

The optimized anti-CD20 monoclonal antibody ublituximab bypasses natural killer phenotypic features in Waldenström macroglobulinemia

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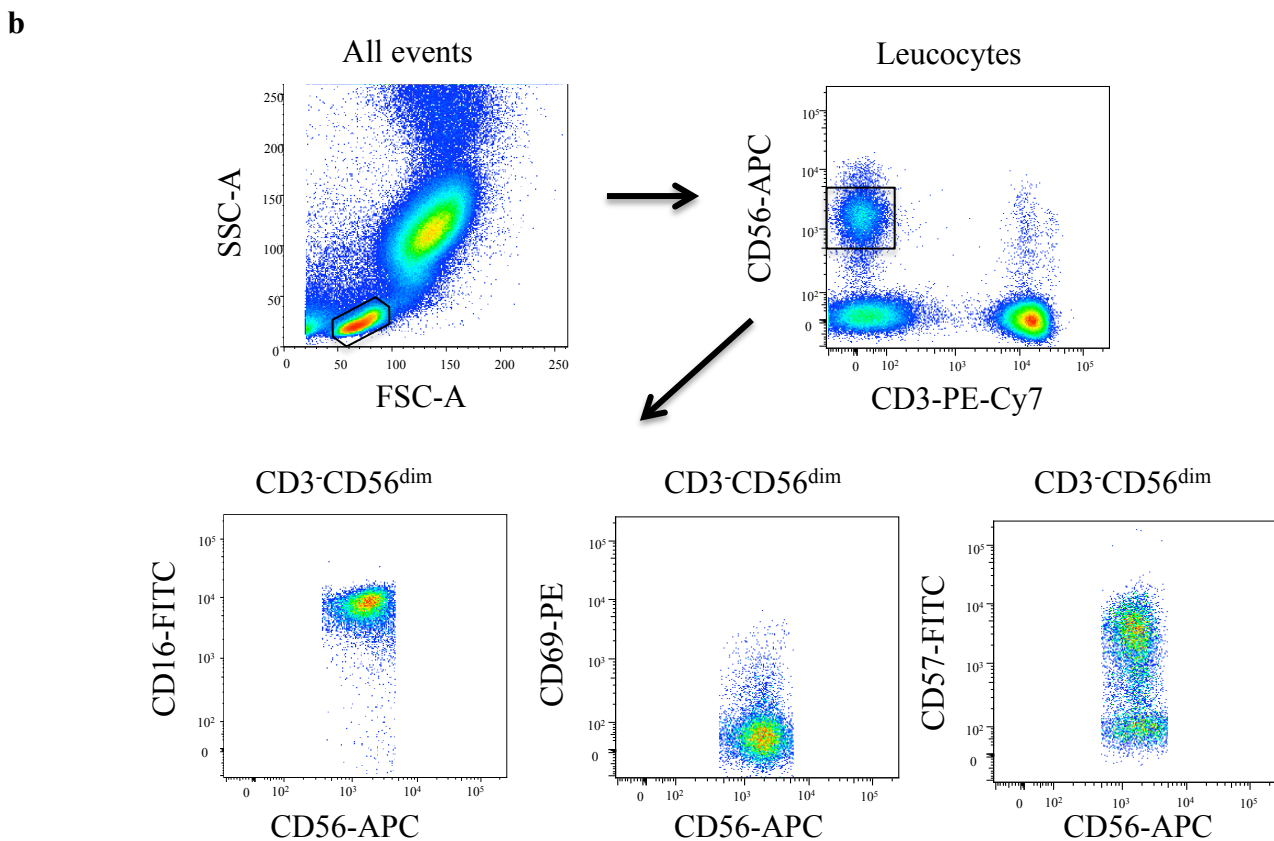
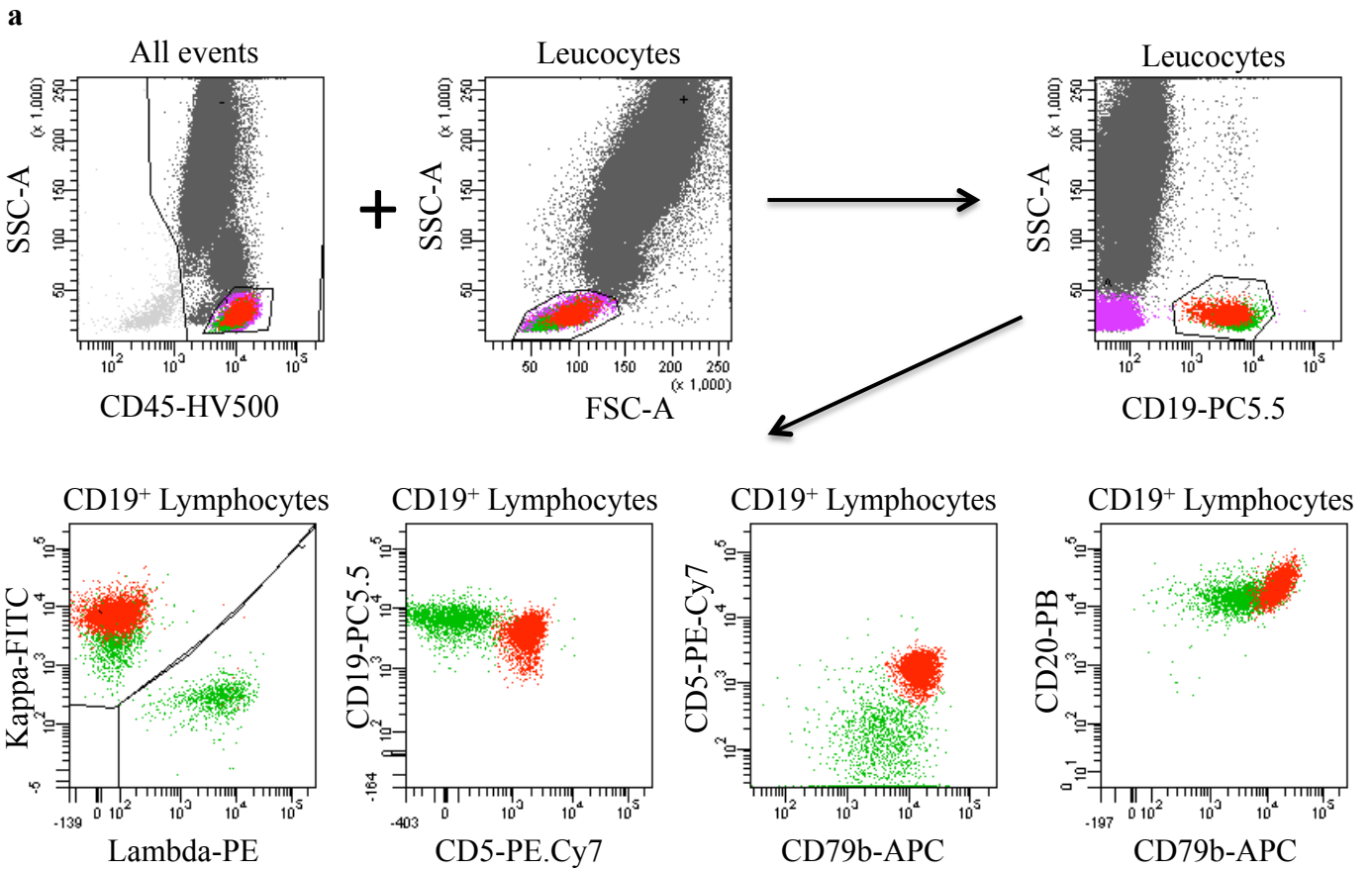
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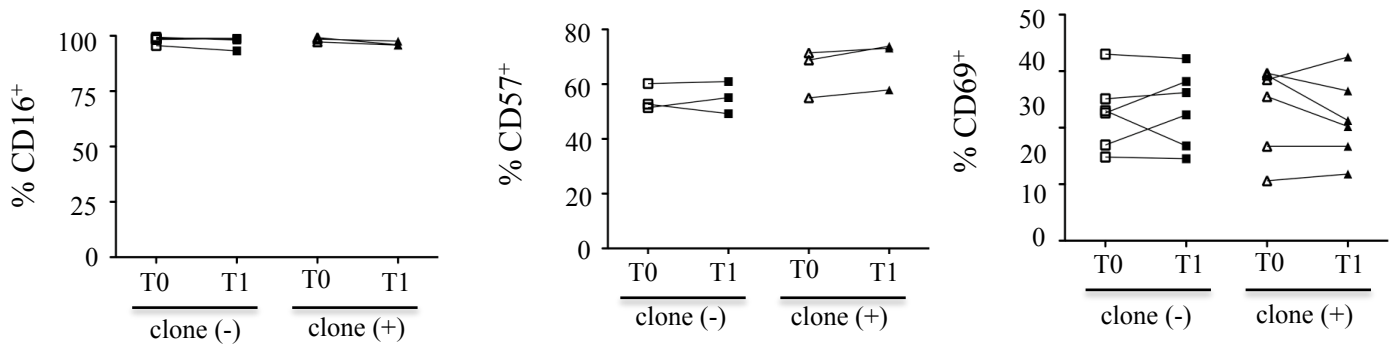
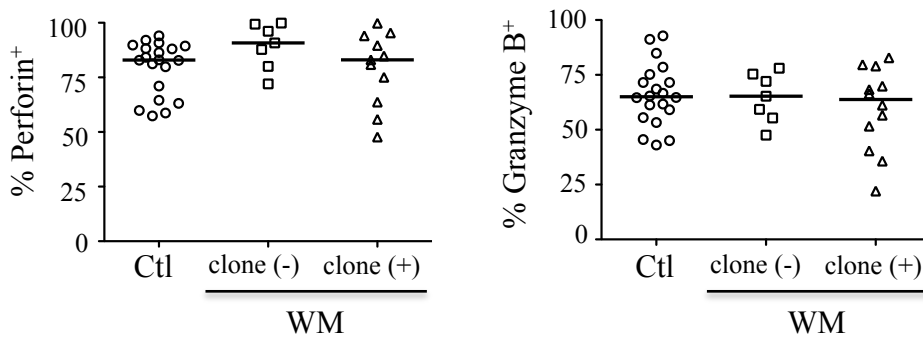
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Supplemental data 1 : Antibodies used in this study

Antibodies	Clone #	Fluorochrome	Compagny
NK Cell-surface staining			
Anti-CD3	UCHT1	ECD	Beckman Coulter
Anti-CD8	T8	FITC	Beckman Coulter
Anti-CD16	3G8	FITC	Beckman Coulter
Anti-CD56	N901	PC7	Beckman Coulter
Anti-CD69	TPI.55.3	PE	Beckman Coulter
Anti-CD159a/NKG2A	Z199	APC	Beckman Coulter
Anti-NKG2D	ON72	APC	Beckman Coulter
Anti-CD335/NKp46	BAB281	PE	Beckman Coulter
Anti-ILT-2/CD85j	GHI/75	PE	Beckman Coulter
Anti-CD19	SJ25C1	PerCP Cy5.5	BD Biosciences
Anti-DNAM-1	DX11	FITC	BD Biosciences
Anti-LAIR1	DX26	FITC	BD Biosciences
Anti-2B4	C1.7	PE	BD Biosciences
Anti-CD57	HNK-1	FITC	BD Biosciences
Anti-CD107a	H4A3	FITC	BD Biosciences
Anti-NKG2C	134591	PE	R&D systems
Anti-NKp80/KLFR1	239127	PE	R&D systems
Anti-CD337/NKp30	AF29-4D12	APC	Miltenyi Biotec
Intra-cellular staining			
Anti-Perforin	δG9	FITC	BD Biosciences
Anti-Granzyme-B	GB11	FITC	BD Biosciences
B Cell-surface staining			
Anti-CD19	J3-119	PC5.5	Beckman Coulter
Anti-CD20	B9H9 (HRC20)	PB	Beckman Coulter
Anti-FMC7	FMC7	FITC	Beckman Coulter
Anti-CD10	ALB1	AF750	Beckman Coulter
Anti-CD45	HI30	HV500	BD Biosciences
Anti-CD5	L17F12	PE-Cy7	BD Biosciences
Anti-CD23	EBVCS-5	APC	BD Biosciences
Anti-CD79b	SN8	APC	BD Biosciences
Anti-CD38	HB7	PE	BD Biosciences
Anti Kappa/Lambda	polyclonal	FITC/PE	Dako



Supplemental data 2. Determination of a B-cell circulating clone in WM. Lymphocytes were isolated from peripheral blood samples by gating on the forward (FSC)/side (SSC) scatters and CD45/SSC. **(a)** The CD19⁺ B-lymphocytes were evaluated for the B-cell surface markers (Supplemental data 1) for the determination of the Matutes/Moreau score, which was less than or equal to 3 for all patients. In this representative case (patient #7, Table 1), the B-cell circulating clone, shown in red, was Kappa⁺CD5⁺CD79b⁺CD20⁺. This B-cell clone corresponded to 50% of the peripheral B-lymphocytes and to 10% of the total lymphocytes. Residual normal B lymphocytes are shown in green. **(b)**. The NK cells were evaluated on the CD3⁺CD56⁺ lymphocytic gate for the determination of a panel of NK-cell markers (Supplemental data 1), like CD16, CD69 and CD57.

a**b**

Supplemental data 3. Stability of CD16, CD57, and CD69 surface expression and intracellular staining of lytic granule components in NK cells from WM patients with or without circulating clones. **(a)** Cell-surface markers were tested at T₀, corresponding to the first inclusion, and then 12 to 24 months later (T₁) on NK cells from WM patients without (clone (-); squares) or with (clone (+); triangles) circulating B clones. **(b)** Comparison of intra-cellular expression of perforin and granzyme-B in NK cells collected from healthy donors (Ctl) and WM patients without (clone (-); squares) or with (clone (+); triangles) circulating B clones. Horizontal bars represent the median value.