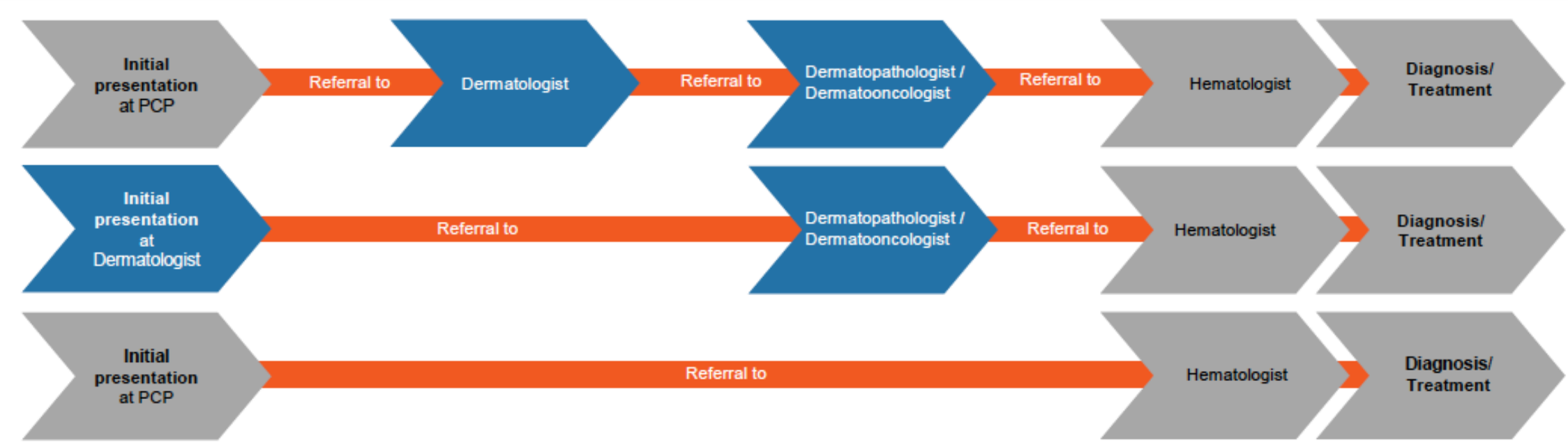
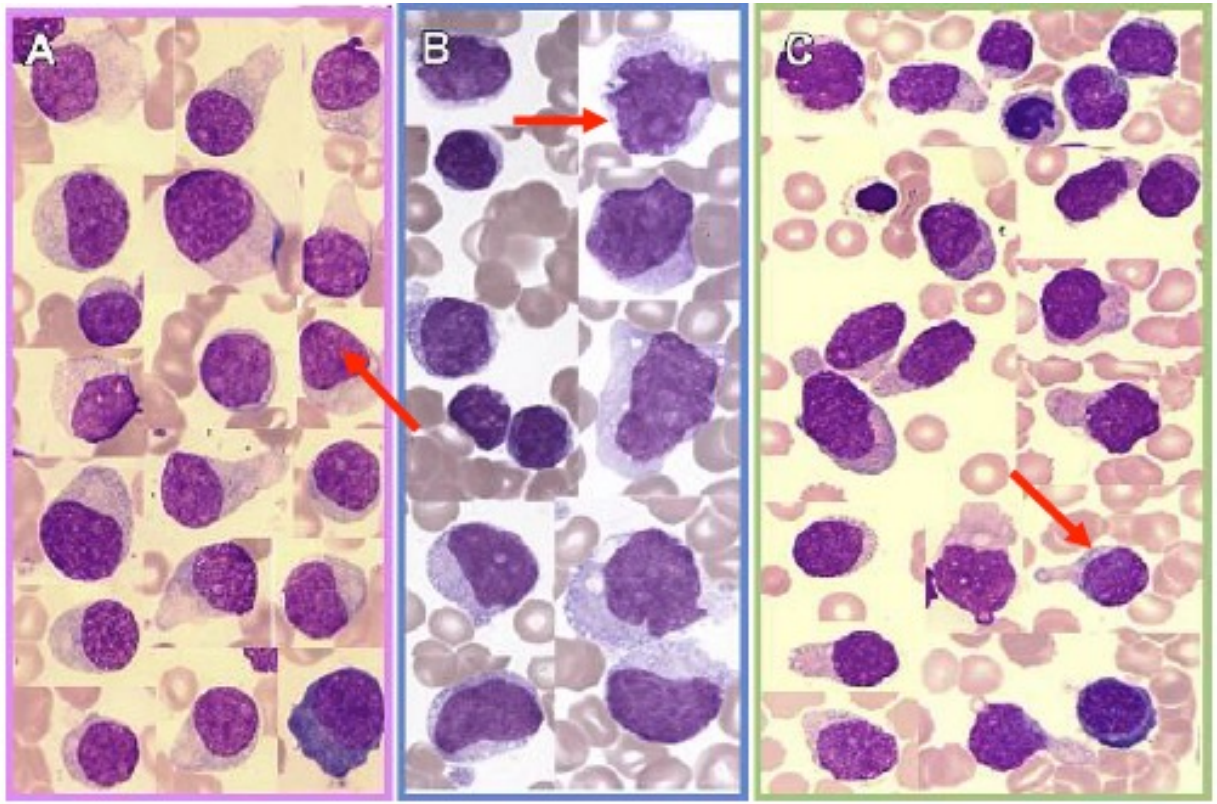
L’algoritmo del citofluorimetrista

Prof. Francesco Buccisano

A multidisciplinary approach may facilitate accurate and timely diagnosis of BPDCN



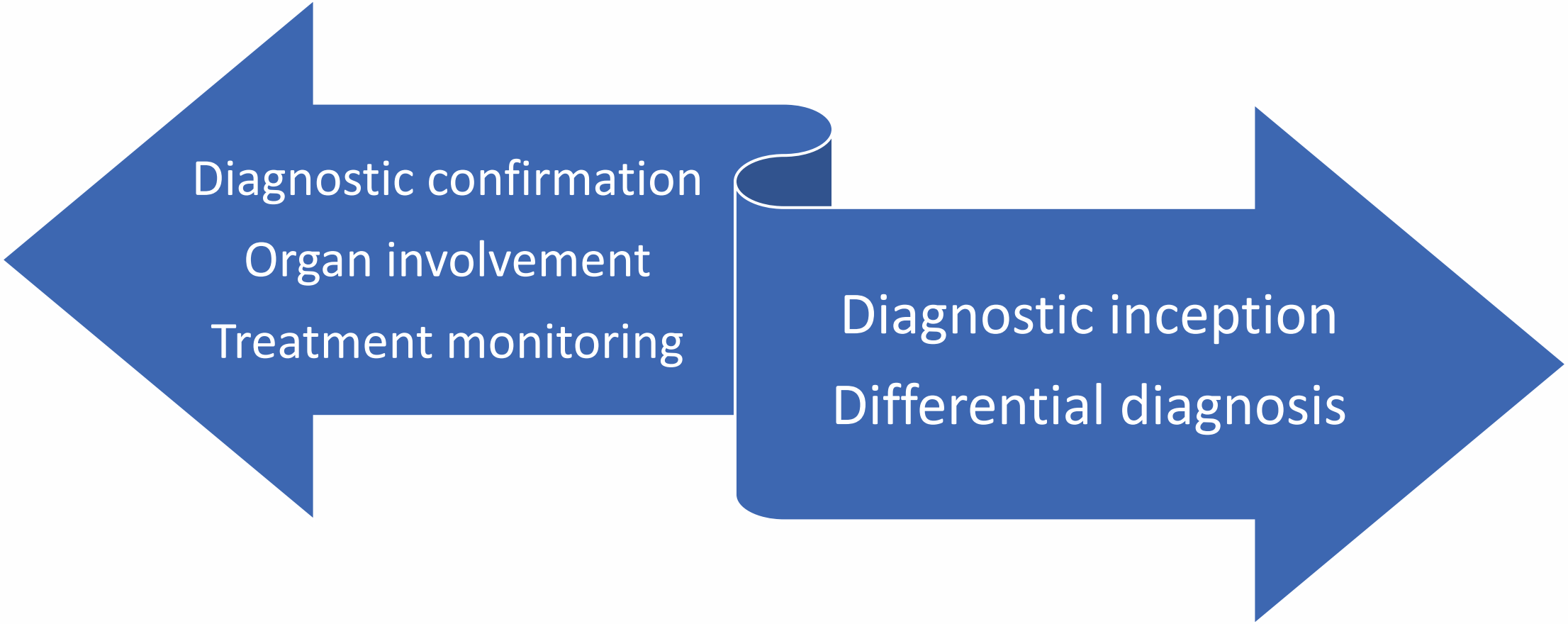
BPDCN diagnosis: morphology



- ✓Intermediate cells with blastic chromatin, inconspicuous nucleoli, and scant cytoplasm (lymphoblast-like)
- ✓Occasional myeloblast-like: larger size, 1 or more prominent nucleoli, abundant cytoplasm
- ✓Circumferential nuclear rimming by vacuoles (pearl necklace appearance) (B arrow)
- ✓Pseudopod cytoplasmic extensions (C arrow)

1. Reichard KK. Surg Pathol Clin. 2013 2. Deconinck E, et al. Hematol Oncol Clin North Am. 2020

The modern role of MFC in BPDCN



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

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

Leukemia (2012) 26, 1908–1975

www.nature.com/leu

Open

ORIGINAL ARTICLE

EuroFlow antibody panels for standardized *n*-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes

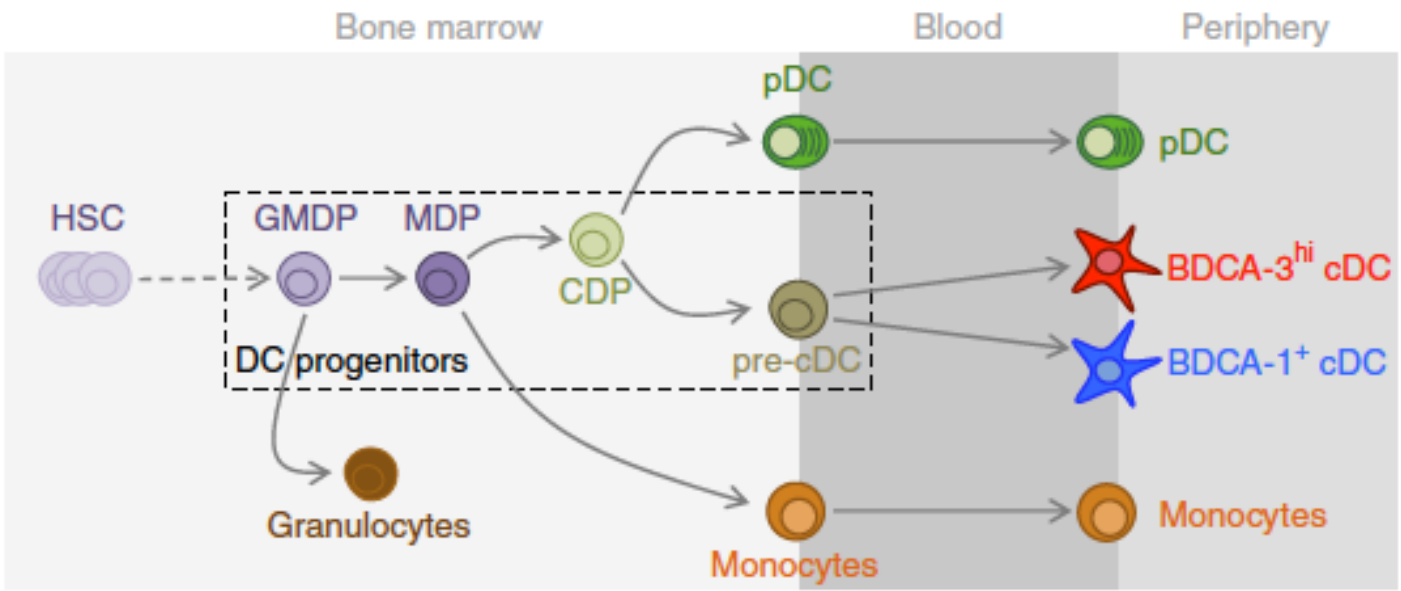
JJM van Dongen[1], L Lhermitte[2], S Böttcher[3], J Almeida[4], VHJ van der Velden[1], J Flores-Montero[4], A Rawstron[5], V Asnafi[2], Q Lécrevisse[4], P Lucio[6], E Mejstrikova[7], T Szczepański[8], T Kalina[7], R de Tute[5], M Brüggemann[3], L Sedek[8], M Cullen[5], AW Langerak[1], A Mendonça[6], E Macintyre[2], M Martin-Ayuso[9], O Hrusak[7], MB Vidriales[10] and A Orfao[4] on behalf of the EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708)

Laboratory diagnostics of hematological malignancies has three major applications:

- Diagnosis
- Prognostic (sub)classification
- Evaluation of treatment effectiveness via detection of MRD.

JJM van Dongen et al, Leukemia 2012

BPDCN: Cell of origin



Plasmacytoid dendritic cells (pDCs) are the most common dendritic cell subset in peripheral blood[1]

- Produced in the bone marrow, circulate in the blood, traffic in skin, and accumulate in lymph nodes when an immune response is triggered[2,3]
- Promote innate antiviral immunity[3]
- Secrete high amounts of type I interferon (IFN; ie, IFN-α, IFN-β) in response to viruses[3]

1. DC hematopoiesis is initiated in the bone marrow (BM). A granulocyte, monocyte and DC progenitor (GMDP) develops into a monocyte and DC progenitor (MDP). MDPs give rise to monocytes and a common DC progenitor (CDP), which loses the potential to produce monocytes.
2. CDPs give rise to plasmacytoid DCs (pDCs), as well as a circulating conventional DC precursor (pre-cDC).
3. Pre-cDCs migrate from the BM through the blood to the periphery to produce the two major subsets of conventional DCs (cDCs) — i.e., BDCA-1+ (CD1c) cDCs and BDCA-3hi (CD303) cDCs.

Breton G et al., Nat. Protoc. 2015 - Taylor J, et al. Blood. 2013 - Laribi K, et al. Biol Blood Marrow Transplant. 2016 - Swiecki M, Colonna M. Nat Rev Immunol. 2015

Classification of BPDCN: WHO 2022 vs. ICC

Table 14. Dendritic cell and histiocytic neoplasms.

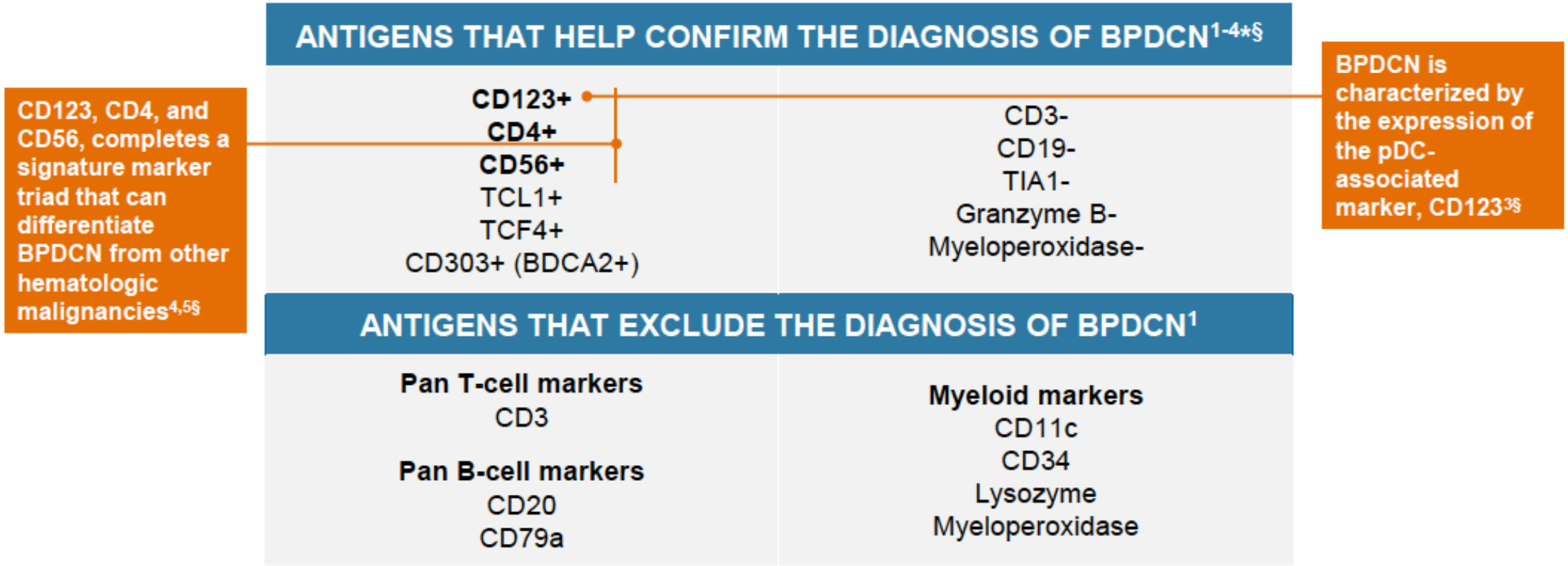
Plasmacytoid dendritic cell neoplasms
Mature plasmacytoid dendritic cell proliferation associated with myeloid neoplasm
Blastic plasmacytoid dendritic cell neoplasm
Langerhans cell and other dendritic cell neoplasms
Langerhans cells neoplasms
Langerhans cell histiocytosis
Langerhans cell sarcoma
Other dendritic cell neoplasms
Indeterminate dendritic cell tumour
Interdigitating dendritic cell sarcoma
Histiocytic neoplasms
Juvenile xanthogranuloma
Erdheim-Chester disease
Rosai-Dorfman disease
ALK-positive histiocytosis
Histiocytic sarcoma

Table 1. Major ICC categories of myeloid neoplasms and acute leukemias

- Myeloproliferative neoplasms
- Myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions
- Mastocytosis
- Myelodysplastic/myeloproliferative neoplasms
- Pre-malignant clonal cytopenias and myelodysplastic syndromes
- Pediatric and/or germline mutation-associated disorders
- Acute myeloid leukemias
- Myeloid proliferations associated with Down Syndrome
- Blastic plasmacytoid dendritic cell neoplasm
- Acute leukemia of ambiguous lineage
- B-lymphoblastic leukemia/lymphoma
- T-lymphoblastic leukemia/lymphoma

Khoury JD et al. Leukemia 2022

BPDCN diagnosis: multiparameter flow-cytometry

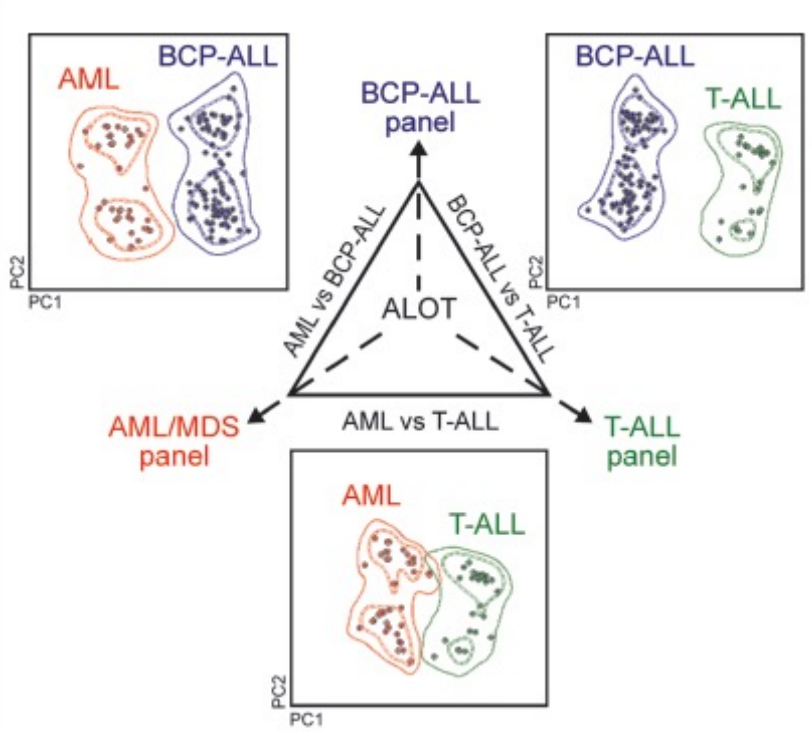


Immunophenotypic diagnostic criteria:

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]

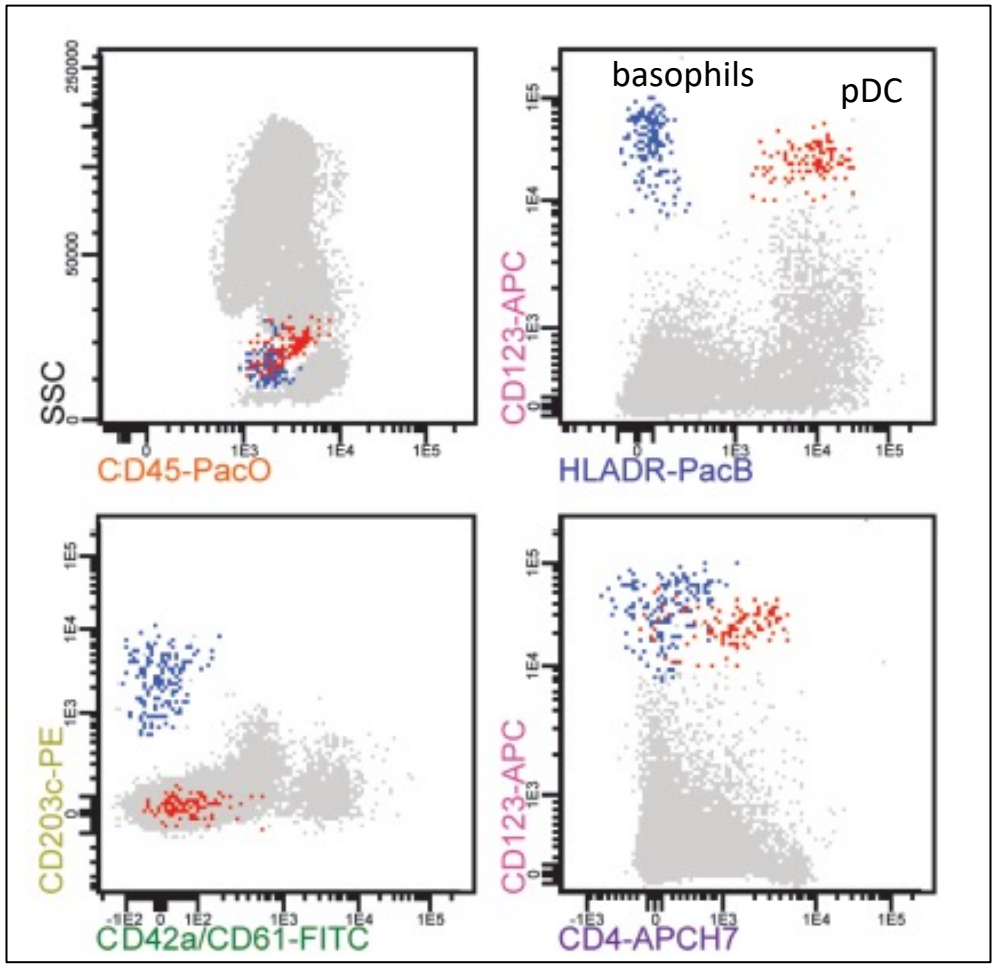
Tube	*PacB*	*PacO*	*FITC*	*PE*	*PerCPCy5.5*	*PECy7*	*APC*	*APCH7*	*Aim*
1	HLADR	CD45	CD16	CD13	CD34	CD117	CD11b	CD10	Diagnosis and classification, neutrophilic maturation, PNH
2	HLADR	CD45	CD35	CD64	CD34	CD117	CD300e (IREM2)	CD14	Diagnosis and classification, monocytic maturation, PNH
3	HLADR	CD45	CD36	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

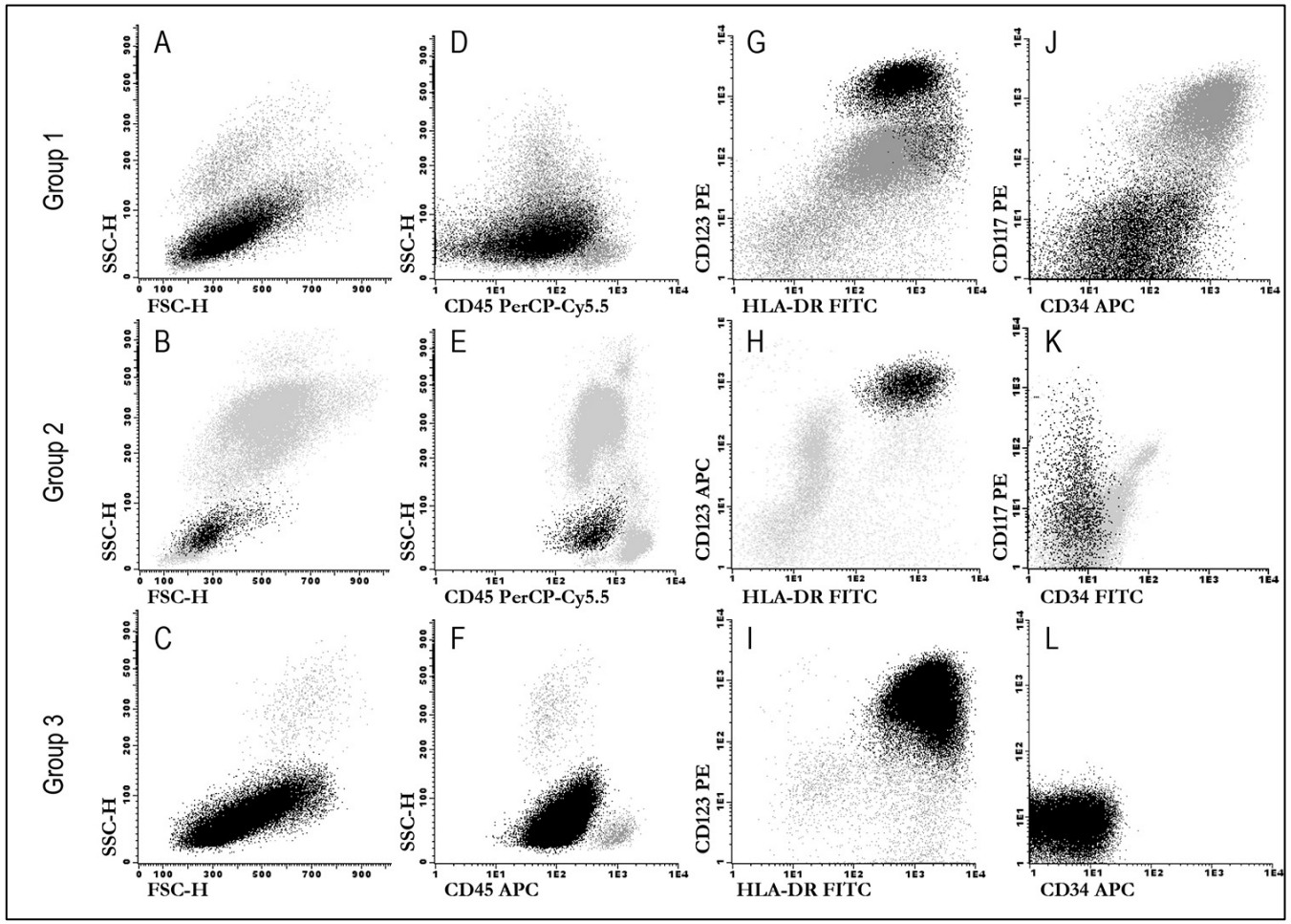
Abbreviations: AML, acute myeloid leukemia; APC, allophycocyanin; BB, backbone; BM, bone marrow; Cy7, cyanin7; FITC, fluorescein isothiocyanate; H7, hilite7; MDS, myelodysplastic syndrome; Nu, nuclear; PacB, pacific blue; PacO, pacific orange; PE, phycoerythrin; PerCPCy5.5, peridinin–chlorophyll–protein–cyanin5.5; 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)



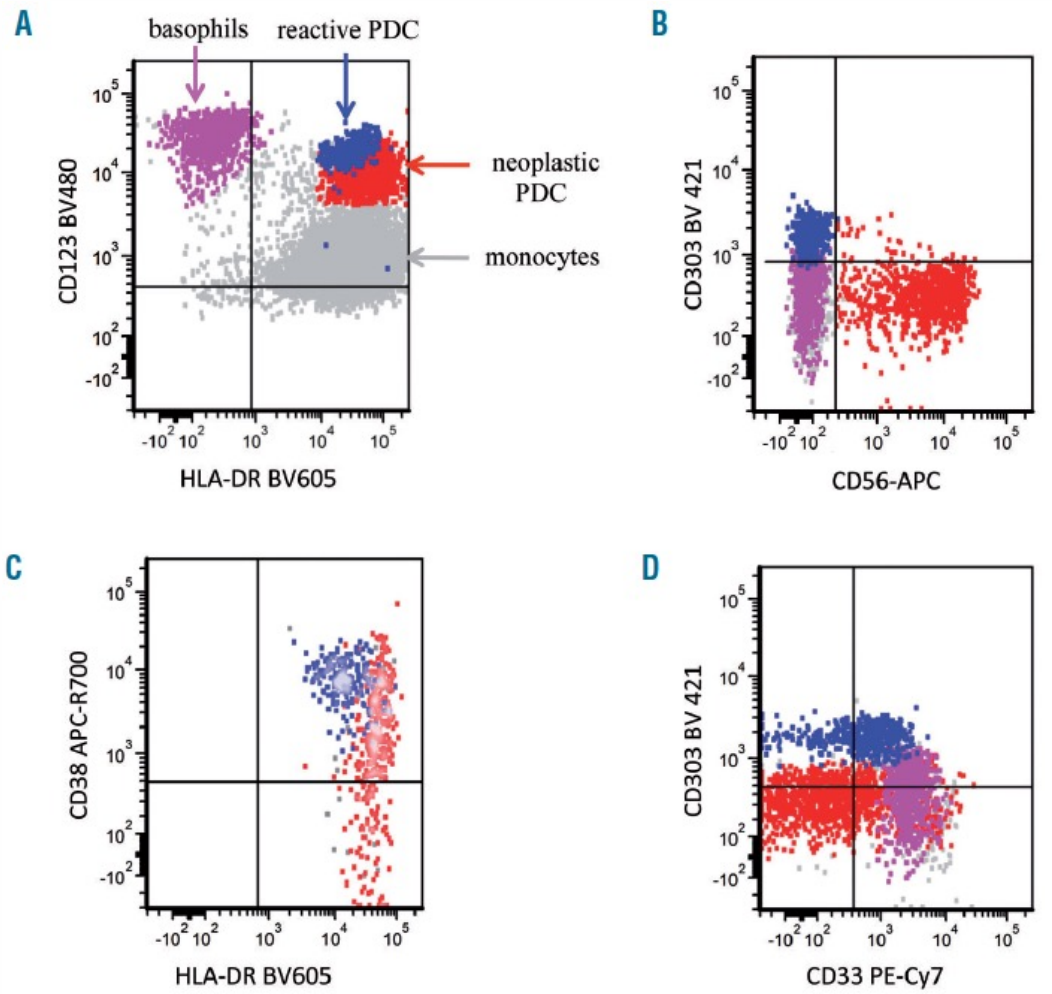


CD45/CD42A-CD61/CD203C/CD34/CD117/CD123/CD4

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

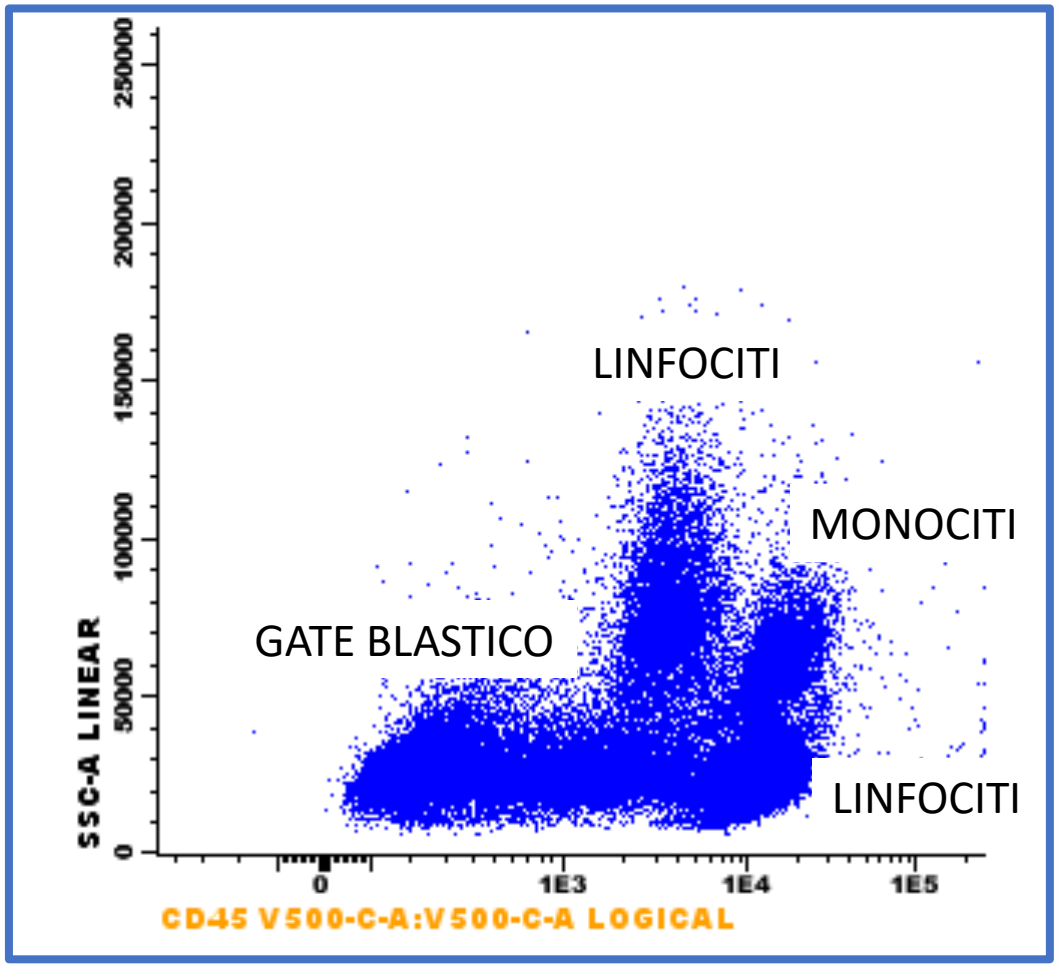
MFC panel construction (MD Anderson proposal)

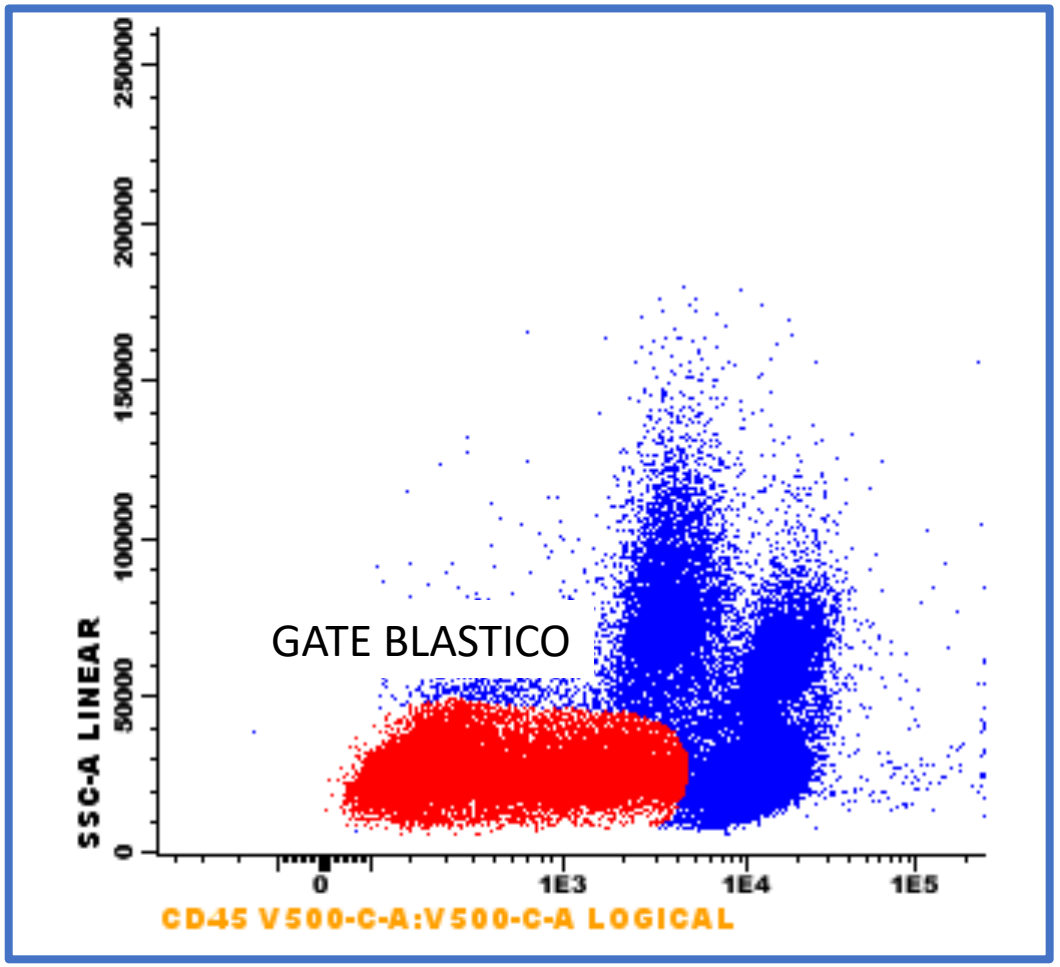
	Antibody list
Panel #1	CD7/CD33/CD19/CD34/CD13/CD2/CD38/CD45
	HLA-DR/CD117/**CD4**/CD34/**CD123**/CD38/CD45
	CD41/CD36/**CD56**/CD34/CD64/HLA-DR/CD14/CD45
	CD5/CD25/CD22/CD34/CD38/CD15/CD45
	TdT/MPO/CD34/CD3/cytoCD3/CD45 (cyto tube)
Panel #2	**HLA-DR/CD64/CD4/CD33/CD56/CD45 CD303/CD123**
Panel #3	**CD2+**/CD4/**CD7-**/**CD38+**/CD45/CD56/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

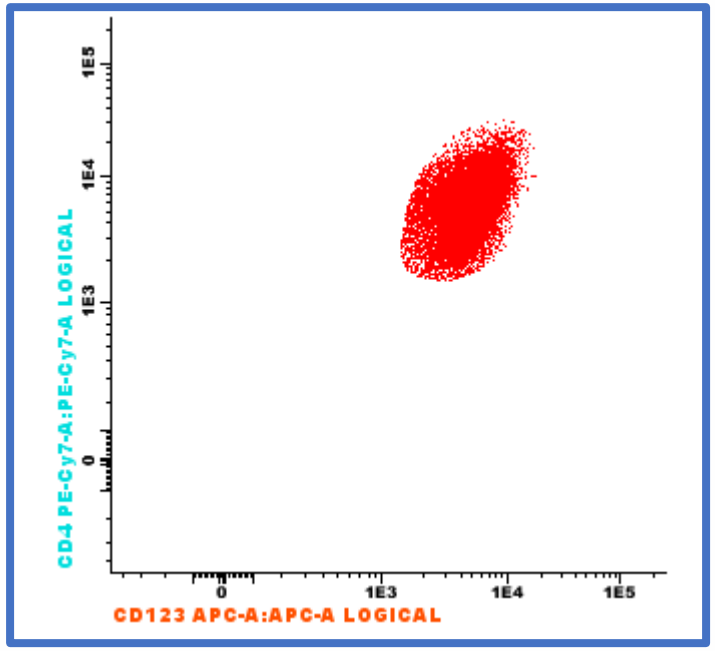


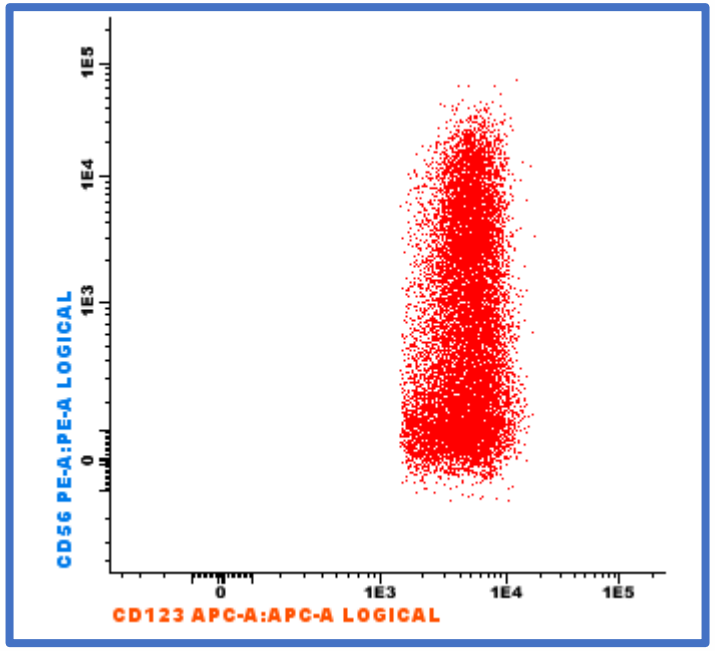
Wang W et al., Haematologica 2021

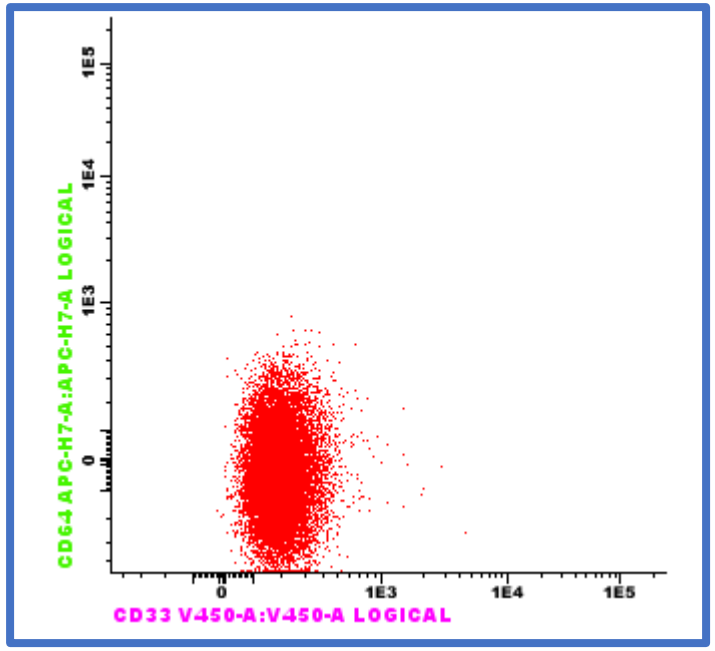
Analytical approach to BPDCN

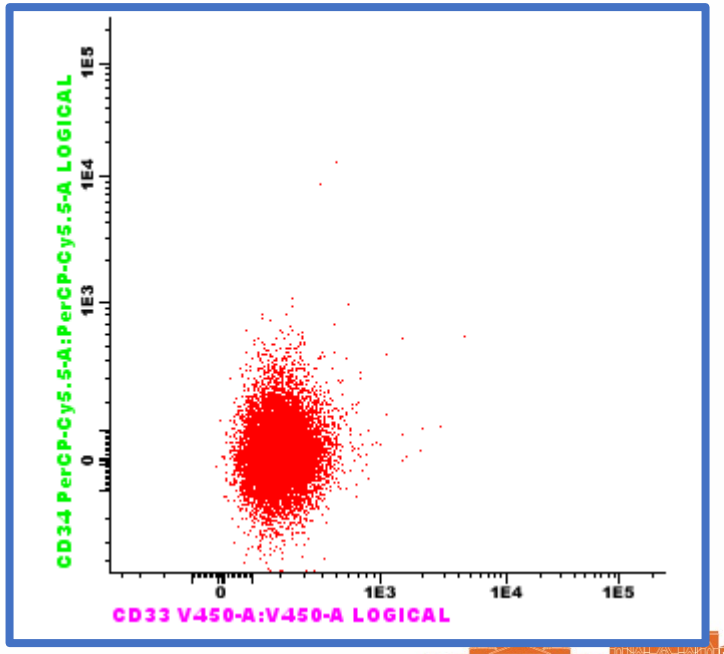




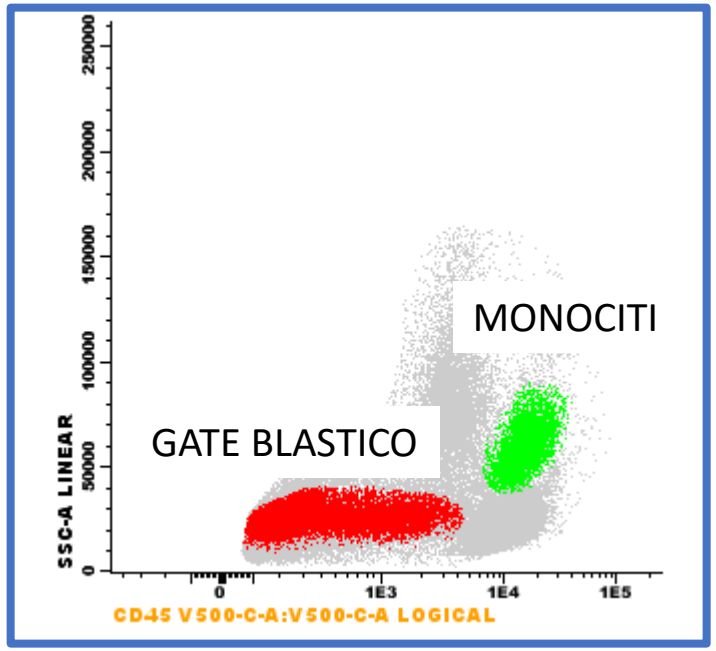

CD4 PE-Cy7-A:PE-Cy7-A LOGICAL
CD123 APC-A:APC-A LOGICAL
0
1E3
1E4
1E5

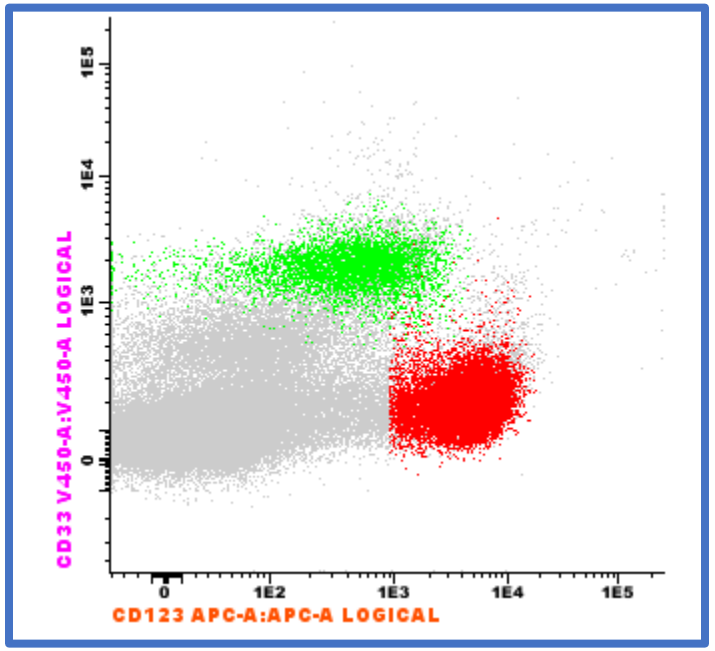

CD56 PE-A:PE-A LOGICAL
CD123 APC-A:APC-A LOGICAL
0
1E3
1E4
1E5

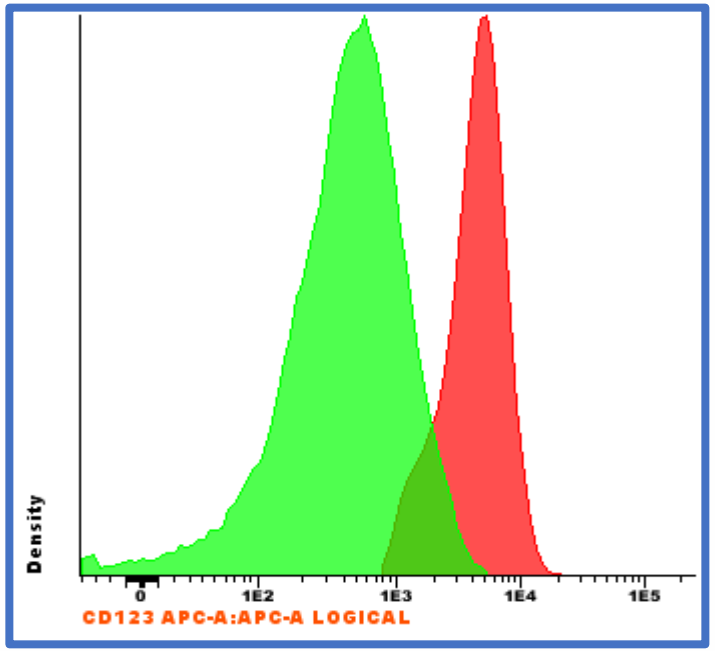

CD64 APC-H7-A:APC-H7-A LOGICAL
CD33 V450-A:V450-A LOGICAL
0
1E3
1E4
1E5


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

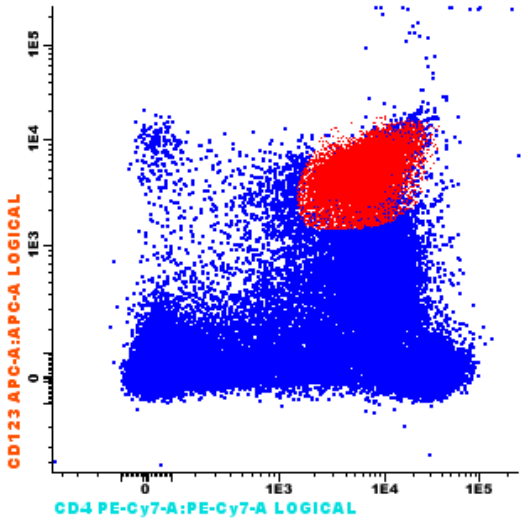
Different degrees of CD123 expression

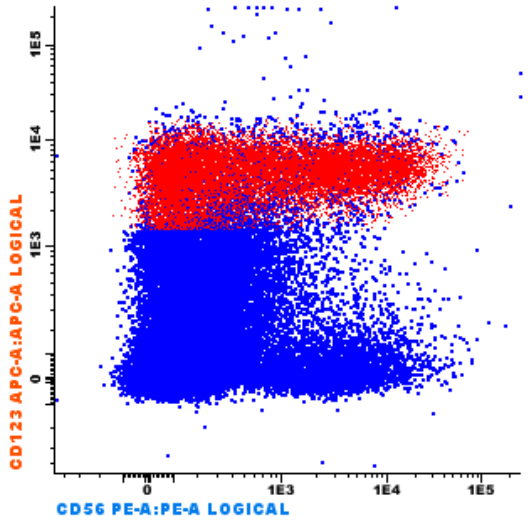


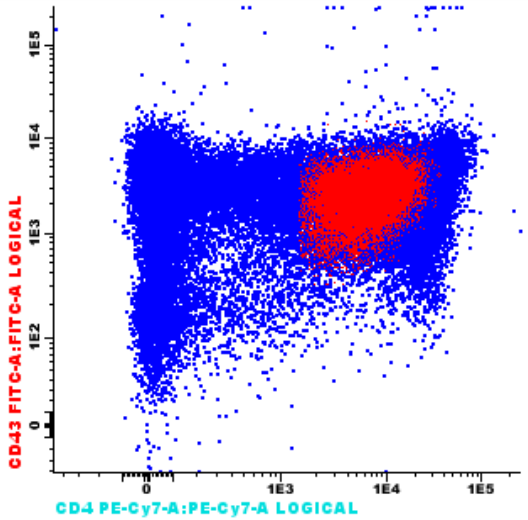


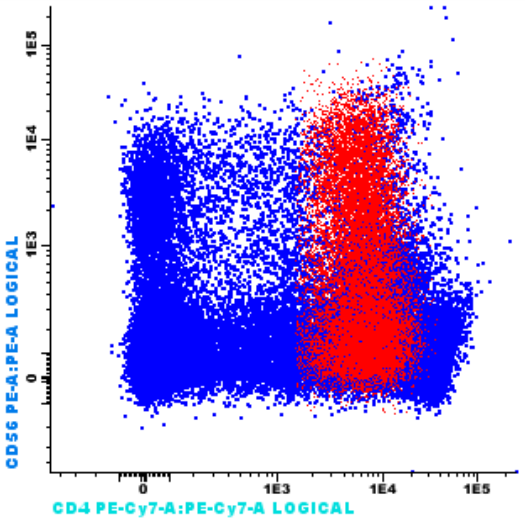


DIAGNOSIS

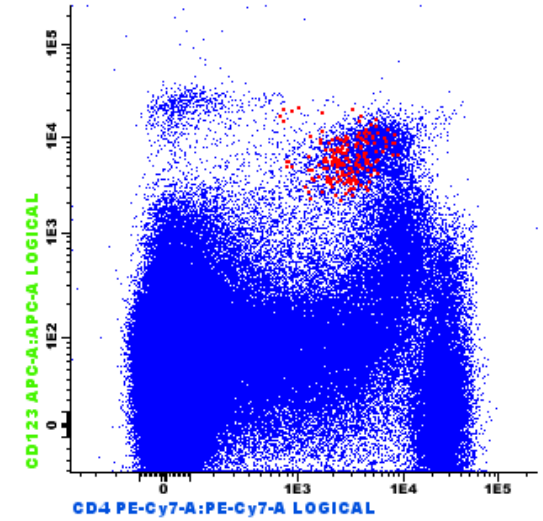


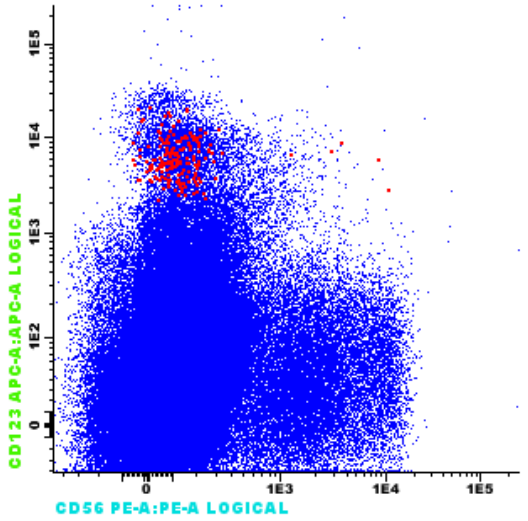


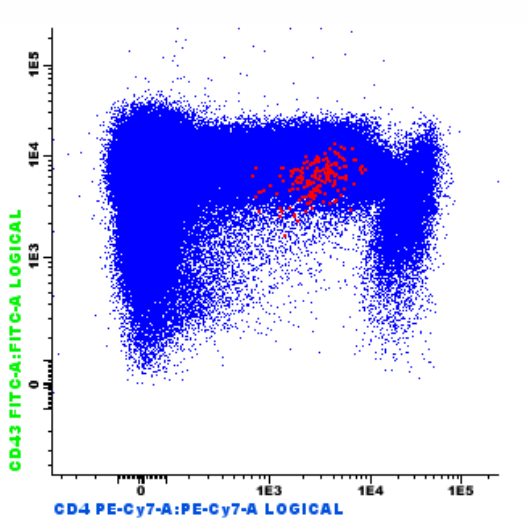


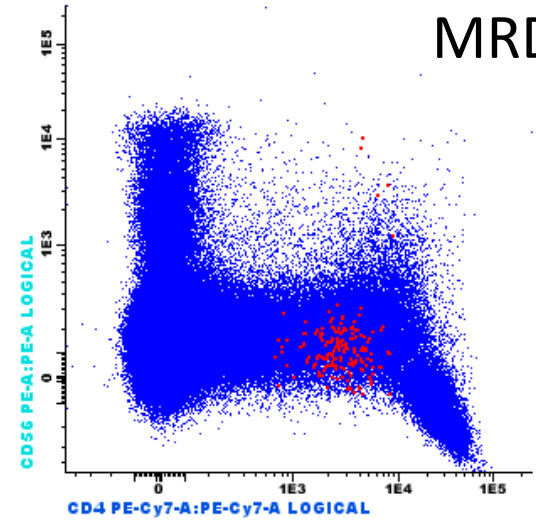


MRD

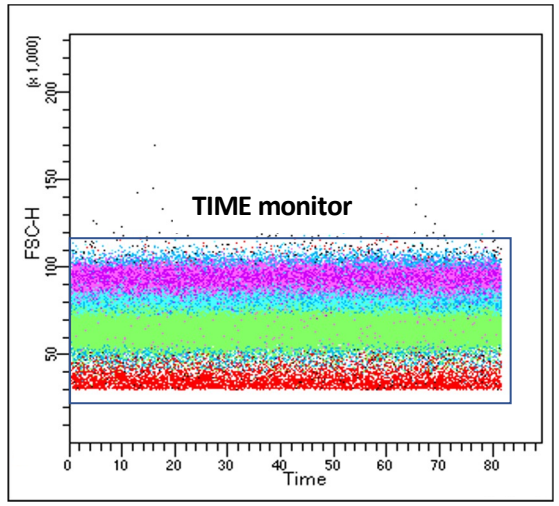


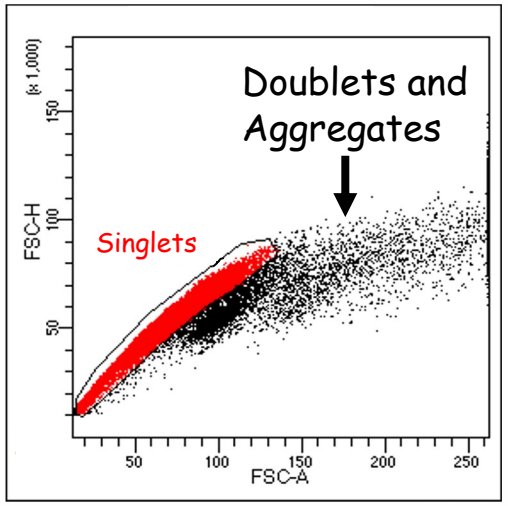






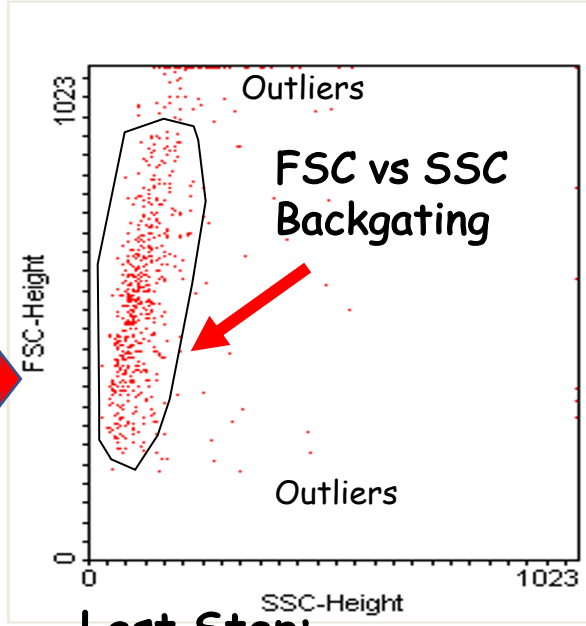
Setting the Right Gating Syntax for Rare Event Analysis: General Rules





Your Own Experiment-Specific Gating Protocol

(Dump channel optional)



First Step: Include the TIME parameter

- To monitor the regularity of the long acquisitions.
- To gate out perturbations.

Second Step: FSC-A vs FSC-H Doublet Discriminator

- To include only cell singlets into analysis.
- Greatly reduces artifacts caused by aggregates.

Third Step: Your Own Logical Gating Protocol

- Any sequential procedure to include relevant cells and to exclude undesired cell populations.

Last Step: FSC vs SSC Backgating

- Fluorescence does not tell you all, and can cheat you.
- Check the rare cell physical parameters consistency.
- Efficiently eliminates parasite events.

Courtesy of Bruno Brando and Arianna Gatti, modified

BPDCN, lab report

- Confirm BPDCN histological report
- Raise the diagnostic suspect of BPDCN in case of suggestive immunophenotype
- Describe the pDC component of blast population in leukemia
- Allow a clear differential diagnosis

Differential diagnosis

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]

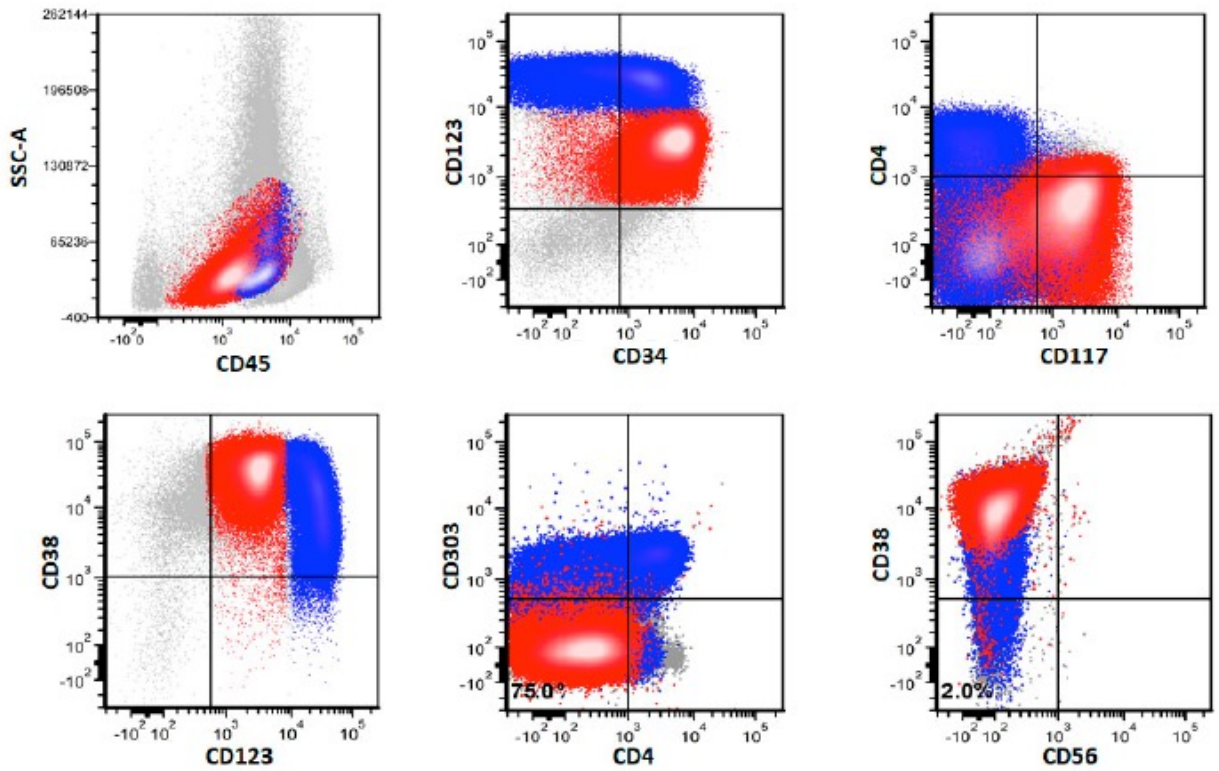
DIFFERENTIAL FOR CUTANEOUS PRESENTATION

- 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

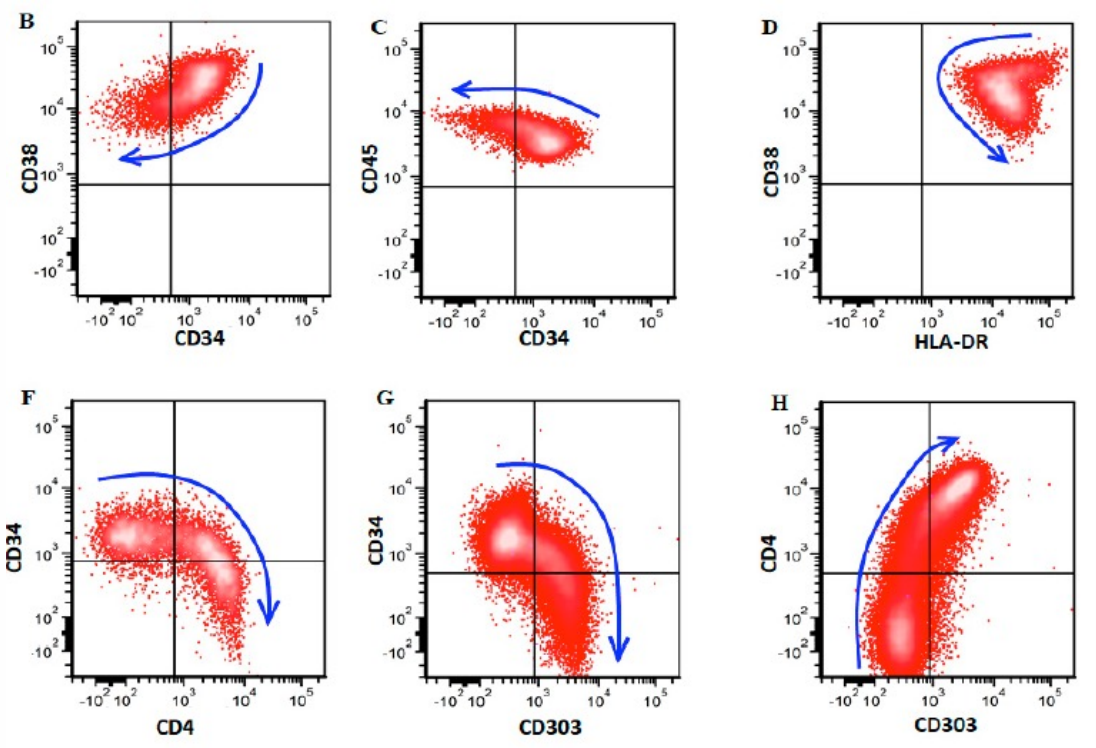
pDC-AML: specific characteristics

- ✓ 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

pDC-AML: biological features



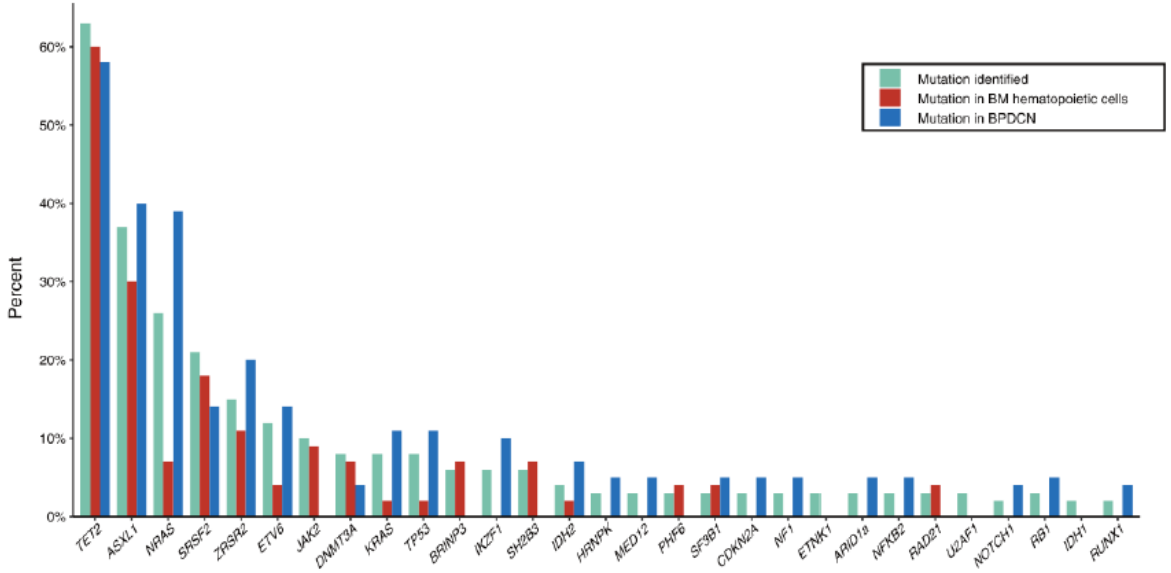
	pDC-AML	BPDCN	P-value
CD34	96%	0	<0.001
CD117	47%	9%	<0.001
CD56	8%	97%	<0.001
CD4	89%	100%	0.039
CD303	100%	44%	0.045
TCL1	12%	98%	<0.001
CD13	33%	0	0.0001
RUNX1	64%	2%	<0.001

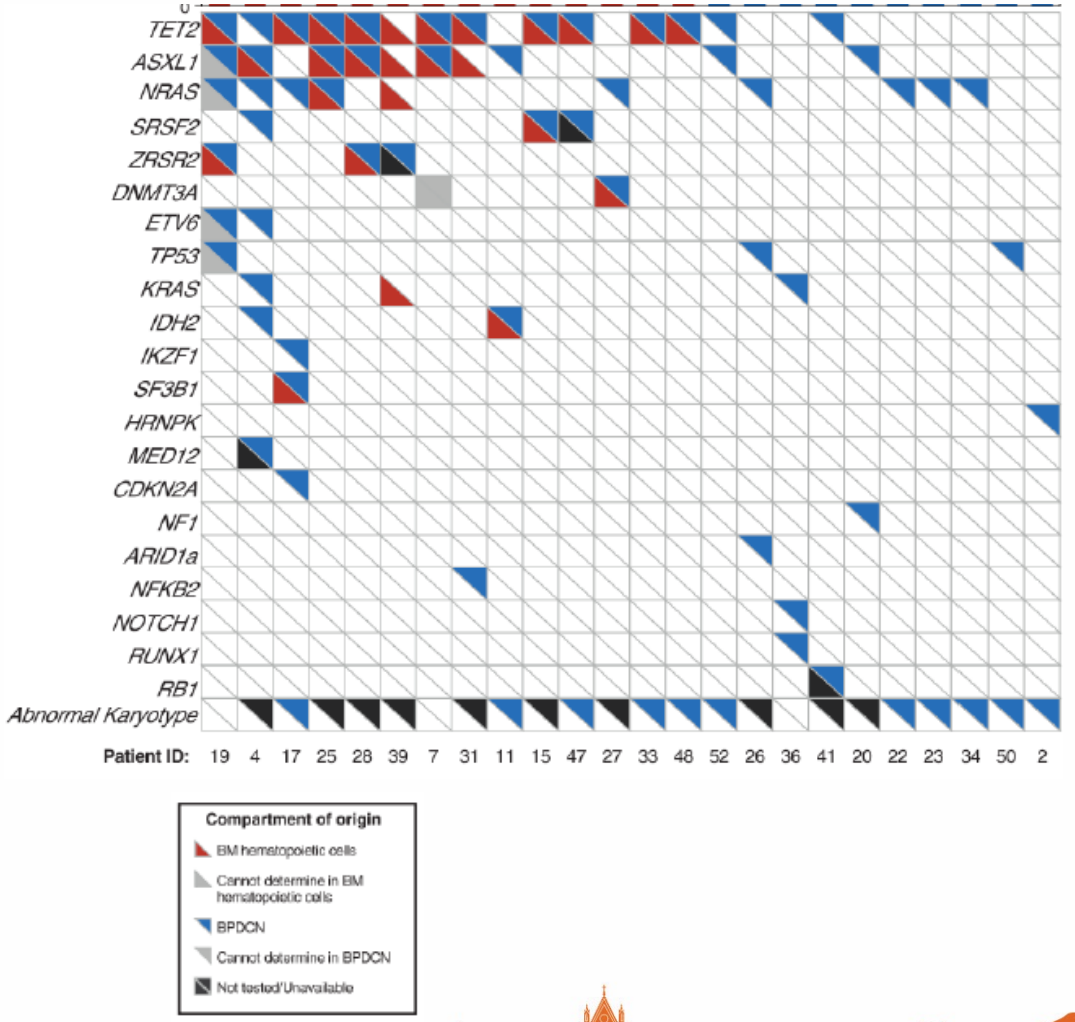
1. Xiao W et al., Blood 2021; 2. Pemmaraju N, Blood 2021; 3. Wang W et al., Cancers 2022

Concomitant hematological diseases

59 patients with BPDCN

- ✓ Median age 69 yrs
- ✓ 12 (24%) had hematopoietic neoplasms (7 MDS, 3 MPN, 2 CMML)
- ✓ 7 before BPDCN (1-9 yrs)
- ✓ 5 concurrently

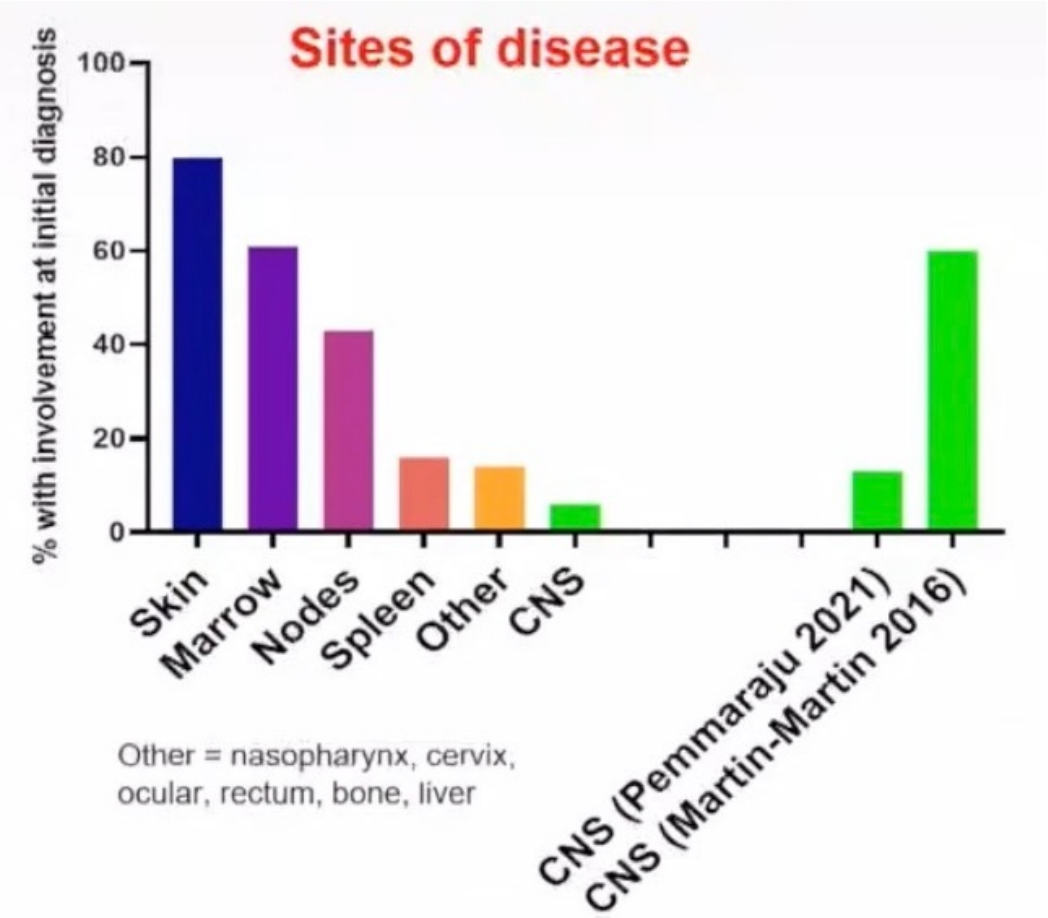




Khanlari M et al., Leukemia 2022

Staging

Clinical presentation



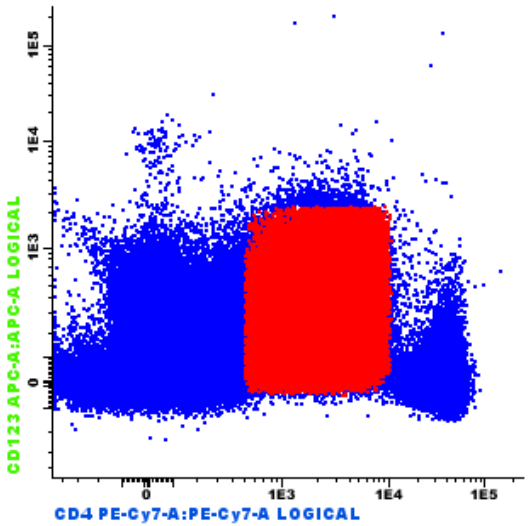
- Referral to an experienced center with expert pathology and clinical trials (preferred)

▼

- 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

BM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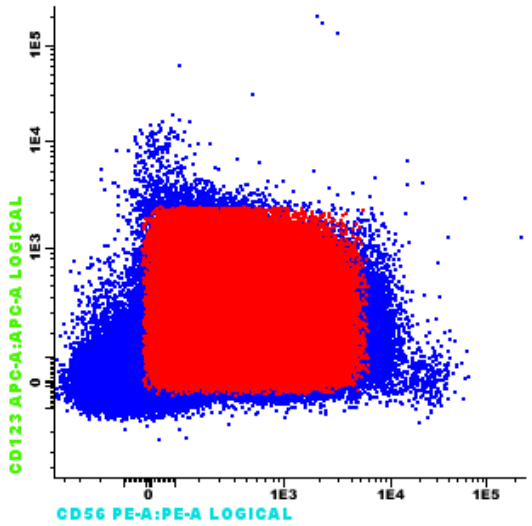
CD4 PE-Cy7-A:PE-Cy7-A LOGICAL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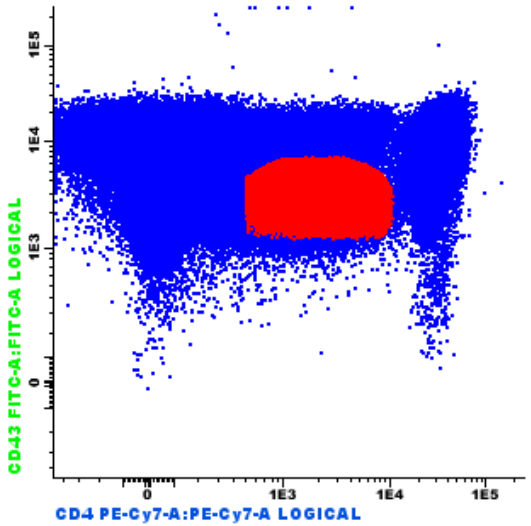
CD56 PE-A:PE-A LOGICAL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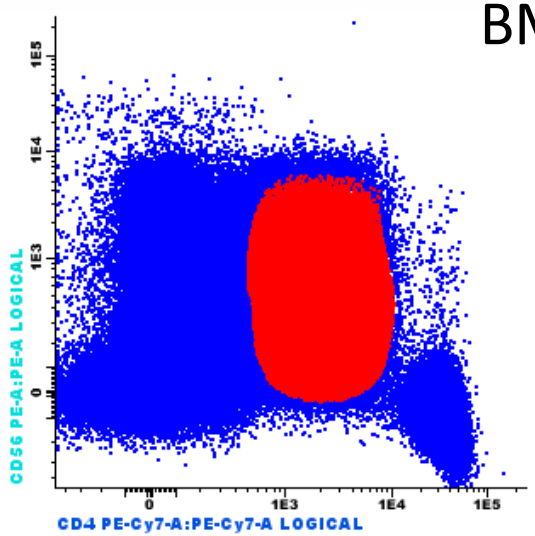
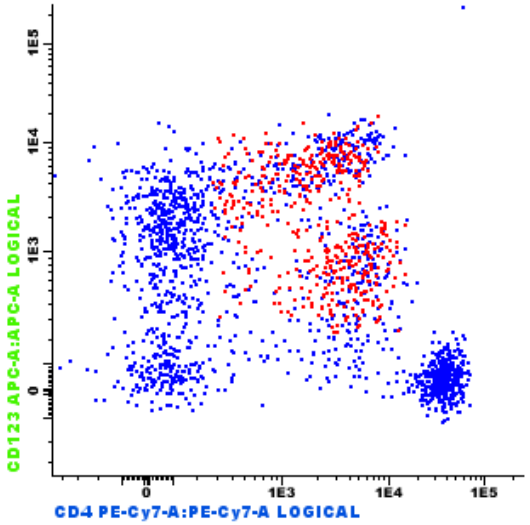
CD4 PE-Cy7-A:PE-Cy7-A LOGICAL


CD56 PE-A:PE-A LOGICAL
CD4 PE-Cy7-A:PE-Cy7-A LOGICAL

CSF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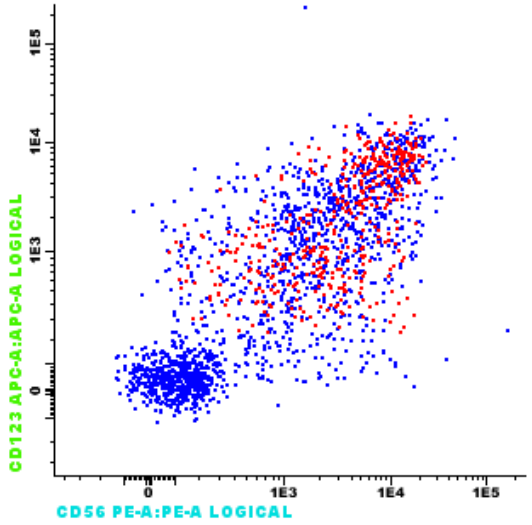
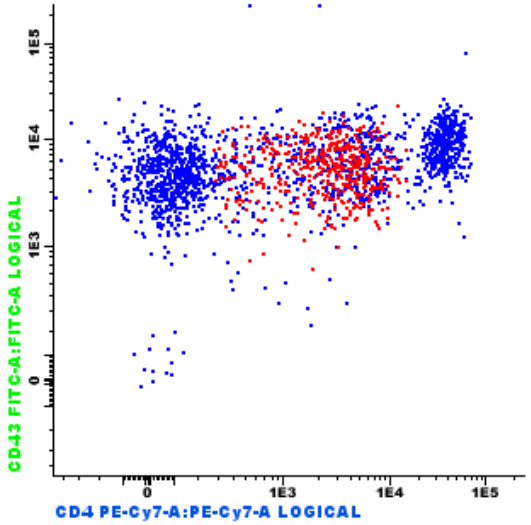

CD123 APC-A:APC-A LOGICAL
CD56 PE-A:PE-A LOGICAL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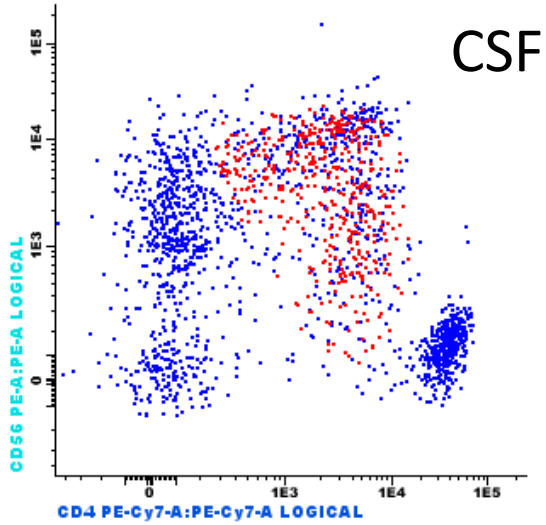
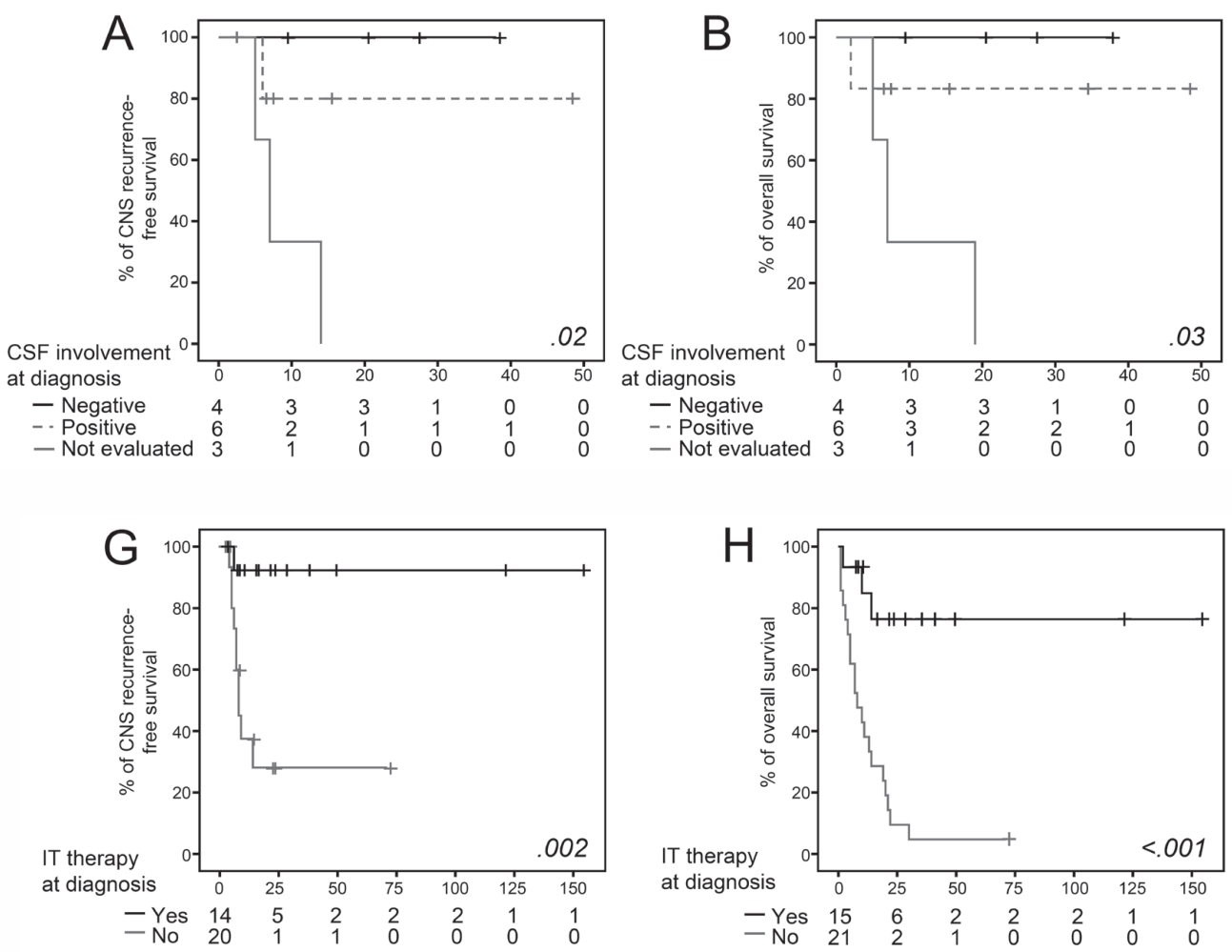
CD4 PE-Cy7-A:PE-Cy7-A LOGICAL


CD56 PE-A:PE-A LOGICAL
CD4 PE-Cy7-A:PE-Cy7-A LOGICAL

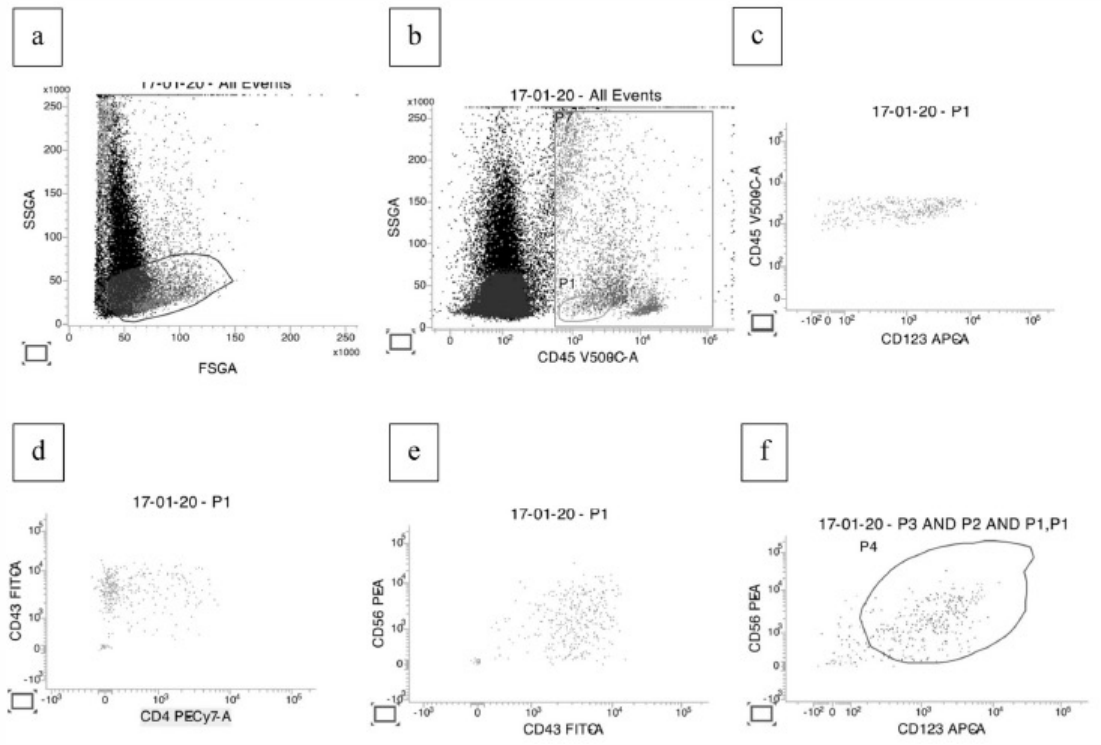
BPDCN with CSF involvement: impact on prognosis (I)

- ✓ 13 BPDCN patients (11 M/ 2 F)
- ✓ Median age 67 yrs (range 11-79)
- ✓ Evaluated by Next-Generation-Flow in different phases of disease
- ✓ The lower cut-off for CNS involvement was defined as a cluster ≥10 events with the appropriate phenotype, based on a 10-parameter tube (≥0.001 cells/µl)
- ✓ Retrospective validation on 23 patients



Martín-Martín L, et al. Oncotarget. 2016

BPDCN with CSF involvement: impact on prognosis (II)



	At diagnosis	At relapse
No.	10	5
Pts tested	6/10 (60%)	5/5 (100%)
Symptoms	2/10 (20%)	0/5 (0)
Cytology+ MFC+	5/6 (83%)	3/5 (60%)
Cytology- MFC+	1/6 (17%)	2/5 (40%)

The morphologic evidence of BPDC and/or a white blood cell count (WBCc) ≥5/microL with less than 10 erythrocytes/ microL was considered as a cytological positive finding.

Buzzatti E et al., Leuk Lymphoma 2022

CONCLUSIONS

- BPDCN is a rare disease that requires a multidisciplinary team to allow a timely diagnosis and staging of disease
- The hematology laboratory has a key role in two ways:
  - Confirm diagnosis and organ involvement during the staging procedure
  - Suggest a possible BPDCN diagnosis in the context of a leukemia screening procedure
  - Discriminate other similar pCD diseases
  - Follow-up treatment efficacy (MRD monitoring)
- Multiparameter Flow Cytometry has a fundamental role due to the elevate sensitivity and specificity

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Patients and their families