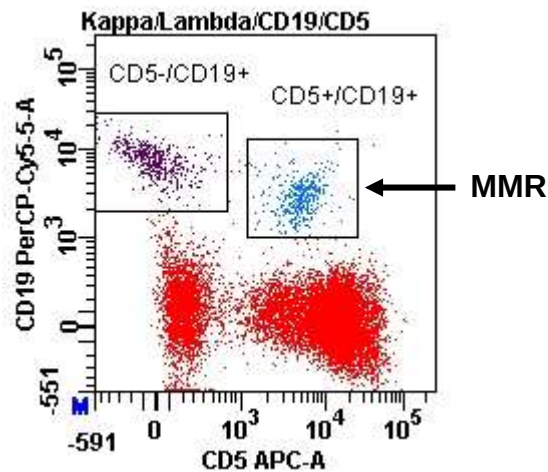
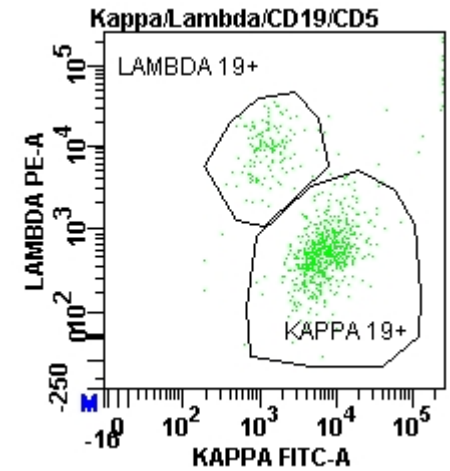
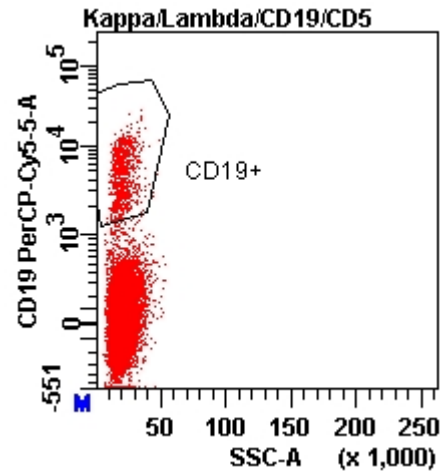
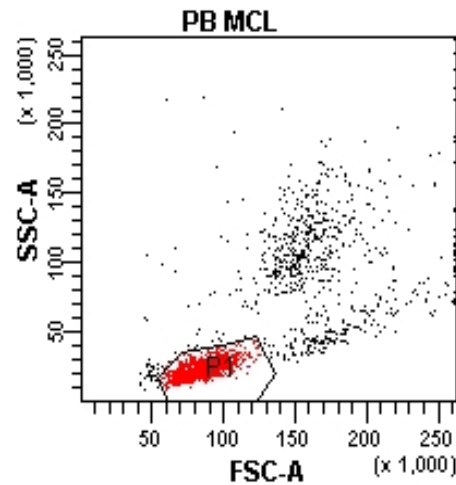
Am J Clin Pathol 2014 142:837-844

CD200 staining by flow cytometry can be useful in the differential diagnosis of B-cell neoplasms and in their detection in the BM.

Utility of CD200 expression and CD20 antibody binding capacity in differentiating chronic lymphocytic leukemia from other chronic lymphoproliferative disorders

Poongodi R et al. Indian J Pathol Microbiol 2018;61:50-7

MMR in MCL



Distinction between Asymptomatic Monoclonal B-cell Lymphocytosis with Cyclin D1 Overexpression and Mantle Cell Lymphoma: From Molecular Profiling to Flow Cytometry

Blanca Espinet^{1,3}, Ana Ferrer^{1,3}, Beatriz Bellosillo^{1,3}, Lara Nonell⁴, Antonio Salar^{2,3}, Concepción Fernández-Rodríguez³, Eulàlia Puigdecamet⁴, Javier Gimeno¹, Mar Garcia-Garcia^{1,3}, María Carmen Vela¹, Elisa Luño¹¹, Rosa Collado¹², José Tomás Navarro⁷, Esmeralda de la Banda⁸, Pau Abrisqueta⁹, Leonor Arenillas^{1,3}, Cristina Serrano¹³, Josep Lloreta^{1,3}, Belén Miñana¹⁰, Andrea Cerutti⁵, Lourdes Florensa^{1,3}, Alberto Orfao¹⁴, Ferran Sanz⁶, Francesc Solé^{1,3}, David Dominguez-Sola¹⁵, and Sergio Serrano^{1,16}

Clin Cancer Res; 20(4) February 15, 2014

Surface CD38/CD200 expression by flow cytometry is useful to discriminate between MALD1 and MCL

MALD1: Monoclonal Asymptomatic Lymphocytosis, cyclin D1-positive

Pazienti che posseggono i criteri diagnostici del Linfoma Mantellare, secondo la classificazione WHO, ma non hanno il decorso clinico aggressivo del MCL

Sono tipicamente caratterizzati da diffusione leucemica, con o senza splenomegalia e assenza di linfadenopatie

Non richiedono terapia

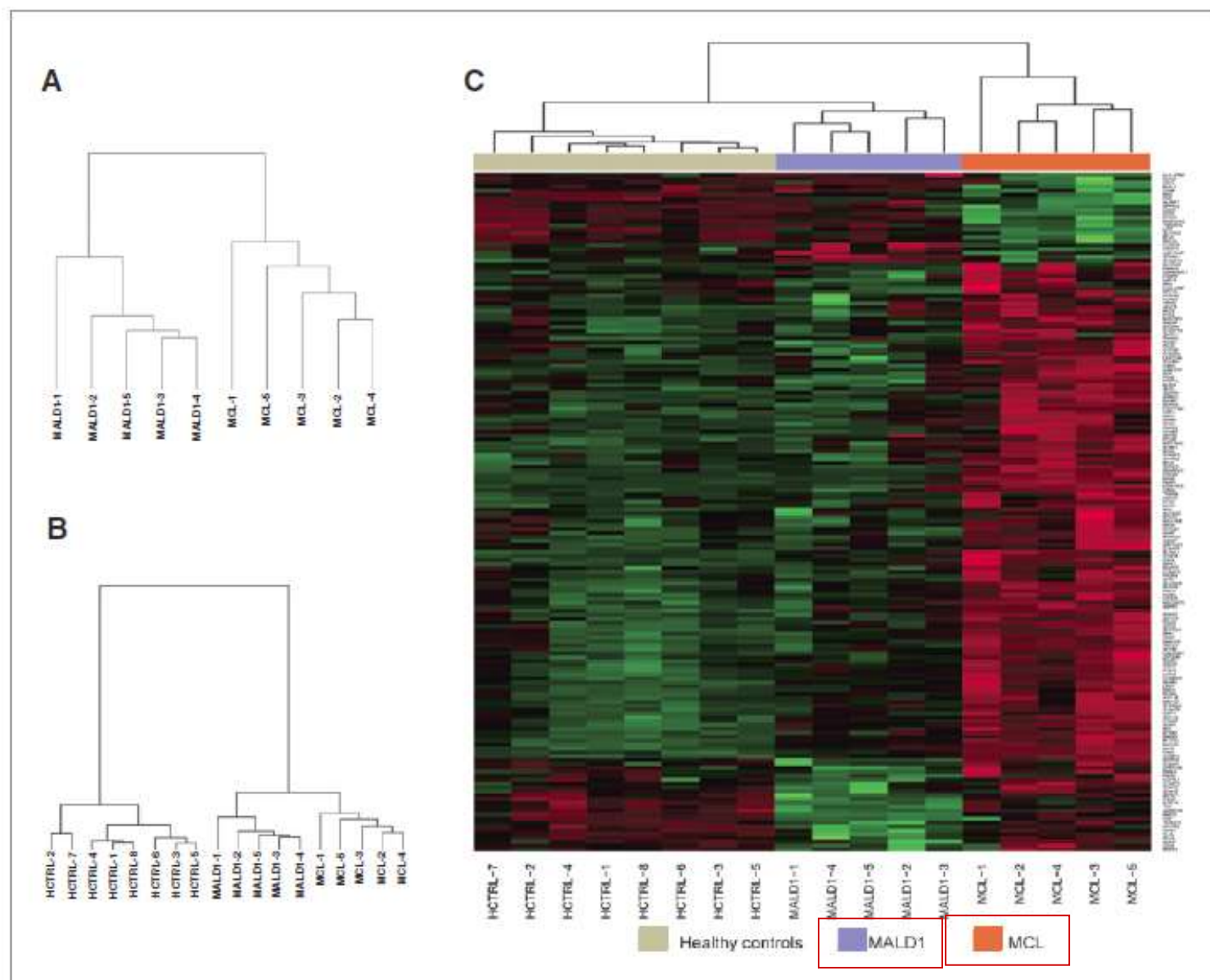
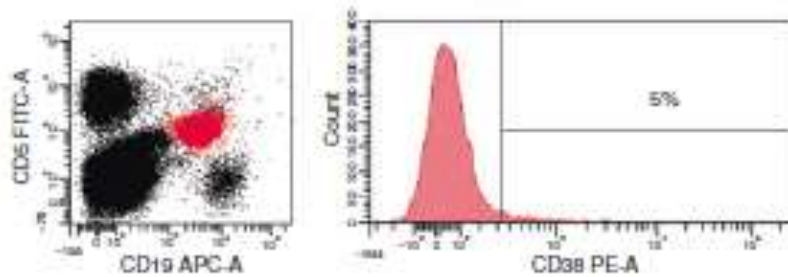
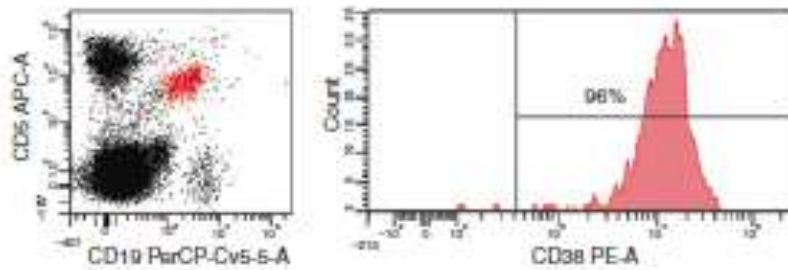
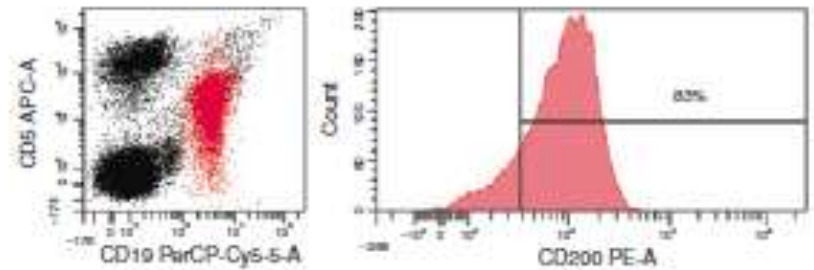


Figure 2. Gene expression analysis of CD19⁺ peripheral blood B cells reveals significant differences between MALD1 and MCL. A, unsupervised hierarchical clustering of the gene expression profiles (GEP) from healthy controls (HCTRL), MALD1, and MCL cases. B, unsupervised hierarchical clustering of GEP from MALD1 and MCL cases separates the 2 entities based on distinct gene signatures. C, heatmap showing the results of the supervised analysis using genes differentially expressed between MALD1 and MCL cases. The list of genes appearing in the heatmap is detailed, in order of appearance, in Supplementary Table S4.

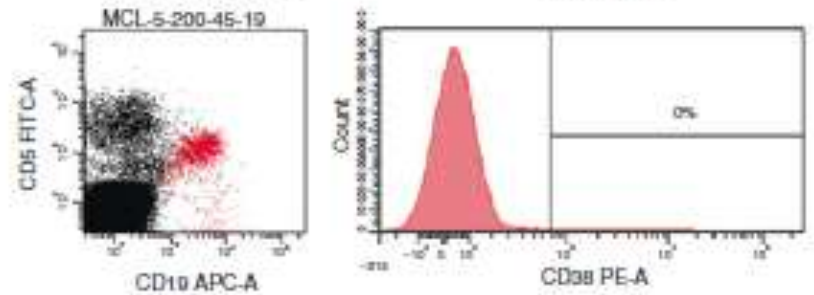
Surface CD38/CD200 expression by flow cytometry is useful to discriminate between MALD1 and MCL



MALD1



MCL



MCL → CD38+CD200-

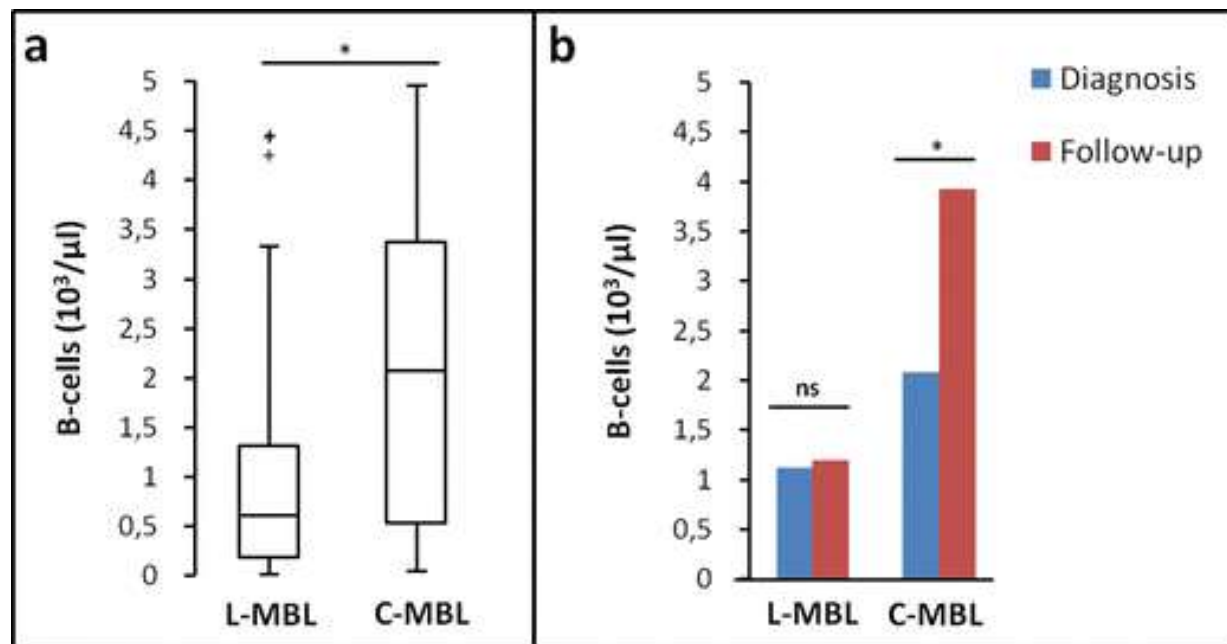
MALD1 → CD38-CD200+

MALD1

- E' descritta per la prima volta un'entità nosologica distinta dal MCL, coinvolta nei processi di attivazione immunitaria, e nei processi Infiammatori, il MALD1
- Espansione di B linfociti monoclonali esprimenti ciclina-D1
- Caratterizzata da decorso benigno
- MCL e MALD1 possono essere distinte in base all'espressione citofluorimetrica di CD38 e CD200
- E' così possibile evitare diagnosi errate e trattamenti chemioterapici non necessari

Lymphoma-like monoclonal B cell lymphocytosis in a patient population: biology, natural evolution, and differences from CLL-like clones

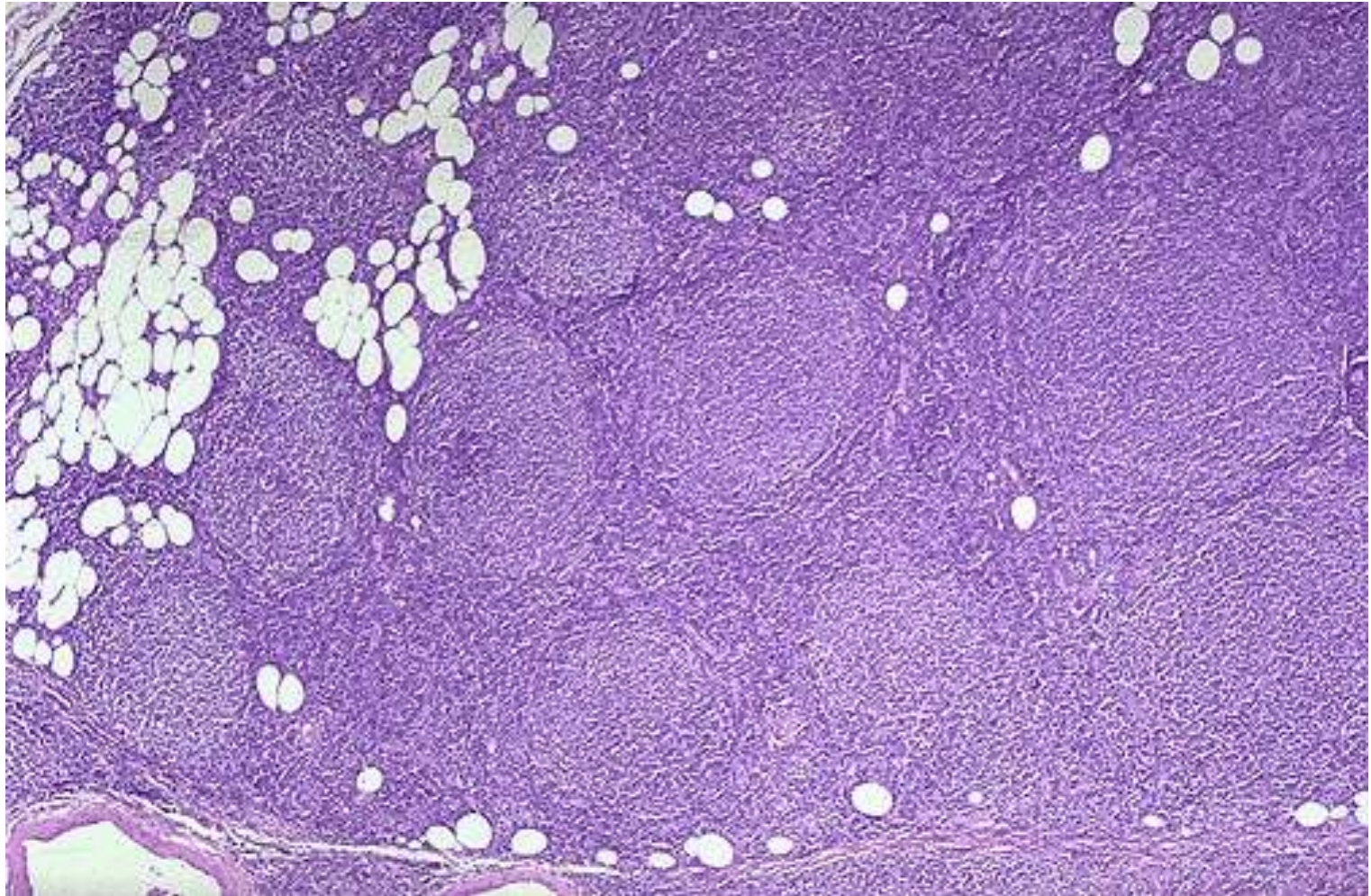
Vander Meeren S., et al [Ann Hematol.](#) 2018 Feb 28. doi: 10.1007/s00277-018-3282-0.



L-MBL: Lymphoma-like MBL

C-MBL: CLL-like MBL

Linfoma Follicolare



Linfoma Follicolare

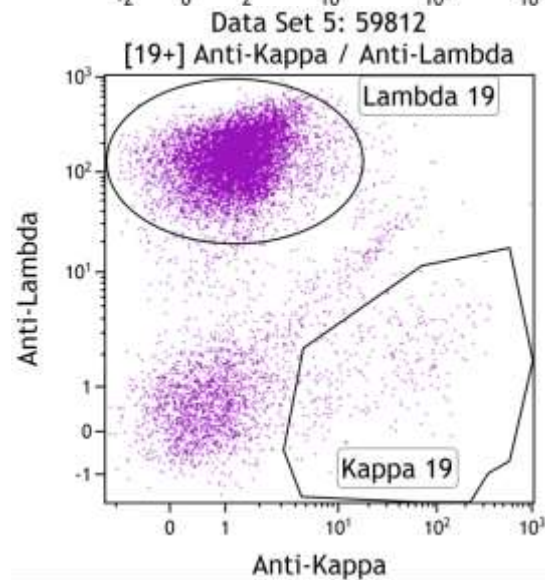
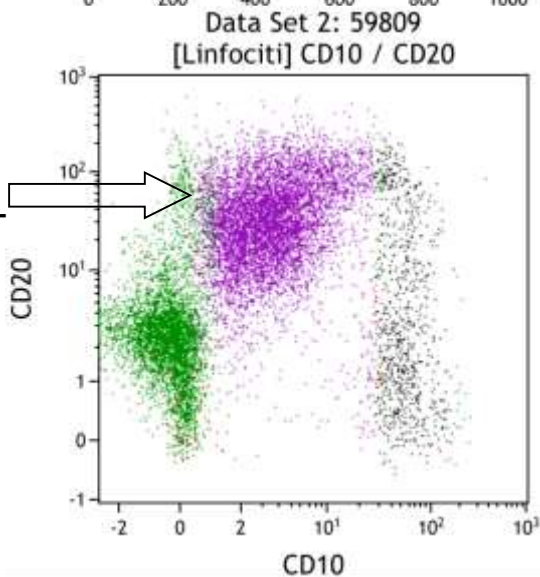
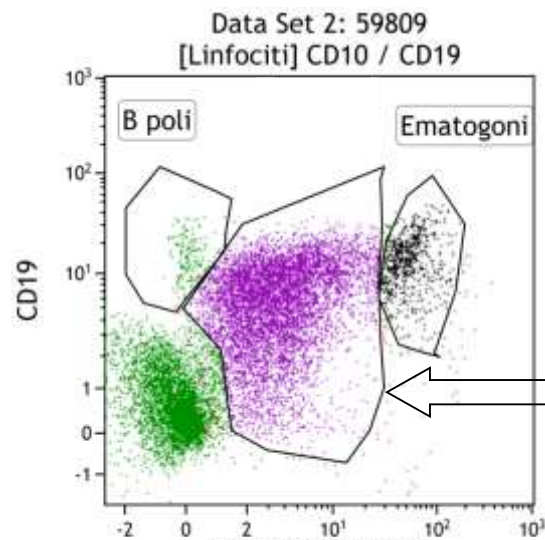
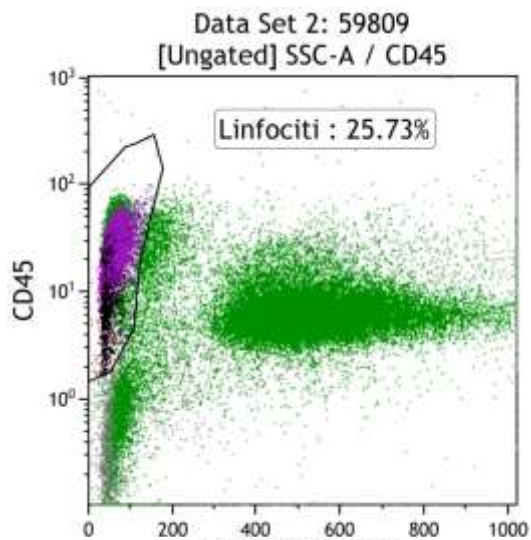
- Linfoma indolente che rappresenta il 30% di tutti i NHL
- Deriva da cellule B del centro germinativo (CD10+)
- Spesso asintomatico, può essere diagnosticato tardivamente quando ha già infiltrato il midollo osseo
- Caratteristico il riarrangiamento cromosomico t(14;18) con attivazione del gene BCL-2

BCL-2

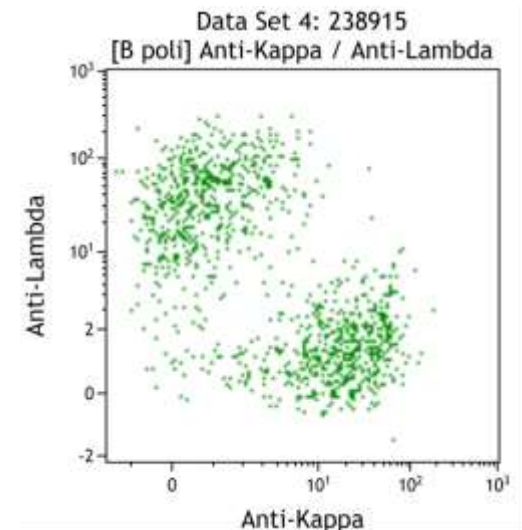
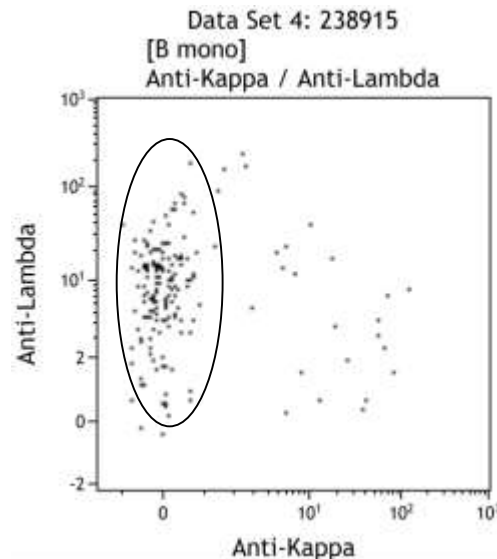
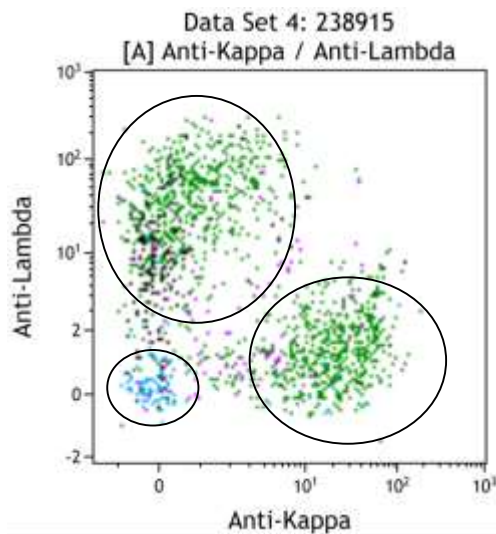
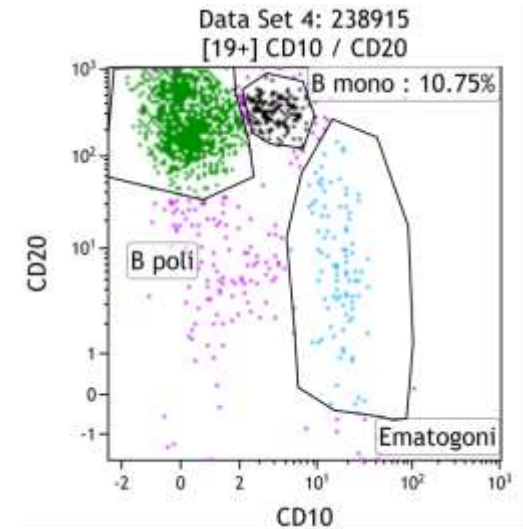
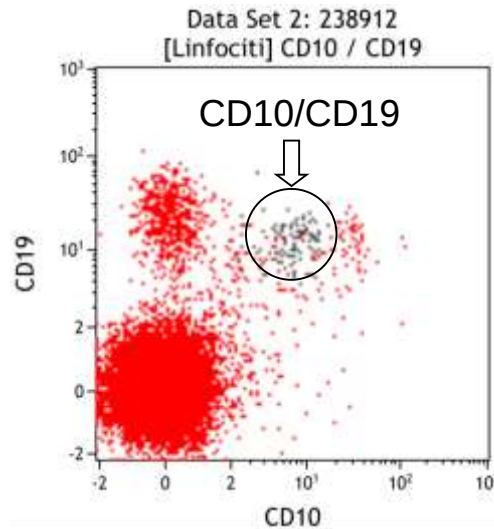
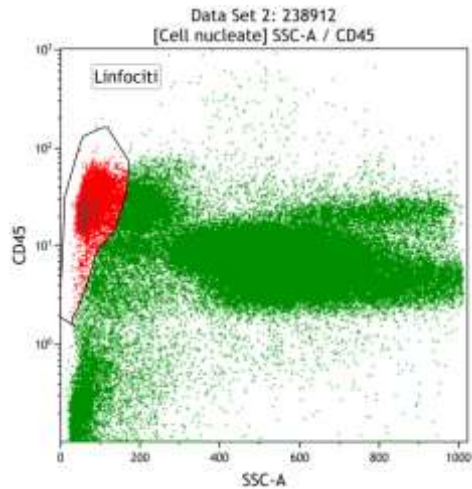
La traslocazione t(14;18) è dimostrabile nel 60-80% dei linfomi follicolari. Il gene Bcl-2 (situato sul cromosoma 18) si fonde con il locus IgH (posto sul cromosoma 14q32) e questa anomala posizione porta ad una alterazione della regolazione dell'espressione della proteina bcl-2 nel clone in cui è avvenuta la traslocazione.

Questa traslocazione è responsabile della sovra-espressione del gene bcl-2 nei linfomi follicolari, con conseguente deregolazione del turn-over delle cellule centrofollicolari che divengono resistenti al processo apoptotico.

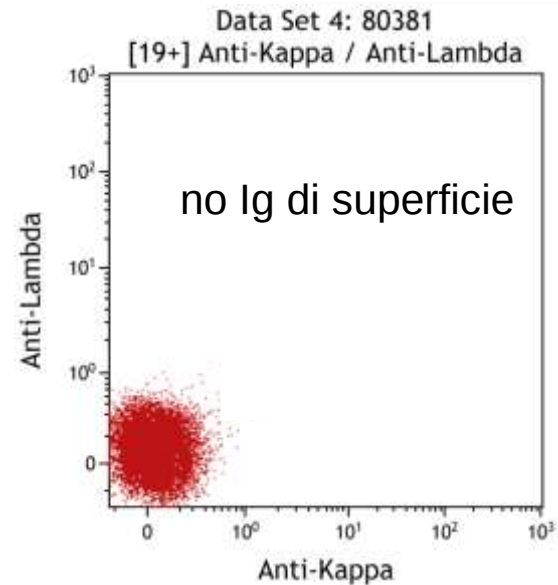
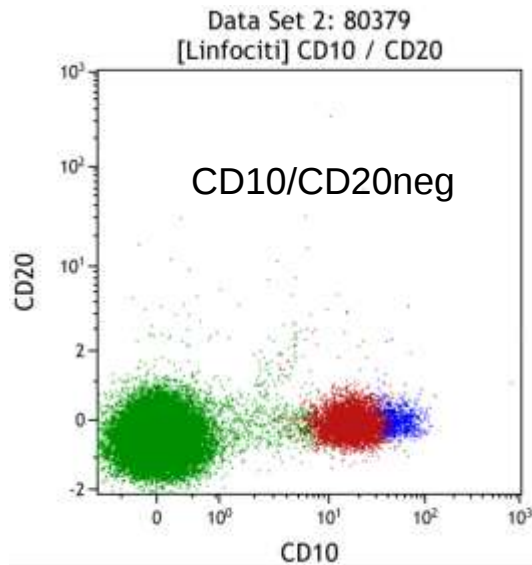
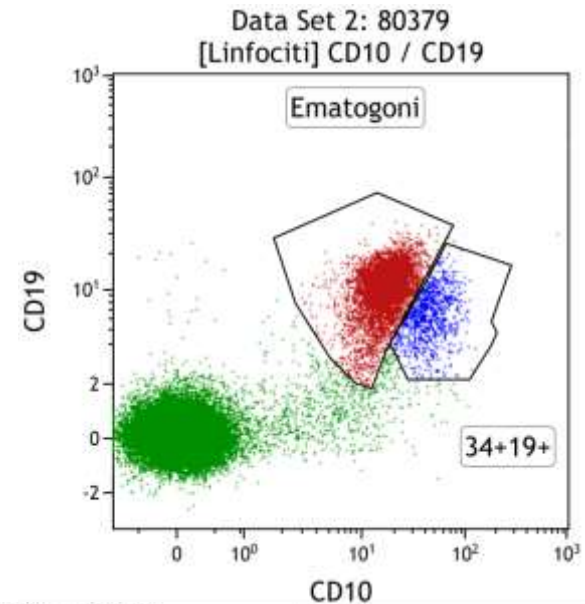
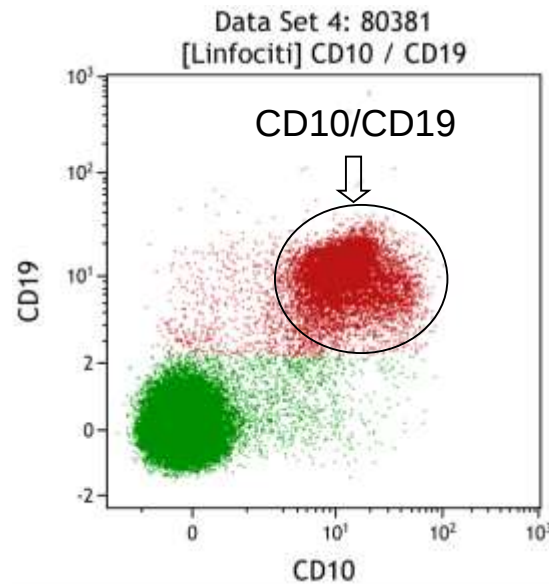
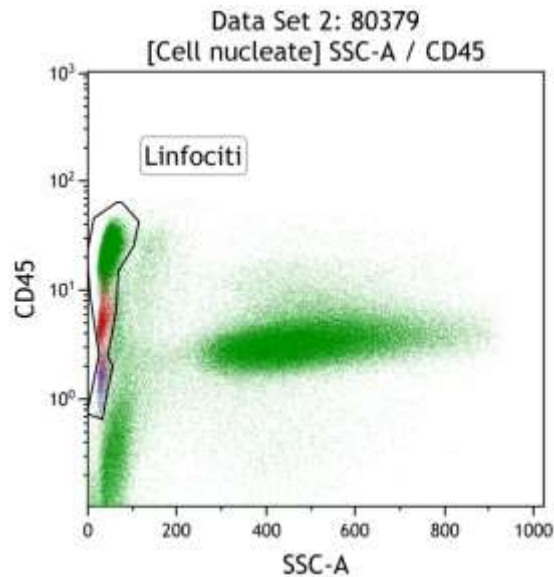
Immunofenotipo BM FL



Risposta alla terapia nel FL



CD10/CD19 non monoclonali



Cyto/FISH lab

Milena Gilestro

Marina Ruggeri

Simona Caltagirone

Elona Saraci

Vittorio Muccio

Daniela Oddolo

Medical staff

Dario Ferrero

Daniele Caracciolo

Luciana Bergui

Marta Coscia

Candida Vitale

Simone Ferrero

Luisa Giaccone

Benedetto Bruno

Prof. Mario Boccadoro