

## Immunophenotypic characterization of reactive and neoplastic plasmacytoid dendritic cells permits establishment of a ten-color flow cytometric panel for initial workup and residual disease evaluation of blastic plasmacytoid dendritic cell neoplasm

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Received: January 16, 2020.

Accepted: March 26, 2020.

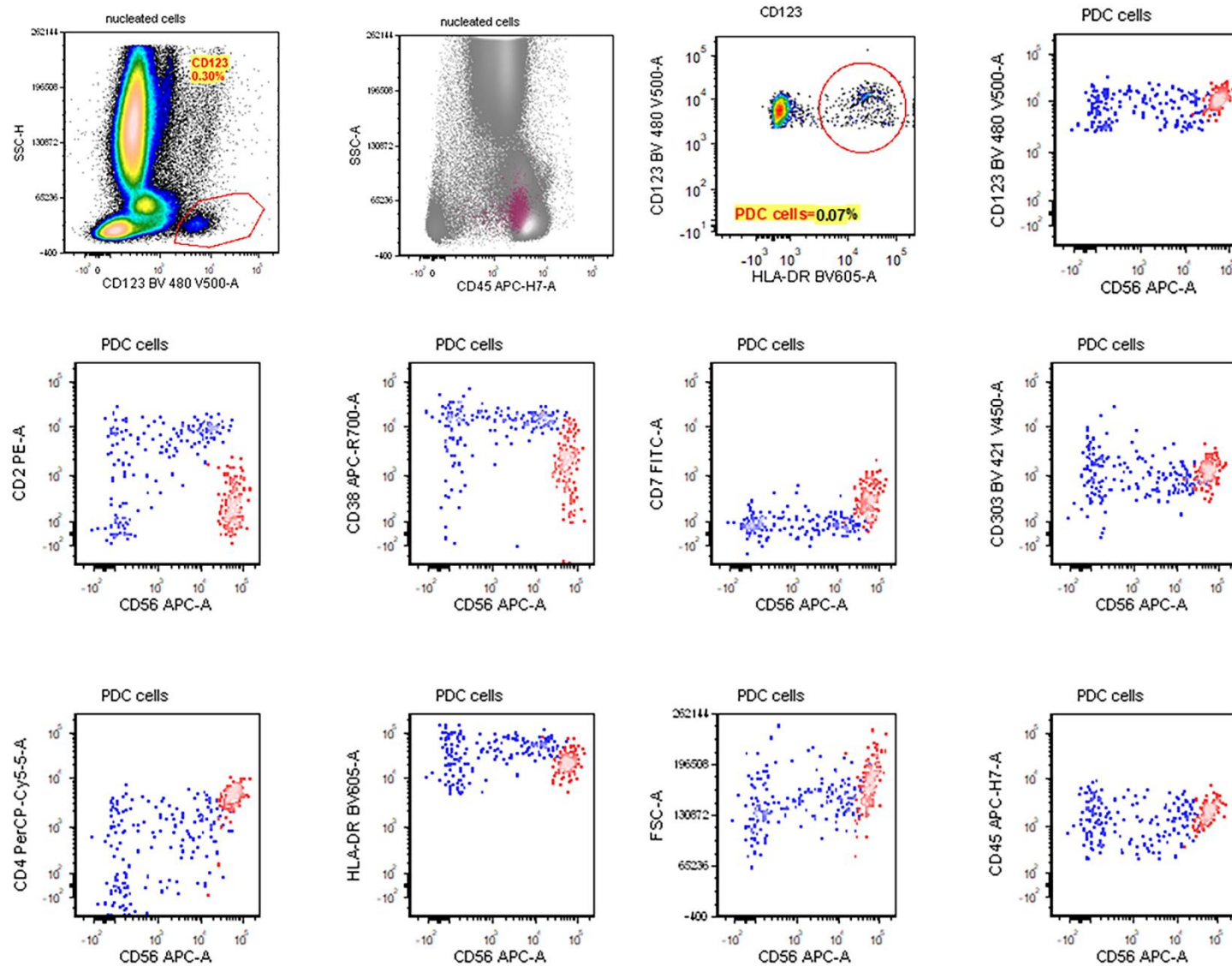
Pre-published: April 2, 2020.

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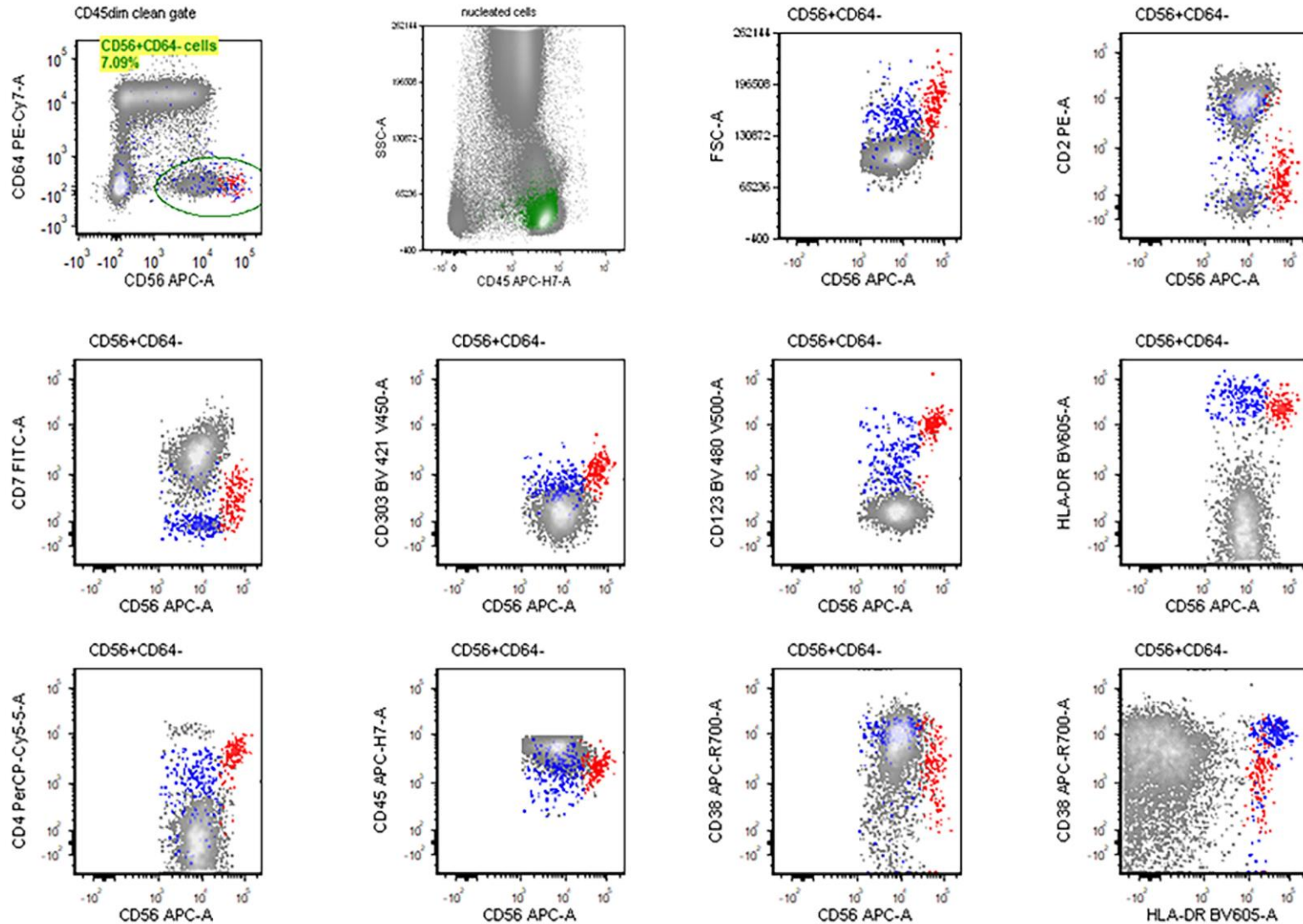
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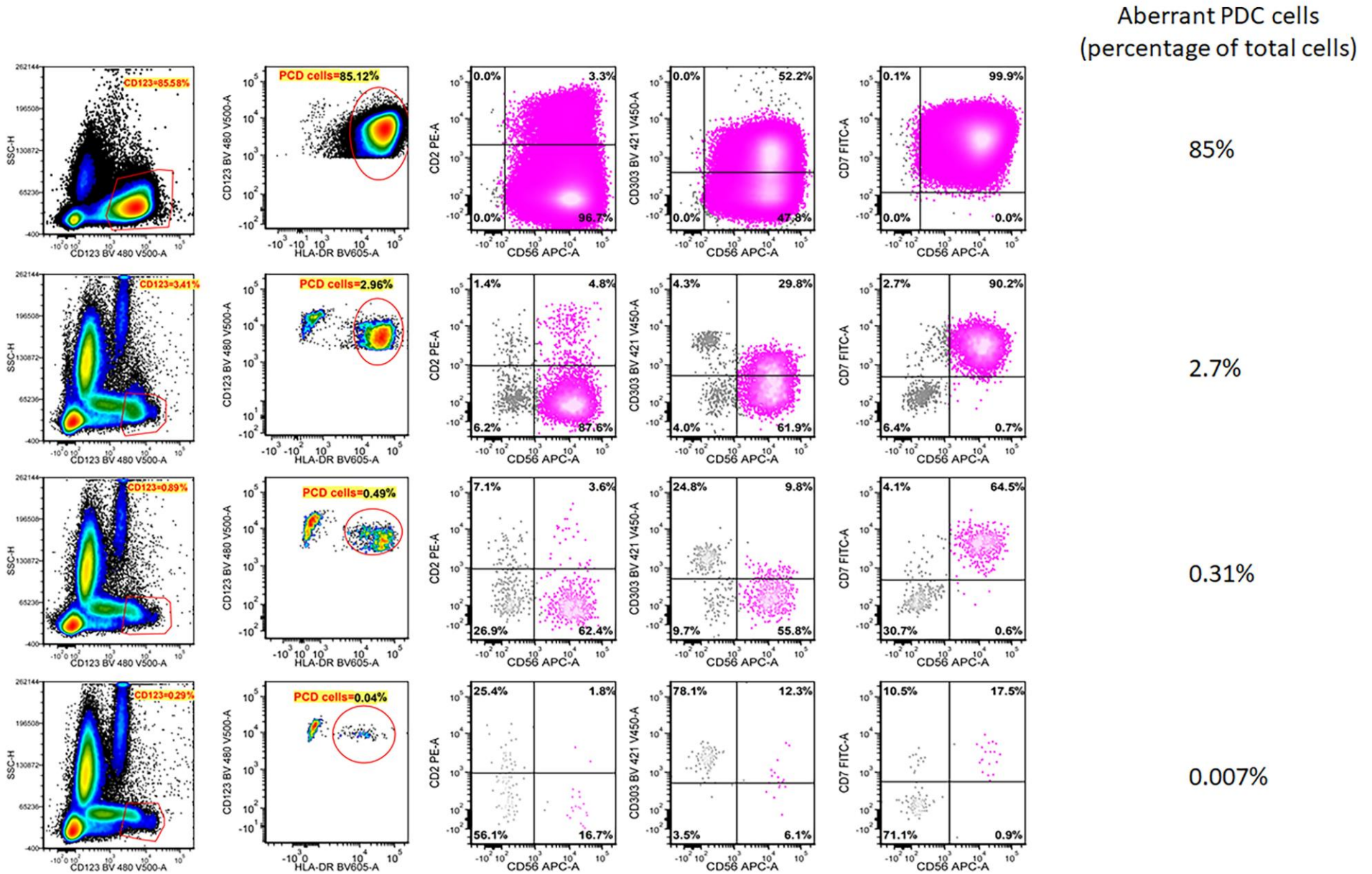
**Supplementary Fig 1:** A case illustration of BPDCN gating and analysis strategy using CD123. CD123/low SSC cells are 0.30% of total nucleated events after removing doublets, debris, platelets and red blood cells, and these cells are reflected on CD45/SSC plot. CD123+/low SSC and HLA-DR+ cells are plasmacytoid dendritic cells (PDC), comprising 0.07% of total events. The CD123+HLADR- cells are basophils. The neoplastic PDCs are highlighted in red and normal PDC are in blue.



**Supplementary Fig 2:** A case illustration of BPDCN gating and analysis strategy using CD56+/CD64-. An alternative gating strategy is also applied in all cases, considering the possibility of loss or down regulation of CD123 post anti-CD123 treatment. The CD56+HLADR+CD64-negative are analyzed. The CD56+HLADR- cells are mostly NK cells, as reflected on CD45/SSC. The neoplastic PDC are highlighted in red and other CD56+HLADR+CD64- cells are in blue. So far, we have not identified any cases with residual BPDCN showing CD123 loss.



**Supplementary Fig 3:** A representative case with serial dilutions shows the sensitivity of this panel is below 0.01%. Pink cells are neoplastic cells.



**Supplementary Table 1: The immunophenotype of BPDCN**

Markers	Positivity (%)
CD2	19% (5/27)
CD3	0% (0/15)
CD4	100% (38/38)
CD5	3% (1/30)
CD7	64% (21/33)
CD13	0% (0/30)
CD14	3% (1/34)
CD15	0% (0/28)
CD19	0% (0/30)
CD22	0% (0/26)
CD25	0% (0/22)
CD33	48% (16/33)
CD34	0% (0/27)
CD36	57% (17/30)
CD38*	88% (30/34)
CD41	0% (0/12)
CD45	100% (39/39)
CD56	97% (36/37)
CD64	0% (0/36)
CD117*	9% (3/34)
CD123*	100%(36/36)
CD303*	44% (7/16)
HLA-DR*	100% (36/36)
MPO	0%(0/16)
TdT	25% (4/16)

**Notes: CD38:** Although 88% (30/34) cases were positive, only 18% (6/34) showed a normal CD38 level, whereas 70%(24/34) showed decreased CD38 and 12% (4/34) showed negative; **CD117:** Only 9% (3/34) cases were positive and all were partially positive; **CD123:** Although all cases were positive, 78% (28/36) showed decreased expression; **CD303:** Although 7 out of 16 (44%) cases were positive, only 1 (6%) showed a relatively normal level when compared to reactive PDCs and overall, most cases showed decreased or negative CD303; **HLA-DR:** All cases were positive and 69% (25/36) showed brighter expression when compared to normal/reactive PDCs.

**Supplementary Table 2: The fluorochromes and antibodies used in the newly designed panel (corresponding to Panel #3 in Table 1)**

Antibody	CD7	CD2	CD4	CD64	CD56	CD38	CD45	CD303	CD123	HLA-DR
Fluorochrome	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	R700	APC-H7	BV421	BV480	BV605
Company	BD	BD	BD	BD	BD	BD	BD	Biolegend	BD	BD
Clone #	4H9	S5.2	SK3	10.1	NCAM16.2	HB7	2D1	201A	7G3	G46.6