SUPPLEMENTARY APPENDIX

Prognostic relevance of CD163 and CD8 combined with EZH2 and gain of chromosome 18 in follicular lymphoma: a study by the Lunenburg Lymphoma Biomarker Consortium

Wendy B.C. Stevens,[1*] Matias Mendeville,[2*] Robert Redd,[3] Andrew J. Clear,[4] Reno Bladergroen,[2] Maria Calaminici,[4] Andreas Rosenwald,[5] Eva Hoster,[6] Wolfgang Hiddemann,[6] Philippe Gaulard,[7] Luc Xerri,[8] Gilles Salles,[9] Wolfram Klapper,[10] Michael Pfreundschuh,[11] Andrew Jack,[12] Randy D. Gascoyne,[13] Yasodha Natkunam,[14] Ranjana Advani,[15] Eva Kimby,[16] Birgitta Sander,[17] Laurie H. Sehn,[13] Anton Hagenbeek,[18] John Raemaekers,[1] John Gribben,[4] Marie José Kersten,[18] Bauke Ylstra,[2] Edie Weller[3] and Daphne de Jong[2]

**WBCS and MM contributed equally to this work*

[1]Department of Hematology, Radboudumc, Nijmegen, the Netherlands; [2]Department of Pathology, VU University Medical Center, Amsterdam, the Netherlands; [3]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA, USA; [4]Centre for Haemato-Oncology, Barts Cancer Institute, University of London, UK; [5]Institute of Pathology, Comprehensive Cancer Center Mainfranken, University of Würzburg, Germany; [6]Department of Medicine III, University Hospital Grosshadern, Munich, Germany; [7]Department of Pathology and Inserm U955, Hôpital Henri Mondor, University Paris-Est, Créteil, France; [8]Département de Biopathologie, Institut Paoli-Calmettes, Marseille, France; [9]Service d'Hématologie,Hospices Civils de Lyon & Université Claude Bernard Lyon-1, UMR CNRS 5239, France; [10]Institute of Pathology, University of Schleswig-Holstein, Kiel, Germany; [11]Medical Clinic I, Saarland University Hospital, Homburg, Germany; [12]Haematological Malignancy Diagnostic Service, St James's University Hospital, Leeds, UK; [13]Department of Pathology & Medical Oncology, Centre for Lymphoid Cancer, British Columbia Cancer Agency, University of British Columbia, Vancouver, Canada; [14]Department of Pathology, Stanford University School of Medicine, CA, USA; [15]Department of Hematology, Stanford University School of Medicine, CA, USA; [16]Department of Medicine, Division of Hematology, Karolinska Institute, Stockholm, Sweden; [17]Department of Laboratory Medicine, Division of Pathology, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden and [18]Department of Hematology, Academic Medical Center, Amsterdam, the Netherlands

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Correspondence: wendy.stevens@radboudumc.nl

Supplementary methods

DNA isolation and Library preparation

FFPE tissue cores were cut vertically into several smaller fragments to increase surface exposure, followed by DNA extraction with a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) as previously described.[1] Double-stranded genomic DNA was quantified using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Carlsbad CA, USA) and 250 ng was fragmented by ultrasonification with a Covaris S2 (Covaris Inc, Woburn MA, USA), with optimized settings for DNA isolated from FFPE tissue.[2] Library preparation of the fragmented DNA was performed with a KAPA Library Preparation kits (KAPA Biosystems, Wilmington MA, USA). Uniquely 8-bp indexed adapters (Roche Nimblegen, Madison WI, USA.) were ligated to the FFPE-extracted DNA followed by purification using AMPure XP beads (Beckman Coulter, Brea CA, USA), which resulted in a fragment size between 150 and 400 basepairs. Subsequently, a PCR amplification was performed with 7 cycles and library yield was assessed by measuring the DNA concentration using an Agilent Bioanalyzer DNA 1000 assay (Agilent Technologies, Santa Clara, CA, USA). Libraries with yield below 50ng were excluded for further analysis.

Shallow whole genome sequencing (WGS) for genome-wide DNA copy number analysis

For shallow WGS, up to 24 barcoded samples libraries were equimolarly pooled and 12.5pM was loaded per lane of a HiSeq Single End Flowcell (Illumina, San Diego CA, USA), followed by cluster generation on a cBot (Illumina, San Diego CA, USA). Sequencing was performed on a HiSeq2000 (Illumina, San Diego CA, USA) in a single-read 50-cycle run mode (SR50).
Shallow WGS reads were analyzed with the Bioconductor package QDNAseq (v1.5.1) [2] which infers copy numbers by a depth of coverage approach without the use of an external reference signal. QDNAseq aligns sequence reads to the human reference genome (GRCh37/hg19) with BWA (v0.7.5), [3] while removing PCR duplicates and reads with mapping qualities below 37 and concurrently dividing the genome into equally sized bins of 30k base pairs. A 2-dimensional Loess correction for GC content and sequence map ability is performed and a blacklist applied based on the 1000 Genomes Project [4] to filter out problematic regions and common regions of germ-line copy number variants.
The resulting copy number profiles were dewaved [5] and segmented. [6] Next, copy number aberrations (CNAs) were called into five discreet categories (homozygous deletion, loss, normal, gain, or amplification) with the Bioconductor package CGHcall (v2.30.0).[7] To reduce dimensions of the data set of 84 000 bins without losing information, CGHregions (v1.26.0; averror setting =

0.0075)[8] was used resulting in 142 chromosomal subregions. A Wilcoxon rank-sum test using 10 000 permutations was performed with CGHtest (v1.1)[9] to compare the distribution of CNAs for each chromosomal subregion. This test includes a permutation-based false discovery rate (FDR) correction for multiple testing. Separate analyses were performed for gains and losses, and chromosomal regions were considered significantly different between cohorts if $P < 0.05$ and $FDR < 0.1$.

Deep targeted sequencing for somatic mutations analysis

For target enrichment, sequence libraries were equimolarly pooled with 8 barcoded samples to a total mass of 1µg DNA. If this amount could not be reached i.e. due to poor DNA quality, a standard of 50ng per patient sample was taken. Libraries were enriched by double hybrid capture for a custom targeted panel using SeqCap EZ choice library capture reagents according to manufacturer's procedures (Roche Nimblegen, Madison WI, USA), covering 122 exons (~50.000 base pairs) of 11 frequently mutated genes in FL (Supplementary table S2). In case a total amount of 1ug DNA could not be reached, the amount of blocking oligonucleotides and EZ enrichment library was adjusted in a linear fashion. Enriched sequence libraries were multiplexed with a maximum of 24 libraries per lane and sequenced on a HiSeq 2000 (Illumina, San Diego CA, USA) in a paired-end 125-cycle mode.

NGS reads were de-multiplexed by Bcl2fastq (Illumina) and adapter sequences trimmed by Cutadapt (v1.6).[8] Subsequently, paired-end reads were aligned to the human reference genome (GRCh37/hg19) with BWA (v0.7.5).[3] Mapped reads were then marked for duplicates with Picard tools (v1.61) [(picard.sourceforge.net)]. Mutation calling was performed with VarScan2 (v2.3.7)[9] according to the following criteria: coverage depth > 20X, average read quality > 20, variant supporting reads >5 and variant allele frequency (VAF) > 10. Mismatches near a stretch of minimally 6 identical nucleotides were neglected. Functional annotation and effect prediction of called variants was performed with SnpEff (v4.1b)[10] Single nucleotide variants (SNVs) and small indels were labeled somatic if impact prediction was ‘high’ or if impact prediction was ‘moderate’ and the variant single nucleotide variant (SNV) was tagged as ‘uncommon’ according to the Single Nucleotide Polymorphism database (dbSNP build 142).[11] This classification eliminated germline SNVs, any synonymous mutation and intronic mutations with low predicted impact. For BCL2, all SNVs except for those with a 'common' dbSNP label were considered aberrant somatic hypermutation (aSHM). All downstream analyses were performed in the programming language R (version 3.2.1) with custom scripts.

Data availability

All sequence data has been uploaded to the European Genome-phenome Archive (EGA; accession number EGAS00001002049)

1. van Essen HF, Ylstra B. High-resolution copy number profiling by array CGH using DNA isolated from formalin-fixed, paraffin-embedded tissues. Methods in molecular biology. 2012;838(329-341.
2. Scheinin I, Sie D, Bengtsson H, et al. DNA copy number analysis of fresh and formalin-fixed specimens by shallow whole-genome sequencing with identification and exclusion of problematic regions in the genome assembly. Genome research. 2014;24(12):2022-2032.
3. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14):1754-1760.
4. Genomes Project C, Abecasis GR, Auton A, et al. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012;491(7422):56-65.
5. van de Wiel MA, Brosens R, Eilers PH, et al. Smoothing waves in array CGH tumor profiles. Bioinformatics. 2009;25(9):1099-1104.
6. Venkatraman ES, Olshen AB. A faster circular binary segmentation algorithm for the analysis of array CGH data. Bioinformatics. 2007;23(6):657-663.
7. van de Wiel MA, Kim KI, Vosse SJ, van Wieringen WN, Wilting SM, Ylstra B. CGHcall: calling aberrations for array CGH tumor profiles. Bioinformatics. 2007;23(7):892-894.
8. van de Wiel MA, Wieringen WN. CGHregions: dimension reduction for array CGH data with minimal information loss. Cancer informatics. 2007;3(55-63.
9. van de Wiel MA, Smeets SJ, Brakenhoff RH, Ylstra B. CGHMultiArray: exact P-values for multi-array comparative genomic hybridization data. Bioinformatics. 2005;21(14):3193-3194.
10. Martin M. Cutadapt removes adapter sequences from high- throughput sequencing reads. EMBnet Journal 2011;17(1):10-12.
11. Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. Nucleic acids research. 2001;29(1):308-311.

Supplementary tables

Table S1: Antibodies used for immunohistochemistry for T-cell subsets, macrophage subsets and tumor cell markers.

Antibody	Company	Working dilution
CD3	Labvision CD3-SP7	20:10 000
CD4	NCL-CD4-268	20:10 000
00CD8	Dako M7103clone CD8/144B	1:500
FOXp3	Abcam	10:1 000
PD1	Abcam	1:100
CD68KP1	Daco code M0814 clone KP1	2:16 000
CD163	Novacastra NCL-L-CD163	20:50 000
P53	Dako code M7001 clone D07	1:3 000
CD20	Dako code M0755 clone L26	10:20 000

Table S2: Custom LLBC hybrid-capture target enrichment panel

Gene	**Target**
KMT2D/MLL2	Entire CDS
CREBBP	Entire CDS
MEF2B	Exons 2, 3, 4, 9
EZH2	Exons 16, 18
EP300	HAT domain (exons 24-30)
BCL2	2800bp around TSS
FAS	Exons 7-9
TNFRSF14	Entire CDS
CARD11	Exons 5-9
TNFAIP3	Entire CDS
MYD88	Exons 3-5

Table S3: number of cases per immunohistochemical markers, which could be scored in the TMA (n=122), in 105 patients all immunohistochemical markers were scored on either core

Marker	No. of patients with core 1 not scored	No. of patients with core 2 not scored	No. of patients with both cores not scored	No. of patients with either core scored
CD3	20	18	12	110
CD4	17	20	12	110
CD8	18	18	13	109
FOXP3	21	18	13	109
PD1	24	19	15	107
P53	20	18	12	110
CD163	20	15	11	111
CD68	18	16	11	111

Table S4: Clinical characteristics of all 122 patients with immunohistochemical and/or molecular markers available.

	Total n = 122	**Early failure** n = 49	**Long remission** n = 73	***p***
Group				0.08
Barts	8 (7%)	6 (12%)	2 (3%)	
GLSG	99 (81%)	39 (80%)	60 (82%)	
LYSA	15 (12%)	4 (8%)	11 (15%)	
Age at diagnosis				0.11
Median (range)	60 (27 - 75)	62 (27 - 75)	58 (32 - 71)	
≤ 60	61 (50%)	21 (43%)	40 (55%)	
Sex				*0.58*
Female	64 (52%)	24 (49%)	40 (55%)	
Grade				*0.43*
Grade 1, 2	90 (74%)	35 (71%)	55 (75%)	
Grade 3A	7 (6%)	4 (8%)	3 (4%)	
Missing	25 (20%)	10 (20%)	15 (21%)	
Stage				*0.41*
Stage I-II	5 (4%)	2 (4%)	3 (4%)	
Stage III	35 (29%)	11 (22%)	24 (33%)	
Stage IV	81 (66%)	36 (73%)	45 (62%)	
Missing	1 (1%)	0	1 (1%)	
B-symptoms				*0.57*
Absent	73 (60%)	28 (57%)	45 (62%)	
Present	47 (39%)	21 (43%)	26 (36%)	
Missing	2 (2%)	0	2 (3%)	
ECOG PS				*0.23*
0	41 (34%)	14 (29%)	27 (37%)	
1	73 (60%)	29 (59%)	44 (60%)	
2	4 (3%)	3 (6%)	1 (1%)	
3	1 (1%)	1 (2%)	0 (0%)	
Missing	3 (2%)	2 (4%)	1 (1%)	
FLIPI risk categories				0.009
low	12 (10%)	2 (4%)	10 (14%)	
intermediate	47 (39%)	14 (29%)	33 (45%)	
high	57 (47%)	31 (63%)	26 (36%)	
missing	6 (5%)	2 (4%)	4 (5%)	
First line therapy				0.52
R-CHOP	106 (87%)	44 (90%)	62 (85%)	
R-CHOP-I	16 (13%)	5 (10%)	11 (15%)	

Abbreviations: Barts: Bartholomew's Hospital Registry London, GLSG: German low-grade Lymphoma Study Group, LYSA: the Lymphoma Study Association, ECOG: Eastern Cooperative Oncology Group, PS: performance score, FLIPI: follicular lymphoma international prognostic index. R-CHOP: rituximab, cyclophosphamide, adriamycin, vincristine and prednisone R-CHVP-I: rituximab, cyclophosphamide, adriamycin, etoposide, prednisolone and interferon-alpha2a

Table S5: distribution of investigated markers in the whole core, interfollicular and intrafollicular compartment in the two subgroups (n=96).
*P25= 25 thpercentile, **P75=75 th percentile

Marker		Early failure				Long remission				p
		P25* (%)	Median (%)	P75** (%)	Range (%)	P25* (%)	Median (%)	P75** (%)	Range (%)	
CD4	whole core	15.2	19.9	26.7	4 - 48.7	14.8	23.4	31.3	5.6 - 53.4	0.12
	interfollicular	17.2	21.8	29.4	3.7 - 49.3	16.9	26.8	34	6.5 - 56.7	0.13
	intrafollicular	10.6	15.6	21.5	3.6 - 47.4	10.9	18.6	28.6	1.3 - 50.8	0.26
CD8	whole core	5.2	7.9	9.5	1.5 - 24.6	7.2	8.6	14	3.8 - 29.4	0.011
	interfollicular	7	10.4	13.3	1.3 - 22.8	8.8	12.4	16.9	4.2 - 29.8	0.024
	intrafollicular	3.1	4.4	6.6	0.9 - 34.4	3.4	5.1	8.8	1.2 - 29.9	0.12
CD3	whole core	26.9	32.2	38.6	13.9 - 72.9	26.6	32.9	45.4	15.9 - 63.6	0.24
	interfollicular	29.6	35	46.5	11.8 - 71.9	32	39.7	49.4	19 - 63.8	0.42
	intrafollicular	18.3	23.4	28.4	12.9 - 77.5	17.9	23.5	35.4	9.4 - 62.5	0.6
FOXP3	whole core	3.7	6.4	8.6	0.4 - 12.4	4.1	5.8	9.1	1.7 - 21.8	>0.99
PD1	whole core	2.9	5.2	8.7	0.3 - 17.5	3.2	4.9	9.1	0.3 - 18.4	0.9
	interfollicular	1,8	3.3	6.4	0.1 - 18.8	2.4	3.8	7.9	0.3 - 18.3	0.6
	intrafollicular	4,3	7.2	11.6	0.2 - 19.6	4.5	7.5	11.7	0.2 - 20.8	0.7
CD68	whole core	3.9	5.8	7.1	2.4 - 14.2	4.5	5.8	7.8	2.8 - 13.7	0.5
	interfollicular	4.4	6.8	8.8	2.5 - 16.7	5.4	6.4	8.6	2.8 - 13.4	0.9
	intrafollicular	3.2	4.4	6.1	1.9 - 14	3.4	4.7	6.7	2.0 - 15	0.37
CD163	whole core	1.4	3.6	5.5	0.2 - 34.7	2.3	5.2	10.1	0.1 - 39.4	0.038
	interfollicular	1.7	4.2	7.9	0.4 - 36.2	3.3	7.4	15.5	0.1 - 39.4	0.031
	intrafollicular	0.8	1.7	2.8	0.1 - 30.8	1.3	1.9	4.7	0.1 - 39.7	0.17
P53	whole core	0.1	0.2	0.7	0 - 19.1	0.1	0.2	0.6	0 - 5.6	0.8
	interfollicular	0	0.2	0.5	0 - 12.6	0.1	0.2	0.5	0 - 3.7	0.8
	intrafollicular	0.1	0.3	0.7	0 - 22.1	0.1	0.4	0.8	0 - 7.1	0.5

Table S6: distribution of investigated markers in the whole core, interfollicular and intrafollicular compartment in the two subgroups (n=105).
*P25= 25 thpercentile, **P75=75 th percentile

Marker		Early failure				Long remission				p
		P25* (%)	Median (%)	P75** (%)	Range (%)	P25* (%)	Median (%)	P75** (%)	Range (%)	
CD4	whole core	15.1	19.9	27.2	4.0-48.7	13.6	22.8	31.2	0.1-53.4	0.29
	interfollicular	17.5	21.8	29.5	3.7-49.3	16.2	26.3	33.8	0.2-56.7	0.28
	intrafollicular	10.4	15.6	21.4	3.6-47.4	9.6	16.1	27.7	0.0-50.8	0.47
CD8	whole core	5.1	7.9	9.5	1.5-24.6	7.1	9.1	14.1	2.9-32.8	0.012
	interfollicular	7.1	10.4	13.5	1.3-22.8	8.6	12.4	17.0	4.2-31.8	0.026
	intrafollicular	3.0	4.4	6.6	0.9-34.4	3.4	4.9	9.0	1.2-41.8	0.12
CD3	whole core	26.9	32.2	38.9	13.9-72.9	26.5	32.9	45.4	15.9-84.6	0.25
	interfollicular	29.8	35	47.2	11.8-71.9	31.7	39.6	50.7	17.7-82.3	0.40
	intrafollicular	18.2	23.4	28.9	12.9-77.5	17.9	23.6	35.4	9.4-87.5	0.57
FOXP3	whole core	3.7	6.3	8.3	0.4-12.4	3.6	5.4	8.5	0.0-21.8	0.70
PD1	whole core	2.8	5.2	8.7	0.3-17.5	3.6	4.4	8.5	0.0-18.4	0.79
	interfollicular	1.7	3.3	6.3	0.1-18.8	2.0	3.7	7.4	0.0-18.3	0.92
	intrafollicular	4.0	7.2	11.6	0.2-20.4	3.9	7.4	10	0.0-20.8	0.88
CD68	whole core	3.9	5.8	7.2	2.4-14.2	4.2	5.8	7.7	0.2-19.6	0.93
	interfollicular	4.4	6.8	9.2	2.5-16.7	4.9	6.4	8.7	0.1-19.7	0.52
	intrafollicular	3.2	4.4	6.1	1.9-14.0	3.1	4.6	6.5	0.3-22.1	0.68
CD163	whole core	1.6	3.6	5.7	0.2-34.7	2.4	5.9	11.1	0.1-39.4	0.027
	interfollicular	1.9	4.5	8.0	0.4-36.2	3.5	8.8	17.0	0.1-39.4	0.021
	intrafollicular	0.8	1.7	2.8	0.1-30.8	1.3	1.8	4.7	0.1-39.7	0.23
P53	whole core	0.0	0.2	0.7	0.0-19.1	0.1	0.2	0.5	0.0-5.6	0.98
	interfollicular	0.0	0.1	0.5	0.0-12.6	0.0	0.1	0.5	0.0-3.7	0.94
	intrafollicular	0.1	0.3	0.7	0.0-22.1	0.1	0.3	0.7	0.0-7.1	0.79

Table S7: Odds ratio (OR) (95% CI) for a 10% change in the IHC markers from univariate analysis, and multivariate analysis without and with the FLIPI of the whole core (n=96).

	Univariate		**Multivariable without FLIPI**		**Multivariable with FLIPI**	
	OR (95% CI)	***p***	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***
%CD4	1.36 (0.92, 2.06)	0.13	1.2 (0.7, 2.2)	0.6	1.21 (0.65, 2.28)	0.5
%CD8	3.86 (1.48, 12.13)	0.011	4.5 (1.1, 21.2)	0.041	3.63 (0.89, 17.08)	0,084
%P53	0.15 (0.0, 1.10)	0.16	0.9 (0.6, 1.1)	0.27	0.84 (0.57, 1.06)	0.23
%PD1	1.00 (0.39, 2.58)	>0.99	1.0 (0.3, 3.3)	>0.99	1.13 (0.32, 4.06)	0.9
%CD163	2.01 (1.11, 4.37)	0.042	1.74 (0.9, 4.2)	0.17	1.69 (0.83, 4.17)	0.19
%CD68	1.33 (0.31, 6.09)	0.7	0.8 (0.1, 5.7)	0.8	1.24 (0.16, 9.38)	0.8
%FOXP3	1.45 (0.47, 4.85)	0.5	0.9 (0.2, 4.1)	0.9	1.13 (0.25, 5.53)	0.9
%CD3	1.30 (0.91, 1.91)	0.16	0.8 (0.4, 1.4)	0.37	0.70 (0.34, 1.39)	0.31
FLIPI, high	0.28 (0.11, 0.66)	0.005			0.31 (0.12, 0.79)	0.016

Table S8: OR (95% CI) for a 10% change in the markers from univariate analysis, and multivariate analysis without and with the FLIPI of the interfollicular compartment (n=96).

	Univariate		**Multivariable without FLIPI**		**Multivariable with FLIPI**	
	OR (95% CI)	***p***	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***
%CD4	1.33 (0.91, 1.99)	0.14	1.37 (0.80, 2.4)	0.26	1.34 (0.77, 2.41)	0.30
%CD8	2.59 (1.16, 6.36)	0.03	3.72 (1.18, 13.52)	< 0.01	3.18 (0.98, 11.81)	0,07
%P53	0.08 (0.00, 1.41)	0.19	0.11 (0.00, 2.26)	0.23	0.08 (0.00, 2.09)	0.20
%PD1	1.10 (0.42, 2.95)	0.85	1.24 (0.35, 4.52)	0.74	1.22 (0.32, 4.7)	0.77
%CD163	1.92 (1.14, 3.63)	0.03	1.67 (0.92, 3.4)	0.12	1.59 (0.85, 3.31)	0.17
%CD68	0.81 (0.23, 2.92)	0.75	0.50 (0.09, 2.56)	0.41	0.68 (0.11, 3.78)	0.66
%CD3	1.15 (0.82, 1.63)	0.42	0.68 (0.37, 1.21)	0.20	0.71 (0.38, 1.27)	0.26
FLIPI, high	0.28 (0.11, 0.66)	< 0.01			0.33 (0.12, 0.83)	< 0.01

Table S9: OR (95% CI) for a 10% change in the markers from univariate analysis, and multivariate analysis without and with the FLIPI of the intrafollicular compartment (n=96).

	Univariate		Multivariable without FLIPI		Multivariable with FLIPI	
	OR (95% CI)	***p***	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***
%CD4	1.36 (0.94, 2.04)	0.12	1.58 (0.90, 2.91)	0.12	1.64 (0.91, 3.11)	0.11
%CD8	2.03 (0.91, 5.94)	0.13	2.28 (0.65, 9.46)	0.22	1.98 (0.53, 8.79)	0.33
%P53	0.27 (0.02, 1.16)	0.16	0.24 (0.02, 1.12)	0.14	0.22 (0.01, 1.18)	0.15
%PD1	1.16 (0.53, 2.55)	0.71	1.41 (0.51, 4.09)	0.51	1.58 (0.53, 4.98)	0.42
%CD163	1.54 (0.82, 3.73)	0.24	1.21 (0.58, 3.16)	0.64	1.26 (0.59, 3.34)	0.58
%CD68	1.72 (0.37, 9.15)	0.50	1.43 (0.20, 11.21)	0.73	2.51 (0.31, 22.75)	0.39
%CD3	1.18 (0.84, 1.72)	0.37	0.58 (0.26, 1.23)	0.16	0.55 (0.23, 1.21)	0.15
FLIPI, high	0.28 (0.11, 0.66)	< 0.01			0.26 (0.10, 0.65)	< 0.01

Table S10: FOXP3 perifollicular patterns by cohort based on agreement scores of three independent pathologists

FOXP3 perifollicular pattern	**Total n=96**	**Early failure n=39**	**Long remission n=57**	**P**
Positive	21 (22%)	10 (26%)	11 (19%)	0.46
Negative	75 (78%)	29 (74%)	46 (81%)	

Table S11: Frequencies and statistics of copy number gains and losses per chromosomal region by subgroup

Table S12: Somatic variants from targeted resequencing

Table S13: Distribution of gene mutation status by subgroup (n=111)

	Total n=111 (%)	Early failure n=47 (%)	Long remission n=64 (%)	OR [95% CI]	*p (unadjusted)*
BCL2					
Mutated	103 (93)	45 (96)	58 (91)	0.43 [0.04 - 2.57]	*0.46*
Unmutated	8 (7)	2 (4)	6 (9)		
KMT2D					
Mutated	80 (72)	35 (74)	45 (70)	0.81 [0.31 - 2.04]	*0.7*
Unmutated	31 (28)	12 (26)	19 (30)		
CREBBP					
Mutated	72 (65)	34 (72)	38 (59)	0.56 [0.23 - 1.35]	*0.17*
Unmutated	39 (35)	13 (28)	26 (41)		
TNFRSF14					
Mutated	33 (30)	13 (28)	20 (31)	1.19 [0.48 – 2.99]	*0.8*
Unmutated	78 (70)	34 (72)	44 (69)		
MEF2B					
Mutated	12 (11)	5 (11)	7 (11)	1.03 [0.26 - 4.42]	*> 0.99*
Unmutated	99 (89)	42 (89)	57 (89)		
EZH2					
Mutated	23 (21)	4 (9)	19 (30)	4.48 [1.34 - 19.59]	*0.008*
Unmutated	88 (79)	43 (91)	45 (70)		
TNFAIP3					
Mutated	9 (8)	2 (4)	7 (11)	2.74 [0.49 - 28.30]	*0.30*
Unmutated	102 (92)	45 (96)	57 (89)		
EP300					
Mutated	7 (6)	2 (4)	5 (8)	1.90 [0.29 - 20.78]	*0.7*
Unmutated	104 (94)	45 (96)	59 (92)		
CARD11					
Mutated	9 (8)	4 (9)	5 (8)	0.91 [0.18 - 4.88]	*> 0.99*
Unmutated	102 (92)	43 (91)	59 (92)		
FAS					
Mutated	4 (4)	4 (9)	0 (0)	0.00 [0.00 – 1.08]	*0.030*
Unmutated	107 (96)	43 (91)	64 (100)		
MYD88					
Mutated	2 (2)	1 (2)	1 (2)	0.73 [0.01 - 58.52]	*> 0.99*
Unmutated	109 (98)	46 (98)	63 (98)		

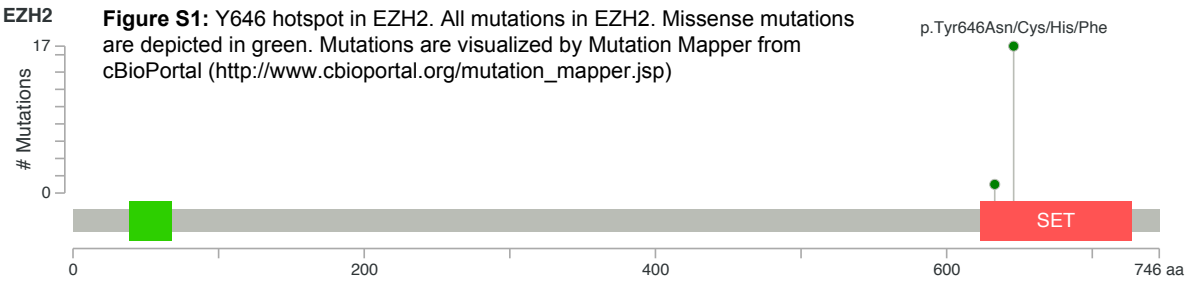

Figure S1: Y646 hotspot in EZH2. All mutations in EZH2. Missense mutations are depicted in green. Mutations are visualized by Mutation Mapper from cBioPortal (http://www.cbioportal.org/mutation_mapper.jsp)