

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	p value	<i>MYC and BCL2 IHC Double expressor</i>			
		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	p value	<i>MYD88 L265P mutation</i>			
		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	p value	Cell-of-origin (Hans' classifier) - DLBCL NOS			
		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	p 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.

Suppl. Table 7. Immune Response Categories between High vs. Low PTX3 groups (10% cut-off).

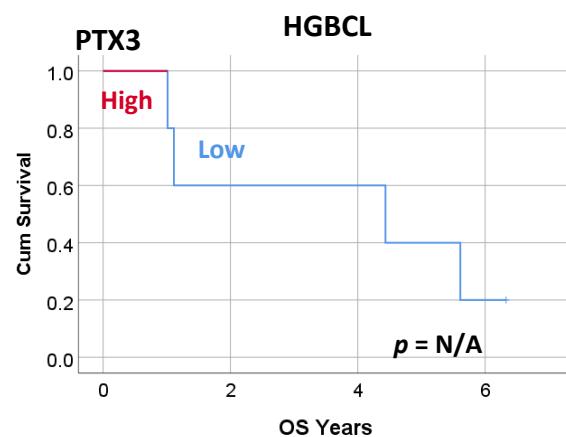
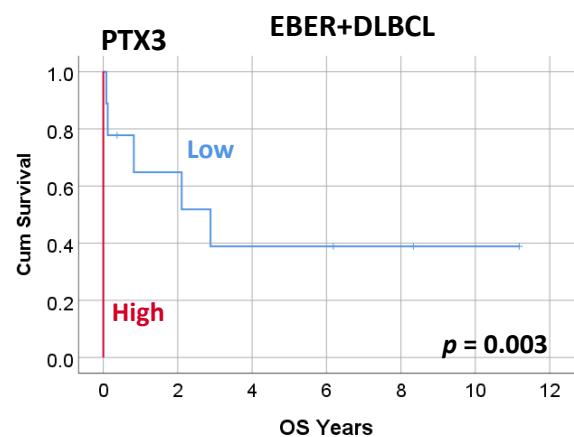
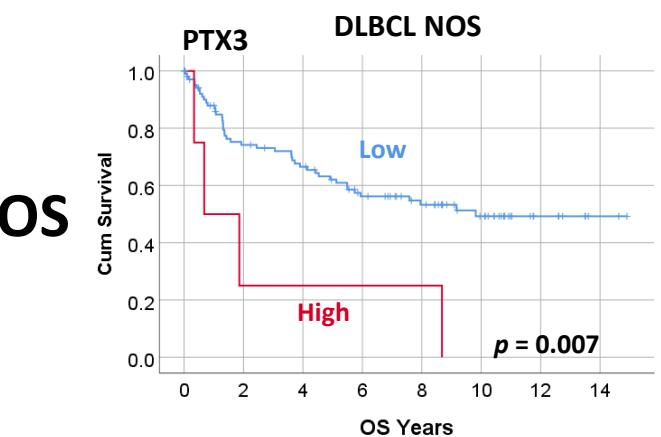
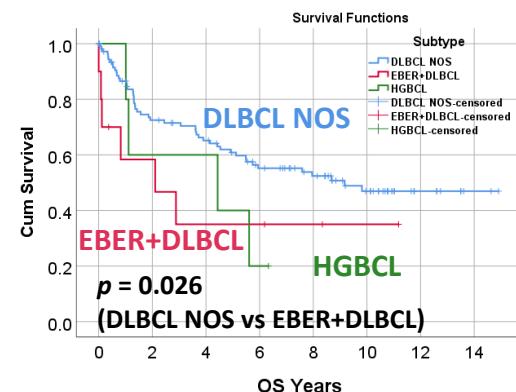
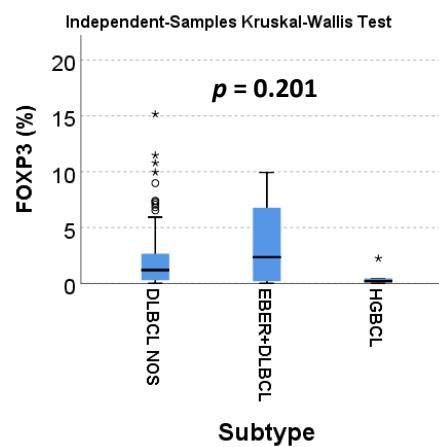
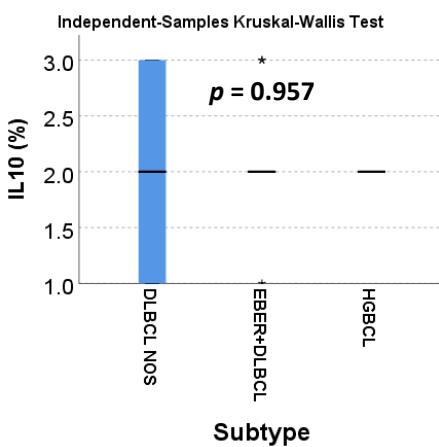
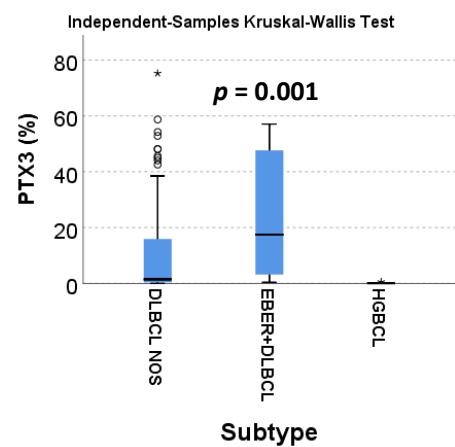
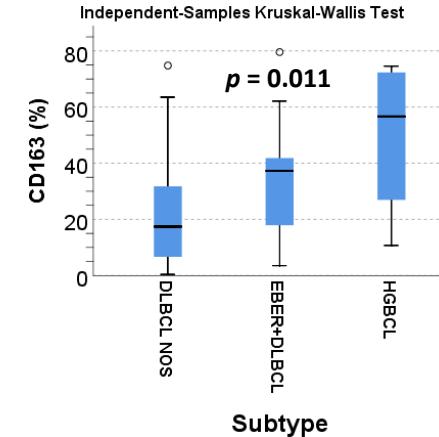
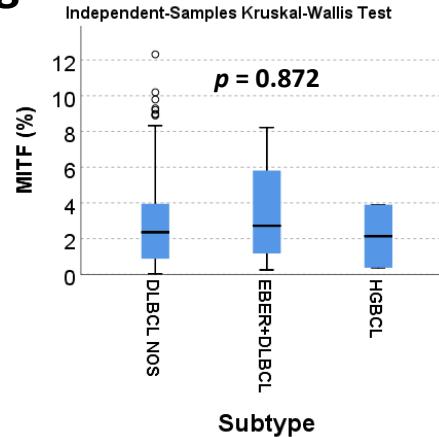
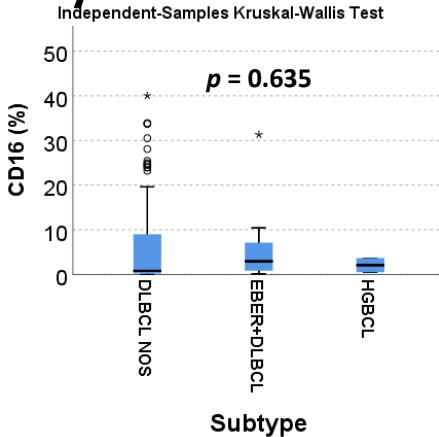
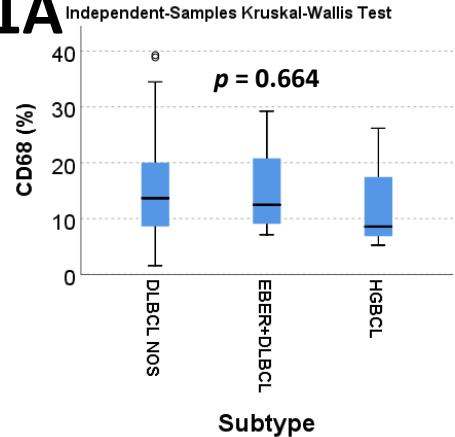
Group	Gene	P value	Num. of IR Cat.	Regulation	Antigen Processing	Interleukins	Adhesion	NK Cell Functions	Complement	Leukocyte Functions	TLR	Senescence	TNF Superfamily	Transporter Functions	Cell Functions	B-Cell Functions	Macrophage Functions	Cytokines	Chemokines	T-Cell Functions	Cytotoxicity	Cell Cycle	Microbial Functions	Pathogen Defense	HK	CT Antigen	Annotation
High PTX3	<i>CYFIP2</i>	0.002	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Innate immune response	
	<i>NUBP1</i>	0.002	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NK	
	<i>CDS3</i>	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	CD molecules, Adaptive immune response	
	<i>IL2RG</i>	0.008	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	Adaptive immune response, CD molecules, Chemokines and receptors	
	<i>IKBKE</i>	0.011	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Innate immune response	
	<i>HMOX1</i>	0.012	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Adaptive immune response, Antigen processing and presentation, Cell Type specific, Cytokines and receptors	
	<i>PEMPF9</i>	0.018	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Innate immune response	
	<i>IGG2B</i>	0.019	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	CD molecules	
	<i>CD48</i>	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Adaptive immune response	
	<i>BTK</i>	0.021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Chemokines and receptors, Inflammatory response	
	<i>CKLF</i>	0.021	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	Macrophage activation, Adaptive immune response, Innate immune response	
	<i>SYK</i>	0.023	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Innate immune response	
	<i>MAP2K1</i>	0.028	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Innate immune response	
	<i>AGK</i>	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Innate immune response	
	<i>BTK</i>	0.032	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Adaptive immune response	
	<i>DUSP5</i>	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Innate immune response	
	<i>HLA-DRA</i>	0.041	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Kidney, Innate immune response, Antigen processing and presentation	
	<i>TAB1</i>	0.041	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Innate immune response	
	<i>LCP1</i>	0.047	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	Leukocyte Functions, Macrophage Functions, T-Cell Functions, T-cell activation	
	<i>C7</i>	0.048	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Complement pathway, Innate immune response	
	<i>ETS1</i>	0.048	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Senescence pathway	
	<i>MYD88</i>	0.128	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Innate immune response, Toll-like receptor	
	<i>LLRLB1</i>	0.14	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NK Cell Functions, Regulation, T-Cell Functions; CD molecules, Regulation of immune response, T-cell proliferation	
Low PTX3	<i>MAGEC1</i>	0.494	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	HK	
	<i>CRBN</i>	0.494	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Innate immune response	
	<i>SLC10A7</i>	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Innate immune response	
	<i>LR</i>	0.092	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	CD molecules, NK cell functions, Regulation of immune response	
	<i>KIR2J1</i>	0.095	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Response to drug	
	<i>ARGP</i>	0.093	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Adhesion, CD molecules, Regulation of immune response	
	<i>ICAM4</i>	0.078	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Macrophage Functions, T-Cell Functions (T-cell activation)	
	<i>FPR1</i>	0.067	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	CT Antigen	
	<i>SPOL1</i>	0.063	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Regulation of immune response	
	<i>AMCA1</i>	0.058	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Regulation of immune response	
	<i>ULBP2</i>	0.047	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Regulation of immune response	
	<i>NTSE</i>	0.048	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	CD molecules, Phagocytosis, Transporter Functions	
	<i>MAGEC2</i>	0.048	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	CT Antigen	
	<i>CLC23</i>	0.05	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	CT Antigen	
	<i>PLA2G1B</i>	0.053	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Positive regulation of immune response	
	<i>IFI44L</i>	0.057	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	Cytokines and receptors, Interleukins	
	<i>GUSB</i>	0.057	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	CD molecules	
	<i>TRFC</i>	0.056	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	CD molecules, Regulation of immune response	
	<i>CD34</i>	0.033	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Basic cell functions, Cell Type specific, CD molecules, NK cell functions	
	<i>NCR1</i>	0.029	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	Anti-inflammatory cytokines, B-cell differentiation, Interleukins	
	<i>LL1</i>	0.026	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	CD molecules, TNF Superfamily members and their receptors	
	<i>TNFSF12A</i>	0.026	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	CD molecules, NK cell functions, Regulation of immune response	
	<i>KIR3DL2</i>	0.022	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Adhesion, CD molecules	
	<i>ITGB4</i>	0.017	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Chemokines and receptors	
	<i>IL2RA1</i>	0.01	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	CD molecules, NK cell functions, Regulation of immune response	
	<i>PPA</i>	0.012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	HK	
	<i>KIN20S1</i>	0.009	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	CD molecules, NK cell functions, Regulation of immune response	
	<i>TDFB2</i>	0.009	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Immunosuppression, Interleukins	

P values: 0.006, 0.069, 0.083, 0.162, 0.175, 0.306, 0.306, 0.306, 0.306, 0.329, 0.329, 0.329, 0.329, 0.527, 0.582, 0.961, 0.961, 1, 1, 1, 1, N/A, N/A

1A

Survival analysis

Training set

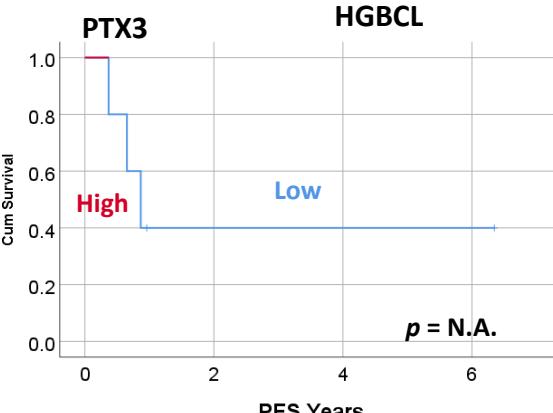
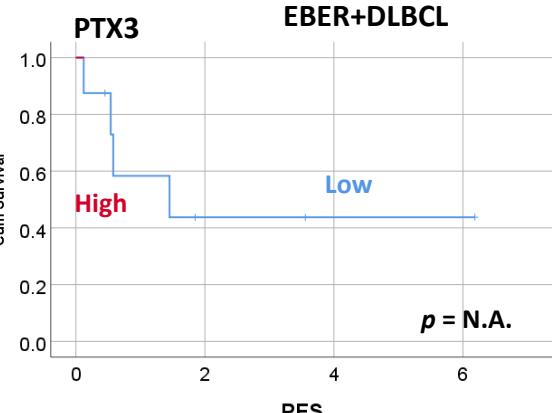
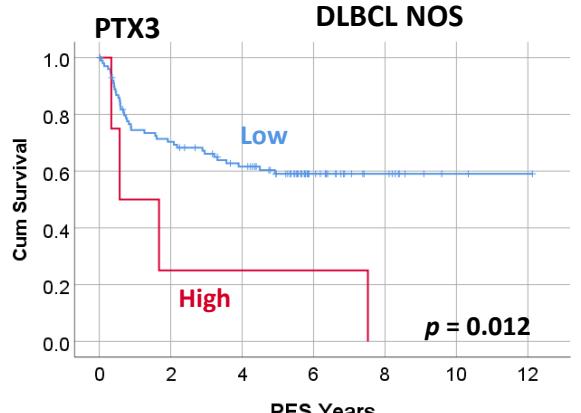


1B

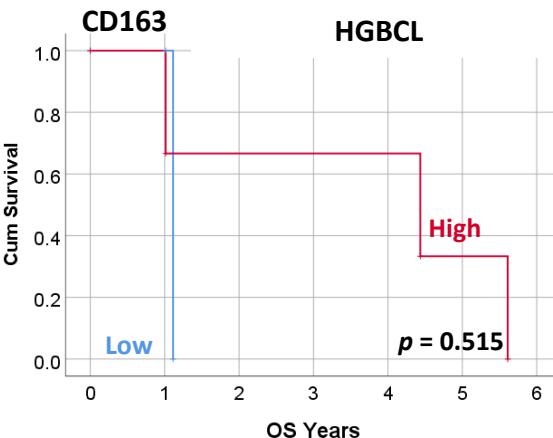
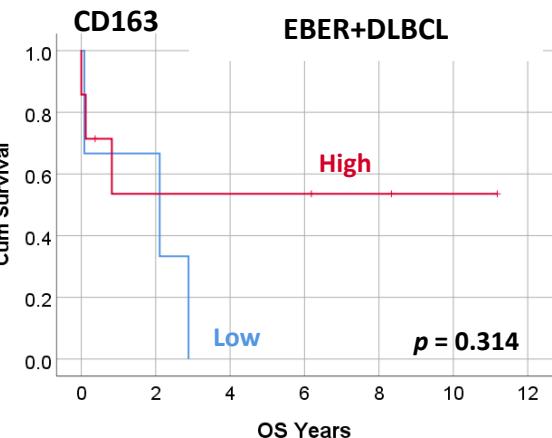
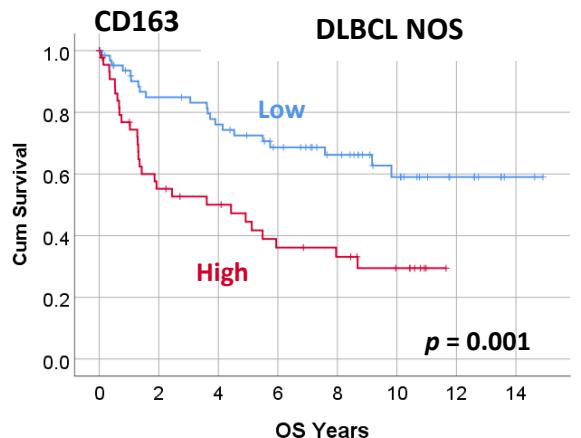
Survival analysis

Training set

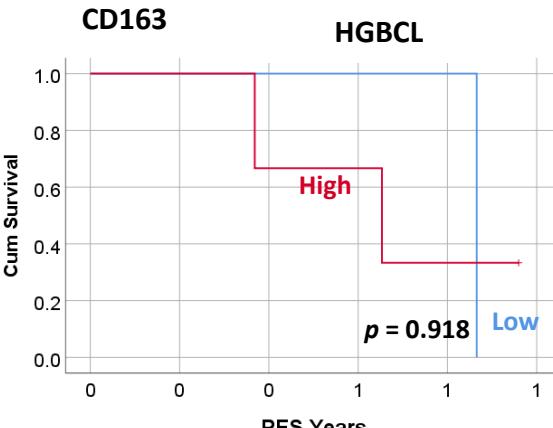
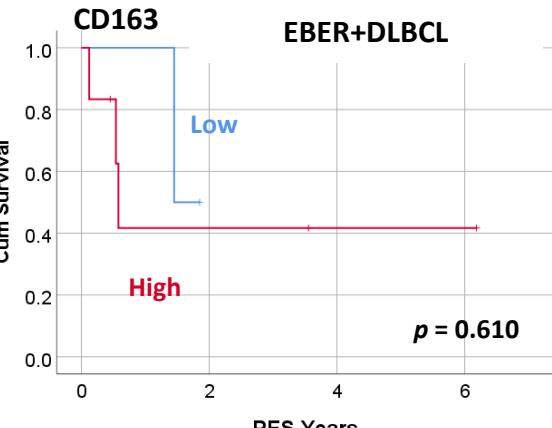
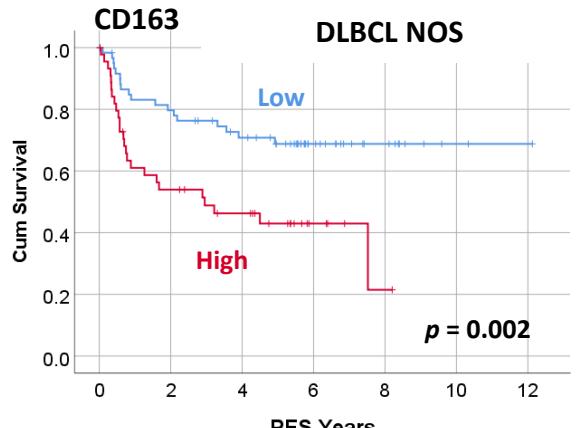
PFS



OS

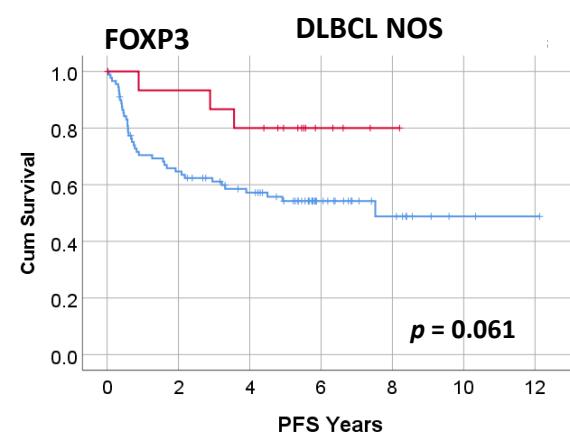
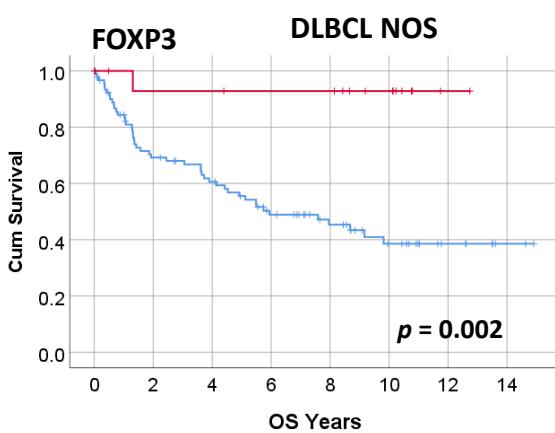
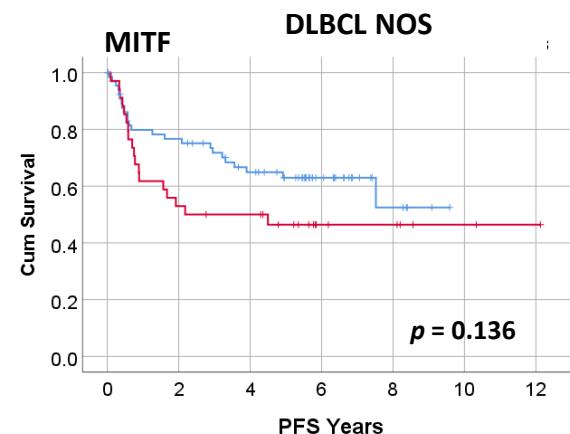
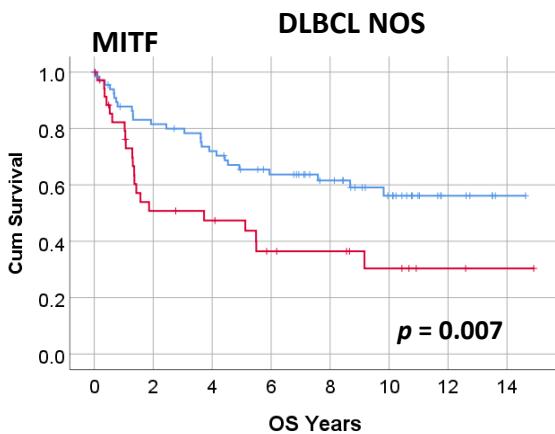
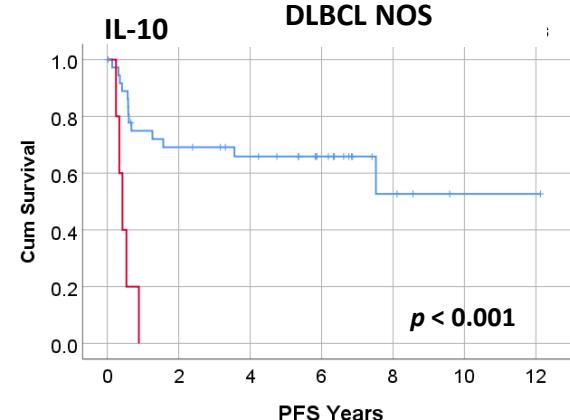
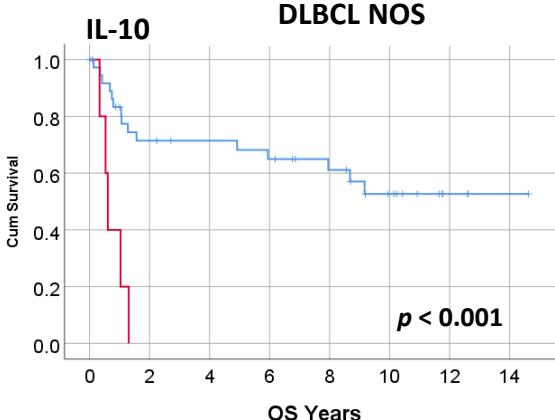


PFS



1C

Survival analysis Training set

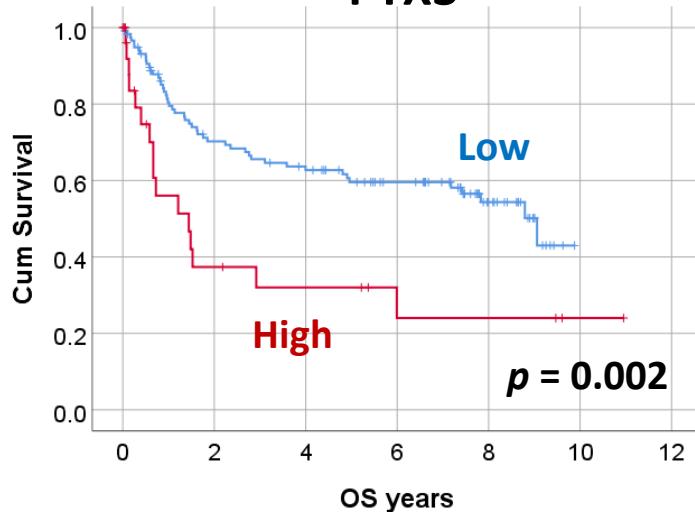


2A

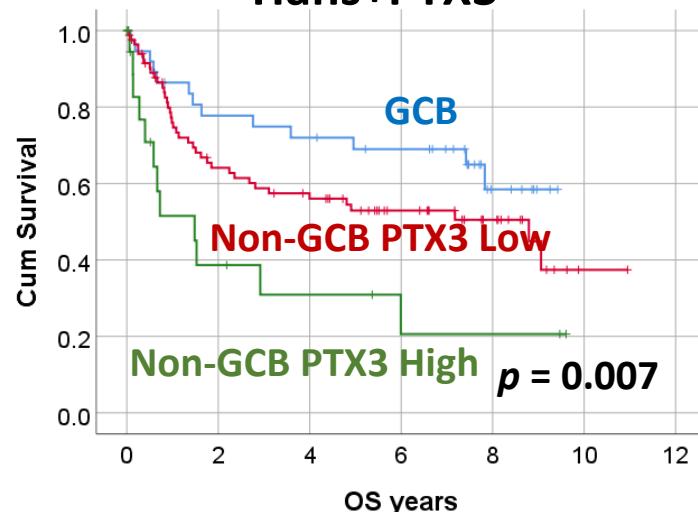
Validation set

Overall Survival

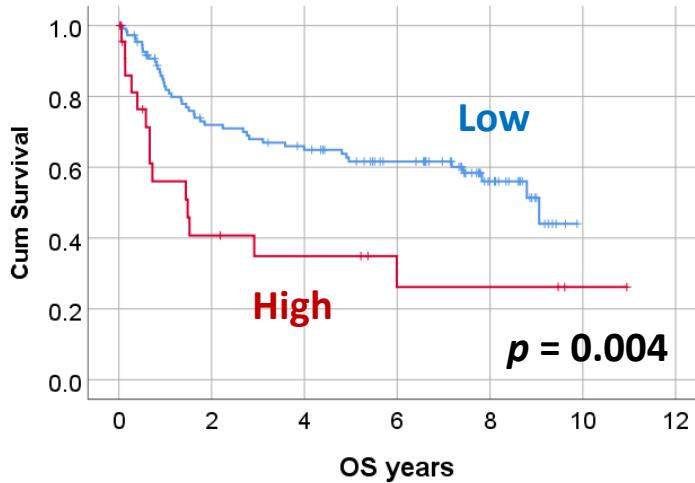
PTX3



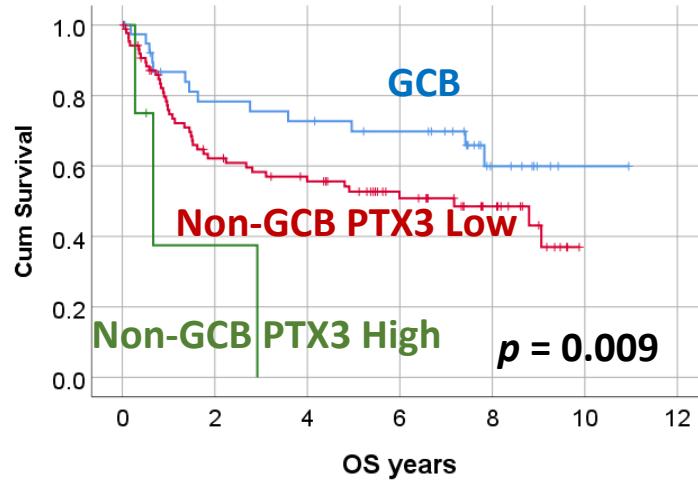
Hans+PTX3



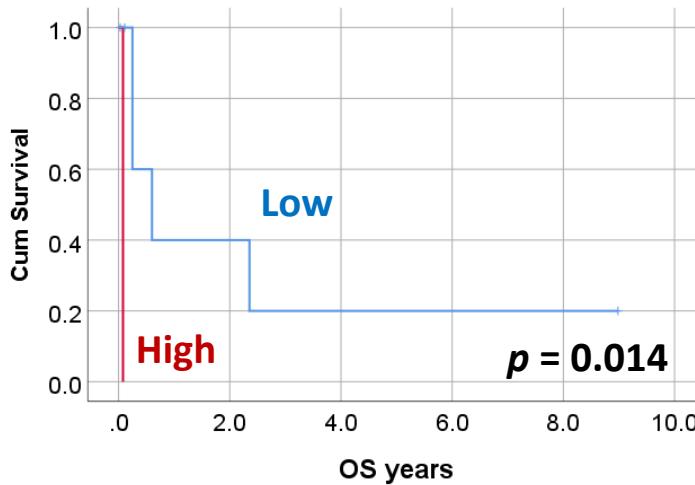
PTX3 (EBER- cases)



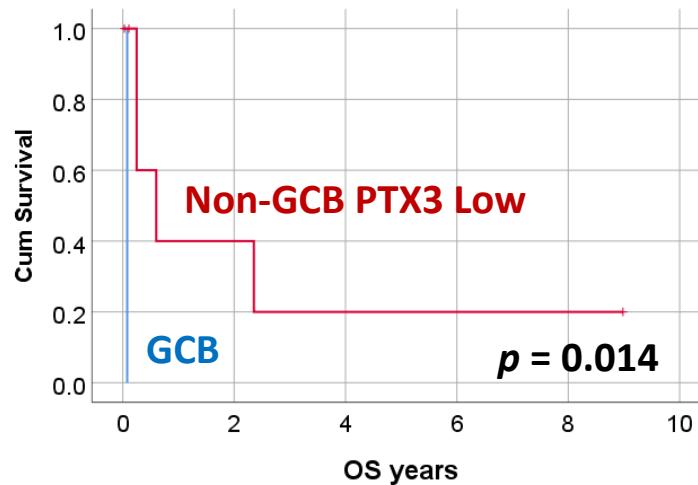
Hans+PTX3 (EBER- cases)



PTX3 (EBER+ cases)



Hans+PTX3 (EBER+ cases)

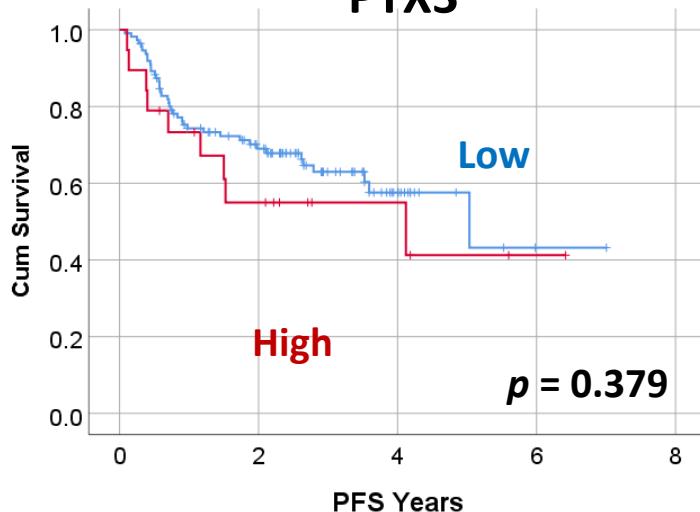


2B

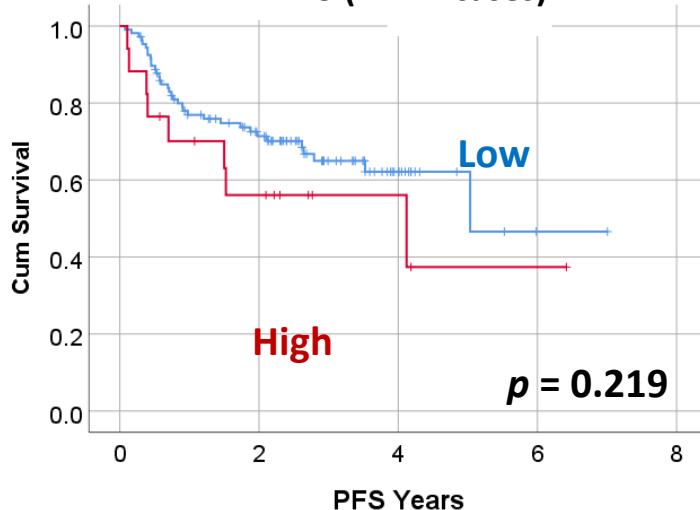
Validation set

Progression Free Survival

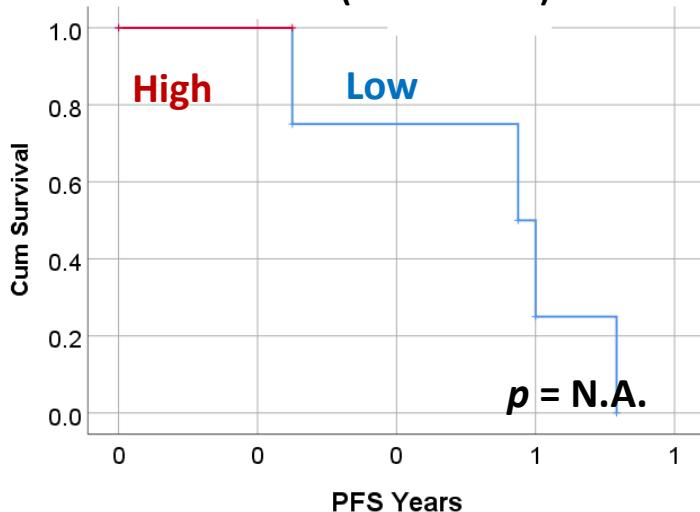
PTX3



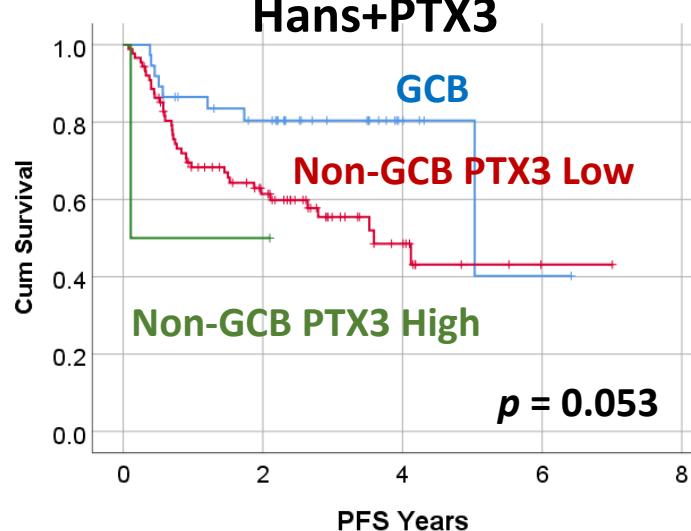
PTX3 (EBER- cases)



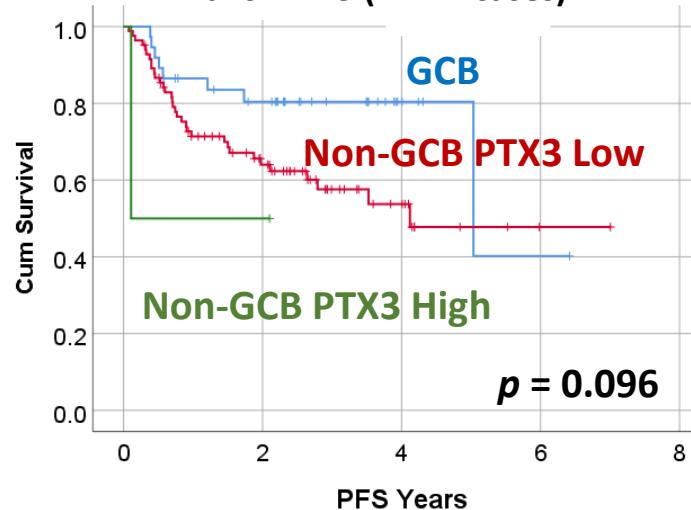
PTX3 (EBER+ cases)



Hans+PTX3



Hans+PTX3 (EBER- cases)



Hans+PTX3 (EBER+ cases)

