SUPPLEMENTARY APPENDIX

Targeted genotyping of circulating tumor DNA for classical Hodgkin lymphoma monitoring: a prospective study

Vincent Camus,[1,2] Mathieu Viennot,[2] Justine Lequesne,[3] Pierre-Julien Viailly,[2] Elodie Bohers,[2] Lucile Bessi,[2] Bénédicte Marcq,[1,2] Pascaline Etancelin,[2,4] Sydney Dubois,[1,2] Jean Michel Picquenot,[2,5] Elena-Liana Veresezan,[2,5] Marie Cornic,[3] Lucie Burel,[3] Justine Loret,[3] Stéphanie Becker,[6] Pierre Decazes,[6] Pascal Lenain,[1] Stéphane Lepretre,[1,2] Emilie Lemasle,[1] Hélène Lanic,[1] Anne-Lise Ménard,[1] Nathalie Contentin,[1] Hervé Tilly,[1,2] Aspasia Stamatoullas[1,2] and Fabrice Jardin[1,2]

[1]Department of Hematology, Center Henri Becquerel; [2]University of Rouen, INSERM U1245, Center Henri Becquerel; [3]Clinical Research Unit, Centre Henri Becquerel; [4]Department of Genetic Oncology, Center Henri Becquerel; [5]Department of Pathology, Center Henri Becquerel and [6]Department of Nuclear Medicine and Radiology, Center Henri Becquerel and QuantIF (Litis EA4108 – FR CNRS 3638), Rouen, France

 doi:10.3324/haematol.2019.237719

Received: October 16, 2019.

Accepted: February 12, 2020.

Pre-published: February 20, 2020.

Correspondence: *VINCENT CAMUS* - vincent.camus@chb.unicancer.fr

Supplemental data

Supplementary methods:

Patients

A universal patient identification number (UPN) was created for each patient to describe results.

DNA extraction

Circulating cell-free DNA (cfDNA) was extracted from 3mL of plasma aliquots with Amp Circulating Nucleic Acid® QI Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA was eluted in 60 to 80 µL of AVE buffer then stored at -80 ° C. Quantification of the double-stranded DNA was performed by fluorometry on Qubit 2.0 (Thermo Fisher Scientific Carlsbad, CA, USA), with Qubit® dsDNA kit HS Assay (Thermo Fisher Scientific, Carlsbad, CA, USA). DNA extraction from FFPE (formalin fixed paraffin embedded) biopsies was performed using the Maxwell® 16 (Promega, Madison, USA) and the Maxwell 16 FFPE Plus LEV DNA Purification® (Promega, Madison, USA) kits. The genomic DNA (gDNA) thus extracted was "repaired" by NEB Next FFPE DNA Repair Mix® (New England Biolab, Ipswich, USA) before being quantified by fluorimetry and stored at -20 ° C.

Chemotherapy regimens and radiotherapy

Patients with favorable localized stage I-II disease received 2 cycles of ABVD (doxorubicin, bleomycin, vinblastin, dacarbazine) followed by 20-Gray involved-field radiotherapy (IFRT); unfavorable localized stage I-II received 4 cycles of ABVD followed by 30-Gray IFRT [10,11]. Advanced stage disease patients < 60 year old received a treatment strategy driven by PET (according to AHL2011 trial results [12]) : all patients received two cycles of upfront BEACOPPescalated (Increased-dose bleomycin, etoposide, doxorubicin, cyclophosphamide,

vincristine, procarbazine, and prednisone), after which PET assessment was performed (PET2). Patients with a positive PET2 scan received the further four cycles of BEACOPPescalated and those with a negative PET2 scan switched to four cycles of ABVD for the remaining induction therapy. In both treatment groups, PET after four cycles of chemotherapy was used to decide whether to continue with the same treatment in those with negative PET(either two cycles of ABVD or two cycles of BEACOPPescalated) or start salvage therapy in patients with positive PET. Regarding elderly (>60 years old) advanced stage patients : they received 6-8 cycles of ABVD with or without bleomycin[13] or another conventional chemotherapy (procarbazine and vinblastine-based regimen) or inclusion in a therapeutic clinical trial according to the physician's decision.

Positron emission tomography (PET) evaluation

PET evaluation at diagnosis and during treatment was performed according to routine local recommendations for the management of patients with a diagnosis of *de novo* cHL. The response to treatment was evaluated with ^{18}F-fluorodeoxyglucose (FDG) PET by visually comparing the metabolic uptake of the area involved in diagnosis versus metabolic uptake of the mediastinum and of the liver, considered as reference organs according to the Deauville score [12]. Two independent readings by two different nuclear medicine physicians were performed. Metabolic response was also assessed according to the Lugano criteria. Complete metabolic response was defined as negative FDG PET (Deauville score at 1, 2or 3) with or without a non-fixing residual mass. Partial metabolic response was defined as FDG PET with Deauville score at 4 or 5 with reduction of intensity uptake compared to the examination carried out at the diagnosis whatever the size of the residual mass, without appearance of new lesions. Metabolic non response was defined by a Deauville score at 4 or 5 without significant change in FDG uptake compared to the diagnostic examination performed without appearance of new lesions. Metabolic progression was defined as a score of 4 or 5 with increased FDG

uptake (SUV) of a hypermetabolic lesion compared to the examination performed at diagnosis or by the appearance of a new FDG-hypermetabolic focus compatible with a lymphoma.

Next-Generation Sequencing

Next-generation sequencing of targeted genes was performed on Ion Torrent Personal Genome Machine ™ (PGM, Thermo Fisher Scientific). The first step is the preparation of the libraries: a multiplex PCR is performed with primers specifically chosen to target the 4 base pair hotspot deletion in gene exon 1 *NFKBIE*, the coding regions of the genes *ITPKB* (exons 2 to 8), *PTPN1* (exons 1 to 10), *TNFAIP3* (exons 2 to 9), SOCS1 (exon 2), exons 12 and 14 of the *STAT6* gene, exons 1 to 3 of the *B2M* gene, exons 15 to 18 of the *XPO1* gene, and exons 1 to 4 of the *GNA13* gene (See **Supplementary Table** 1 for details of amplicons). The design of the PCR primers was performed using the AmpliSeq ™ Designer tool (Thermo Fisher Scientific). 169 primer pairs were divided into 3 pools. This panel covers 12.36 kb of the genome. The minimum and maximum sizes of the amplicons are respectively 125 and 175pb. Adapters, nucleotide sequences involved in the clonal amplification and sequencing steps, are then ligated to the amplicons. The second step is an emulsion PCR, which clonally amplifies each fragment of the library. This is performed in microreactors (lipid droplets) containing the elements necessary for the PCR (library, nucleotides, polymerase) and 20µM spheres called ISP (Ion Sphere Particles), lined with PCR primers complementary to one of the adapters. This step requires precise dosing of the library so as to obtain equimolarity between fragments of the library and microreactors. At the end of the PCR, the ISPs are lined with amplified nucleotide sequences. Several configurations of microreactors can be created. Only the monoclonality configuration (1 sphere, 1 DNA fragment) is desired. The enrichment step then makes it possible to eliminate the ISPs without amplicon, but not the bi or poly clonal ISPs, and to prepare the sequencing matrix that will be loaded on the sequencing chip. The clonal

PCR steps, enrichment and loading of the sequencing chips (deposit of an ISP in a well of a chip), are carried out by the Ion Chef ™ System with the PGM Ion Hi-Q View Chef ™ kit. Sequencing is addition sequencing: the incorporation of a nucleotide complementary to the nucleotide to be identified is associated with the release of a proton, resulting in a pH variation detected by a semiconductor located at the bottom of each well. For all samples (DNA extracted from biopsy or plasma DNA), 3 PCRs were performed, one per primer pool, with a total amount of 100ng DNA for the DNA samples extracted from biopsy and 6µL of PCR test sample for plasma DNA samples (total variable DNA due to different initial concentrations). The products of the 3 PCRs were then pooled, before ligation of the adapters. 22 PCR cycles were performed. For all samples, the library concentration was standardized with the Ion Library Equalizer kit (Thermo Fisher Scientific) according to the manufacturer's recommendations. 8 samples were sequenced per chip (Ion 318 ™ v2 Chip, Life Technologies, Waltham, MA). Bioinformatic analysis of the data was performed by software builders for base-calling (Variant Caller), alignment and quality control (Torrent Suite, Coverage Analysis). According to pathologists, the average percentage of tumor cells in cHL is 3%. Based on a theoretical sensitivity of 1%, and on a minimum number of mutated reads equal to 50, the minimum depth of desired sequencing was set at 5000X. Annotation of variants was created after aggregation of information from the RefSeq gene, RefSeq mRNA, 1000 genomes, ExAc, cg40, ESP, COSMIC (v64), dbSNP, and ClinVar. Variants with depth <100x or with number of mutated reads <6 were eliminated. Variants related to residual technical artifacts were filtered, as well as those with negative SIFT and CADD scores. All variant with > 1% minor allele frequency (MAF) in these databases are considered as SNPs and were not considered as somatic variant. Constitutional SNPs may be present in the plasma of patients with 50% or 100% VAF, but we did not consider them as to be positive results in our somatic mutations detection process. We had access to peripheral blood mononuclear

cells (PBMC) germline DNA for each patient in case of any ambiguities for variant classification after consulting the reference databases. Regarding technical detection limits, taking into account previous experiences in our laboratory, we considered a lower limit of detection of 0.5% VAF making it possible to eliminate more than 95% of the low frequency variants probably corresponding to background noise. For ctDNA analysis after two cycles of chemotherapy, we performed a blind search for new variants and a manual check of all mutations that were present at diagnosis on Integrative Genomics Viewer (IGV). Samples at diagnostic and after C2 were all treated in the same way. The desired depth was the same as for the diagnostic sample.

Statistical analysis

The characteristics of the sample were described using numbers and percentages for qualitative variables, and by mean, median, standard deviation and extreme values for quantitative variables. Comparison of characteristics according to the mutational profile of patients was established by a Chi2 test (or Fisher's exact test) for qualitative variables, and a non-parametric Wilcoxon Mann Whitney test for quantitative variables. Plasma DNA concentrations at diagnosis and median VAFs of plasma variants at diagnosis were compared according to different clinical and biological characteristics by the Wilcoxon Mann Whitney test. Their correlation with continuous variables was measured by the Pearson and Spearman coefficients, so as to highlight first a possible linear relationship, then monotonous otherwise. Overall survival (OS) in months was calculated from the date of diagnosis to the date of death from any cause or the date of last follow-up while alive. Progression-free survival (PFS) in months was calculated from the date of diagnosis until disease progression, relapse or death from any cause or the last patient follow-up. Estimates of survival were calculated using the Kaplan-Meier method. The median VAF comparison of the variants detected in the biopsy and matched plasma were

evaluated by the non-parametric Wilcoxon signed-rank test. The level of significance retained for each test is 5%. Statistics were performed with R software v3.3.2.

Supplementary Table 1 : Chromosomal localization of Hodgkin-Panel amplicons

Chromosome	gene	Start region	Stop region
chr1	*ITPKB*	226822316	226822405
chr1	*ITPKB*	226822405	226822505
chr1	*ITPKB*	226822491	226822602
chr1	*ITPKB*	226822585	226822688
chr1	*ITPKB*	226825374	226825456
chr1	*ITPKB*	226827245	226827341
chr1	*ITPKB*	226827330	226827412
chr1	*ITPKB*	226829536	226829625
chr1	*ITPKB*	226829544	226829677
chr1	*ITPKB*	226829643	226829739
chr1	*ITPKB*	226829729	226829826
chr1	*ITPKB*	226829785	226829872
chr1	*ITPKB*	226834784	226834884
chr1	*ITPKB*	226834878	226834977
chr1	*ITPKB*	226834939	226835026
chr1	*ITPKB*	226834997	226835087
chr1	*ITPKB*	226836367	226836447
chr1	*ITPKB*	226836427	226836498
chr1	*ITPKB*	226923200	226923294
chr1	*ITPKB*	226923263	226923358
chr1	*ITPKB*	226923344	226923436
chr1	*ITPKB*	226923379	226923477
chr1	*ITPKB*	226923441	226923538
chr1	*ITPKB*	226923501	226923600
chr1	*ITPKB*	226923546	226923633
chr1	*ITPKB*	226923622	226923723
chr1	*ITPKB*	226923716	226923818
chr1	*ITPKB*	226923738	226923837
chr1	*ITPKB*	226923838	226923938
chr1	*ITPKB*	22692 52	226923957
chr1	*ITPKB*	226923992	226924092
chr1	*ITPKB*	226923999	226924100
chr1	*ITPKB*	226924114	226924211
chr1	*ITPKB*	226924169	226924279

chr1	*ITPKB*	226924278	226924367
chr1	*ITPKB*	226924356	226924446
chr1	*ITPKB*	226924434	226924522
chr1	*ITPKB*	226924515	226924603
chr1	*ITPKB*	226924601	226924697
chr1	*ITPKB*	226924623	226924726
chr1	*ITPKB*	226924740	226924841
chr1	*ITPKB*	226924754	226924858
chr1	*ITPKB*	226924886	226925012
chr1	*ITPKB*	226925028	226925155
chr12	*STAT6*	57493815	57493912
chr12	*STAT6*	57496604	57496704
chr15	*B2M*	45003707	45003801
chr15	*B2M*	45003792	45003889
chr15	*B2M*	45007590	45007673
chr15	*B2M*	45007662	45007755
chr15	*B2M*	45007744	45007830
chr15	*B2M*	45007819	45007902
chr15	*B2M*	45007891	45007982
chr15	*B2M*	45008469	45008549
chr16	*SOCS1*	11348660	11348754
chr16	*SOCS1*	11348771	11348873
chr16	*SOCS1*	11348773	11348894
chr16	*SOCS1*	11348888	11348973
chr16	*SOCS1*	11348906	11348995
chr16	*SOCS1*	11348995	11349095
chr16	*SOCS1*	11349015	11349152
chr16	*SOCS1*	11349158	11349246
chr16	*SOCS1*	11349164	11349297
chr16	*SOCS1*	11349181	11349283
chr16	*SOCS1*	11349301	11349392
chr17	*GNA13*	63010304	63010390
chr17	*GNA13*	63010380	63010466
chr17	*GNA13*	63010452	63010539
chr17	*GNA13*	63010528	63010618
chr17	*GNA13*	63010609	63010678
chr17	*GNA13*	63010667	63010748
chr17	*GNA13*	63010737	63010829
chr17	*GNA13*	63010818	63010907
chr17	*GNA13*	63010898	63010968
chr17	*GNA13*	63014328	63014410
chr17	*GNA13*	63014399	63014473
chr17	*GNA13*	63049550	63049641
chr17	*GNA13*	63049630	63049699
chr17	*GNA13*	63049687	63049758

chr17	*GNA13*	63049742	63049834
chr17	*GNA13*	63049821	63049943
chr17	*GNA13*	63049844	63049923
chr17	*GNA13*	63052337	63052437
chr17	*GNA13*	63052440	63052572
chr17	*GNA13*	63052454	63052551
chr17	*GNA13*	63052511	63052609
chr17	*GNA13*	63052601	63052700
chr2	*XPO1*	61715668	61715750
chr2	*XPO1*	61715739	61715827
chr2	*XPO1*	61715816	61715891
chr2	*XPO1*	61717742	61717815
chr2	*XPO1*	61717804	61717886
chr2	*XPO1*	61717873	61717942
chr2	*XPO1*	61719158	61719225
chr2	*XPO1*	61719210	61719279
chr2	*XPO1*	61719268	61719349
chr2	*XPO1*	61719408	61719493
chr2	*XPO1*	61719475	61719556
chr2	*XPO1*	61719513	61719591
chr2	*XPO1*	61719589	61719667
chr20	*PTPN1*	49127098	49127198
chr20	*PTPN1*	49177838	49177931
chr20	*PTPN1*	49177920	49178009
chr20	*PTPN1*	49181498	49181586
chr20	*PTPN1*	49181581	49181656
chr20	*PTPN1*	49184885	49184978
chr20	*PTPN1*	49184959	49185036
chr20	*PTPN1*	49191006	49191082
chr20	*PTPN1*	49191072	49191145
chr20	*PTPN1*	49191103	49191196
chr20	*PTPN1*	49194885	49194973
chr20	*PTPN1*	49194962	49195031
chr20	*PTPN1*	49195028	49195123
chr20	*PTPN1*	49195113	49195197
chr20	*PTPN1*	49195157	49195248
chr20	*PTPN1*	49195664	49195745
chr20	*PTPN1*	49195734	49195828
chr20	*PTPN1*	49195826	49195910
chr20	*PTPN1*	49196218	49196313
chr20	*PTPN1*	49196257	49196350
chr20	*PTPN1*	49196338	49196431
chr20	*PTPN1*	49196383	49196468
chr20	*PTPN1*	49197739	49197817
chr20	*PTPN1*	49197817	49197911

chr20	*PTPN1*	49197909	49198002
chr20	*PTPN1*	49199140	49199240
chr20	*PTPN1*	49199204	49199309
chr6	*NFKBIE*	44232727	44232827
chr6	*TNFAIP3*	138192300	138192387
chr6	*TNFAIP3*	138192376	138192464
chr6	*TNFAIP3*	138192453	138192529
chr6	*TNFAIP3*	138192518	138192595
chr6	*TNFAIP3*	138192584	138192671
chr6	*TNFAIP3*	138195940	138196035
chr6	*TNFAIP3*	138196024	138196112
chr6	*TNFAIP3*	138196101	138196184
chr6	*TNFAIP3*	138196778	138196855
chr6	*TNFAIP3*	138196844	138196915
chr6	*TNFAIP3*	138196904	138196982
chr6	*TNFAIP3*	138197108	138197187
chr6	*TNFAIP3*	138197172	138197265
chr6	*TNFAIP3*	138197258	138197334
chr6	*TNFAIP3*	138198185	138198266
chr6	*TNFAIP3*	138198244	138198315
chr6	*TNFAIP3*	138198305	138198389
chr6	*TNFAIP3*	138198379	138198470
chr6	*TNFAIP3*	138199540	138199618
chr6	*TNFAIP3*	138199607	138199689
chr6	*TNFAIP3*	138199645	138199736
chr6	*TNFAIP3*	138199736	138199821
chr6	*TNFAIP3*	138199810	138199907
chr6	*TNFAIP3*	138199918	138200005
chr6	*TNFAIP3*	138199936	138200031
chr6	*TNFAIP3*	138200012	138200111
chr6	*TNFAIP3*	138200081	138200171
chr6	*TNFAIP3*	138200168	138200266
chr6	*TNFAIP3*	138200269	138200357
chr6	*TNFAIP3*	138200332	138200414
chr6	*TNFAIP3*	138200372	138200461
chr6	*TNFAIP3*	138200450	138200536
chr6	*TNFAIP3*	138201146	138201226
chr6	*TNFAIP3*	138201215	138201294
chr6	*TNFAIP3*	138201267	138201345
chr6	*TNFAIP3*	138201332	138201422
chr6	*TNFAIP3*	138202114	138202204
chr6	*TNFAIP3*	138202193	138202293
chr6	*TNFAIP3*	138202293	138202380
chr6	*TNFAIP3*	138202307	138202402
chr6	*TNFAIP3*	138202401	138202494

Supplementary Table 2 : Somatic variants detected by high throughput DNA sequencing of biopsy (gDNA) and plasma (ctDNA) samples at diagnosis (baseline) and after 2 cycles of chemotherapy (C2). The coverage (sequencing depth) is expressed in number (n) of reads analyzed.

Universal Patient identification number (UPN)	**Somatic mutation**		**Genomic DNA (biopsy)**		**ctDNA (plasma)**			
	Gene	variant	VAF (%)	concentration (ng/mL)	VAF (%)	Coverage baseline (n)	Coverage Post C2 (n)	haploid genome equivalents per ml (hGE/ml)
UPN1	*SOCS1*	P.Y203S	0.56	15	0.62	4509	6126	37.63
UPN2	*B2M*	p.M1K	1.68	11.2	1.14	3519	3061	58.37
	NFBKBIE	p.Y254FS	2.24		0.94	4946	3565	
	SOCS1	p.R69FS	3.06		0.77	2178	836	
	B2M	p.L10FS	1.4		undetectable	undetectable	undetectable	
UPN3	*B2M*	UTR5	1.13	14.6	8.6	3589	2785	1677.88
	NFKBIE	P.Y254FS	4.63		21.4	3632	2872	
	STAT6	P.N417S	1.34		12.4	5375	3948	
	ITPKB	P.A290G	1.9		14.9	6748	5707	
UPN4	*ITPKB*	P.G260R	2.51	8.9	undetectable	undetectable	undetectable	35.18
	STAT6	P.N421K	1.47		undetectable	undetectable	undetectable	
	B2M	P.C100X	2.06		0.6	4173	4637	

	SOCS1	P.F79I	2.63		undetectable	undetectable	undetectable	
	XPO1	P.E571K	1.23		0.8	4998	3643	
	ITPKB	P.R331P	1.42		undetectable	undetectable	undetectable	
	ITPKB	P.A298P	1.33		undetectable	undetectable	undetectable	
	ITPKB	P.L8V	1.43		undetectable	undetectable	undetectable	
	SOCS1	P.L166Q	1.6		0.52	4635	5332	
	SOCS1	P.P123A	1.37		undetectable	undetectable	undetectable	
	SOCS1	p.G122fs	undetectable		1.9	9604	9358	
UPN5	*NFKBIE*	P.Y254FS	2.77	10.8	0.6	4759	4912	39.11
	SOCS1	P.P165FS	2.4		undetectable	undetectable	undetectable	
	B2M	P.L10F	undetectable		0.52	3089	3593	
	ITPKB	P.P37L	undetectable		0.74	2307	2352	
	ITPKB	P.P36S	undetectable		0.95	2267	2351	
	B2M	p.Q22X	0.56		undetectable	undetectable	undetectable	
UPN6	no variant		no variant	4.5	no variant	no variant	no variant	0
UPN7	*TNFAIP3*	P.E132X	1.89	18.9	10.1	4358	4435	531.34
	SOCS1	P.R169S	4.34		23.25	4196	4321	
	SOCS1	P.160_167DEL	3.25		17.16	3637	4354	
	SOCS1	P.138_143DEL	undetectable		0.66	7739	8167	
UPN8	*XPO1*	P.E571G	1.6	16.3	11.6	3288	3792	651.67
	SOCS1	P.P165FS	2.09		9.9	2587	5880	
	ITPKB	P.C5FS	2.9		5.5	1750	3165	
	ITPKB	P.S16R	2.77		5.4	1747	3183	
	ITPKB	P.S410N	3.62		12.9	8237	14053	
	TNFAIP3	P.G440X	0.86		4.2	5174	5443	
	TNFAIP3	P.L227FS	0.96		8.8	4324	5120	

	ITPKB	P.A272T	2.15		7.87	4334	6821	
UPN9	*SOCS1*	P.H54FS	0.73	12.9	1.9	1022	2014	64.56
	NFKBIE	P.Y254FS	undetectable		0.84	4257	4650	
UPN10	*B2M*	P.M1T	2.35	4.2	2.4	3587	loss of follow-up	944.16
	STAT6	P.D519N	2.43		4.3	3406	loss of follow-up	
	STAT6	P.N417Y	1.92		5.1	6442	loss of follow-up	
	TNFAIP3	P.C627FS	2.16		4.75	8160	loss of follow-up	
	TNFAIP3	P.R410FS	2.37		4.7	3948	loss of follow-up	
	ITPKB	P.M257FS	1.63		9.2	109	loss of follow-up	
	SOCS1	P.S116R	2.76		2.2	4092	loss of follow-up	
UPN11	no variant		no variant	12.9	no variant	no variant	no variant	0
UPN12	*STAT6*	P.N417Y	3.14	6.2	1.92	5414	4115	39.48
	TNFAIP3	P.E297X	1.6		0.53	3936	3395	
	B2M	P.L13R	3.04		undetectable	undetectable	undetectable	
	GNA13	P.K121X	2.2		0.96	2598	2790	
	SOCS1	P.H4FS	0.54		0.54	4604	4069	
	SOCS1	UTR3	0.74		undetectable	undetectable	undetectable	
	SOCS1	P.H87FS	0.6		undetectable	undetectable	undetectable	
	XPO1	P.E571K	undetectable		0.57	4769	2433	
UPN13	*B2M*	P.M1T	1.43	5.8	7.7	3704	4237	251.52
	SOCS1	P.S29FS	3.4		4.6	2347	4129	
	SOCS1	P.V199A	1.51		2.61	5443	6438	
	SOCS1	P.H136L	0.69		1.28	9971	8716	
UPN14	*SOCS1*	P.Y64X	1.68	10.6	4.5	491	1187	2684.93
	SOCS1	P.F58L	undetectable		2.4	702	2355	
	SOCS1	p.H54D	undetectable		2.08	864	2807	
UPN15	no variant		no variant	11.4	no variant	no variant	no variant	0
UPN16	*B2M*	P.Y98N	1.63	9.6	2.5	3388	3461	135.81
	SOCS1	P.S106R	3.88		2.8	3592	3214	
	B2M	P.L15FS	1.98		1.9	2857	2329	

	TNFAIP3	P.G482FS	1.71		1.1	8334	6403	
	SOCS1	P.Q131E	1.89		2.64	9806	7334	
	SOCS1	UTR5	1.77		1.76	4832	4010	
UPN17	*ITPKB*	P.K232FS	2.6	18.9	10.6	4221	5043	830.87
UPN18	*STAT6*	P.N417Y	1	12.6	undetectable	undetectable	undetectable	9.67
	SOCS1	P.T100I	0.81		undetectable	undetectable	undetectable	
	SOCS1	P.M1I	0.9		undetectable	undetectable	undetectable	
	GNA13	P.Q27X	0.7		undetectable	undetectable	undetectable	
	SOCS1	P.P165S	undetectable		0.93	3563	4988	
UPN19	no variant		no variant	11	no variant	no variant	no variant	0
UPN20	*SOCS1*	P.Q108H	2.02	11.5	4.5	1716	2043	210.02
	SOCS1	P.A120P	0.58		4.25	2305	2921	
	SOCS1	P.L93FS	1.05		1.8	3769	4356	
	~~*SOCS1*~~	~~P.L12Q~~	~~1.03~~		~~0.87~~	-	-	
	SOCS1	P.E91G	1.06		2.02	3662	4365	
	SOCS1	P.I67FS	undetectable		0.83	2056	2344	
	TNFAIP3	P.S592FS	undetectable		4.9	4945	7336	
	XPO1	P.E571K	undetectable		3.3	3918	2973	
	ITPKB	P.408_411DEL	undetectable		5.13	7699	7786	
	B2M	P.L12Q	1.03		0.87	3234	4029	
UPN21	TNFAIP3	p.R162fs	undetectable	12.6	1.77	1300	dead before C2	271.4
UPN22	*STAT6*	P.D419Y	undetectable	16.6	2.28	4500	3940	89.61
	TNFAIP3	P.R87X	undetectable		0.86	6628	6637	
	SOCS1	UTR5	0.52		0.88	5138	4625	
	SOCS1	UTR5	0.52		0.95	5073	4609	
UPN23	*NFKBIE*	P.Y254FS	undetectable	11.9	2.6	4094	3758	244.86
	STAT6	P.N417S	undetectable		3	4421	3728	
	SOCS1	P.E91G	undetectable		6.2	3594	2794	
	SOCS1	P.A132FS	undetectable		3.7	8830	7532	
	TNFAIP3	P.L324FS	0.59		1.2	4112	2736	

	SOCS1	P.G139D	undetectable		0.74	9000	7527	
UPN24	B2M	P.D73N	undetectable	17	0.53	3764	3369	38.21
	ITPKB	P.A70Q	undetectable		0.77	2349	5242	
UPN25	*STAT6*	P.D419Y	1.37	18.4	9.4	4327	4831	703.18
	STAT6	P.N417Y	0.86		8.7	4337	4835	
	TNFAIP3	SPLICING	1.43		3.4	1842	1505	
	ITPKB	P.V192I	0.74		4	5718	6027	
UPN26	SOCS1	P.138_143DEL	undetectable	17	1.38	9927	9168	122.95
UPN27	*B2M*	P.L7X	5.5	17.5	1.3	4224	2430	246.11
	STAT6	P.D419G	7.9		2.5	4878	2770	
	XPO1	P.E571K	25.6		10.5	4565	1919	
	ITPKB	P.G511D	7.1		2.2	8206	5855	
	SOCS1	P.N5FS	3.02		1.17	3895	3018	
	SOCS1	P.G122FS	6.7		1.1	9829	6930	
	TNFAIP3	P.K417FS	7.44		0.7	5290	4741	
UPN28	*B2M*	UTR5	undetectable	15.4	0.78	3314	2496	51.53
UPN29	no variant		no variant	15.4	no variant	no variant	no variant	0
UPN30	*STAT6*	P.N417Y	0.82	10	undetectable	undetectable	undetectable	13.55
	STAT6	P.D419N	0.82		undetectable	undetectable	undetectable	
	B2M	P.A8FS	1.3		undetectable	undetectable	undetectable	
	SOCS1	P.R159FS	0.61		undetectable	undetectable	undetectable	
UPN31	*TNFAIP3*	SPLICING	NA	NA	0.6	7682	3991	525.16
	XPO1	P.E571K			4.4	3463	1657	
	GNA13	P.D222N			4.1	3087	1255	
	GNA13	P.M375K			2.7	9709	4296	
	B2M	P.R3FS			3.5	4060	1770	
	ITPKB	P.R132P			4.8	6930	4459	
	SOCS1	P.T185FS			0.82	4010	2511	
UPN32	*NA*	NA	NA	NA	no variant	no variant	no variant	0
UPN33	*TNFAIP3*	P.L303X	NA	NA	2.91	7149	5244	359.41

UPN34	*GNA13*	SPLICING	NA	NA	7.81	627	1633	2473.11
	SOCS1	P.A3P			4.34	1499	2816	
	B2M	P.Y86X			10.84	1302	2584	
	B2M	P.L84FS			12.14	1302	2585	
	B2M	P.W80DEL			12.16	1301	2581	
	STAT6	P.D419G			6.95	2212	3277	
	NFKBIE	P.Y254FS			4.18	2442	3793	
UPN35	*STAT6*	P.G416V	NA	NA	3.51	4903	3730	472.03
	STAT6	P.N417Y			3.96	4903	3723	
	ITPKB	P.R286FS			2.1	6151	4196	
	SOCS1	P.S109X			0.56	4271	2512	
UPN36	*NA*	NA	NA	NA	no variant	no variant	no variant	0
UPN37	*B2M*	P.V9E	NA	NA	1.89	4030	3211	109.72
	TNFAIP3	NA			0.69	3910	2776	
	NFKBIE	P.Y254FS			1.14	5112	3367	
UPN38	*PTPN1*	SPLICING	NA	NA	7.91	4552	4331	1434.85
	SOCS1	P.F58L	NA		3.83	940	1505	
	SOCS1	P.Q131E	NA		6.36	6147	6410	
	SOCS1	P.G133V	NA		6.4	6141	6387	
	SOCS1	P.P198F	NA		4.02	4830	4958	
	SOCS1	P.L204V	NA		3.85	4842	4973	
	SOCS1	P.207_210DELINSLEUGL	NA		4.1	4836	4974	
	SOCS1	UTR3	NA		3.98	4850	4964	
	B2M	P.L7X	NA		2.27	1632	1789	
	NFKBIE	P.Y254FS	NA		3.17	2125	3071	
UPN39	*NA*	NA	NA	NA	no variant	no variant	no variant	0
UPN40	*NA*	NA	NA	NA	no variant	no variant	no variant	0
UPN41	*NA*	NA	NA	NA	no variant	no variant	no variant	0
UPN42	*SOCS1*	P.Q6X	NA	NA	1.99	3088	3552	1592
UPN43	*NA*	NA	NA	NA	no variant	no variant	blood collection not performed	0

UPN44	SOCS1	P.S143FS	NA	NA	0.71	10071	8299	165.26
	TNFAIP3	SPLICING			1.57	5726	6286	
	ITPKB	P.A241P			1.91	7318	5385	
	SOCS1	P.S116N			0.87	5504	4379	
	B2M	P.E97X			0.61	6724	5100	
	GNA13	P.E26FS			0.74	673	708	
UPN45	TNFAIP3	P.C57FS	NA	NA	6.57	4561	5526	181.3
	GNA13	P.H118Y			0.63	3012	3740	
	PTPN1	P.L294F			0.51	6941	5054	
	PTPN1	P.R428K			0.54	4000	2692	
UPN46	*NA*	NA	NA	NA	no variant	no variant	no variant	0
UPN47	TNFAIP3	P.T161FS	NA	NA	0.9	1558	3415	44.81
	ITPKB	P.M14I			0.58	2080	1789	
	SOCS1	P.V2E			0.74	4992	4664	
	GNA13	P.R260X			0.75	5462	7065	
UPN48	*GNA13*	SPLICING	NA	NA	4.51	4549	4380	130.75
	SOCS1	P.87_90DEL			1.83	5086	3758	
	B2M	SPLICING			0.54	4839	4788	
	SOCS1	P.R109W			2.45	3101	2069	
UPN49	*NA*	NA	NA	NA	no variant	no variant	no variant	0
UPN50	*NA*	NA	NA	NA	no variant	no variant	no variant	0
UPN51	*NA*	NA	NA	NA	no variant	no variant	no variant	0
UPN52	*PTPN1*	P.L294F	NA	NA	0.66	5169	5473	30.17
UPN53	*NA*	NA	NA	NA	no variant	no variant	no variant	0
UPN54	*SOCS1*	UTR3	NA	NA	2.16	6410	blood collection not performed	261.22
	SOCS1	P.F79L			0.53	1133	blood collection not performed	
UPN55	*NA*	NA	NA	NA	no variant	no variant	no variant	0
UPN56	*STAT6*	P.D419G	NA	NA	6.1	8070	6401	158.14
	STAT6	P.N417D			6	8079	6398	
	B2M	P.M1K			3	2295	3182	

	TNFAIP3	P.D134FS			2.3	5168	4644	
	SOCS1	P.H129FS			2.3	10619	9624	
	SOCS1	P.125_129DEL			1.21	10529	9661	
	SOCS1	P.F58L			1.45	3150	2785	
	B2M	P.L12P			1.68	4362	3191	
UPN57	STAT6	P.G416R	NA	NA	0.79	5043	8454	47.02
	SOCS1	P.D63Y			1.14	968	2101	
UPN58	*SOCS1*	P.N110FS	NA	NA	5.4	3481	dead before C2	683.26
	SOCS1	P.R103FS			5.3	4734	dead before C2	
	SOCS1	P.M1I			1.2	4240	dead before C2	
UPN59	*SOCS1*	P.S116R	NA	NA	2.7	4451	3532	747.41
UPN60	*NA*	NA	NA	NA	no variant	no variant	no variant	0

Supplementary Table 3: Comparison between the ctDNA results of our classical Hodgkin Lymphoma (cHL) cohort and data from the study by Spina et al. [6]

	Our study		Spina et al. *Blood* 2018					
number of patients	60 untreated patients		First cohort : 15 patients with biopsy / plasma ctDNA comparison					
			second cohort : 80 untreated patients and 32 refractory / relapsed patients					
Samples	genomic DNA (FFPE biopsy) and plasma ctDNA		genomic DNA from microdissecated HRS cells and plasma ctDNA					
NGS methods	PGM® : ampliseq		Illumina® : CAPP-seq					
Genes panel	coding sequence and splice sites (or hotspots) of 9 genes		coding exons and splice sites (or hotspots) of 77 genes					
	ctDNA of 60 untreated patients		ctDNA of 15 untreated patients		ctDNA of 80 untreated patients		ctDNA of 32 relapsed/refractory patients	
Number of mutated patients by gene in ctDNA samples	n =	(%)	n =	(%)	n =	(%)	n =	(%)
XPO1	6	(10)	1	(7)	9	(11.2)	4	(12.5)
GNA13	8	(13.3)	4	(27)	15	(18.7)	9	(28.1)
SOCS1	31	(51.7)	ND	ND	ND	ND	ND	ND
ITPKB	14	(23.3)	8	(53)	22	(27.5)	6	(18.8)
B2M	20	(33.3)	4	(27)	13	(16.2)	4	(12.5)
STAT6	14	(23.3)	12	(80)	30	(37.5)	9	(28.1)
NFKBIE	8	(13.3)	1	(7)	5	(6.2)	3	(9.4)
PTPN1	3	(5)	ND	ND	ND	ND	ND	ND
TNFAIP3	19	(31.7)	8	(53)	28	(35)	8	(25)

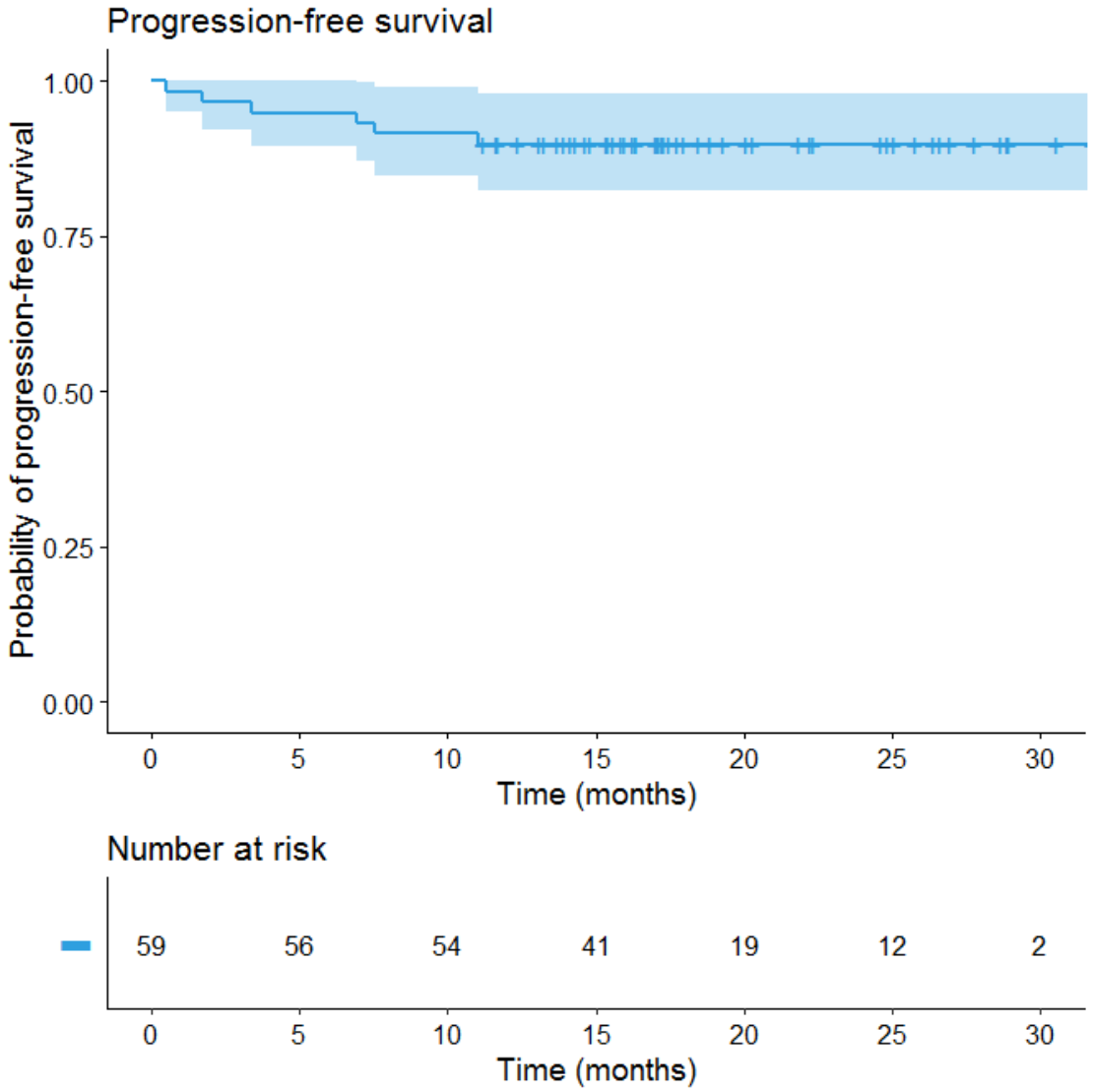
Abbvreviations : NGS : next generation sequencing ; ctDNA : circulating tumor DNA ; FFPE : formalin fixed paraffin embedded ; HRS cells : Hodgkin and Reed-Sternberg cells ; ND : not done

Supplementary Table 4: Correlations between the mutational profile at diagnosis and the clinico-biological characteristics of patients

	ITPKB			SOCS1			TNFAIP3			XPO1			STAT6			NFKBIE			B2M		
Somatic mutations at diagnosis	**mutated**	**unmutated**	**p**	**mutated**	**unmutated**	**p**	**mutated**	**unmutated**	**p**	**mutated**	**unmutated**	**p**	**mutated**	**unmutated**	**p**	**mutated**	**unmutated**	**p**	**mutated**	**unmutated**	**p**
Sex																					
Female	9 (64.3%)	19 (41.3%)	0.23	14 (45.2%)	14 (48.3%)	1	11 (57.9%)	17 (41.5%)	0.36	6 (100%)	22 (40.7%)	**0.007**	7 (50%)	21 (45.7%)	1	5 (62.5%)	23 (44.2%)	0.45	12 (60%)	16 (40%)	0.23
Male	5 (35.7%)	27 (58.7%)		17 (54.8%)	15 (51.7%)		8 (42.1%)	24 (58.5%)		0 (0%)	32 (59.3%)		7 (50%)	25 (54.3%)		3 (37.5%)	29 (55.8%)		8 (40%)	24 (60%)	
median age (range) [years]	32.5 [20-60]	34.5 [21-86]	0.44	33 [20-86]	48 [21-81]	0.2	34 [20-80]	32 [21-86]	0.95	30.5 [20-39]	34 [21-86]	0.39	30.5 [21-48]	35.5 [20-86]	0.11	28 [23-38]	34 [20-86]	0.092	30.5 [20-60]	37.5 [21-86]	0.14
Bulky disease (>=10 cm)																					
Yes	2 (14.3%)	7 (15.2%)	1	7 (22.6%)	2 (6.9%)	0.15	3 (15.8%)	6 (14.6%)	1	1 (16.7%)	8 (14.8%)	1	1 (7.1%)	8 (17.4%)	0.67	2 (25%)	7 (13.5%)	0.59	5 (25%)	4 (10%)	0.14
No	12 (85.7%)	39 (84.8%)		24 (77.4%)	27 (93.1%)		16 (84.2%)	35 (85.4%)		5 (83.3%)	46 (85.2%)		13 (92.9%)	38 (82.6%)		6 (75%)	45 (86.5%)		15 (75%)	36 (90%)	
Ann Arbor stage																					
Stage I-II	7 (50%)	24 (52.2%)	1	11 (35.5%)	20 (69%)	**0.02**	7 (36.8%)	24 (58.5%)	0.2	2 (33.3%)	29 (53.7%)	0.42	5 (35.7%)	26 (56.5%)	0.29	5 (62.5%)	26 (50%)	0.71	10 (50%)	21 (52.5%)	1
Stage III-IV	7 (50%)	22 (47.8%)		20 (64.5%)	9 (31%)		12 (63.2%)	17 (41.5%)		4 (66.7%)	25 (46.3%)		9 (64.3%)	20 (43.5%)		3 (37.5%)	26 (50%)		10 (50%)	19 (47.5%)	
Number of involved nodal areas																					
≥4	7 (50%)	8 (17.4%)	**0.034**	11 (35.5%)	4 (13.8%)	0.075	8 (42.1%)	7 (17.1%)	0.078	3 (50%)	12 (22.2%)	0.16	4 (28.6%)	11 (23.9%)	0.73	4 (50%)	11 (21.2%)	0.098	9 (45%)	6 (15%)	**0.027**
<4	7 (50%)	38 (82.6%)		20 (64.5%)	25 (86.2%)		11 (57.9%)	34 (82.9%)		3 (50%)	42 (77.8%)		10 (71.4%)	35 (76.1%)		4 (50%)	41 (78.8%)		11 (55%)	34 (85%)	
B symptoms																					
Yes	10 (71.4%)	23 (50%)	0.22	18 (58.1%)	15 (51.7%)	0.82	13 (68.4%)	20 (48.8%)	0.25	4 (66.7%)	29 (53.7%)	0.68	9 (64.3%)	24 (52.2%)	0.62	4 (50%)	29 (55.8%)	1	11 (55%)	22 (55%)	1
No	4 (28.6%)	23 (50%)		13 (41.9%)	14 (48.3%)		6 (31.6%)	21 (51.2%)		2 (33.3%)	25 (46.3%)		5 (35.7%)	22 (47.8%)		4 (50%)	23 (44.2%)		9 (45%)	18 (45%)	
IPS (Hasenclever)																					
0-2	8 (57.1%)	31 (67.4%)	0.7	17 (54.8%)	22 (75.9%)	0.15	11 (57.9%)	28 (68.3%)	0.62	3 (50%)	36 (66.7%)	0.65	9 (64.3%)	30 (65.2%)	1	8 (100%)	31(59.6%)	**0.042**	12 (60%)	27 (67.5%)	0.77
3-5	6 (42.9%)	15 (32.6%)		14 (45.2%)	7 (24.1%)		8 (42.1%)	13 (31.7%)		3 (50%)	18 (33.3%)		5 (35.7%)	16 (34.8%)		0 (0%)	21 (40.4%)		8 (40%)	13 (32.5%)	
Histologic subtype																					
Sclero-nodular subtype	11 (78.6%)	31 (67.4%)	0.52	25 (80.6%)	17 (58.6%)	0.11	16 (84.2%)	26 (63.4%)	0.14	5 (83.3%)	37 (68.5%)	0.66	12 (85.7%)	30 (65.2%)	0.19	8 (100%)	34 (65.4%)	0.091	16 (80%)	26 (65%)	0.37
other subtypes	3 (21.4%)	15 (32.6%)		6 (19.4%)	12 (41.4%)		3 (15.8%)	15 (36.6%)		1 (16.7%)	17 (31.5%)		2 (14.3%)	16 (34.8%)		0 (0%)	18 (34.5%)		4 (20%)	14 (35%)	

abbreviations: BMI = body mass index; IPI : international prognostic index; NA : not available; aaIPI = age adjusted IPI; cHL : classical Hodgkin lymphoma

Supplementary Figure 1 : Progression-free survival (PFS) of the global cohort of patients.



Supplementary Figure 2 : Overall survival (OS) of the global cohort of patients.