

Immune-checkpoint expression in Epstein-Barr virus positive and negative plasmablastic lymphoma: a clinical and pathological study in 82 patients

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Supplemental Table 1. List of Antibodies used in this series.

Antibodies	clone	Source	Dilution
CD45	2B11	Dako Glostrup, Denmark	1:100
CD20	L26	Dako, Glostrup, Denmark	1:250
CD79a	JBC117	Dako, Glostrup, Denmark	1 :50
PAX5	SP34	Ventana Medical Sytems, Tucson, USA	prediluted
CD138	B-A38	Ventana Medical Sytems, Tucson, USA	prediluted
Ig Kappa	polyclonal	Ventana Medical Sytems, Tucson, USA	prediluted
Ig Lambda	polyclonal	Ventana Medical Sytems, Tucson, USA	prediluted
IgG	polyclonal	Ventana Medical Sytems, Tucson, USA	prediluted
IgA	polyclonal	Ventana Medical Sytems, Tucson, USA	prediluted
IgM	polyclonal	Ventana Medical Sytems, Tucson, USA	prediluted
IgD	polyclonal	Ventana Medical Sytems, Tucson, USA	prediluted
CD10	56C6	Novocastra-Leica microsystems	1:100
BCL6	PG-B6p	Dako, Glostrup, Denmark	1:30
CD30	Ber-H2	Dako, Glostrup, Denmark	1:40
IRF4/MUM1	MUM1p	Dako, Glostrup, Denmark	1:25
EMA	E29	Dako, Glostrup, Denmark	1:50
CD30	Ber-H2	Dako, Glostrup, Denmark	1:40
BCL2	SP66	Ventana Medical Sytems, Tucson, USA	prediluted
ALK	ALK1	Dako, Glostrup, Denmark	1:25
HHV8	13B10	Ventana Medical Sytems, Tucson, USA	prediluted
Ki67	MIB-1	Dako, Glostrup, Denmark	1:100
PD-L1	SP142	Ventana Medical Sytems, Tucson, USA	1:100
PD-1	NAT105	Abcam	1:100
IDO	1A3	Abcam	1:250
DC-SIGN	5D7	Abcam	1:100
CD163	10D6	Novocastra	1:100
FOXP1	SP133	Ventana Medical Sytems, Tucson, USA	prediluted
GCTE1	RAM341	Abcam	1:100
Cyclin D1	SPA4R	Dako, Glostrup, Denmark	prediluted
CD56	MRQ-42	Ventana Medical Sytems, Tucson, USA	prediluted

Supplemental Table 2. Immune checkpoint score.

Immune checkpoint score		% of cells positive for immune checkpoint marker		
		<10% of immune cells ⁺ = 0 <5% of tumor cells ⁺ = 0	from 10% to 30% of immune cells ⁺ = 1 from 5 to 30% of tumor cells ⁺ = 1	≥ 30 % of immune cells ⁺ or of tumor cells ⁺ = 2
Intensity of staining	- or + = 0	0	1	2
	++ = 1	1	2	3
	+++ = 2	2	3	4

Legend: Immune checkpoint (ICP) score was calculated by adding the percentage of cells positive for ICP marker and the intensity of the staining. Low ICP score included patients' samples with ICP score ≤ 2 . Moderate ICP score included patient's samples with ICP score =2. High ICP score included patients' samples score with ICP score ≥ 3 .

Supplemental Table 3. Survival analysis of plasmablastic lymphoma patients.

		Number of patients	Median progression free survival time , months (SE)	2-year Survival (95% confidence interval)	Log rank p-value	Multivariate Hazard Ratio (confidence interval)	Adjusted Cox p-value **
Age	<60 y.o	20/42	NR	56% (30%-75%)	0,0676	-	
	>=60 y.o	22/42	12 (7)	28% (10%-49%)			
Sex	Female	11/42	14.5 (12)	48% (12%-78%)	0,5028	-	
	Male	31/42	13 (8)	38% (20%-55%)			
Site of involvement	Nodal	8/42	13 (8)	25% (4%-56%)	0,466	-	
	Extranodal						
	Oral	11/42	NR	90% (47%-99%)	0,0057	NS	
	Digestif	7/42	NR	57% (17%-84%)	0,6418	-	
	Others	16/42	3 (5)	17% (3%-41%)	0,0135	3.9(1.4-10.6)	0.009
LDH increased	No	13/25	20 (10)	48% (17%-73%)	0,7475	-	
	Yes	12/25	15 (2)	42% (12%-70%)			
Stage	1-2	19/36	NR	63% (35%-82%)	0,0032	5.1 (1.7-15.6)	0,005
	3-4	17/36	2.5 (4)	20% (4% -46%)			
Bone marrow or marrow aspiration infiltration	No	23/30	40 (NC)	58% (33% -77%)	0,001	-	
	Yes	7/30	1 (1)	0% NC			
HIV seropositive	No	19/40	14 (5)	19% (4%-43%)	0,03	NS	NS
	Yes	21/40	NR	60% (37%-78%)			
Post-transplantation	No	38/40	15 (5)	43% (26%-59%)	0,9262	-	
	Yes	2/40	14 (NC)	0% (NC)			
Other ID context	No	38/40	15 (5)	45% (27%-61%)	0,279	-	
	Yes	2/40	1 (NC)	0% (NC)			
No ID context known	No	23/40	NR	52% (30%-71%)	0,0663	-	
	Yes	15/40	13 (10)	23% (5%-50%)			
Therapy	None	9/38	1 (0.2)	33% (8%-62%)	0,3476	-	
	CHOP or CHOP like	12/38	40 (13)	53% (19%-78%)			
	RCHOP or RCHOP like	5/38	NR	80% (20%-97%)			
	EPOCH	4/38	3 (4)	0% (NC)			
	Others chemotherapies*	5/38	3 (6)	25% (1%-67%)			
	Autologous hematopoietic stem cell transplantation	1/38	NR	100% (NC)			
	Radiotherapy alone	2/38	3 (4)	50% (1%-91%)			
Therapy Response	None/failure	18/31	1 (1)	0% (NC)	<0.0001	-	
	Partial response	6/31	14 (6)	22% (4%-61%)			
	Complete response	7/31	NR	88% (59%-97%)			
EBV	Negative	21/42	13 (8)	22% (5%-46%)	0,0498	0.3 (0.1-0.8)	0,022
	Positive	21/42	NR	58% (33%-77%)			
B-cell markers	CD20	Negative	38/41	14.5 (1)	40% (23%-57%)	0,5467	-
		Positive	3/41	NR	67% (5%-95%)		
	CD79a	Negative	15/39	13 (7)	38% (15%-62%)	0,5716	-
		Positive	24/39	20 (5)	43% (20%-65%)		
PAX5	Negative	26/32	15 (NC)	46% (26%-64%)	0,3496	-	
	Positive	6/32	1 (5)	25% (1%-65%)			

CD138/VS38		Negative	0/42	NC	NC	NC	NS	NS
		Positive	42/42	15 (4)	41% (24%-57%)			
Light chain restriction	Kappa	Negative	22/35	12 (8)	20% (6%-41%)	0,0491	NS	NS
		Positive	13/35	NC	76% (42%-91%)			
	Lambda	Negative	18/35	40 (4)	59% (33%-78%)	0,2732	-	
		Positive	17/35	14 (2)	19% (4%-44%)			
GC/non GC markers	CD10	Negative	30/32	15 (15)	48% (29%-65%)	0,03		
		Positive	2/32	0.25 (NC)	0% (NC)			
	BCL6	Negative	18/24	1.25 (5)	39% (17%-60%)	0,2153		
		Positive	6/24	NR	56% (7%-88%)			
	GCET1	Negative	20/20	NR	51% (27%-71%)	NC		
		Positive	0/20	NC	NC			
	MUM1	Negative	0/31	NC	NC	NC		
		Positive	31/31	15 (12)	46% (27%-63%)			
	CD56	Negative	25/26	15 (NC)	48% (26%-66%)	0,8520		
		Positive	1/26	NC	100% (NC)			
	FOXP1	Negative	16/21	NC	60% (31%-80%)	0,1487		
		Positive	5/21	14 (15)	20% (1%-58%)			
MYC protein		Negative	6/26	3 (NC)	50% (11%-80%)	0,6974	-	
		Positive	20/26	15 (NC)	50% (25% -70%)			
CD30		Negative	24/35	20 (14)	46% (24%-65%)	0,6961	-	
		Positive	11/35	14 (4)	40% (11%-68%)			
EMA		Negative	7/23	3 (NC)	50% (11%-80%)	0,9903	-	
		Positive	16/23	14 (2)	33% (11%-57%)			
BCL2		Negative	12/16	2 (1)	42% (15%-67%)	0,8166	-	
		Positive	4/16	15 (13)	50% (6%-84%)			
CD56		Negative	13/14	13 (5)	36% (10%-63%)	0,7483	-	
		Positive	1/14"	NR	0% (NC)			
ALK		Negative	12/12	12 (5)	29% (7%-56%)	NC	-	
		Positive	0/12	NC	NC			
Immune checkpoint	PD-L1 ⁺ immune cells	Low PD-L1 score	9/24	7.5 (7)	22% (3%-51%)	0,0756	-	
		High/moderate PD-L1 score	15/24	NC	63% (32%-83%)			
	PD-1 ⁺ immune cells	Low PD-1 score	9/20	14.5 (9)	25% (4%-56%)	0,2968	-	
		High/moderate PD-1 score	11/20	NC	55% (23%-78%)			
Gene rearrangement	MYC	Negative	14/22	14.5 (17)	45% (18%-69%)	0,1855	-	
		Positive	8/22	NR	70% (22%-92%)			
	BCL2	Negative	19/19	14.5 (NC)	48% (23%-69%)	NC	-	
		Positive	0/19	NC	NC			
	BCL6	Negative	18/19	14.5 (1)	44% (20%-66%)	0,3775	-	
		Positive	1/19	NR	100% (NC)			

Legend: Event-free survival was compared using log rank test. *Others chemotherapies:

velcade, dexamethasone and methotrexate followed by rituximab (n=1); velcade,

dexamethasone and liposomal doxorubicin (n=1); velcade and dexamethasone (n=4); rituximab associated with bendamustine (n=1); velcade, thalidomide and dexamethasone (n=2); VP16, mitoxantrone and ifosfamide (n=1); and vincristine, adriamycine and dexamethasone (n=1). **Initial Cox model includes all covariates with logrank p-value<0.05 and less than 20% of missing values. After backward-stepwise selection, only covariate with p<0.05 were kept in the final Cox model. NR, not reached; NC, not calculable; NS, not significant; -, not included in Cox model.