

SUPPLEMENTARY MATERIAL

Distribution of markers in reactive tonsils

The distribution of markers in reactive tonsils is relevant for understanding the functions of cells in immune responses and because DLBCL is a hematological neoplasia originating from germinal centers of lymphoid follicles. CD68+ and MITF+ cells (macrophages) were widely distributed in control tissue. CD16+ cells were scarce and only identified in the lymphoepithelium²⁹. CD163+ cells predominated in the interfollicular regions. PTX3+ cells had a macrophage morphology in all areas, while positive centroblasts were also found in the germinal centers. IL10+ cells were scarce in all areas. Double immunohistochemistry showed a mutually exclusive distribution between CD163 with CD16 and partially exclusive distribution with MITF.

Suppl. Table 1. Correlation between the clinicopathological characteristics and the survival of the patients.

Characteristics	OS				PFS		
	p value	HR	95% CI		p value	HR	95%
Histological							
CD3+	-	-	-	-	-	-	-
CD5+	0.830	1.1	0.5	2.6	0.268	1.6	0.7
CD20+	-	-	-	-	-	-	-
CD10+	0.195	0.7	0.4	1.2	0.039	0.5	0.2
BCL6+	0.904	1.0	0.6	1.7	0.519	1.2	0.7
MUM1+	0.016	2.1	1.2	3.8	0.150	1.6	0.8
Non-GCB (Hans classifier)	0.06* ²	1.7	1.0	3.0	0.036	2.0	1.0
BCL2+	0.002	2.9	1.5	5.6	0.024	2.2	1.1
EBV EBER+	0.09* ¹	2.1	0.9	4.9	0.244	1.8	0.7
RGS1-High	0.308	1.4	0.8	2.5	0.589	0.8	0.5
MYC-High**	0.245	1.4	0.8	2.3	0.307	1.4	0.8
Molecular							
<i>BCL2</i> t+ (split FISH)	0.911	1.0	0.4	2.2	0.735	0.9	0.3
<i>MYC</i> t+	0.679	1.2	0.5	2.6	0.173	1.7	0.8
<i>BCL2</i> IHC+ and <i>MYC</i> -IHC-High (double expressor)	0.002	2.4	1.4	4.1	0.008	2.2	1.2
<i>MYC</i> t+ and/or <i>MYC</i> -IHC-High**	0.050	1.8	1.0	3.2	0.421	1.3	0.7
<i>MYD88</i> L265P mutation	0.915	1.0	0.5	2.3	0.642	0.8	0.3
Clinical							
Age >60	0.013	2.3	1.2	4.4	0.388	1.3	0.7
Sex, male	0.763	0.9	0.6	1.5	0.975	1.0	0.6
Location							
Nodal (+spleen)		reference				reference	
Waldeyer's ring	0.377	0.7	0.3	1.5	0.612	0.8	0.4
Gastrointestinal	0.056	0.4	0.1	1.0	0.177	0.5	0.2
Other Extranodal	0.663	1.1	0.6	2.0	0.625	1.2	0.6
LDH High	0.002	2.7	1.5	5.2	0.003	3.0	1.5
High sIL-2R	0.042	2.9	1.0	8.0	0.149	2.1	0.8
High performance status	0.100	2.0	0.9	4.6	0.515	1.3	0.6
Extranodal >1 site	0.000	3.9	2.0	8.0	0.034	2.2	1.1
Stage III-IV	0.002	2.3	1.4	3.9	0.002	2.5	1.4
B symptoms	0.272	1.4	0.7	2.8	0.154	1.7	0.8
IPI High Intermediate + High	<0.001	3.6	2.0	6.4	0.002	2.6	1.4
Treatment							
RCHOP		reference				reference	
RCHOP-like	0.268	1.4	0.8	2.6	0.784	1.1	0.6
Other	0.140	2.2	0.8	6.1	0.255	2.0	0.6
Clinical Response (CR)	<0.001	4.8	2.7	8.3	0.000	22.2	11.1

** The OS was calculated for cases with follow-up equal or below to 5 years.

*1, in the Kaplan-meier analysis, Breslow test, $p = 0.022$

*2, in the Kaplan-meier analysis, Tarone-Ware test $p = 0.048$.

Suppl. Table 2.1. Main pathological characteristics (validation series).

Characteristics	Frequency (%)	
Histological		
CD3+	0/159	0.0
CD5+	24/154	15.6
CD20+	149/154	95.5
CD10+	40/153	26.1
BCL6+	116/151	76.8
MUM1+	133/152	87.5
Non-GCB (Hans classifier)	109/152	71.7
BCL2+	126/151	83.4
EBV EBER+	11/152	7.2
RGS1-High	44/130	33.8

Suppl. Table 2.2. Main clinical characteristics (validation series).

Clinical features	Frequency (%)	
Age >60	116/159	73.0
Sex, male	86/159	54.1
Location		
Nodal (+spleen)	60/159	37.7
Waldeyer's ring	13/159	8.2
Gastrointestinal	24/159	15.1
Other Extranodal	62/159	39.0
LDH High	89/156	57.1
High sIL-2R (>530)	114/149	76.5
High performance status	34/141	24.1
Extranodal >1 site	34/132	25.8
Stage III-IV	81/146	55.5
B symptoms	40/140	28.6
IPI High Intermediate + High	62/138	44.9
Treatment		
RCHOP	108/149	72.5
RCHOP-like	36/149	24.2
Other	5/149	3.4
Clinical Response (CR)	98/136	72.1

Suppl. Table 3. Correlation between markers with Double expressor status

Marker	<i>MYC and BCL2 IHC Double expressor</i>				
	<i>p</i> value	Positive		Negative	
		Mean	±STD	Mean	±STD
CD68	0.491	14.6	8.2	15.6	8.7
CD16	0.019	7.1	8.1	5.6	10.4
MITF	0.814	2.7	2.3	3.2	3.1
CD163	0.174	24.1	17.9	19.7	17.7
PTX3	0.626	11.6	17.4	10.5	16.9
IL10	0.279	12.2	11.4	7.9	9.7
FOXP3	0.078	1.4	1.5	2.7	3.3
CSF1R	0.609	23.6	22.9	27.2	26.7
RGS1	0.680	13.3	17.1	8.3	9

BCL2 IHC positive (50%), MYC IHC >22% (digital quantification).

Mann-Whitney U Test (Non-parametric test).

Suppl. Table 4. Correlation between markers with MYD88 L265P mutational status.

Marker	<i>MYD88 L265P mutation</i>				
	<i>p</i> value	Positive		Negative	
		Mean	±STD	Mean	±STD
CD68	0.874	16.5	11.2	15.3	8.3
CD16	0.392	3.1	4.9	6.6	10.1
MITF	0.251	1.9	1.8	3.1	2.9
CD163	0.841	22.7	18.6	20.8	17.9
PTX3	0.813	11.2	14.4	11.3	17.3
IL10	0.388	11.7	9.1	9.3	10.6
FOXP3	0.677	1.1	0.8	2.4	3.0
CSF1R	0.621	19.0	14.0	27.8	26.8
RGS1	0.871	7.6	8.8	10	12.3

Mann-Whitney U Test (Non-parametric test).

Suppl. Table 5. Correlation between markers with cell-of-origin (DLBCL NOS only).

Marker	Cell-of-origin (Hans' classifier) - DLBCL NOS				
	<i>p</i> value	GCB		Non-GCB	
		Mean	±STD	Mean	±STD
CD68	0.034	13.2	8.3	16.4	7.9
CD16	<0.001	2.3	5.5	8.8	11.1
MITF	0.326	2.8	2.8	3.3	2.9
CD163	0.018	17.2	18.9	23.2	16.4
PTX3	0.036	7.1	12.6	13.5	18.5
IL10	<0.001	3.2	3.9	12.7	11.4
FOXP3	0.567	2.4	3.5	2.1	2.3
CSF1R	0.252	22.7	23.2	27.6	24.7
RGS1	0.247	6.1	7.0	9.2	10.9

Mann-Whitney U Test (Non-parametric test).

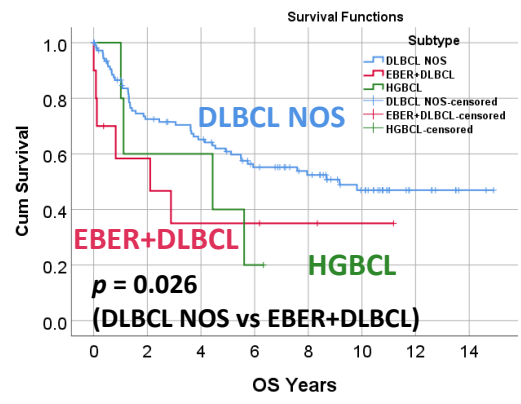
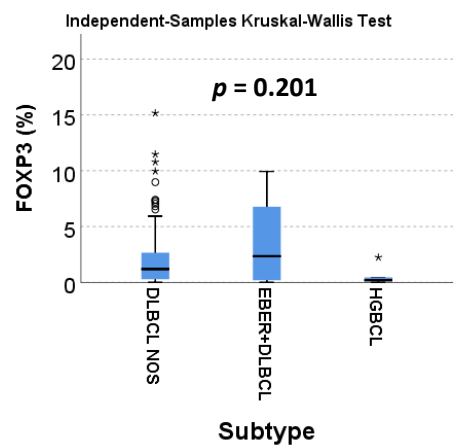
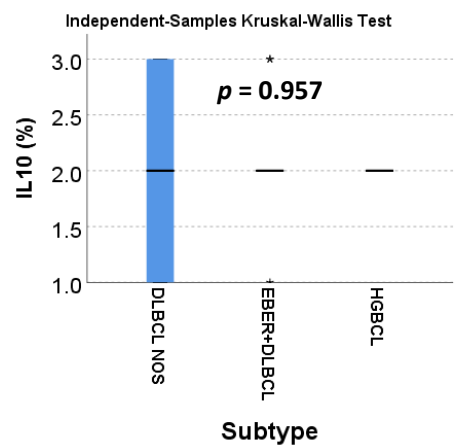
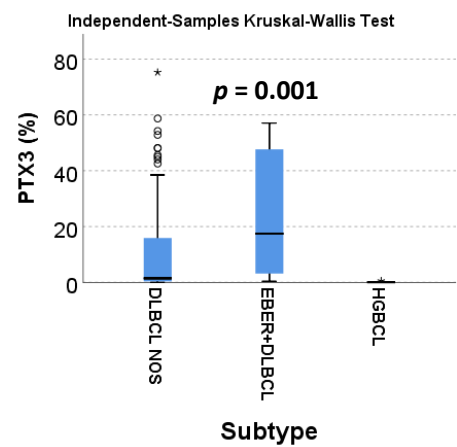
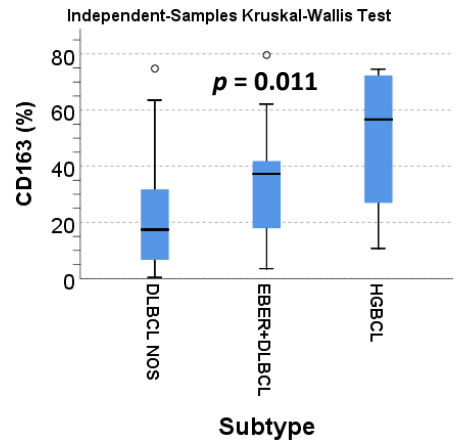
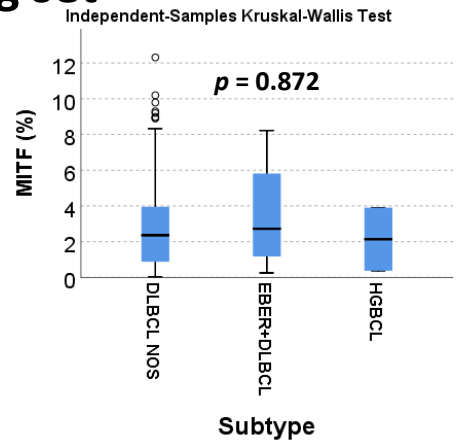
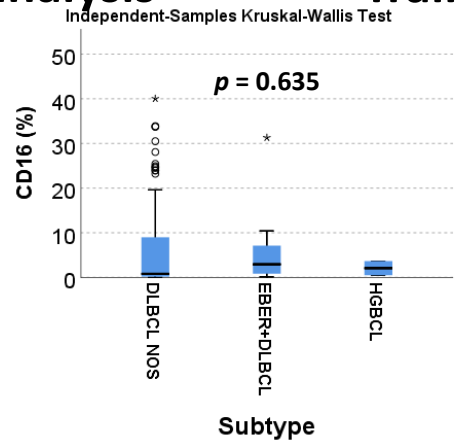
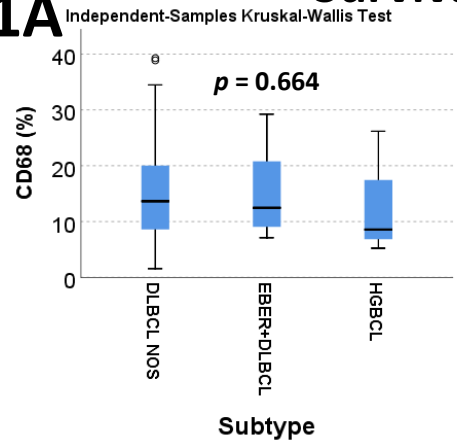
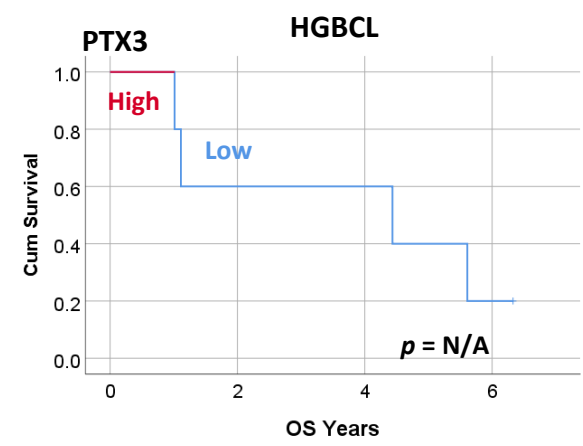
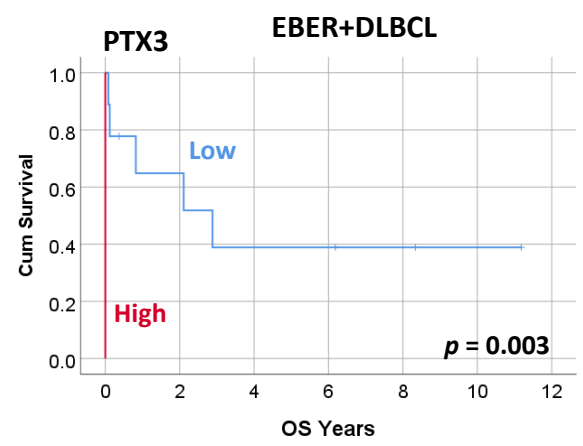
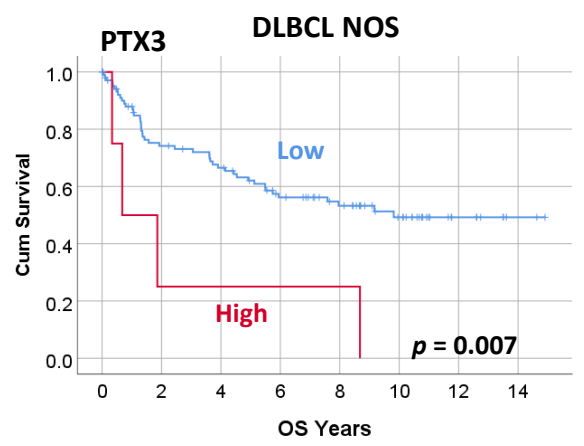
Suppl. Table 6. Correlation between markers with PD-L1.

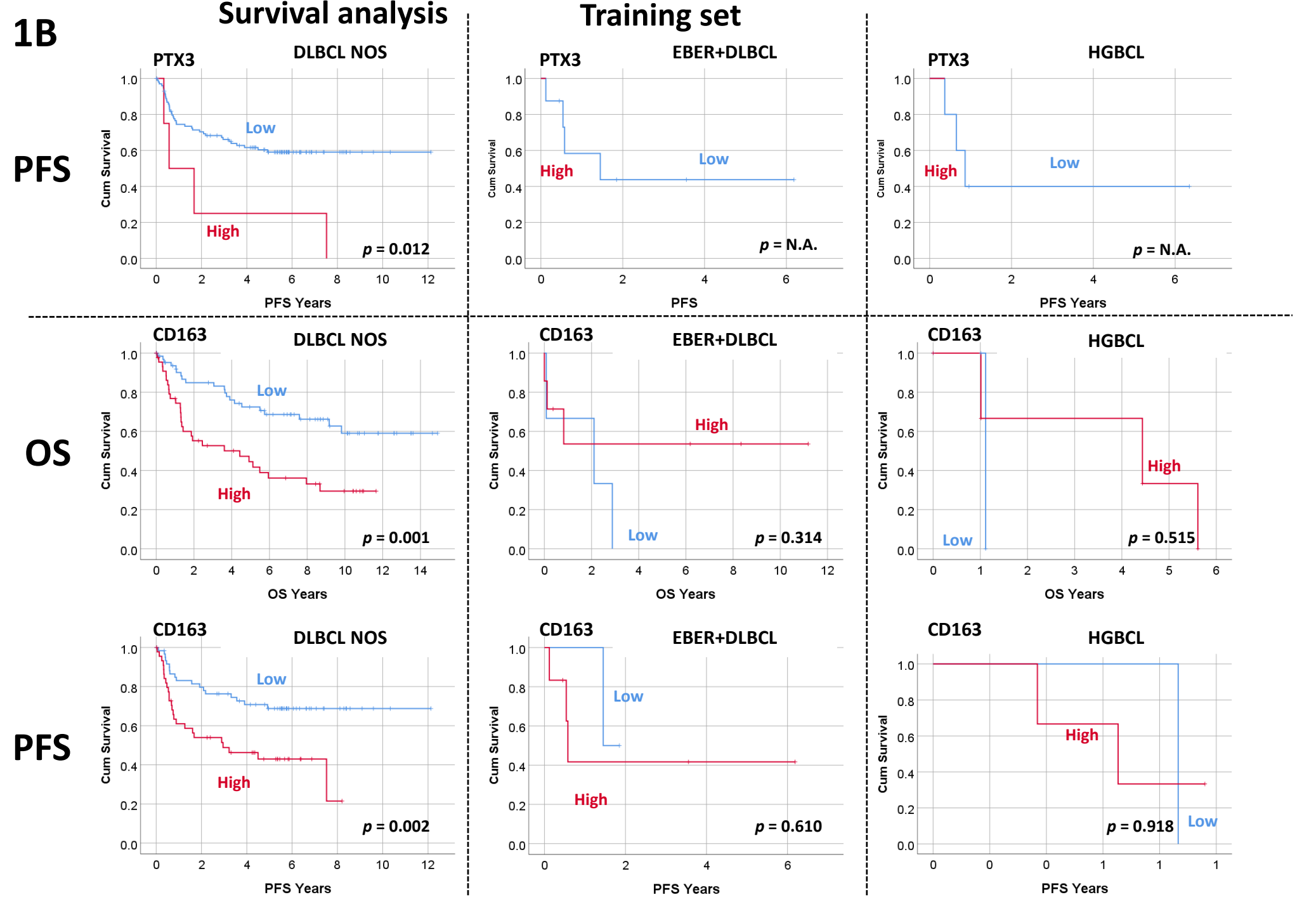
	PD-L1	
	Correlation coefficient	<i>p</i> value
CD68	0.185	0.184
CD16	0.440	0.002
MITF	0.237	0.109
CD163	0.329	0.016
PTX3	0.103	0.463
IL10	0.468	0.001
FOXP3	-0.148	0.291
CSF1R	0.126	0.367
RGS1	0.432	0.002

Bivariate correlation.

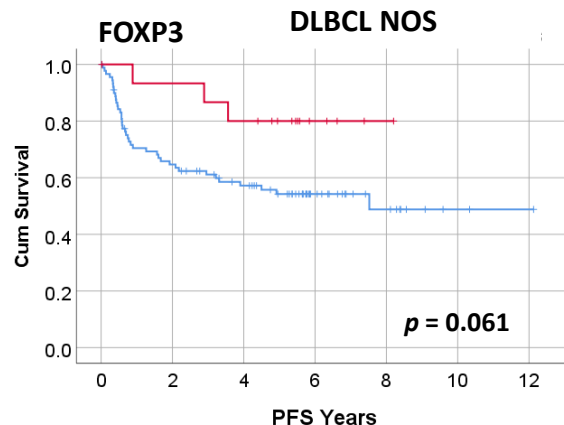
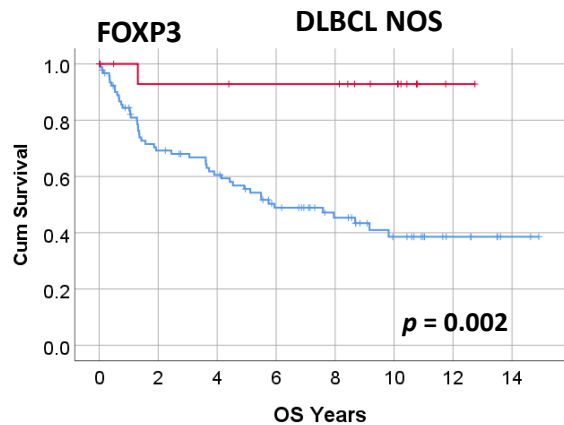
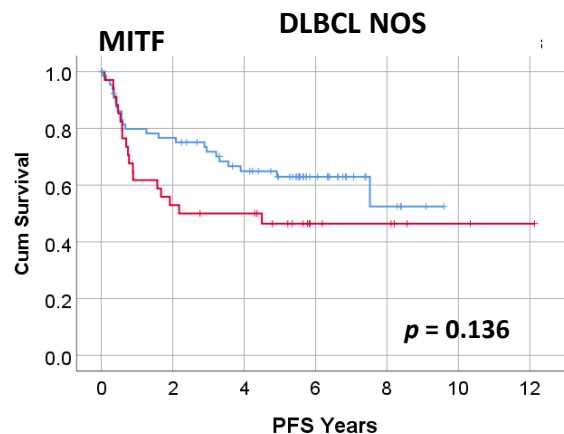
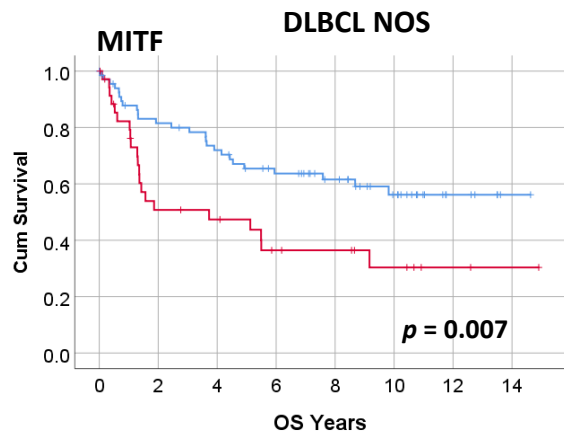
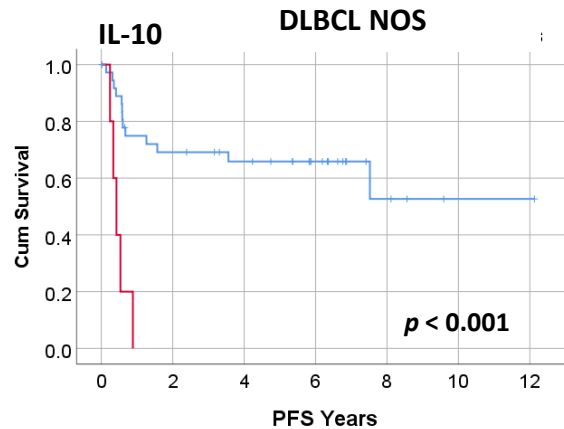
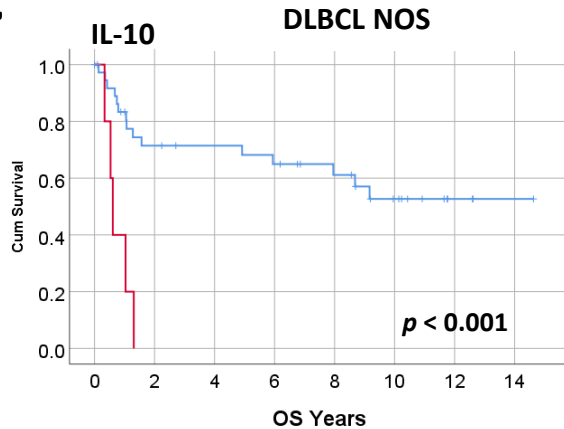
1A

Survival analysis Training set

**OS**



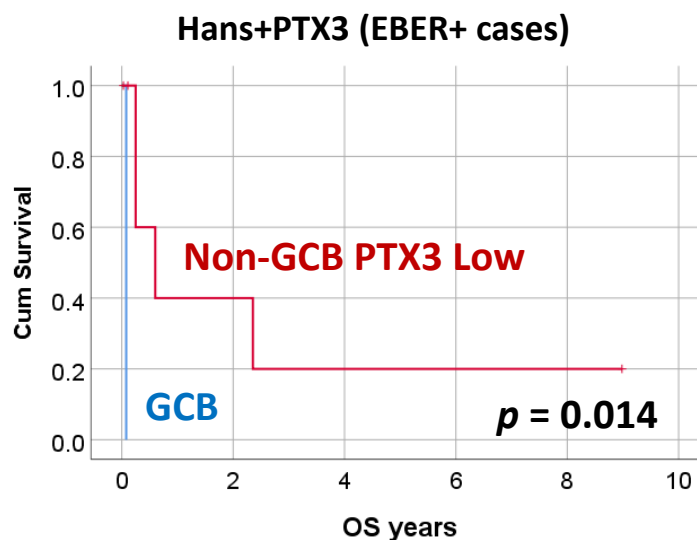
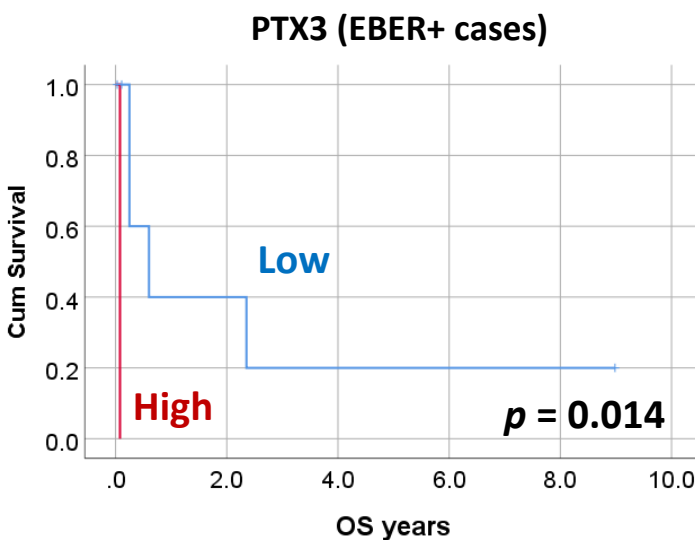
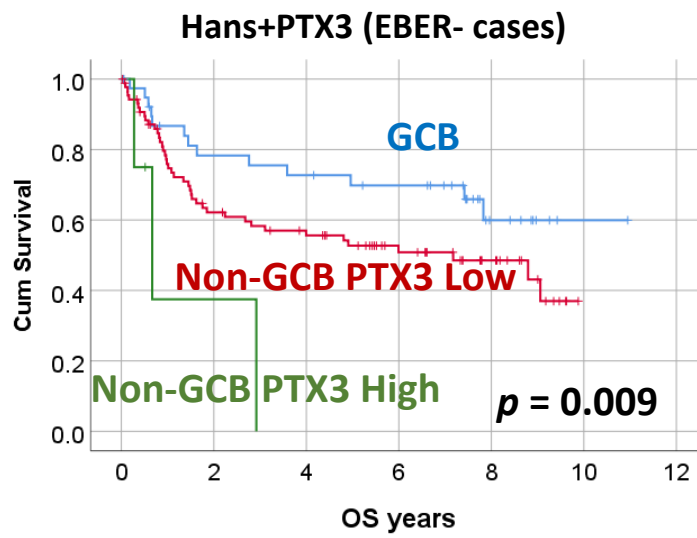
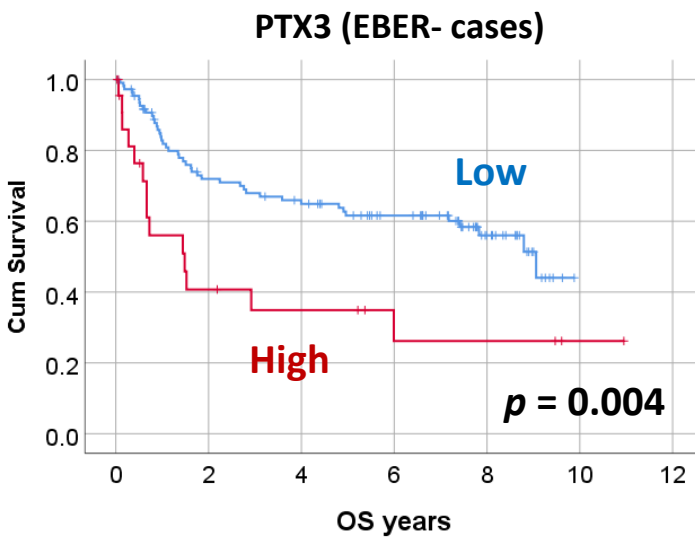
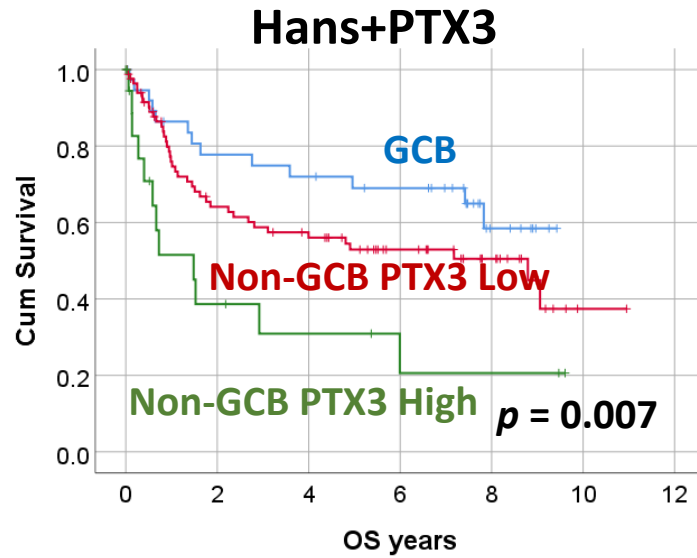
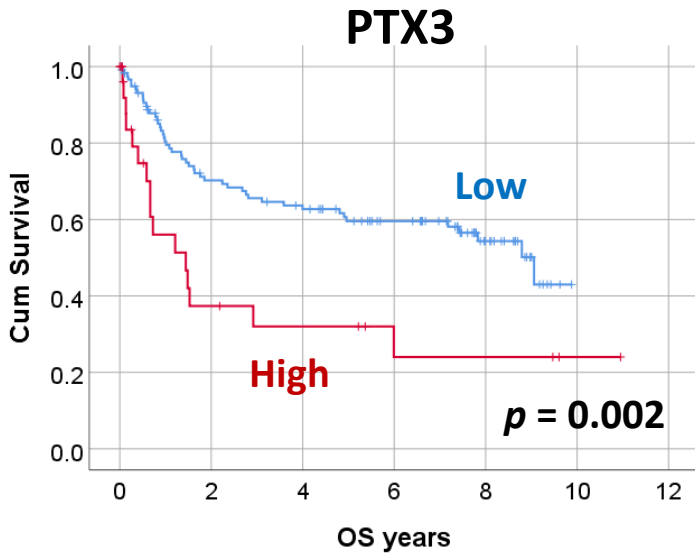
1C Survival analysis Training set



2A

Validation set

Overall Survival



2B

Validation set

Progression Free Survival

