MASTOCITOSI E BPDCN: LE DIAGNOSI DIFFICILI

BOLOGNA, ROYAL HOTEL CARLTON
12 OTTOBRE 2026

Pittfalls nella diagnostica citofluorimetrica

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Disclosure: Francesco Buccisano

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
JAZZ PHAMACEUTICALS					X	X	
NOVARTIS					X		
MENARINI STEMLINE					X		
ASTELLAS					X		
SERVIER					X		

Pitfalls in MFC diagnostics of BPDCM and SM

- Historical rarity of disease, Many nomenclature changes (WHO 2008, WHO 2016)
- Difficulty in clinico-pathologic recognition/diagnosis
- Morphology not characteristic, unspecified reason for testing
- Minority population, fibrosis, hemodilution
- Concomitant hematological disease (~20%)
- Unmet technical requirement and gap of knowledge

Diagnostic/clinical «awareness» of BPDCN

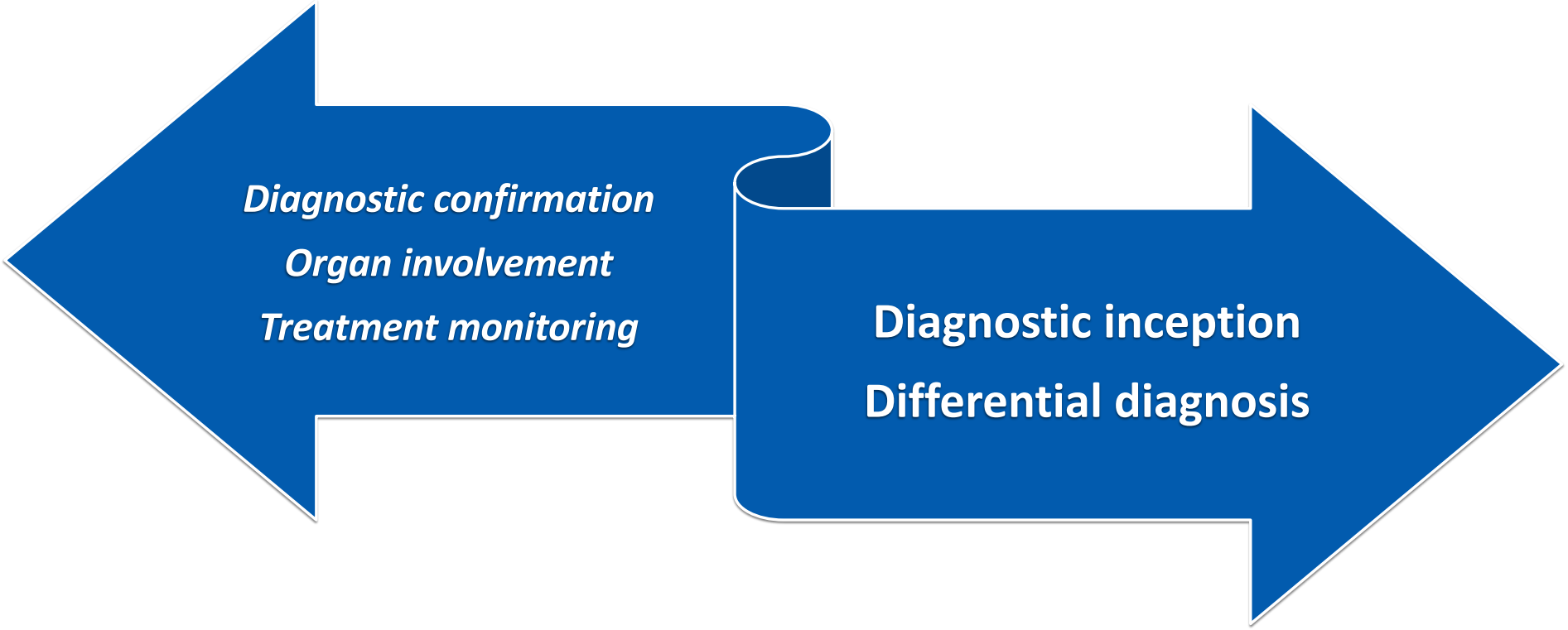
	Replies	Percentages
Do you have a BPDCN immunophenotypic panel (CD4-CD56-CD123-BDCA2/CD303) that you use on a regular basis?	✓ Yes ✓ No ✓ Only in selected cases	33 17 50
Your immunophenotypic panel includes markers that exclude BPDCN, such as: CD3, CD19, myeloperoxidase, CD11c, CD14, CD64?	✓ Yes ✓ No	0 100
Does your histochemical panel include CD4, CD56, CD123, BDCA2, TCL1, or excludes: MPO, CD3, CD20 o CD19, CD163, Lysozyma?	✓ Yes ✓ No	57 43
Do you routinely perform CD123 in acute myeloid leukemia at diagnosis?	✓ Yes ✓ No	59 41
Do you routinely perform a cerebral fluid exam at diagnosis?	✓ Yes ✓ No	70 30
Do you use a different therapeutic approach between BPDCN patients with leukemic spread or without?	✓ Yes ✓ No	26 74
What type of chemotherapy do you use? AML-oriented, ALL-oriented or NHL-oriented?	✓ AML-like ✓ ALL-like ✓ NHL-like	39 44 17

NHL, non-Hodgkin lymphoma.

41% do not perform routinely CD123 at diagnosis, 50% test BPDCN only in selected cases

Valentini C et al., Blood Adv 2021 (letter)

The role of MFC in BPDCN



BPDCN diagnosis: multiparameter flow-cytometry

CD123, CD4, and CD56, completes a signature marker triad that can differentiate BPDCN from other hematologic malignancies[4,5§]

BPDCN is characterized by the expression of the pDC-associated marker, CD123[3§]

ANTIGENS THAT HELP CONFIRM THE DIAGNOSIS OF BPDCN[1-4*§]	
CD123+ **CD4+** **CD56+** TCL1+ TCF4+ CD303+ (BDCA2+)	CD3- CD19- TIA1- Granzyme B- Myeloperoxidase-

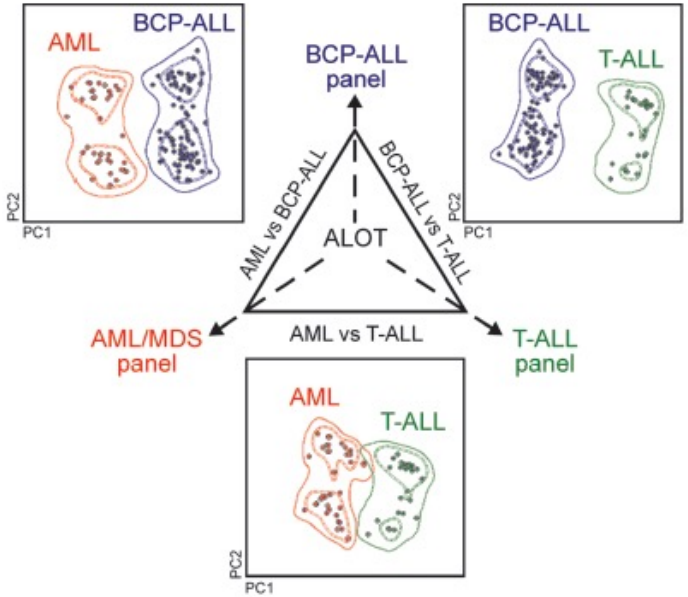
ANTIGENS THAT EXCLUDE THE DIAGNOSIS OF BPDCN[1]	
Pan T-cell markers CD3 **Pan B-cell markers** CD20 CD79a	**Myeloid markers** CD11c CD34 Lysozyme Myeloperoxidase

Immunophenotypic diagnostic criteria:

Expression of CD123 and one other pDC marker (TCF4, TCL1, CD303) in addition to CD4 and/or CD56, or,
Expression of any three pDC markers and absent expression of all expected negative markers

Khoury JD et al. Leukemia 2022

MFC panel construction (Euroflow approach)



AML vs BCP-ALL	
CD19	32%
CyMPO	27%
CyCD79a	20%
CD45	8%
CyCD3	6%

AML vs T-ALL	
CyMPO	26%
CD7	26%
CyCD3	26%
CD34	15%
SmCD3	6%

BCP-ALL vs T-ALL	
CD19	23%
CyCD3	22%
CD7	22%
CyCD79a	14%
CD34	9%

Table 14. The EuroFlow AML/MDS antibody panel[a,b]

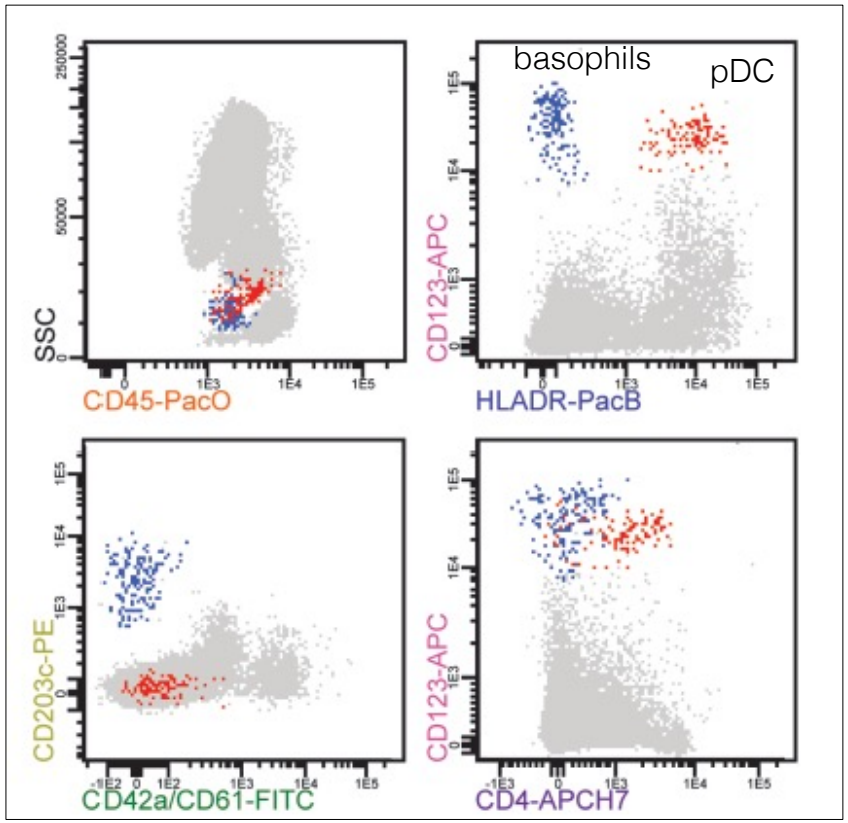
Tube	*PacB*	*PacO*	*FITC*	*PE*	*PerCPCy5.5*	*PECy7*	*APC*	*APCH7*	*Aim*
1	HLADR	CD45	CD16	CD13	CD34	CD117	CD11b	CD10	Diagnosis and classification, neutrophilic maturation, PNH
2	HLADR	CD45	CD35	CD64	CD34	CD117	CD300e (IREM2)	CD14	Diagnosis and classification, monocytic maturation, PNH
3	HLADR	CD45	CD36	CD105	CD34	CD117	CD33	CD71	Diagnosis and classification, erythroid maturation
4	HLADR	CD45	NuTdT	CD56	CD34	CD117	CD7	CD19	Aberrant expression of lymphoid markers, abnormal B lymphoid maturation
5	HLADR	CD45	CD15	NG2	CD34	CD117	CD22	CD38	Aberrant marker expression, stem cells
6	HLADR	CD45	CD42a and CD61	CD203c	CD34	CD117	CD123	CD4	Diagnosis and classification of AML Megakaryocytic, basophilic, and plasmacytoid dendritic cell lineages
7	HLADR	CD45	CD41	CD25	CD34	CD117	CD42b	CD9	Characterization of megakaryoblastic leukemia, and systemic mastocytosis

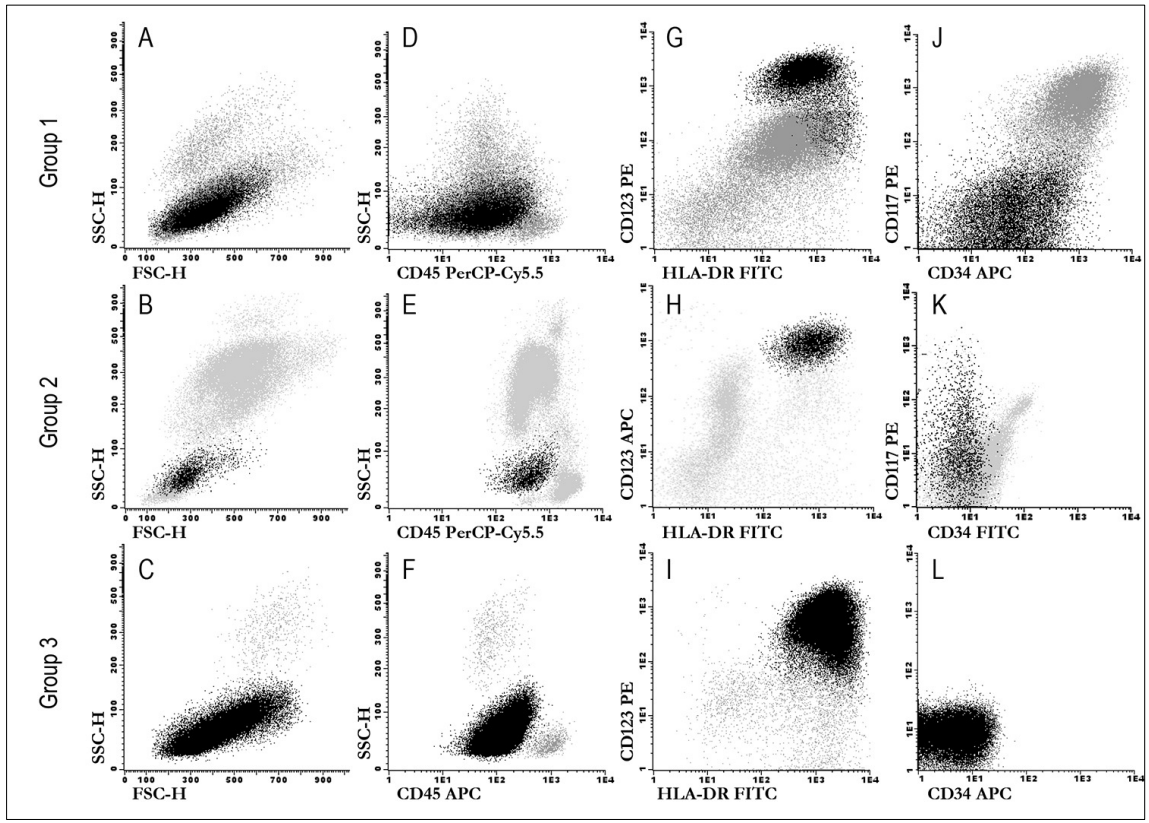
Abbreviations: AML, acute myeloid leukemia; APC, allophycocyanin; BB, backbone; BM, bone marrow; Cy7, cyanin7; FITC, fluorescein isothiocyanate; H7, hilite7; MDS, myelodysplastic syndrome; Nu, nuclear; PacB, pacific blue; PacO, pacific orange; PE, phycoerythrin; PerCPCy5.5, peridinin–chlorophyll–protein–cyanin5.5; PNH, paroxysmal nocturnal hemoglobinuria. [a]Further information about markers and hybridomas is provided in the Appendix. [b]A total of 96 BM samples were evaluated for selection of the BB markers. An additional 84 BM AML samples were evaluated with this version (final) of the panel.

6	HLADR	CD45	CD42a and CD61	CD203c	CD34	CD117	CD123	CD4	Diagnosis and classification of AML Megakaryocytic, basophilic, and plasmacytoid dendritic cell lineages

Van Dongen JJ et al., Leukemia 2012

MFC panel construction (Euroflow approach)



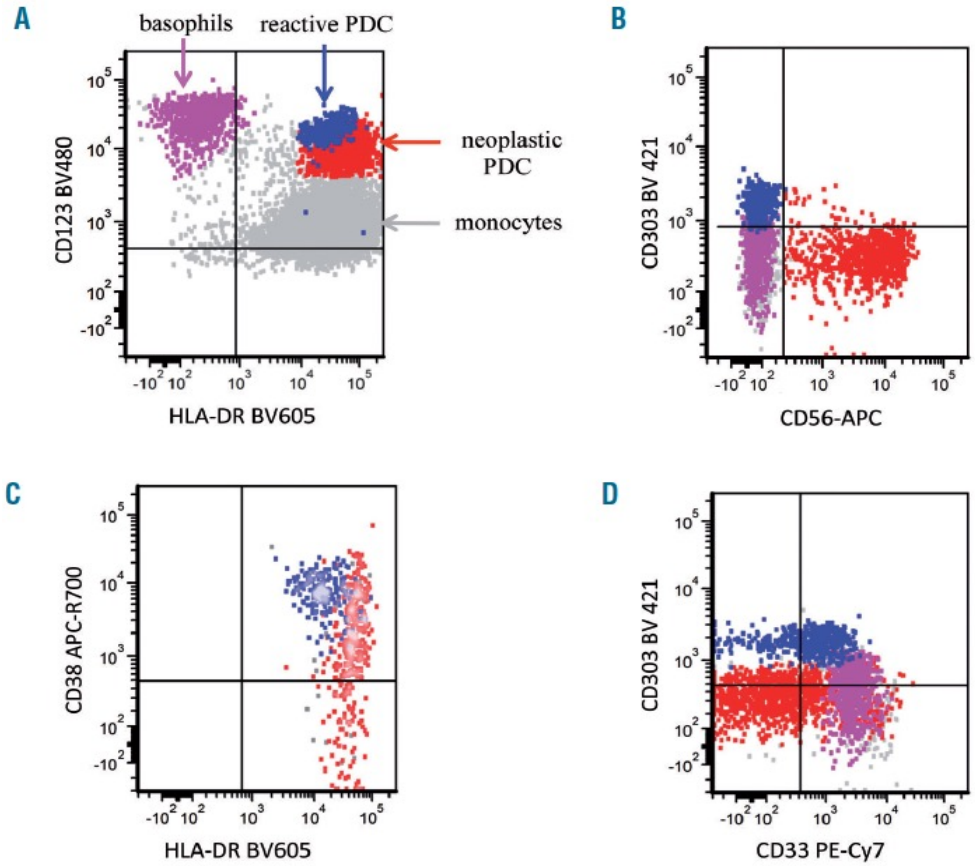


CD45/CD42A-CD61/CD203C/CD34/CD117/CD123/CD4

Van Dongen JJ et al., Leukemia 2012; Martìn-Martìn L et al., Oncotarget 2015

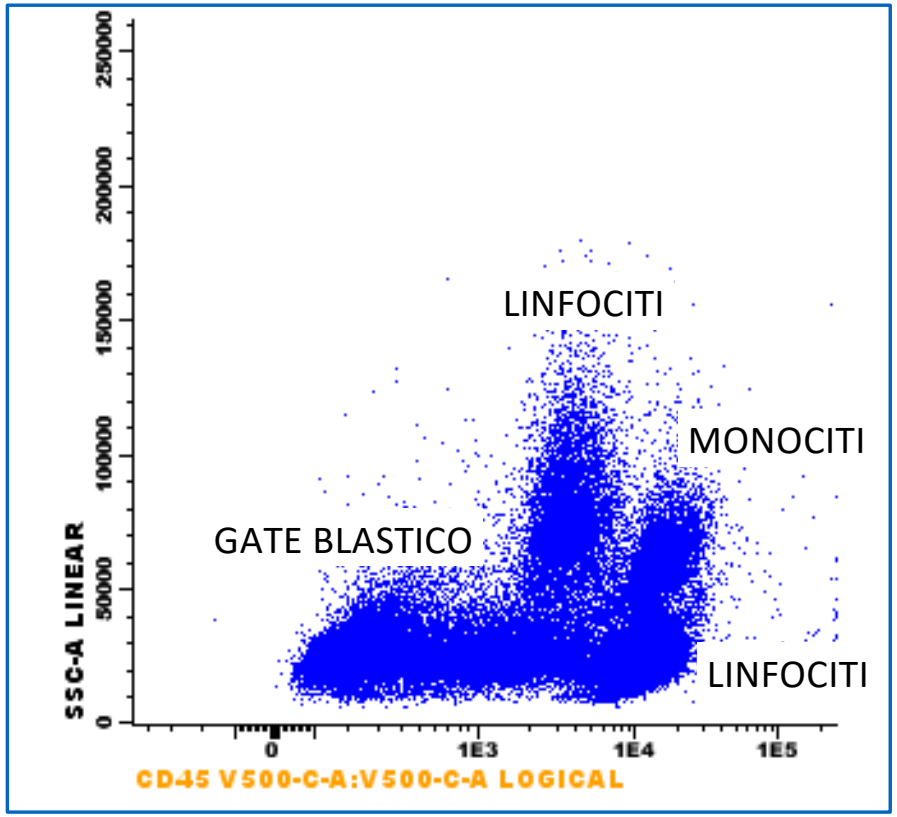
MFC panel construction (MD Anderson proposal)

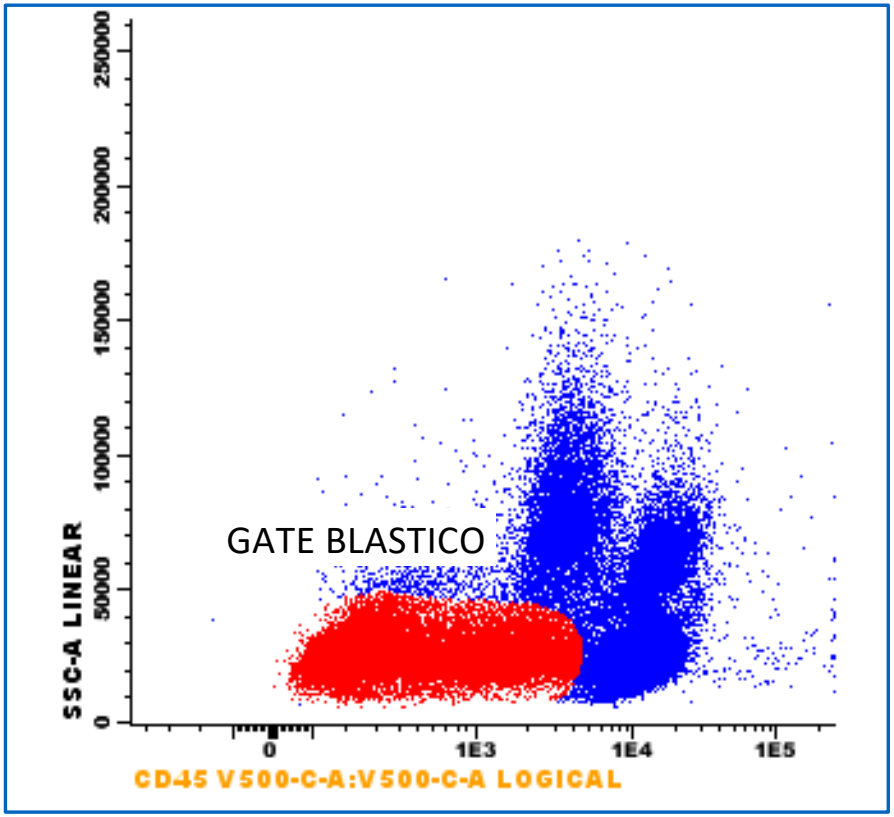
	Antibody list
Panel #1	CD7/CD33/CD19/CD34/CD13/CD2/CD38/CD45
	HLA-DR/CD117/**CD4**/CD34/**CD123**/CD38/CD45
	CD41/CD36/**CD56**/CD34/CD64/HLA-DR/CD14/CD45
	CD5/CD25/CD22/CD34/CD38/CD15/CD45
	TdT/MPO/CD34/CD3/cytoCD3/CD45 (cyto tube)
Panel #2	**HLA-DR/CD64/CD4/CD33/CD56/CD45 CD303/CD123**
Panel #3	**CD2+**/CD4/**CD7-**/**CD38+**/CD45/CD56/ CD64/CD123/CD303/ HLA-DR

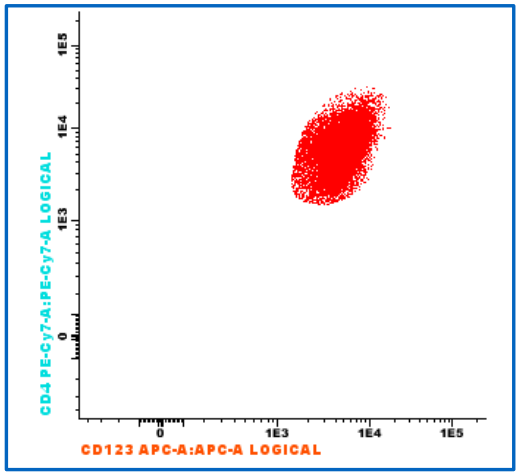


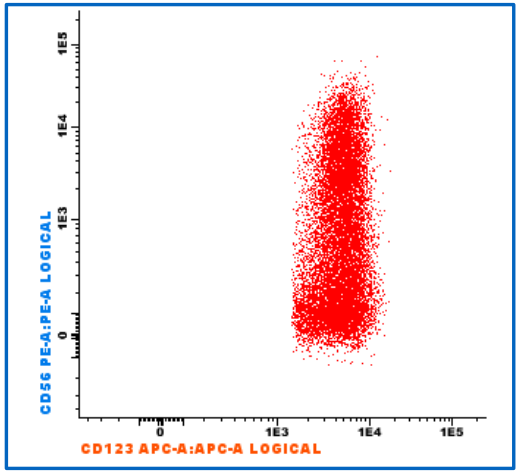
Wang W et al., Haematologica 2021

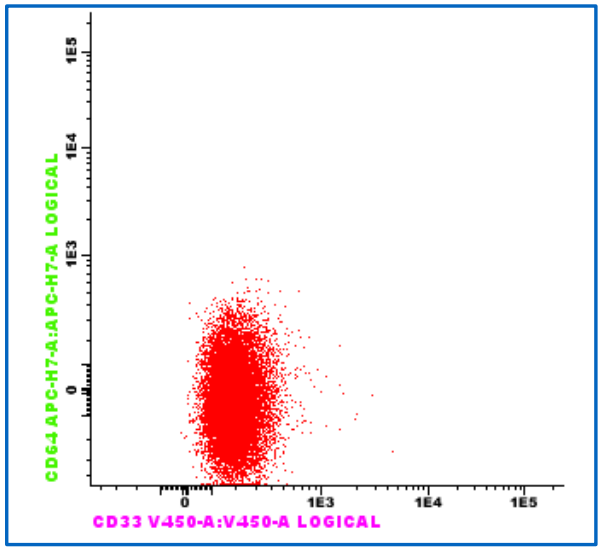
Analytical approach to BPDCN

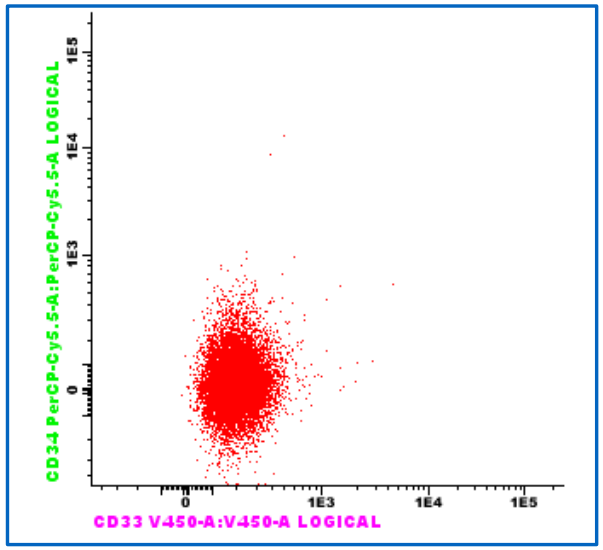




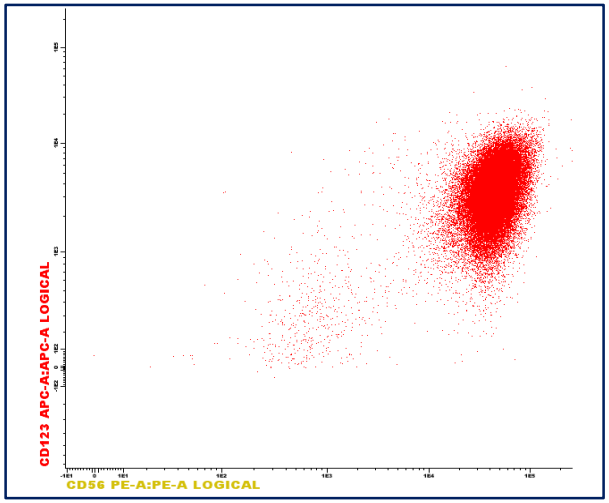

CD4 PE-Cy7-A:PE-Cy7-A LOGICAL
CD123 APC-A:APC-A LOGICAL
0
1E3
1E4
1E5

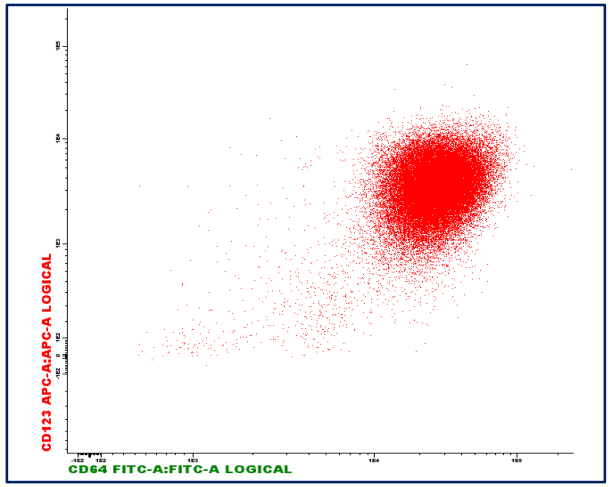

CD56 PE-A:PE-A LOGICAL
CD123 APC-A:APC-A LOGICAL
0
1E3
1E4
1E5


CD64 APC-H7-A:APC-H7-A LOGICAL
CD33 V450-A:V450-A LOGICAL
0
1E3
1E4
1E5

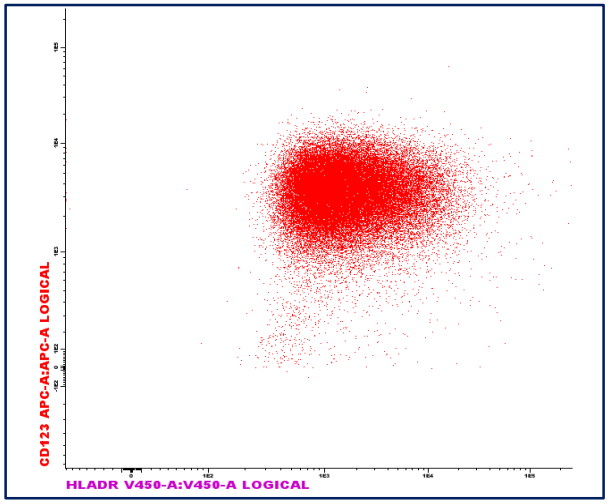

CD34 PerCP-Cy5.5-A:PerCP-Cy5.5-A LOGICAL
CD33 V450-A:V450-A LOGICAL
0
1E3
1E4
1E5

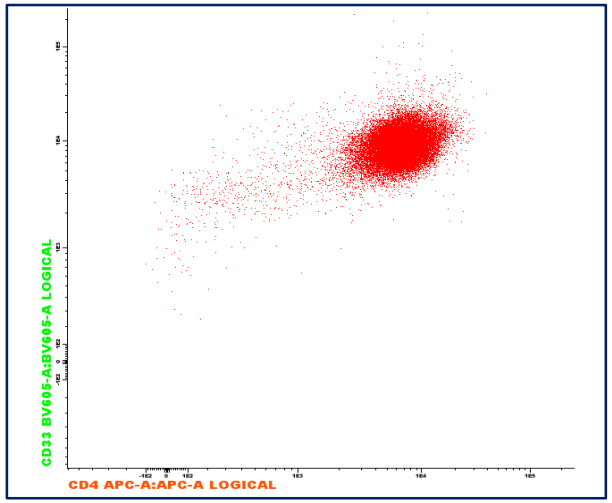
BPDCN





Bright CD64 positivity

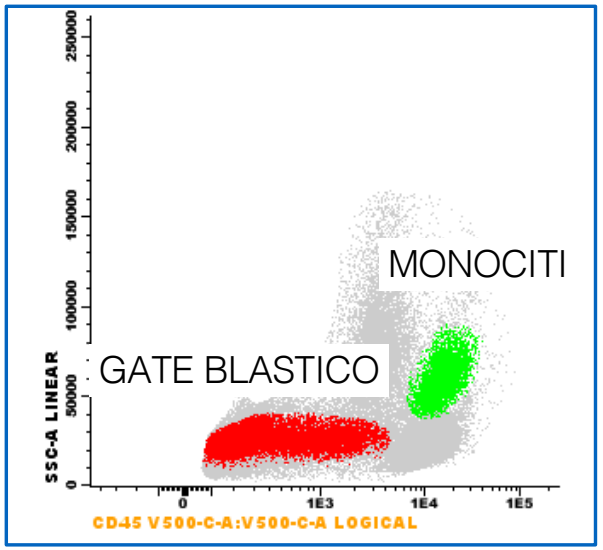


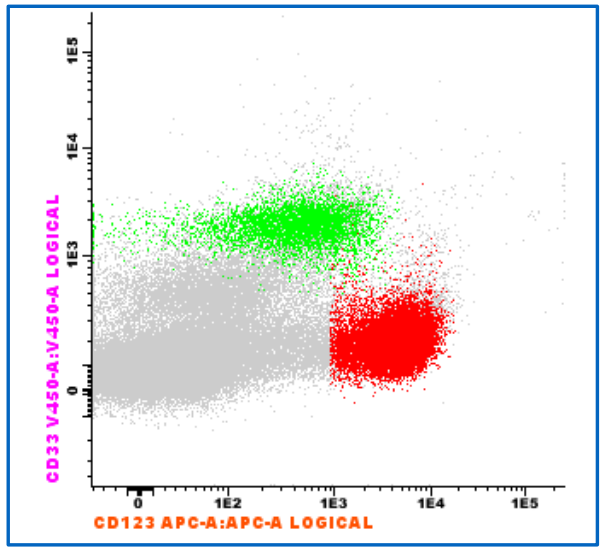


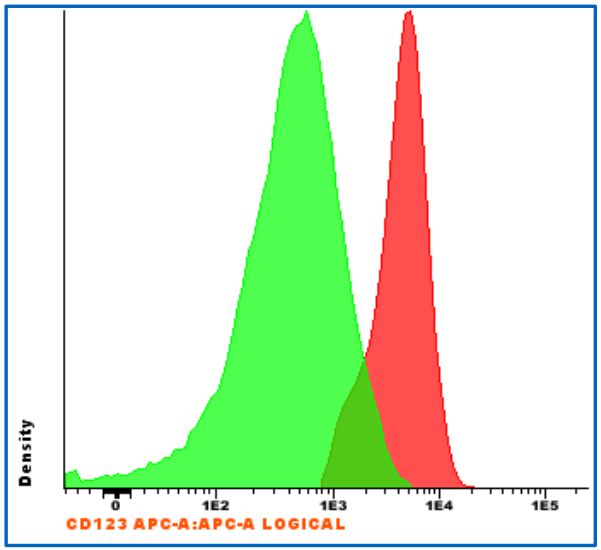
Bright CD33 positivity

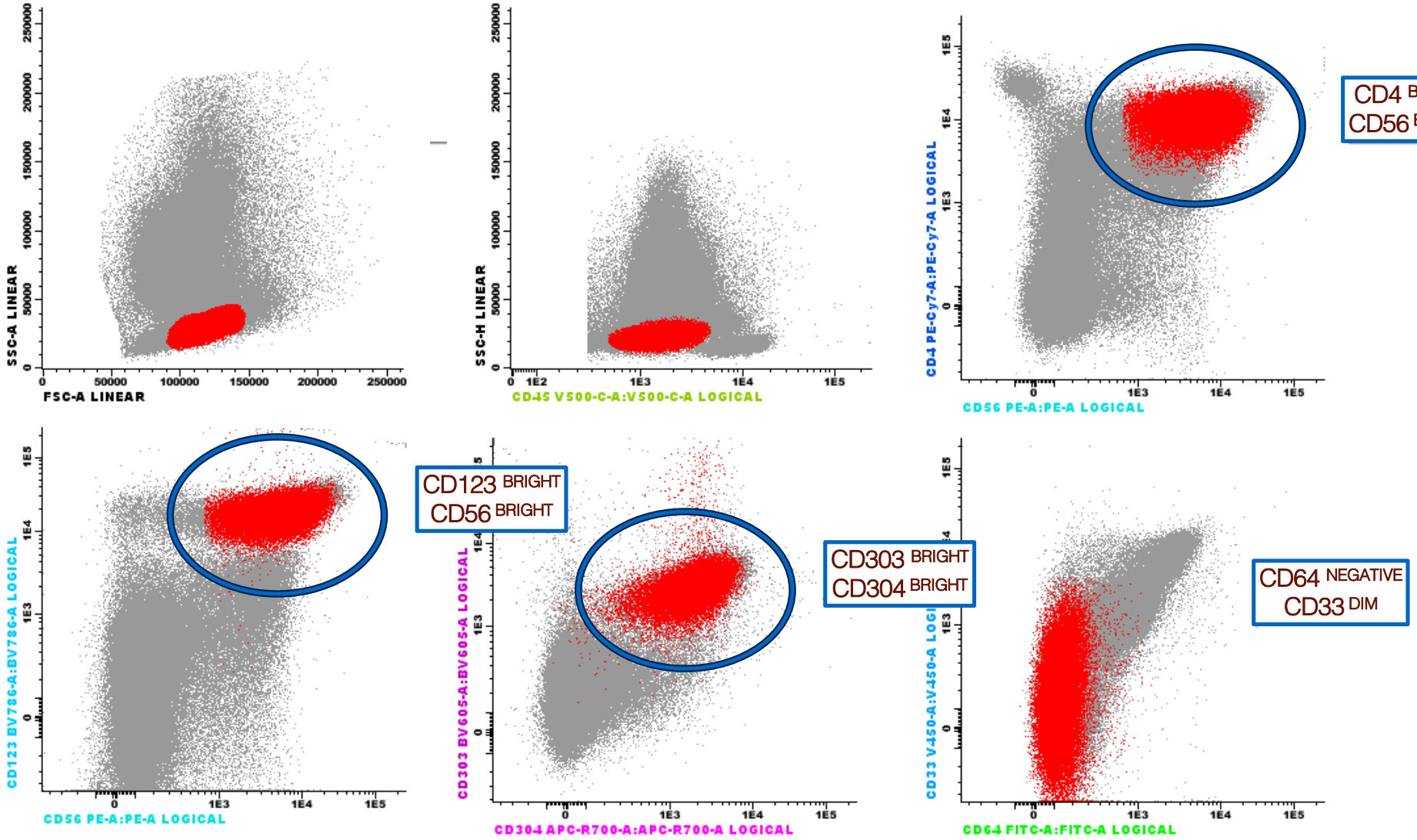
pCD-like AML

Different degrees of CD123 expression









Caso clinico basato sull'esperienza personale del medico

CD34 / CD304 / HLA-DR / CD64 / CD4 / CD33 / CD56 / CD45 / CD303 / CD123

BPDCN Is Often Mistaken for Other Hematologic Malignancies

DIFFERENTIAL FOR LEUKEMIC PRESENTATION

- Acute myeloid leukemias (AML)[1,2]
- Non-Hodgkin lymphoma (NHL)[1,2]
- Chronic myelomonocytic leukemia (CMML)[1]
- Aggressive NK-cell leukemia/lymphoma (ANKCLL)[1,3]

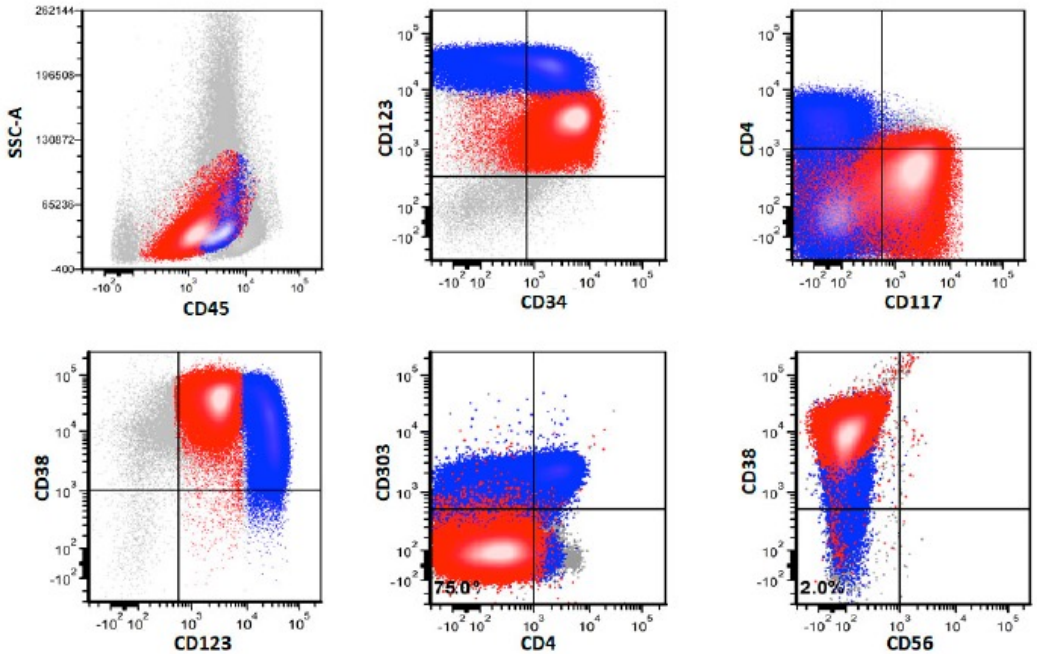
DIFFERENTIAL FOR CUTANEOUS PRESENTATION

- CD56+ acute myeloid leukemias (AML)[1,2]
- Cutaneous T-cell lymphoma (CTCL)[1,3]
- Extranodal NK/T-cell lymphoma (NK-TCL)[1,3]
- Subcutaneous panniculitis-like T-cell lymphoma (SPTCL)[1]

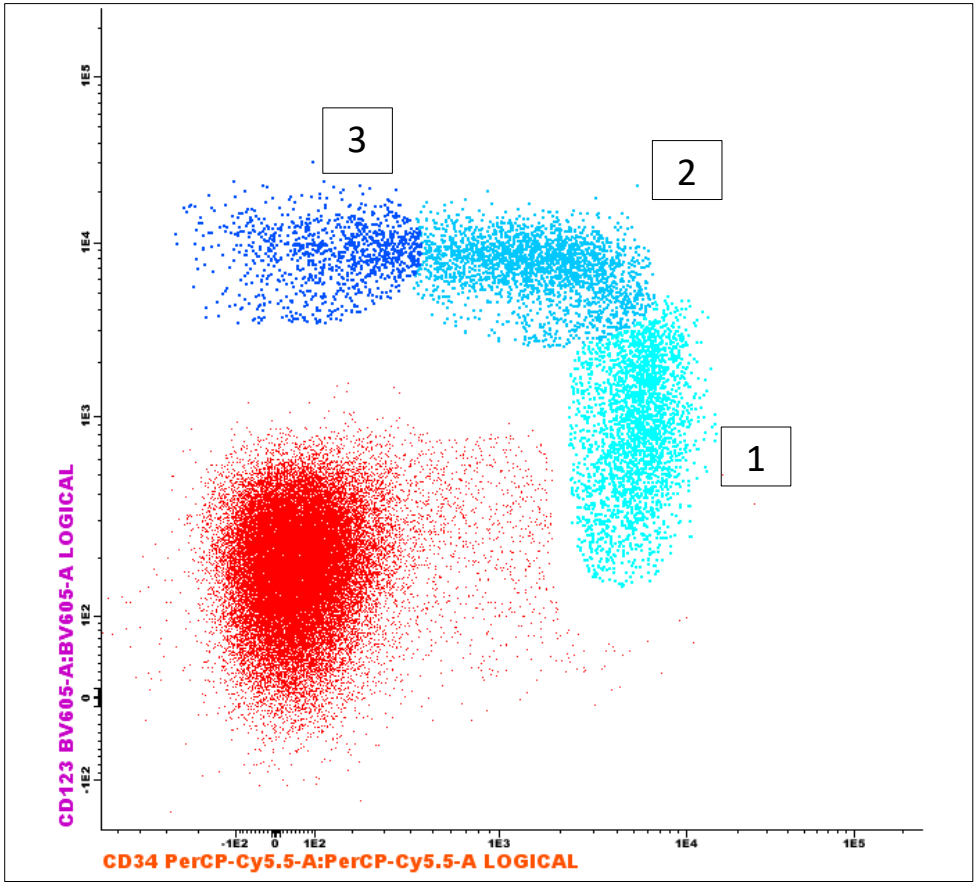
1. Goyal A, et al. In: Carter JB, et al. eds. Atlas of Cutaneous Lymphomas: Classification and Differential Diagnosis. Cham, Switzerland: Springer International; 2015 2. Bueno C, et al. Haematologica. 2004 3. Pagano L, et al. Br J Haematol. 2016

pDC-AML: specific characteristics

- ✓AML with ≥2% plasmacytoid dendritic cells (pDC) has been recently described as pDC-AML
- ✓Low incidence (~5% of AML)
- ✓pDC expansion with frequent RUNX1 mutations
- ✓Poor clinical outcome



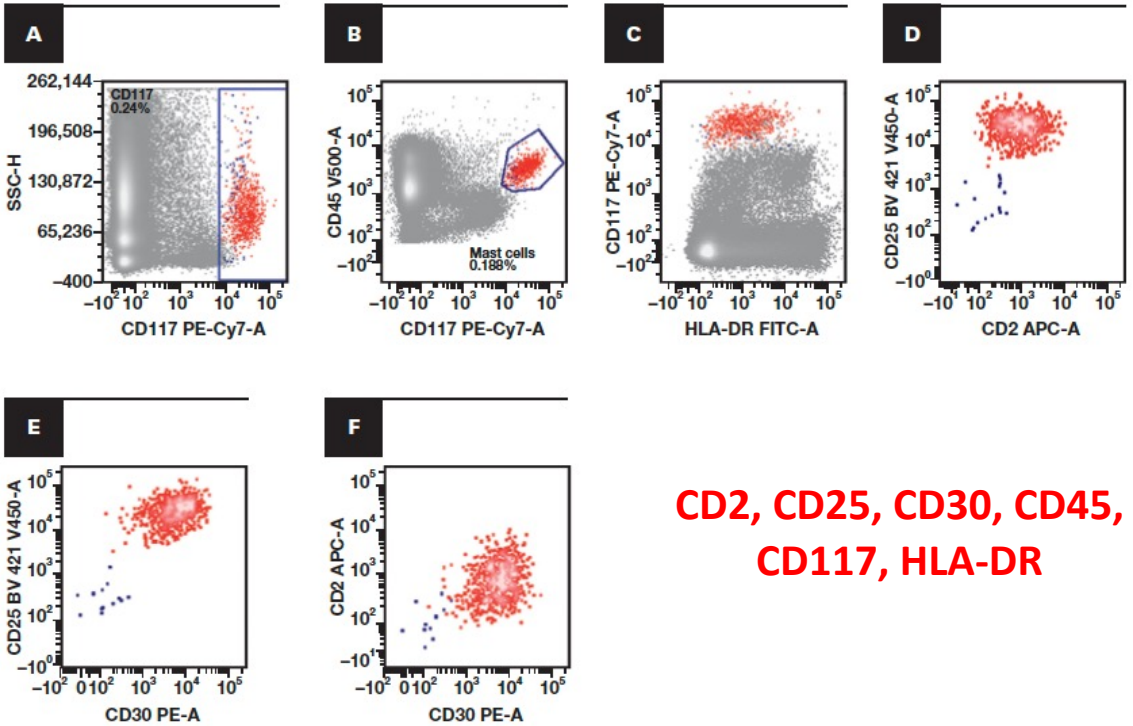
1. Xiao W et al., Blood 2021; 2. Pemmaraju N, Blood 2021; 3. Wang W et al., Cancers 2022



1. Immature BPDCN (Cyan): $CD34^{POS}$ $CD123^{DIM}$
2. Intermediate BPDCN (light blue): $CD34^{BRIGHT}$ $CD123^{BRIGHT}$
3. Mature BPDCN (blue): $CD34^{NEG}$ $CD123^{BRIGHT}$

Sensitivity of MFC in detection of abnormal mastcells

- 1 million cells or all sample in the tube at a median flow rate.
- Mast cells were gated based on CD117(bright+)/side scatter (A), followed by CD117+CD45+ gating (B)
- Nonspecific binding and no overlap with myeloblasts were further ensured by examining the CD117/HLA-DR plot (C).
- The mast cells were then analyzed for the expression of CD2, CD25, and CD30 (D).
- An expression of 10% or more positive cells compared with control was considered positive for that marker



CD2, CD25, CD30, CD45, CD117, HLA-DR

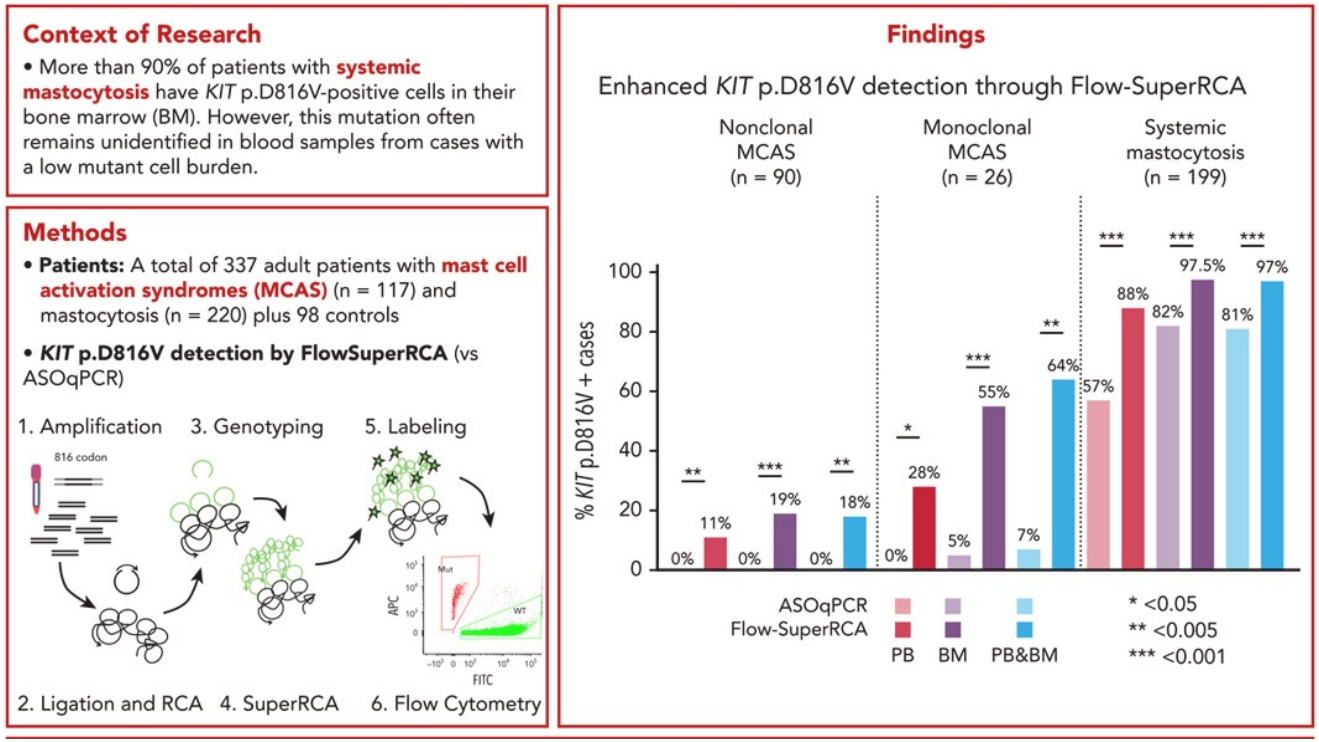
TABLE 2 Mast Cell Immunophenotype Comparison Between Systemic Mastocytosis and Reactive/Normal Mast Cells, by Flow Cytometry

	All SM, No. (%) (n = 87)[a]			Reactive, No. (%) (n = 40)			*P* value
	Uniformly positive	Partially positive	Positive total	Uniformly positive	Partially positive	Positive total	
CD2	41 (47)	27 (31)	68 (78)	0 (0)	0 (0%)	0 (0)	<.0001
CD25	83 (95)	2 (2)	85 (98)	3 (8)	2 (5)	5 (13)	<.0001
CD30	48 (55)	30 (34)	78 (90)	0 (0)	5 (13)	5 (13)	<.0001
1 abnormality	—	—	87 (100)	—	—	10 (25)	<.0001
2 or 3 abnormalities	—	—	80 (92)	—	—	0 (0)	<.0001

Nwogbo OV et al., Am J Clin Pathol 2024

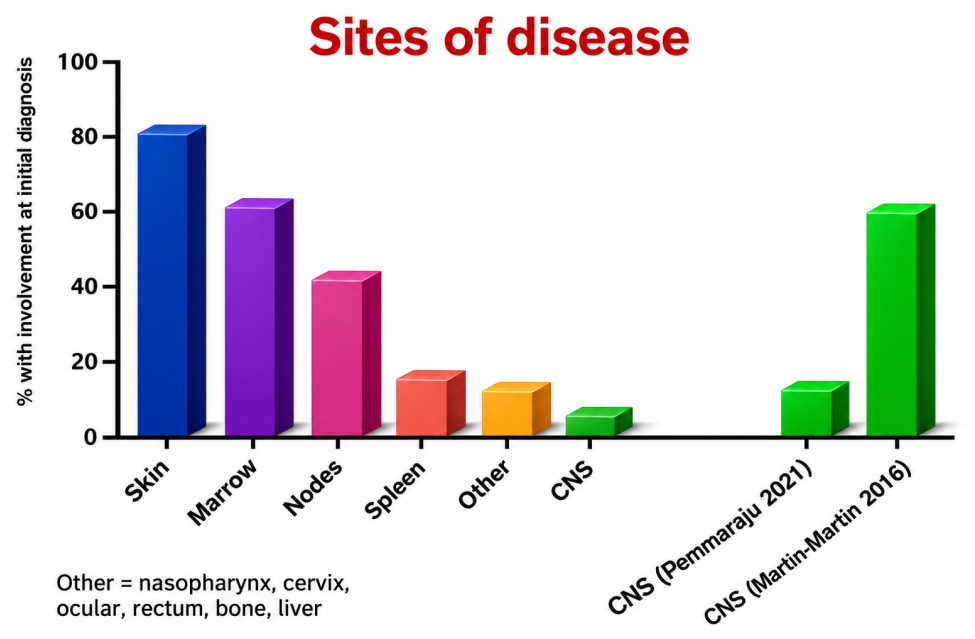
Improved diagnostic screening and classification of clonal mast cell diseases by ultrasensitive KIT p.D816V detection

These results confirm the high specificity and ultra sensitivity of the Flow-SuperRCA assay for the detection of *KIT* p.D816V, emerging as a well-suited whole-blood and whole-BM test for diagnostic screening and classification of patients with MCAS and mastocytosis



Navarro-Navarro P et al., Blood 2025

Clinical presentation



- Referral to an experienced center with expert pathology and clinical trials (preferred)

▼

- Evaluation:
  - Dermatology with skin biopsy
  - Hematology
    - Bone marrow aspirate/biopsy
    - Flow-cytometry, including CD123, CD4, CD56
    - Karyotype/cytogenetics
    - Myeloid panel DNA-seq
  - PET/CT scan
  - Lumbar puncture with prophylactic IT chemo

Taylor J, et al. Blood. 2019

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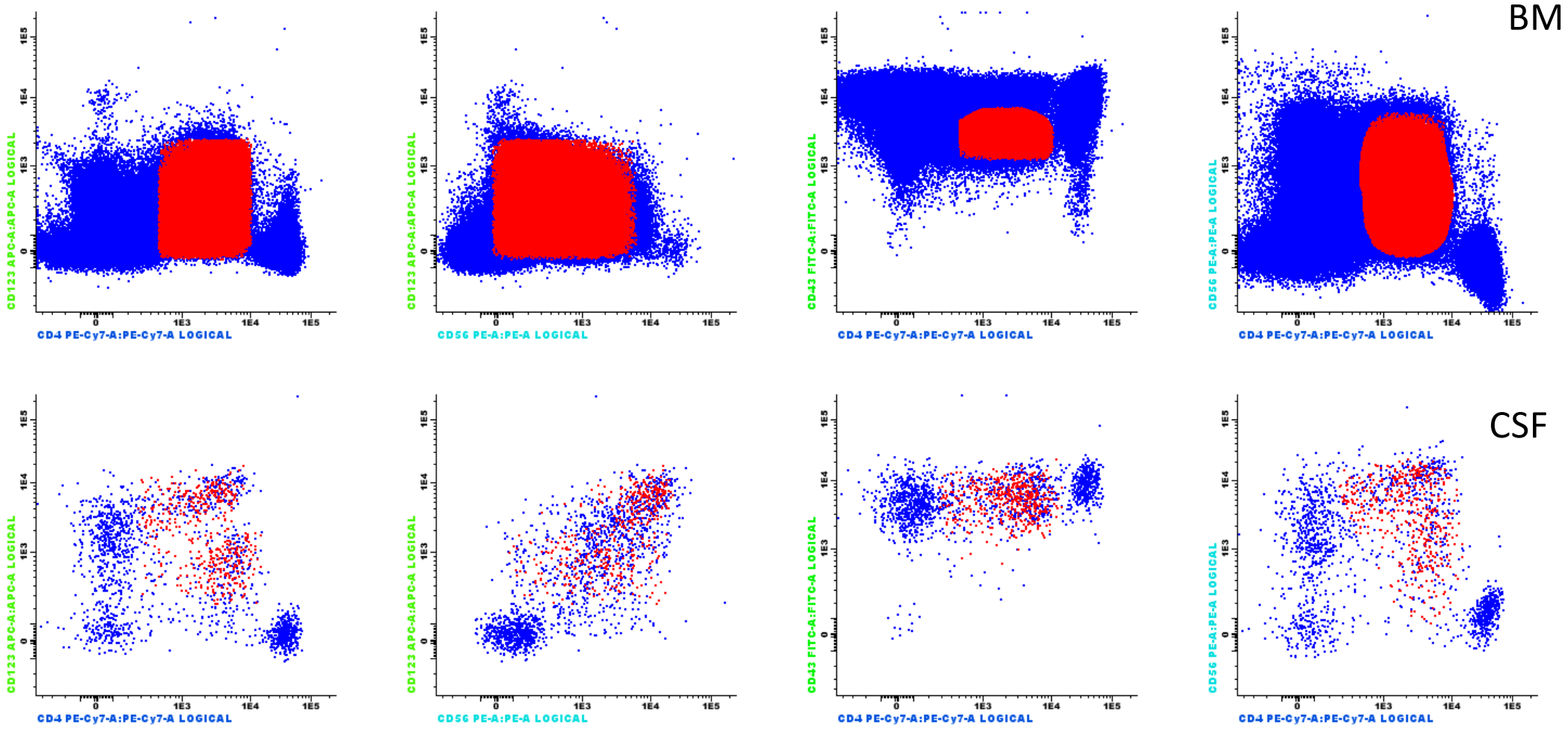
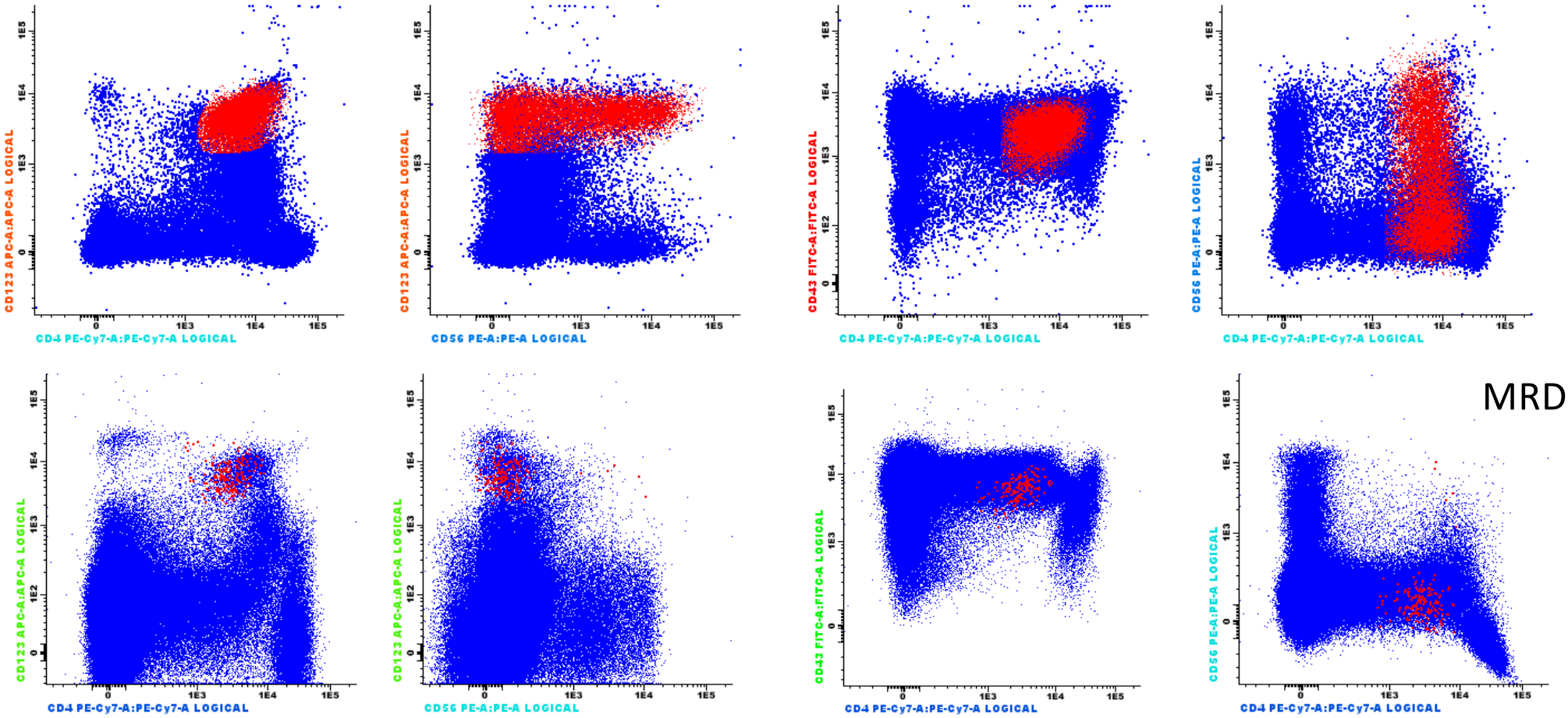
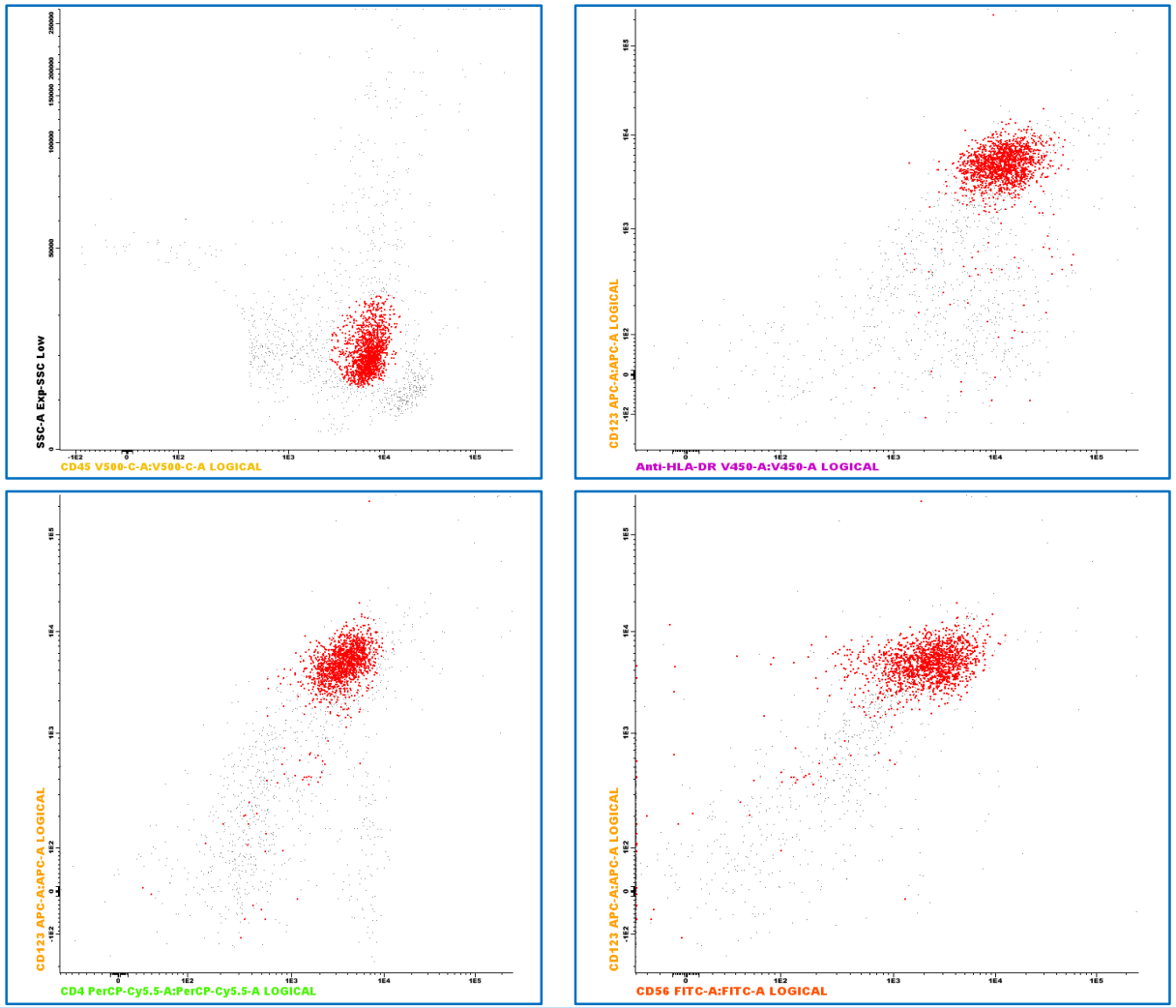


MASTOCITOSI E BPDCN: LE DIAGNOSI DIFFICI

DIAGNOSIS





BPDCN cutaneous infiltrate

Courtesy of A. Gatti

CONCLUSIONS

- ✓ BPDCN and SM are rare diseases that require a multidisciplinary team to allow a timely diagnosis and staging of disease
- ✓ The hematology laboratory has a key role in two ways:
  - Suggest a possible diagnosis in the context of a leukemia screening procedure
  - Confirm diagnosis and organ involvement during the staging procedure
- ✓ Unmet reagent setting and gap of knowledge need to be addressed