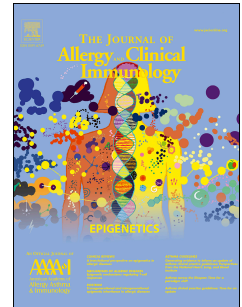


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Lymphoproliferative Disease in Wiskott-Aldrich Syndrome. Analysis of the French Registry of Primary Immunodeficiencies

Morgane Cheminant, MD, PhD, Nizar Mahlaoui, MD, PhD, Céline Desconclois, MD, Danielle Canioni, MD, PhD, Loïc Ysebaert, MD, PhD, Loïc Dupré, PhD, Zilton Vasconcelos, PhD, Marion Malphettes, MD, Despina Moshous, MD, PhD, Bénédicte Neven, MD, PhD, Pierre-Simon Rohrlach, MD, PhD, Marc Bernard, MD, Yves Bertrand, MD, PhD, Alain Fischer, MD, PhD, Felipe Suarez, MD, PhD



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2 French Registry of Primary Immunodeficiencies

3

4 **Authors:** Morgane Cheminant¹⁻³ MD, PhD, Nizar Mahlaoui²⁻⁵ MD, PhD, Céline
 5 Desconclois⁶ MD, Danielle Canoni^{2,7} MD, PhD, Loïc Ysebaert⁸ MD, PhD, Loïc
 6 Dupré⁹ PhD, Zilton Vasconcelos^{9,10} PhD, Marion Malphettes¹¹ MD, Despina
 7 Moshous²⁻⁵ MD, PhD, Bénédicte Neven²⁻⁵ MD, PhD, Pierre-Simon Rohrlich¹² MD,
 8 PhD, Marc Bernard¹³ MD, Yves Bertrand¹⁴ MD, PhD, Alain Fischer^{2-5,15} MD, PhD
 9 and Felipe Suarez¹⁻³ MD, PhD

10

11 **Affiliations:** ¹Clinical Hematology, Necker University Hospital, AP-HP, Paris,
 12 France; ²Paris Descartes University, Sorbonne Paris Cité, Imagine Institute, Paris,
 13 France, ³French National Reference Center for Primary Immune Deficiencies
 14 (CEREDIH), Necker University Hospital, AP-HP, Paris, France; ⁴Pediatric Immuno-
 15 Hematology and Rheumatology Unit, Necker University Hospital, AP-HP, Paris,
 16 France; ⁵INSERM UMR 1163, Laboratory of Human Genetics of Infectious Diseases,
 17 Necker Branch, Paris, France; ⁶Biological Hematology, Kremlin Bicêtre University
 18 Hospital, AP-HP, Paris, France; ⁷Pathology Department, Necker University Hospital,
 19 Paris, AP-HP, Paris, France; ⁸Clinical Hematology, Institut Universitaire du Cancer
 20 Toulouse-Oncopôle, Toulouse, France; ⁹Center for Pathophysiology of Toulouse
 21 Purpan, INSERM UMR1043, CNRS UMR5282, Paul Sabatier University, Toulouse,
 22 France; ¹⁰Fernandes Figueira Institute, Fiocruz, Rio de Janeiro, Brazil; ¹¹Immunology
 23 Department, Saint-Louis University Hospital, AP-HP, Paris, France; ¹²Nice
 24 University Hospital, University of Côte d'Azur, Nice, France; ¹³Clinical Hematology,
 25 Pontchaillou University Hospital, Rennes, France ; ¹⁴Department of Immuno-Hemato-

26 Pediatrics, Claude Bernard University, IHOP, Lyon, France; ¹⁵Collège de France,
27 Paris, France.

28

29 **Correspondence:** Prof. Felipe Suarez, Department of Clinical Hematology, Necker
30 Hospital, 149 rue de Sèvres, 75015 Paris, France.

31 Phone number: +33(0)144495368

32 Fax number: +33(0)144494020

33 E-mail: felipe.suarez@aphp.fr

34

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46

47 **Capsule summary**

48 Aggressive mature B-cell LPD can occur in WAS as in XLT pediatric and adult
49 patients. Immunochemotherapy is applicable to all WAS/XLT patients, while
50 transplantation still remains controversial for adult patients.

51

52 **Keywords**

53 Wiskott-Aldrich syndrome, X-linked thrombocytopenia, primary immune deficiency,
 54 lymphoproliferative disease, lymphoma

55

56 **Abbreviations**

alloSCT	allogeneic stem cell transplantation
CNS	central nervous system
CR	complete response
DLBCL	diffuse large B-cell lymphoma
CHOP	doxorubicin, cyclophosphamide, vincristine, prednisone
EBV	Epstein-Barr virus
EBER	Epstein-Barr virus-encoded RNA
GCB	germinal center B-cell
GVHD	graft-versus-host disease
IQR	interquartile range
KS	Kaposi's sarcoma
PTLD-like	LPD with histological features analogous to post-transplant LPD
LPL	lymphoplasmacytic non-Hodgkin lymphoma
LPD	lymphoproliferative disease
PR	partial response
PID	primary immune deficiency
PD	progressive disease
SRT	severe refractory thrombocytopenia
SD	stable disease
WASP	Wiskott-Aldrich protein
WAS	Wiskott-Aldrich syndrome
XLT	X-linked thrombocytopenia

57

58 *To the Editor*

59 Wiskott-Aldrich syndrome (WAS) is a rare X-linked primary immune deficiency
 60 (PID) caused by mutations in the WAS gene. The clinical triad of
 61 microthrombocytopenia, eczema and immunodeficiency defines the classical WAS
 62 phenotype, whereas the so-called “attenuated form” X-linked thrombocytopenia
 63 (XLT) manifests with thrombocytopenia alone with or without mild eczema. An
 64 increased risk of lymphoproliferative disease (LPD) in WAS/XLT has been described
 65 as early as 1961¹ and confers a poor prognosis to patients^{2–5}. However, the precise
 66 incidence of LPD in WAS remains unknown.

67 Through a retrospective study within the national registry of the CEREDIH,
 68 the French National Reference Center for PID (**Supplementary data**), we identified
 69 13 WAS/XLT patients who developed LPD before allogeneic stem cell
 70 transplantation (alloSCT) among the 189 WAS and 46 XLT patients treated between
 71 1988 and October 2017. Considering that WAS/XLT patients were at risk before
 72 undergoing alloSCT, crude rate and incidence ratio of LPD in untransplanted patients
 73 were 8.4% and 511 per 1×10^5 patient-years [95%CI 272 – 873] respectively, higher
 74 than in the general population (7.5 per 1×10^5 patient-years [95%CI 7.1 – 7.8],
 75 $p < 0.001$). Crude rates (4.3% [95%CI, 0.5 – 14] for the XLT cohort and 5.8% [95%CI,
 76 2.9 – 10.2] for the WAS cohort) and incidence ratio (334 [95%CI 40 – 1200] and 531
 77 per 1×10^5 patient-years [95%CI 265 – 943], respectively) of LPD were similar in
 78 XLT and WAS patients. The cumulative incidence of LPD was 1.9% at age 10 and
 79 9.9% at age 30 as shown by competing risk analysis. After age 40, the risk of LPD
 80 persists in untransplanted WAS/XLT patients as 3 cases occurred at age 43.2, 50.3
 81 and 50.6 (**Figure A**). The median age at diagnosis of WAS/XLT was similar in the
 82 entire cohort and in the subgroup of patients who developed LPD (1 year [IQR 0 –

3]). Among the 13 patients who developed LPD, two were previously diagnosed with XLT. WAS scores prior to LPD onset were 3 (n=2), 4 (n=3), 5 (n=5) and 5-SRT (n=1). Four patients with a score 5 had autoimmune cytopenia and 2 developed Kaposi's sarcoma (KS). In contrast, no patients were diagnosed with KS in the WAS/XLT patients without LPD. Four patients had received immunosuppressive therapy, all prior to the diagnosis of LPD. Nine patients were under immunoglobulin replacement therapy and 6 had been splenectomized (**Supplementary Table 1**). Genetic diagnosis of WAS/XLT was available for 195 patients (83%), including 10 who developed LPD. Five splice-site mutations, 1 nonsense mutation, 1 missense mutation, 2 insertions and 1 deletion were identified (**Figure B**). All these mutations, except the missense mutation, are predictive of a severe WAS phenotype. Patients with LPD were less likely to have missense mutations than patients without LPD (10% vs. 60.3% respectively, $p=0.002$). WASP expression was evaluated by immunohistochemistry on tumor samples of 11 patients. In six samples, WASP expression was absent in both T and B cells. In the 5 remaining patients, WASP was expressed on T cells of the microenvironment but was negative on tumor large B cells (**Figure C and Supplementary Table 2**), suggesting the presence of revertant T cells. We were able to further assess the WASP expression by flow cytometry and western blot in one of them, patient 10, that we found bimodal. These results suggest that a corrective mutation has occurred in T cell progenitors and that revertant CD8⁺ T cells preferentially expanded in the bone marrow (**Supplementary Fig. 1**). The main characteristics of LPD in WAS/XLT patients are described in the **table**. The median age at diagnosis of LPD was 22 years [IQR 6.2 – 24.7]. All LPD except two presented as disseminated disease, mostly with central nervous system (CNS) involvement in 6/13 patients (46%). Twelve cases were evaluable for centralized

pathology review and were classified according to the 2016 revised edition of the WHO classification of lymphoid neoplasms⁶. Six cases were classified as diffuse large B-cell lymphomas (DLBCL) (four of non-germinal center B-cells (GCB) and two of GCB phenotype). Age-of-onset of DLBCL patients ranged from 22 to 50 years. Five cases were classified as LPD with histological features analogous to post-transplant LPD (PTLD-like). These PTLD-like cases were monomorphic in three cases and polymorphic in two cases (**Figure C**). Age-of-onset of PTLD-like cases ranged from 2 to 6 years, except one patient diagnosed at the age of 50. One case diagnosed at age 44 was classified as an IgG-associated lymphoplasmacytic non-Hodgkin lymphoma (LPL) lacking *MYD88* mutation. One case diagnosed in 1988 could not be reviewed and was considered as an unclassified B-cell lymphoma based on pathology report. Epstein Barr-virus (EBV) detection, as defined by the EBV-encoded RNA (EBER) positivity in tumor cells, was positive in 8 cases (**Table**).

One patient, diagnosed post-mortem with a CNS B-cell lymphoma, was untreated and died of lymphoma (**Supplementary Fig. 2**). Two patients with EBV-associated LPD only received anti-CD20 monoclonal antibody leading to disease progression and death for one and to complete response (CR) in the other patient. Ten patients received intensive chemotherapy, combined with rituximab for eight of them. No unexpected toxicity was recorded. Six of them achieved a CR, and two achieved a stable disease (SD). Two patients progressed on therapy and died of disease. One patient in SD received methotrexate and cytarabine followed by an autologous SCT as salvage therapy and achieved a CR. He is alive and well 16.3 years after the diagnosis of LPD. Five patients, four of whom were in CR and one in SD, underwent an alloSCT. Two patients died of transplant related toxicity and three are alive and well 24, 73 and 115 months after alloSCT. One patient relapsed 4 months after

transplantation and is alive in CR after salvage chemo-radiotherapy. No post-transplant lymphoproliferative disease occurred, thus highlighting the protective role of alloSCT. Among the 8 non-transplanted patients, four of them were not transplanted because of PD. Four adult patients, who had achieved CR, were not transplanted because of their advanced age (44 for one and over 50 years for two) or lack of matched donor. These patients are alive and well 29, 44, 70 and 196 months after the end of treatment for LPD (**Table and Supplementary Fig. 2**). In total, 7/13 patients are alive in continuous CR 24 to 196 months after the diagnosis of LPD, three of whom after alloSCT. The estimated 12- and 24-months survival rates after the diagnosis of LPD were 67.1% and 42% respectively (**Supplementary Fig. 3**). Thirty-eight percent (5/13) of WAS/XLT patients with LPD vs. 39% (87/222) without LPD received an alloSCT (**Supplementary Table 3**).

Overall, our findings suggest that older untransplanted WAS, as well as XLT patients⁵, remain at risk for aggressive mature B-cell LPD. The percentage of LPD is indeed similar irrespective of the degree of severity of the immune deficiency, emphasizing that the distinction between XLT and WAS might therefore be irrelevant. A high frequency of mutations leading to WASP defective expression was observed. However, in half of the patients, the discordant WASP expression between T lymphocytes and large tumor B cells, suggests that revertant T cells cannot, at least in some cases, prevent occurrence of LPD⁷. Intensive immunochemotherapy was well tolerated and patients who reach a CR may have a long-term survival. Despite the retrospective nature of this study, survival appears higher than the poor outcomes previously reported for WAS patients with LPD^{1,2,5}, which may reflect the wider use of rituximab and intensive chemotherapy in our series. The occurrence of malignancy is usually considered an indication for alloSCT⁸. Nevertheless four patients diagnosed

after age 40 are alive in continuous CR without alloSCT. AlloSCT seems justified whenever feasible in young patients, but should be weighed against transplantation risks in older patients⁹. Prospective studies are unrealistic for such a rare disease, but collaborative observational trials would be helpful to set optimized therapeutic approach.

For detailed methods, please see the Methods section in the Online Repository.

Morgane Cheminant, MD, PhD¹⁻³

Nizar Mahlaoui, MD, PhD²⁻⁵

Céline Desconclois, MD⁶

Danielle Canioni, MD, PhD^{2,7}

Loïc Ysebaert, MD, PhD⁸

Loïc Dupré, PhD⁹

Zilton Vasconcelos, PhD^{9,10}

Marion Malphettes, MD¹¹

Despina Moshous, MD, PhD²⁻⁵

Bénédicte Neven, MD, PhD²⁻⁵

Pierre-Simon Rohrlich, MD, PhD¹²

Marc Bernard, MD¹³

Yves Bertrand, MD, PhD¹⁴

Alain Fischer, MD, PhD^{2-5,15}

Felipe Suarez, MD, PhD¹⁻³

From: ¹Clinical Hematology, Necker University Hospital, AP-HP, Paris, France;

²Paris Descartes University, Sorbonne Paris Cité, Imagine Institute, Paris, France,

³French National Reference Center for Primary Immune Deficiencies (CEREDIH),
 Necker University Hospital, AP-HP, Paris, France; ⁴Pediatric Immuno-Hematology
 and Rheumatology Unit, Necker University Hospital, AP-HP, Paris, France;
⁵INSERM UMR 1163, Laboratory of Human Genetics of Infectious Diseases, Necker
 Branch, Paris, France; ⁶Biological Hematology, Kremlin Bicêtre University Hospital,
 AP-HP, Paris, France; ⁷Pathology Department, Necker University Hospital, Paris,
 AP-HP, Paris, France; ⁸Clinical Hematology, Institut Universitaire du Cancer
 Toulouse-Oncopôle, Toulouse, France; ⁹Center for Pathophysiology of Toulouse
 Purpan, INSERM UMR1043, CNRS UMR5282, Paul Sabatier University, Toulouse,
 France; ¹⁰Fernandes Figueira Institute, Fiocruz, Rio de Janeiro, Brazil; ¹¹Immunology
 Department, Saint-Louis University Hospital, AP-HP, Paris, France; ¹²Nice
 University Hospital, University of Côte d'Azur, Nice, France; ¹³Clinical Hematology,
 Pontchaillou University Hospital, Rennes, France ; ¹⁴Department of Immuno-Hemato-
 Pediatrics, Claude Bernard University, IHOP, Lyon, France; ¹⁵Collège de France,
 Paris, France.

E-mail: felipe.suarez@aphp.fr

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229 **Table:** WAS gene mutation types, clinical manifestations of WAS/XLT syndrome,
 230 LPD characteristics and outcome in WAS/XLT patients.

Patient	WAS/XLT clinical manifestations	Score	WAS mutation type	WASP expression by IHC	Age at onset of LPD (y)	Extranodal manifestations	Histologic subtype	Allo - SCT	Overall outcome, age at last follow-up (cause of death)
1	KS	5	Nonsense	Neg	4	CNS, Lung	B-cell PTL-like, EBV+	No	Died, 5 y (progressive LPD)
2	AIHA	5	NA	Discordant (pos LT, neg LB)	22	CNS	DLBCL non GCB type, EBV-	MUD	Died, 23 y (sepsis)
3	AI pancytopenia	5	Missplicing	Neg	22	Bone (knee)	DLBCL GCB type, EBV-	MUD	A/W, 24 y
4	Infections	3	Missplicing	Discordant (pos LT, neg LB)	24	Lung, bone	DLBCL non GCB type, EBV-	No	Died, 25 y (progressive LPD)
5	KS, AIHA	5	Deletion	Neg	2	CNS	B-cell PTL-like, EBV+	Haplo	A/W, 12 y
6	Life-threatening infections	4	Missplicing	Neg	6	CNS, lung	B-cell PTL-like, EBV+	No	Died, 8 y (progressive LPD)
7	Life-threatening infections	4	NA	NA	14	CNS	Unclassified B-cell LPD	No	Died, 15 y (progressive LPD)
8	AIHA	5	NA	Neg	4	Digestive tract	B-cell PTL-like, EBV+	MUD	Died, 12 y (Chronic GVHD)
9	Life-threatening infections	4	Insertion	Discordant (pos LT, neg LB)	50	CNS	B-cell PTL-like, EBV+	No	A/W, 55 y
10	Thrombocytopenia	1	Missplicing	Pos CD8+ T cells (Suppl Fig. 1)	50	Bone Marrow	DLBCL non GCB type, EBV+	No	A/W, 53 y
11	SRT	5-SRT	Insertion	Discordant (pos LT, neg LB)	22	No	DLBCL GCB type, EBV-	MMUD	Alive, 23 y (GVHD)
12	Infections	3	Missplicing	Discordant (pos LT, neg LB)	43	No	Lymphoplasmacytic lymphoma, EBV+	No	A/W, 44 y
13	Thrombocytopenia	1	Missense	Neg	27	No	DLBCL, non GCB type, EBV+	No	A/W, 44 y

231 WAS: Wiskott-Aldrich syndrome; XLT: X-linked-thrombocytopenia; LPD: lymphoproliferative
232 disease; NA: not available; KS: Kaposi's sarcoma; AIHA: autoimmune hemolytic anemia; SRT: severe
233 refractory thrombocytopenia; CNS: central nervous system; PTLN-like: LPD with features analogous
234 to post transplant LPD; EBV: Epstein-Barr virus; DLBCL: diffuse large B-cell lymphoma; GCB: of
235 germinal center B-cell origin; MUD: matched unrelated donor; haplo: haplotype-mismatched
236 (haploidentical) related donors; MMUD: mismatched unrelated donor; A/W: alive and well; GVHD:
237 graft versus host disease; WASP: Wiskott-Aldrich syndrome protein; IHC: immunohistochemistry; LT:
238 T lymphocytes; LB: B lymphocytes; Neg: negative; Pos: positive.

FIGURE LEGEND

A: Cumulative incidence of lymphoma, transplantation and death unrelated to lymphoma or transplantation, as competing risk in WAS/XLT patients. Lymphoma indicates lymphoproliferative disease; transplantation indicates allogeneic stem-cell transplantation.

B: Schematic representation of the WAS gene and protein domain. The 12 exons are colored. Lines between colored exons represent intronic regions. The untranslated regions of the firsts and last regions are shown in black. The domain structure of WASP is shown below the respective exons. Types and localizations of mutations identified in WAS/XLT patients who developed a LPD are indicated.

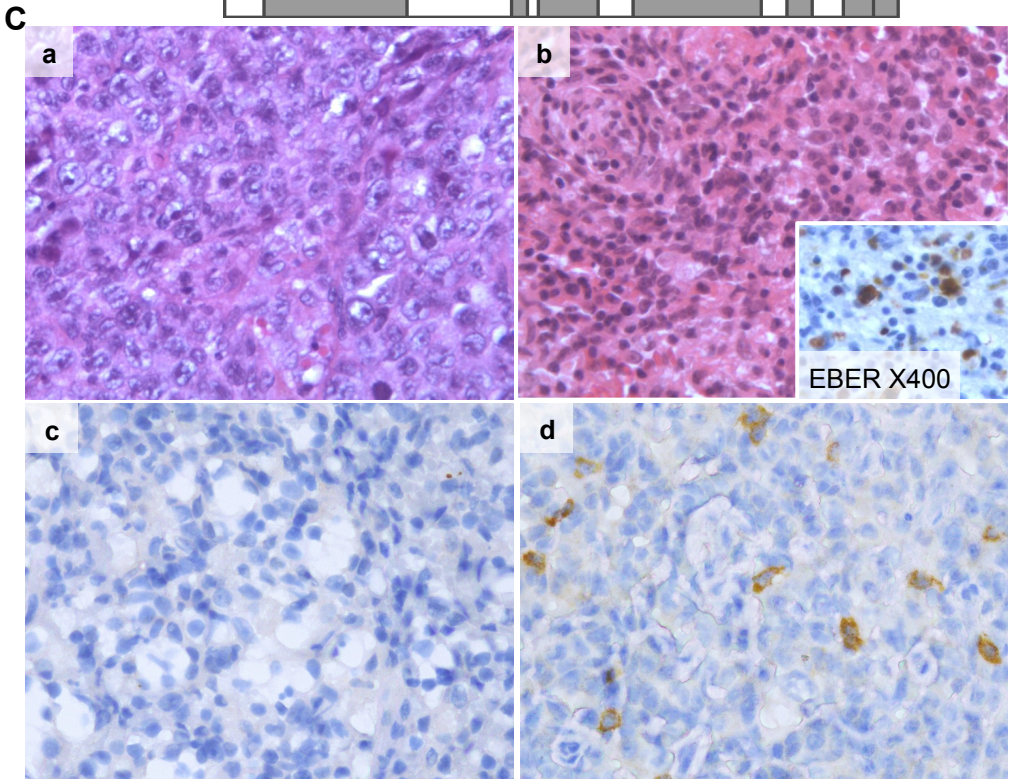
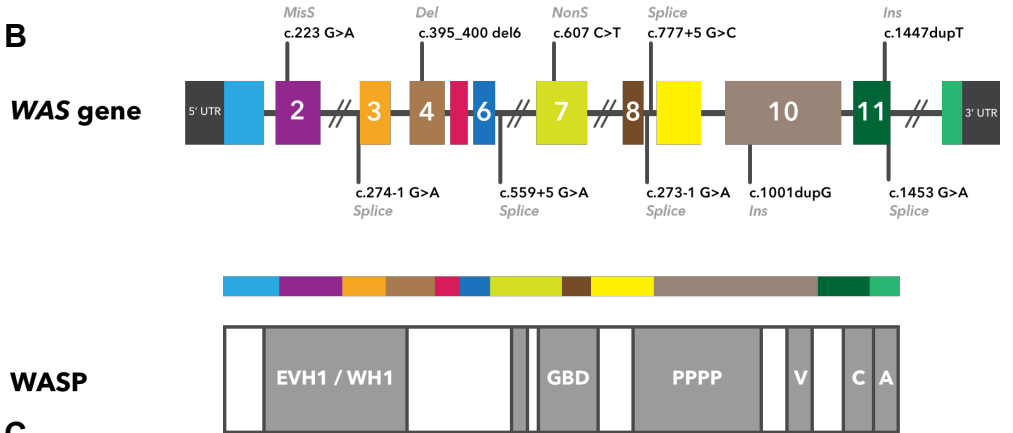
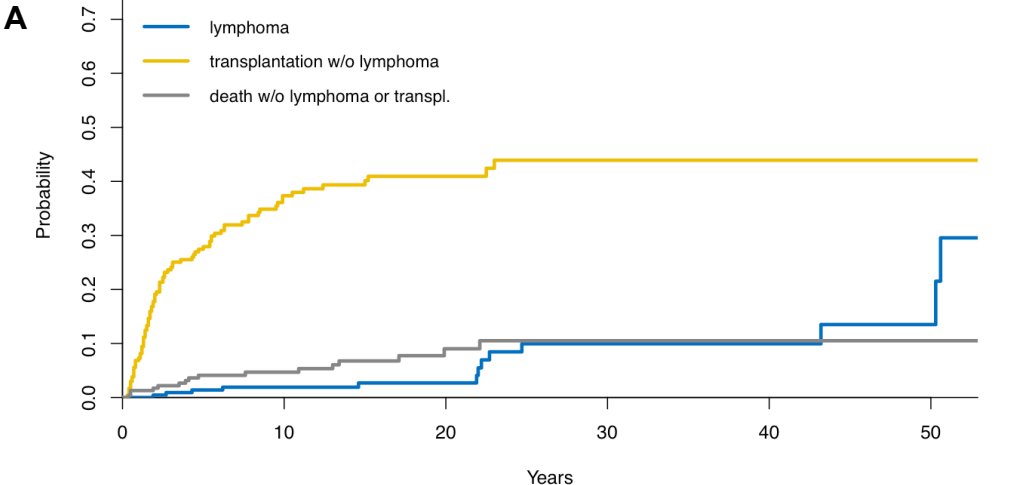
C: Diagnosis of lymphoproliferative disease on biopsy and WASP expression. **a.** DLBCL type LPD: effacement of architecture by a diffuse infiltrate of large neoplastic cells (a, HES x400). Tumor cells do not express EBV-encoded RNA. **b.** Polymorphic LPD with histological features analogous to post-transplant LPD: effacement of architecture by a polymorphic infiltrate (b, HES x400). Tumor cells express EBV-encoded RNA (EBER, x400). **c.** In five samples, WASP expression was absent in both T and B cells (c). **d.** In the 5 remaining patients, WASP was expressed on normal small T cells of the microenvironment but was negative on tumor large B cells (d).

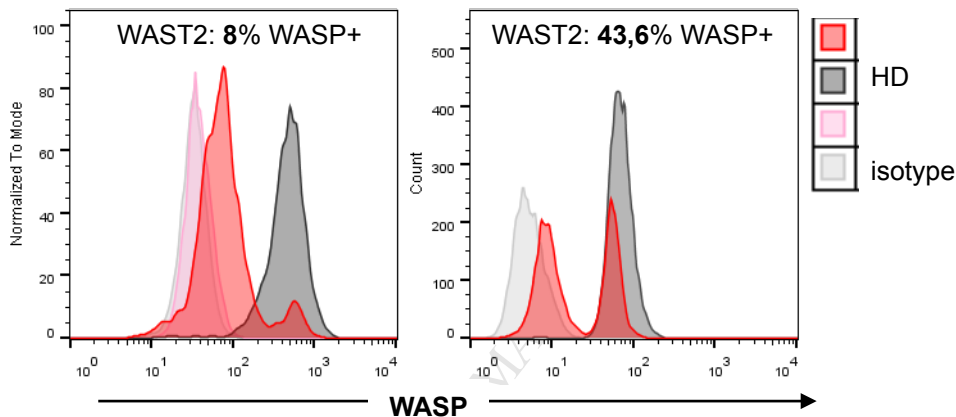
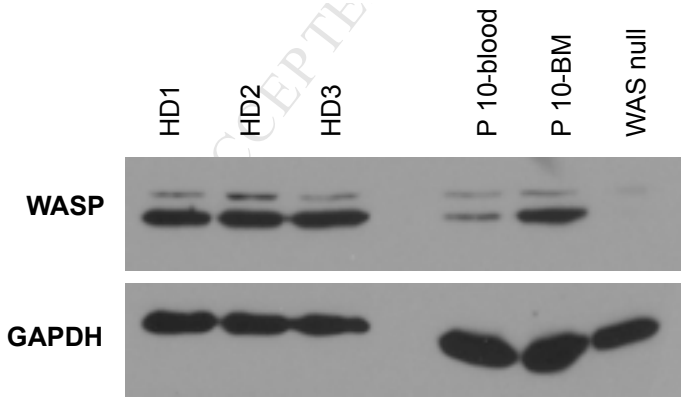
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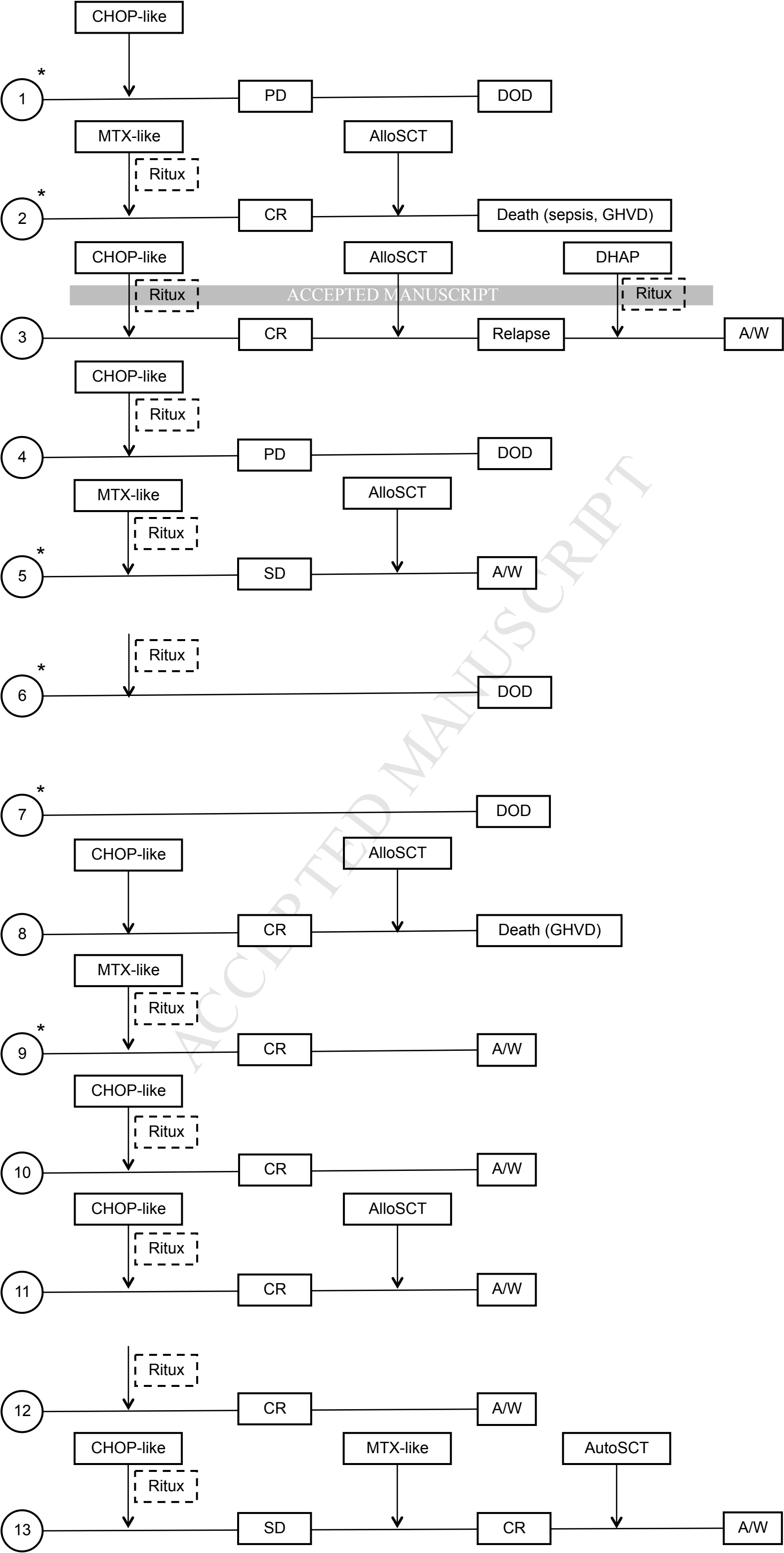
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Authorship contributions

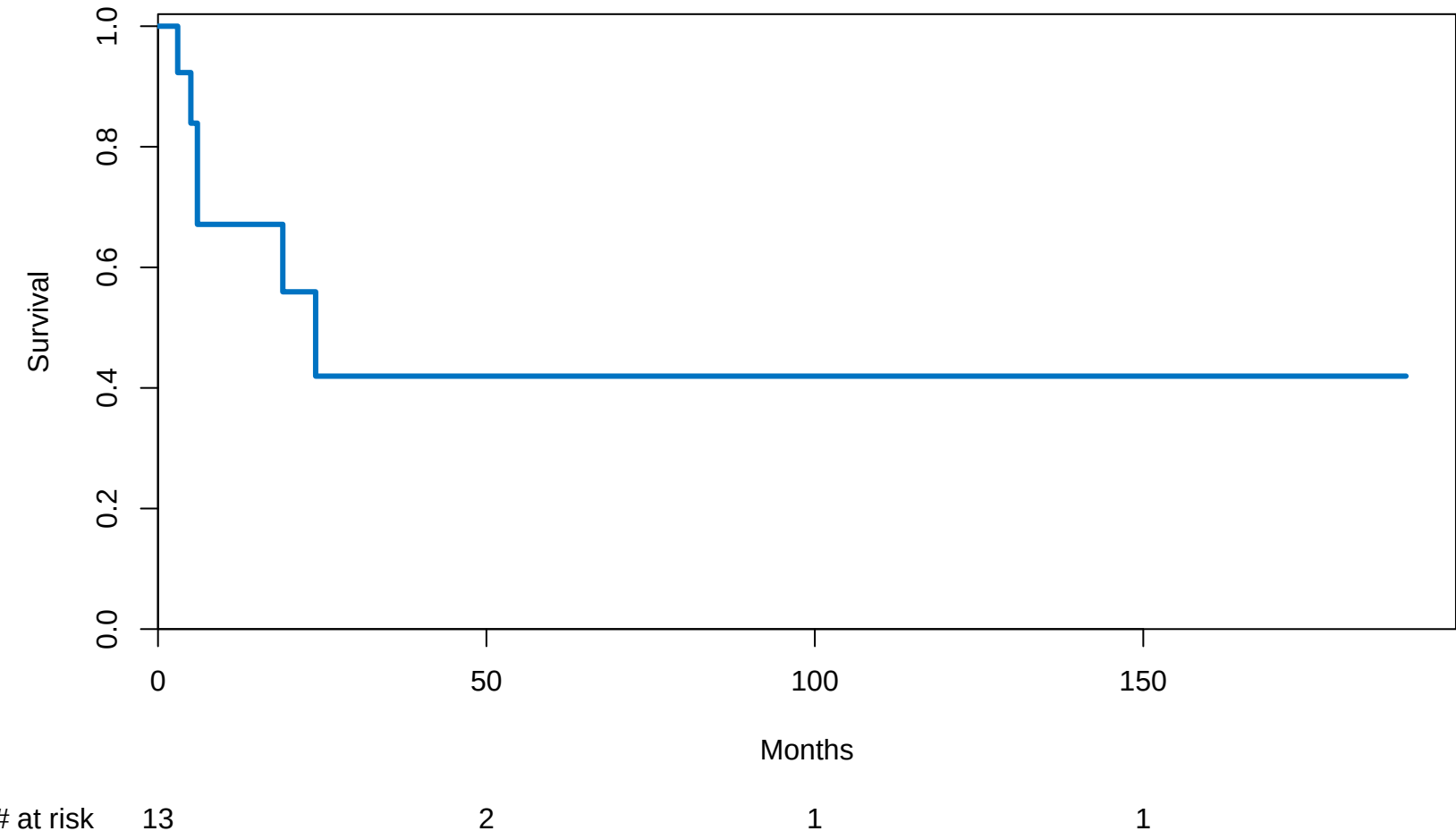
MC, NM, AF and FS conceptualized, supervised the study and wrote the manuscript. MC, NM, AF and FS provided data and data analysis. CD identified WAS gene mutations. DC reviewed the biopsy specimens and analyzed WASP expression. LD and ZV analyzed WASP expression by flow cytometry and Western blot. MC, NM and FS contributed to statistical interpretation. MM, YB, MB, LY, LD, DM, BN, PSR, MC, NM, AF and FS provided patient material and clinical data.



A**PBMC-CD8****BoneMarrow-CD8****B**



Survival after lymphoma (censored)



ACCEPTED MANUSCRIPT

Lymphoproliferative Disease in Wiskott-Aldrich Syndrome. Analysis of the French Registry of Primary Immunodeficiencies

SUPPLEMENTAL DATA

Description of the French National Reference Center for Primary Immune Deficiencies (CEREDIH) Registry

The French National Reference Center for Primary Immune Deficiencies (CEREDIH) is a national network of academic centers in metropolitan France. The CEREDIH registry is population based, and its methodology has been described (The French national registry of primary immunodeficiency diseases. *Clin. Immunol.* 2010;135(2):264–272). Patients were enrolled retrospectively and prospectively. Data are collected by annual on-site visits by CEREDIH members. Collected information includes among others, demographics, date of clinical and molecular diagnosis, date of disease onset and occurrence of malignancies.

SUPPLEMENTARY TABLES

Supplementary table 1: Clinical manifestations of patients with WAS/XLT with lymphoproliferative disease, ranked according to their severity score

Score	N patients (n=13)	Clinical manifestations	Median age at LPD (y)	Treatment received before LPD
5	6	AIHA (n=4) Kaposi (n=2) SRT (n=1)	13	CYC – MTX – TPO agonist Rituximab – Azathioprine Docetaxel – CTC
4	3	Life-threatening infections (n=3)	14	-
3	2	Infections (n=2)	34	-
2	0	-	-	-
1	2	Micro-thrombocytopenia	39	-

AIHA: autoimmune hemolytic anemia; SRT: severe refractory thrombocytopenia; LPD: lymphoproliferative disease; CYC: cyclophosphamide; TPO: thrombopoietin; CTC: corticosteroids

Supplementary table 2: WAS gene mutations and WASP protein expression by immunohistochemistry on lymphoma biopsies among the patients with WAS/XLT who developed a lymphoproliferative disease.

Patients	WAS mutation	Type	Expected WASP expression	WASP expression by IHC
1	Exon 7 c.607C>T	Nonsense (p.Gln203 Stop Q203X)	Reduced and truncated	Neg
2	NA	NA	NA	Discordant (pos LT, neg LB)
3	Intron 8 c.777+5 G>C	Missplicing (del exon 8)	Neg	Neg
4	Intron 8 c.777+1 G>A	Missplicing (del exon 8)	Neg	Discordant (pos LT, neg LB)
5	Exon 4 c.395_400 del6	Del in frame (p.Asp132_Glu133)	Unknown	Neg
6	Intron 2 c.274-1 G>A	Missplicing (del exon 3)	Neg	Neg
7	NA	NA	NA	NA
8	NA	NA	NA	Neg
9	Exon 10 c.1001dupG	Insertion frameshift (Stop aa 335)	Reduced and truncated	Discordant (pos LT, neg LB)
10	Exon 11 c.1453 G>A	Missplicing (del exon 11)	Reduced and truncated	Pos CD8+ T cells (Cf Suppl Fig. 1)
11	Exon 11 c.1447dupT	Insertion frameshift (Stop aa 494)	Reduced and truncated	Discordant (pos LT, neg LB)
12	Intron 6 c.559+5 G>A	Missplicing (fs stop aa 190/normal)	Reduced	Discordant (pos LT, neg LB)
13	Exon 2 c.223G>A	Missense	Heterogeneous (Normal to negative)	Neg

WAS: Wiskott-Aldrich syndrome gene; WASP: Wiskott-Aldrich syndrome protein; NA: not available; IHC: immunohistochemistry; LT: T lymphocytes; LB: B lymphocytes; Neg: negative.

Supplementary table 3: Contingency table of patients with WAS/XLT with vs. without lymphoproliferative disease according to allogeneic hematopoietic stem cell transplantation

	No LPD	LPD	Total
No alloSCT	135	8	143
AlloSCT	87	5	92
Total	222	13	235

LPD: lymphoproliferative disease; alloSCT: allogeneic hematopoietic stem-cell transplantation.

SUPPLEMENTARY FIGURES

Supplementary figure 1: Bimodal WASP expression in patient 10. Flow cytometry analysis of WASP expression revealed low to negative expression of WASP in blood and bone marrow–derived lymphocytes, except for a minor fraction of blood CD8+ and about 50% of marrow CD8+ T cells that express WASP at close to normal levels. (A). Western blot analysis shows strongly reduced expression of WASP in blood–derived lymphocytes, while bone marrow–derived lymphocytes express about half the normal amount of WASP (B).

Supplementary figure 2: Treatment and clinical evolution among the patients with WAS/XLT who developed a lymphoproliferative disease.

*: central nervous system involvement; CHOP: doxorubicin, cyclophosphamide, vincristine, prednisone; Ritux: rituximab; MTX: high-dose methotrexate; DHAP: dexamethasone, high-dose cytarabine, oxaliplatin; CR: complete response; PD : progressive disease; SD: stable disease; A/W: alive and well; DOD: died of disease; alloSCT: allogeneic stem-cell transplantation; GVHD: graft versus host disease.

Supplementary figure 3: Analysis of survival after cancer treatment in WAS/XLT patients who developed a LPD.