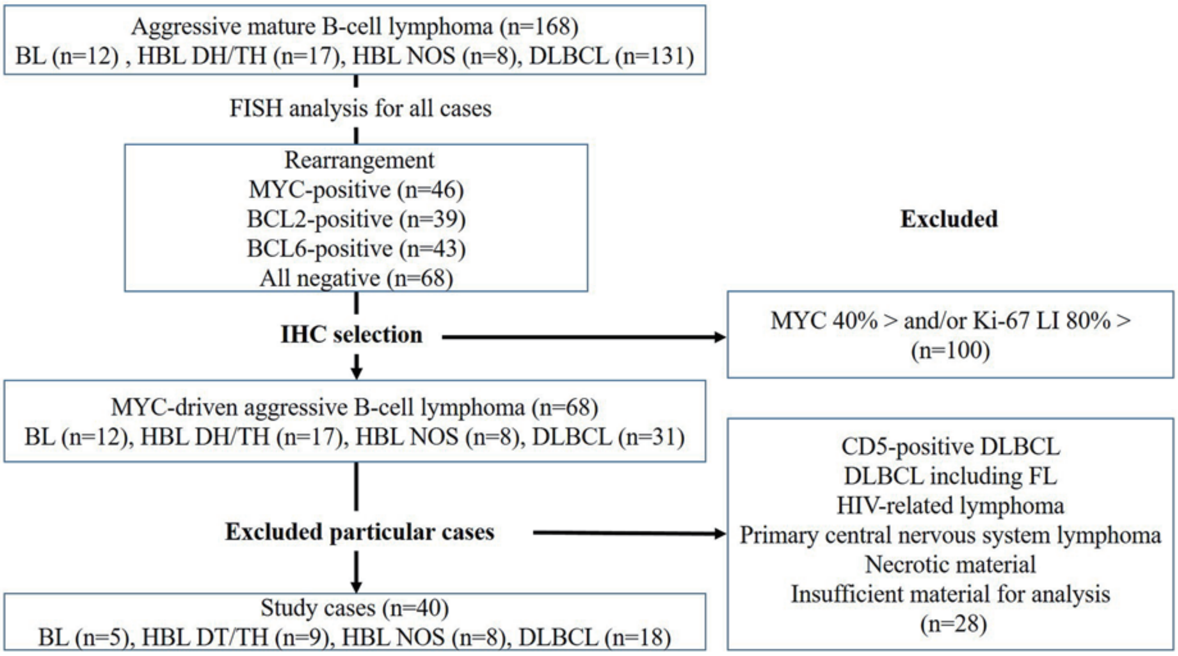
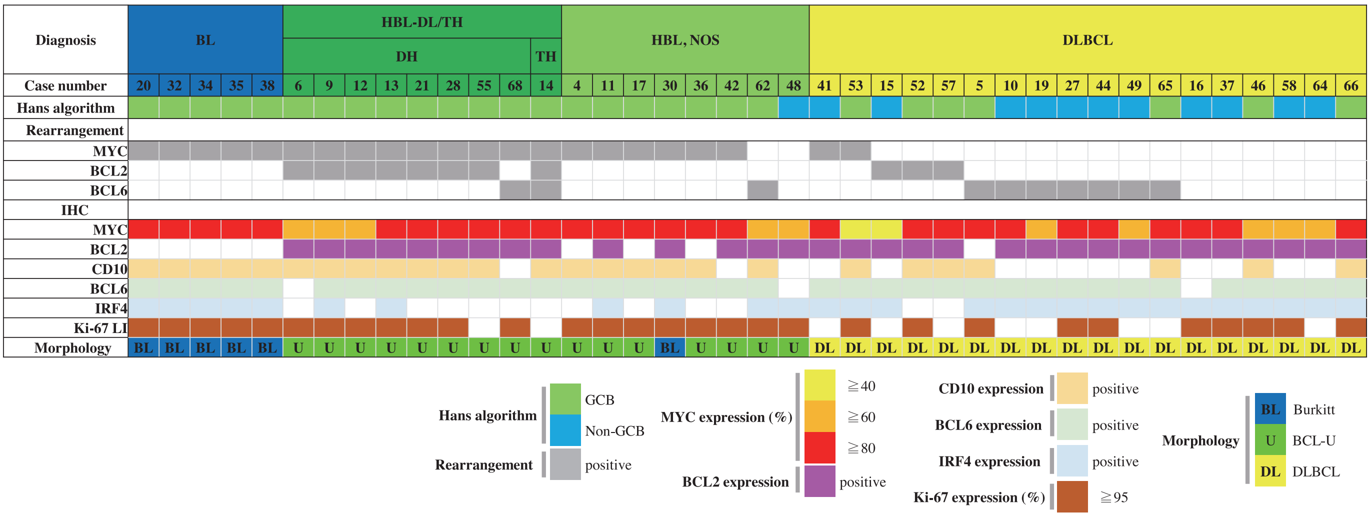
Supplementary Materials



Supplementary Fig. S1. Flow diagram for case selection
The information of case selection in our study by IHC selection and exception of particular cases.
FISH: fluorescence *in situ* hybridization, Ki-67 LI: Ki-67 labeling index, BL: Burkitt lymphoma, HBL-DH/TH: high-grade B-cell lymphoma double hit/ triple hit, HBL NOS: high-grade B-cell lymphoma, not otherwise classified, DLBCL: diffuse large B-cell lymphoma, HIV: human immune deficiency virus, IHC: immunohistochemistry.



Supplementary Fig. S2. IHC and rearrangement results of all cases
According to the Hans algorithm, 28 cases (70%) were assigned to the GCB type and 12 cases (30%) were assigned to the non-GCB type. After exclusion of Burkitt lymphoma, 31 (89%) of 35 cases were double expressors. *MYC*, *BCL2* and *BCL6* rearrangements were found in 22 (55%), 11 (28%) and 10 (25%) of 40 cases. BL: Burkitt lymphoma, HBL-DH/TH: high-grade B-cell lymphoma double hit/ triple hit, HBL NOS: high-grade B-cell lymphoma, not otherwise classified, DLBCL: diffuse large B-cell lymphoma, IHC: immunohistochemistry, GCB: germinal center B-cell lymphoma. BCL-U: B-cell lymphoma, unclassifiable with features intermediate between DLBCL and Burkitt lymphoma, DLBCL: diffuse large B-cell lymphoma.

Supplementary Table S1. Target regions of our panel

Gene (Accession No.)	Chr.	Exon	Exonregion Start		End	Targetregion Start		End	Coveroftargetregion(%)
MYC (NM_002467)	8	1	128748315	to	128748869	128748829	to	128748879	100
		2	128750494	to	128751265	128750483	to	128751275	94.19
		3	128752642	to	128753680	128752631	to	128753214	100
MAX (NM_002382)	14	1	65569022	to	65569057	65569011	to	65569067	66.07
		2	65568264	to	65568290	65568253	to	65568300	100
		3	65560426	to	65560533	65560415	to	65560543	100
		4	65544631	to	65544754	65544620	to	65544764	100
		5	65541842	to	65543381	65543183	to	65543391	83.65
MAX (NM_001271068)		3	65550457	to	65551017	65550887	to	65551027	100
TP53 (NM_000546)	17	5	7578371	to	7579554	7578371	to	7579554	95.63
		6	7578177	to	7578289	7578177	to	7578289	100
		7	7577499	to	7577608	7577499	to	7577608	100
		8	7577019	to	7577155	7577019	to	7577155	100
CCND3 (NM_001760)	6	5	41902671	to	41903845	41903671	to	41903951	100
CDKN2A (NM_00077)	9	2	21970901	to	21971207	21970901	to	21970991	100
BTG1 (NM_001731)	12	1	92539164	to	92539673	92539164	to	92539312	100
		2	92534054	to	92538223	92537856	to	92538223	100
BTG2 (NM_006763)	1	1	203274664	to	203274876	203274735	to	203274876	95.04
		2	203276232	to	203278729	203276227	to	203276553	97.06
ID3 (NM_002167)	1	1	23885618	to	23886285	23885607	to	23885927	95
		2	23885426	to	23885510	23885440	to	23885520	100
TCF3 (NM_001136139)	19	17	1612206	to	1612432	1612290	to	1612380	100

The information of exon regions, target regions of each gene and the coverage of target regions by each amplicon. Chr: chromosome.

Supplementary Table S2. PCR composition and condition

(A)

PCR Component	Amount
Qiagen Multiplex PCR master mix (Qiagen)	12 µl
Ion ampliseq custom panel primer pool (Thermo Fisher)	8 µl
Diluted FFPE DNA	5 µl (40 ng)
Total	25 µl

(B)

	PCR condition		
	First denaturation	(95 ºC)	15 min
23 cycles	Denaturation	(94 ºC)	30 sec
	Annealing	(60 ºC)	90 sec
	Extension	(68 ºC)	15 sec
	Final extension	(72 ºC)	10 min

(***A***) The information of the amounts of DNA polymerase, DNA extracted from FFPE and composition of the PCR primers. (***B***) PCR conditions, including the cycle number, and the degree and time of denaturation, annealing and extension. PCR: polymerase chain reaction, FFPE: formalin-fixed and paraffin-embedded

Supplementary Table S3. Limit of detection (Raji)

	VAF	100%	20%	5%
MYC	p.V20A	58.7	9.5	2.7
			8.0	2.7
	p.S21T	56.7	8.3	2.1
			7.1	1.7
	p.E54D	45.2	8.5	2.3
			9.2	2.6
	p.A59V	50.4	11.7	3.5
			11.6	3.6
TP53	p.R213Q	37.7	5.1	1.2
			6.5	1.5
	p.Y234H	59.7	10.7	2.7
			10.1	3.1

The precise percentage (%) of effected VAF of *MYC* (p.V20A, p.S21T, p.E54D, p.A59V) and *TP53* (p.R213Q, p.Y234H). VAF: variant allele fraction.

Supplementary Table S4. Pathological and genetic status/prediction-function

MSKCC impact High/Medium/ Low	FATHMM impact Disease-Oncogenic (Oncogenic)/ Neutral	FATHMM impact Cancer-associated variant (Cancer)/ non-cancer-associated variant (Non-Cancer)	InterVar Pathogenesis/ Likely pathogenesis/Uncertain significance/Benign

Case number	Diagnosis	Rearrangement			Hans algorithm	IHC					Ki-67 (%)	ID3 (NP_ 002158)	BTG2 (NP_ 006754)	CCND3 (NP_ 001751)	MYC (NP_ 002458)	CDKN2A (NP_ 000068)	BTG1 (NP_ 001722)	MAX (NP_ 002373)	TP53 (NP_ 0000537)	TCF3 (NP_ 001129611)
		MYC	BCL2	BCL6		MYC (%)	BCL2	CD10	BCL6	IRF4										
4	HBL NOS	+	-	-	GCB	90	-	+	+	-	99							D37G (Medium/ Oncogenic/Non-cancer/Uncertain significance)		
5	DLBCL	-	-	+	GCB	99	-	+	+	+	99					A148T (Medium/ Neutral/Cancer/ Benign)			R196* (NA/NA/ NA/Pathogenic)	
6	HBL-DH	+	+	-	GCB	70	+	+	-	-	95				S53I (Medium/ Oncogenic/Non-cancer/Likely pathogenic) P72T (Medium/ Oncogenic/Non-cancer/Likely pathogenic) T73I (Medium/ Oncogenic/Non-cancer/Likely pathogenic) K158N (Medium/ NA/Non-cancer/ Uncertain significance)		L26fs (NA)			
9	HBL-DH	+	+	-	GCB	80	+	+	+	+	99									
10	DLBCL	-	-	+	non-GCB	99	+	-	+	+	90						H74D (Medium/ Oncogenic/Non-cancer/Uncertain significance)			
11	HBL NOS	+	-	-	GCB	90	+	+	+	+	99								M246I (Medium/ Oncogenic/ Cancer/Likely pathogenic)	
12	HBL-DH	+	+	-	GCB	60	+	+	+	-	99									N551K (Medium/ Oncogenic/Non-cancer/Likely pathogenic)
13	HBL-DH	+	+	-	GCB	80	+	+	+	+	99									
14	HBL-TH	+	+	+	GCB	90	+	+	+	-	80				P17A (Medium/ Oncogenic/Non-cancer/Uncertain significance) Y27F (Medium/ Oncogenic/Non-cancer/Uncertain significance) Y47C (Medium/ Oncogenic/Non-cancer/Uncertain significance)					

Case number	Diagnosis	Rearrangement			Hans algorithm	IHC					Ki-67 (%)	ID3 (NP_002158)	BTG2 (NP_006754)	CCND3 (NP_001751)	MYC (NP_002458)	CDKN2A (NP_000068)	BTG1 (NP_001722)	MAX (NP_002373)	TP53 (NP_0000537)	TCF3 (NP_001129611)
		MYC	BCL2	BCL6		MYC (%)	BCL2	CD10	BCL6	IRF4										
15	DLBCL	-	+	-	non-GCB	40	+	-	+	+	85									
16	DLBCL	-	-	-	non-GCB	90	+	-	-	+	95								R273H (Medium/Oncogenic/Cancer/Pathogenic)	
17	HBL NOS	+	-	-	GCB	80	-	+	+	-	99									
19	DLBCL	-	-	+	non-GCB	60	+	-	+	+	90									
20	BL	+	-	-	GCB	90	-	+	+	+	99	L70P (High/Oncogenic/Cancer/Uncertain significance)								
21	HBL-DH	+	+	-	GCB	90	+	+	+	-	95									
27	DLBCL	-	-	+	non-GCB	80	+	-	+	+	95		L20F (Low/Oncogenic/Non-cancer/Uncertain significance) G117D (Medium/Oncogenic/Non-cancer/Uncertain significance)		D63N (Medium/Oncogenic/Non-cancer/Uncertain significance)					
28	HBL-DH	+	+	-	GCB	80	+	+	+	-	95								Y234D (Medium/Oncogenic/Cancer/Likely pathologic) K139N (Medium/Oncogenic/Cancer/Uncertain significance)	
30	HBL NOS	+	-	-	GCB	90	+	+	+	+	99	E68fs(NA) V67L(Low/Oncogenic/Non-cancer/Uncertain significance)		L292fs (NA)	E54D (Low/Oncogenic/Non-cancer/Likely pathogenic) G152A (Medium/NA/Non-cancer/Uncertain significance)					
32	BL	+	-	-	GCB	90	-	+	-	+	99	Q63* (NA)							D281E (Medium/Oncogenic/Cancer/Uncertain significance)	
34	BL	+	-	-	GCB	90	-	+	+	+	99	L78fs (NA)		Q276* (NA)	T73S (Medium/Oncogenic/Non-cancer/Likely pathogenic)					
35	BL	+	-	-	GCB	90	-	+	+	+	99				Q50H (Low/Oncogenic/Non-cancer/Uncertain significance) E54D (Low/Oncogenic/Non-cancer/Likely pathogenic) F153C (Medium/NA/Non-cancer/Uncertain significance) S264R (Medium/NA/Non-cancer/Uncertain significance)	A148T (Medium/Neutral/Cancer/Benign)				

Case number	Diagnosis	Rearrangement			Hans algorithm	IHC					Ki-67 (%)	ID3 (NP_002158)	BTG2 (NP_006754)	CCND3 (NP_001751)	MYC (NP_002458)	CDKN2A (NP_000068)	BTG1 (NP_001722)	MAX (NP_002373)	TP53 (NP_0000537)	TCF3 (NP_001129611)
		MYC	BCL2	BCL6		MYC (%)	BCL2	CD10	BCL6	IRF4										
36	HBL NOS	+	-	-	GCB	80	-	+	+	-	99	L64F (Medium/NA/Non-cancer/Uncertain significance)			S21N (Low/Oncogenic/Non-cancer/Uncertain significance)					
37	DLBCL	-	-	-	non-GCB	80	+	-	+	+	95									
38	BL	+	-	-	GCB	80	-	+	+	+	95	Y76* (NA)			P72T (Medium/Oncogenic/Non-cancer/Likely pathogenic)					
41	DLBCL	+	-	-	non-GCB	99	+	-	+	+	90									E555fs (NA)
42	HBL NOS	+	-	-	GCB	80	+	-	+	-	95				E54D (Low/Oncogenic/Non-cancer/Likely pathogenic)					
44	DLBCL	-	-	+	non-GCB	80	+	-	+	+	95		A126-L128delinsV (NA) A129fs (NA) S149F (Medium/Oncogenic/Non-cancer/Uncertain significance)						A138V (Medium/Oncogenic/Cancer/Uncertain significance)	
46	DLBCL	-	-	-	GCB	70	+	+	+	+	95		L10F (Low/Oncogenic/Non-cancer/Uncertain significance)			A148T (Medium/Neutral/Cancer/Benign)	K162I (Medium/Oncogenic/Non-cancer/Uncertain significance) L37M (Medium/Oncogenic/Non-cancer/Uncertain significance)			
48	HBL NOS	-	-	-	non-GCB	70	+	-	-	+	95	L64V (Low/Oncogenic/Non-cancer/Uncertain significance)	L10F (Low/Oncogenic/Non-cancer/Uncertain significance) G62D (Medium/Oncogenic/Non-cancer/Uncertain significance) R69C (Medium/Oncogenic/Non-cancer/Uncertain significance) Q140* (NA)				G132S (Low/Oncogenic/Non-cancer/Uncertain significance)			
49	DLBCL	-	-	+	non-GCB	60	+	-	+	+	80									
52	DLBCL	-	+	-	GCB	80	+	+	+	-	98				P75T (Medium/Oncogenic/Non-cancer/Uncertain significance)				R273G (Medium/Oncogenic/Cancer/Likely pathogenic)	
53	DLBCL	+	-	-	GCB	50	+	+	+	+	98									
55	HBL-DH	+	+	-	GCB	80	+	+	+	-	80									
57	DLBCL	-	+	-	GCB	90	+	+	+	-	80									D561fs (NA)
58	DLBCL	-	-	-	non-GCB	70	+	-	+	+	95								Y234C (Medium/Oncogenic/Cancer/Likely pathogenic)	

Case number	Diagnosis	Rearrangement			Hans algorithm	IHC						ID3 (NP_002158)	BTG2 (NP_006754)	CCND3 (NP_001751)	MYC (NP_002458)	CDKN2A (NP_000068)	BTG1 (NP_001722)	MAX (NP_002373)	TP53 (NP_0000537)	TCF3 (NP_001129611)
		MYC	BCL2	BCL6		MYC (%)	BCL2	CD10	BCL6	IRF4	Ki-67 (%)									
62	HBL NOS	-	-	+	GCB	70	+	+	+	+	95								R283C (Low/Oncogenic/Cancer/Likely pathogenic)	
64	DLBCL	-	-	-	non-GCB	70	+	-	+	-	90		A82T (Medium/Oncogenic/Non-cancer/Uncertain significance)							
65	DLBCL	-	-	+	GCB	80	+	+	+	+	90									
66	DLBCL	-	-	-	GCB	80	+	+	+	+	95					A148T (Medium/Neutral/Cancer/Benign)			A181P (Medium/Oncogenic/Cancer/Likely pathogenic)	
68	HBL-DH	+	-	+	GCB	80	+	-	+	-	99									

The case number, clinical diagnosis, type of rearrangement, Hans algorithm, results of IHC and FISH and amino acid changes with functional impact (MutationAssessor and FATHMM) are included in this table. IHC: immunohistochemistry, FISH: fluorescence *in situ* hybridization.