

Immunosuppressive Microenvironment and Efficacy of PD-1 Inhibitors in Relapsed/Refractory Classic Hodgkin Lymphoma: Checkpoint Molecules Landscape and Macrophage Populations

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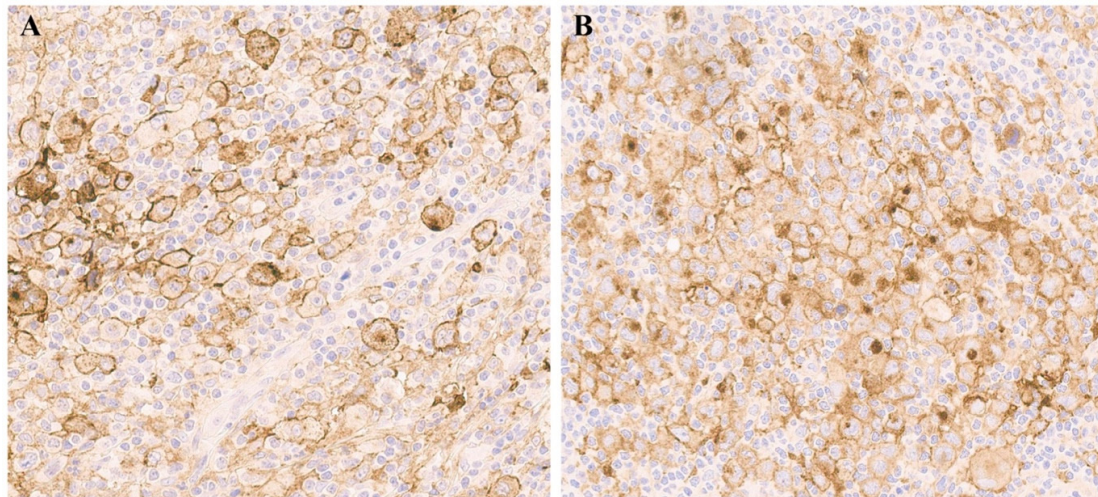


Figure S1. PD-L1 expression in representative cases with different progression-free survival (PFS), primary biopsies: (A) PFS of 61 months, (B) PFS of 2.9 months. [Original magnification: (A,B) $\times 400$].

	PD-1	LAG-3	CTLA-4	TIM-3	TIGIT	CD68	CD163	M2
M2								$r = 1$
CD163							$r = 1$	$r = 0.2$ $p = 0.17$
CD68						$r = 1$	$r = 0.62$ $p < 0.01$	$r = 0.14$ $p = 0.47$
TIGIT					$r = 1$	$r = 0.16$ $p = 0.4$	$r = 0.25$ $p = 0.19$	$r = 0.01$ $p = 0.98$
TIM-3				$r = 1$	$r = 0.28$ $p = 0.14$	$r = 0.22$ $p = 0.21$	$r = 0.39$ $p < 0.01$	$r = 0.05$ $p = 0.73$
CTLA-4			$r = 1$	$r = -0.21$ $p = 0.12$	$r = -0.07$ $p = 0.7$	$r = -0.17$ $p = 0.35$	$r = -0.49$ $p < 0.01$	$r = -0.18$ $p = 0.2$
LAG-3		$r = 1$	$r = 0.1$ $p = 0.44$	$r = 0.16$ $p = 0.23$	$r = 0.01$ $p = 0.97$	$r = 0.16$ $p = 0.38$	$r = 0.04$ $p = 0.75$	$r = 0.01$ $p = 0.93$
PD-1	$r = 1$	$r = 0.31$ $p = 0.02$	$r = 0.23$ $p = 0.08$	$r = 0.02$ $p = 0.87$	$r = 0.3$ $p = 0.11$	$r = 0.05$ $p = 0.77$	$r = -0.08$ $p = 0.53$	$r = 0.08$ $p = 0.57$

Figure S2. Correlation between assessed markers in primary biopsies. Abbreviations: M2–M2-macrophages, r –correlation coefficient.

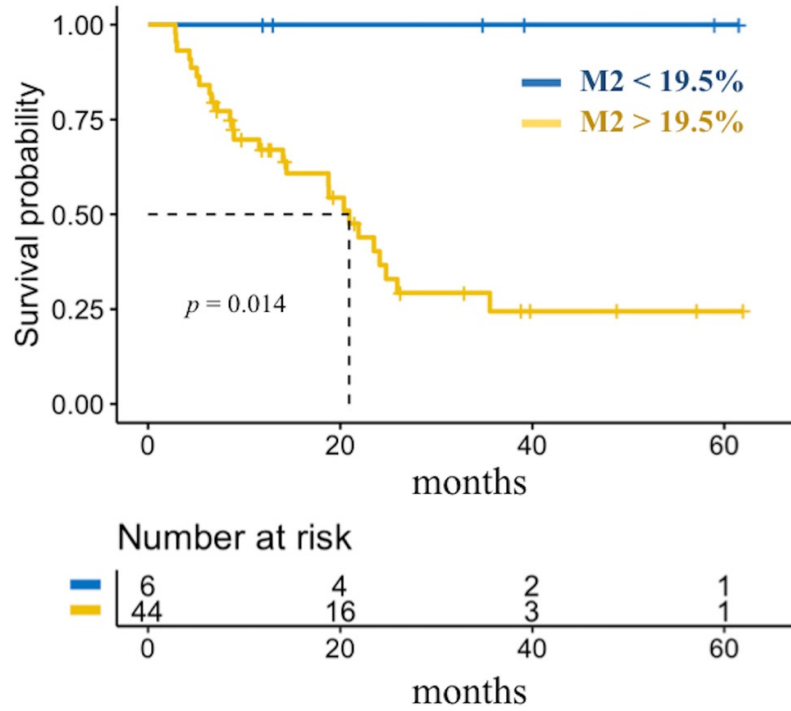


Figure S3. Progression-free survival depending on M2 level in primary biopsies. Abbreviations: M2–M2-macrophages. The dotted line indicates median of survival.

Table S1. Optimal immunohistochemical protocol parameters.

Immunohistochemistry of Single Staining										
Antibody	Clone	C	AR, T, Min, ER1/2	PB, Min	PrAb, Min	PP, Min	Pol, Min	DAB, Min	H, Min	
CD68	PG-M1, Dako	1:100	100°C, 30, ER2	5	15	8	8	10	5	
CD163	10D6, Leica	1:400	100°C, 30, ER1	5	15	8	8	10	5	
PD-1	NAT105, ab52587	1:50	100°C, 30, ER2	5	20	8	8	10	5	
LAG-3	EPR20261, ab209236	1:500	100°C, 30, ER2	5	30	8	8	10	5	
TIM-3	EPR22241, ab241332	1:800	100°C, 30, ER2	5	30	8	8	10	5	
TIGIT	BLR047F, ab243903	1:200	100°C, 30, ER1	5	30	8	8	10	5	
CTLA-4	CAL49, ab237712	1:600	100°C, 30, ER2	5	30	8	8	10	5	
Immunohistochemistry of double staining										
Antibody	Clone	C	AR, T, Min, ER1/2	PB, Min	PrAb, Min	mHRP, Min	rAP, Min	DAB/Red, Min	H, Min	
Antibody Cocktail: CD163/c-maf	10D6, Leica EPR16484, ab199424	1:125	100°C, 40, ER2	5	30	8	20	10/ 5	5	

Abbreviations: C–concentration, AR–antigen retrieval, PB–peroxidase block, PrAb–primary antibody, PP–post primary, Pol–polymer, mHRP–mouse horseradish peroxidase, rAP–rabbit alkaline phosphatase, DAB–3,3'-diaminobenzidine, H–hematoxylin.