Expression of special AT-rich sequence-binding protein 1 is an independent prognostic factor in cutaneous T-cell lymphoma

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Abstract. Cutaneous T-cell lymphoma (CTCL) is a group of slowly progressive, lymphoproliferative disorders characterized by localization of neoplastic T lymphocytes to the skin. The most common type of CTCL is mycosis fungoides which has a mild clinical course with slow and long progression. The rate of progression is generally slow and takes many years but often remains unpredictable. Special AT-rich sequence-binding protein-1 (SATB1) is a global chromatin organizer which controls gene expression by folding and remodeling chromatin, but which also regulates the level of histone methylation and acetylation, important in differentiation and apoptosis. The aim of the present study was to determine if SATB1 may be considered a prognostic and predictive factor of CTCL. The results showed that moderate and high expression of SATB1 correlate with significantly better prognosis of CTCL patients. Moreover, we showed that downregulation of SATB1 in Jurkat cells caused their resistance to activation-induced cell death. In conclusion, SATB1 expression appears to be a strong candidate as a prognostic factor confirming the inner heterogeneous features of CTCLs.

Introduction

Cutaneous T-cell lymphoma (CTCL) is a general term for many types of skin lymphomas and it accounts for 71% of the 3,884 cutaneous lymphomas diagnosed in the United States between 2001 and 2005 (1). Incidence rates (IRs) for all CTCLs in the US population have been estimated to be 4.1-7.7/1,000,000 person-years (1,2). This group of cutaneous lymphomas includes mycosis fungoides (MF), Sézary syndrome (SS), lymphomatoid papulosis (LyP) and cutaneous anaplastic large cell lymphoma. MF is the most common type of CTCL, characterized by slow progression and with no effective cure (3). In the present study, phototherapy with psoralen plus ultraviolet A (PUVA) with or without biologic therapy have a significant meaning. In the early stages, it is used as a monotherapy, and, in later stages, it is combined with interferon or retinoids. Immunomodulatory treatment is used to reduce side-effects, i.e. interferon α, bexarotene, deacetylase inhibitors, denileukin diftitox and methotrexate (4-6). The SS, another frequently occurring and the most aggressive CTCL, is characterized by erythroderma, lymphadenopathy and neoplastic T cells (Sézary cells) in the peripheral blood. The accumulation of these malignant cells contributes to the resistance to apoptosis, in particular, activation-induced cell death (7). This type of disease has a fast clinical course with an unfavorable patient prognosis and comprises ~15% of the total MF/SS population (8).

Early diagnosis, in all types of lymphoma, is of major clinical significance as it makes treatment possible and it may also inhibit further progression of the disease. The main mechanism of the effect of the drugs is associated with induction of apoptosis in cancer cells (9). Furthermore, some drugs promoted cell growth and differentiation or modulation of immune response to the cancer cells. Therapy is highly important, but the correct classification of cancer allows for the best treatment methods to be selected (10,11).

At present, scientists optimize diagnostic methods and search for new specific markers for primary cutaneous lymphomas, but there is a lack of progression markers and clinical course remains unpredictable in the majority of cases. One marker may be a special AT-rich sequence-binding protein-1 (SATB1), a global chromatin organizer cloned in 1992 (12), which appears to be a potential prognostic marker of CTCL (11). SATB1 controls gene expression by folding and remodeling chromatin and regulating the level of histone methylation and acetylation (13,14). SATB1 is expressed primarily in thymocytes, but a very low expression level has also been found in osteoblasts and testis (15,16). Moreover, SATB1 has been reported to be overexpressed in numerous human tumors, including bladder, prostate and rectal cancer as well as in nasopharyngeal carcinoma (17-20). It is considered

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an indicator of unfavorable prognosis in breast cancer, as the nuclear expression of SATB1 correlates with metastasis to the lymph nodes (20-24). It is known that Sézary cells are deficient in the expression of SATB1, but Wang *et al* (7) suggested that restoring SATB1 expression in Hut78 cells (*in vitro* model of Sézary cells) may induce spontaneous cell death and may sensitize cells to the treatment. This suggests that a deficiency in SATB1 expression plays an important role in SS pathogenesis by causing apoptosis resistance. However, little is known regarding the possible role of SATB1 in the prognosis of CTCL patients. Our previous study on a relatively small group of patients with MF suggested that the low level of SATB1 results in an unfavorable prognosis (11).

The aim of the present study was to determine if SATB1 may be considered a prognostic and predictive factor of CTCL. In addition, we also examined the effect of SATB1 downregulation on apoptotic cell death induction in Jurkat cells.

Materials and methods

Patient selection and staging approach. The studied group consisted of 60 patients with cutaneous lymphoma, including 57 with MF, 2 with SS and 1 with LyP. Written informed consent was obtained from each patient before the tissue sample acquisition, and approval of the study was granted by the institution's Ethics Committee (no. 215/2008). Samples were fixed in 10% buffered formalin and embedded in paraffin block. All histopathological results were standardized according to the WHO classification (2008) using an immunohistochemical diagnostic panel of antibodies, CD3, CD4, CD7, CD8, CD20, CD30, CD45RO, and the studies conducted confirmed monoclonal growth of the neoplasm using the PCR method. Patients were staged according to TNMB and subsequently according to the ISCL/EORTC proposal. However, to investigate the significance of SATB1 protein as a prognostic factor we concentrated only on T-classification.

SATB1 immunohistochemical staining and quantitation. The classical immunohistochemical reaction was carried out with the use of rabbit monoclonal antibodies against the SATB1 protein (Abcam) and EnVision™ FLEX Mini kit, High pH (Dako) on 5-μm paraffin sections placed on the SuperFrost Plus microscopic slides (Thermo Fisher Scientific). The slides were examined using Eclipse E800 microscope (Nikon) with NIS-Elements 3.30 image analysis system and CCD camera (DS-5Mc-U1; Nikon) (Fig. 1). The expression intensities of SATB1 were measured along the expression path as per an intensity scoring scale (0-10) using NIS-Elements 3.30 software (Nikon). A 10-point intensity scoring scale was used considering maximum expression as 10 and minimum expression as 0. Patients with none or low SATB1 expression (0-2) were considered SATB1-negative, whereas patients with moderate (3-4) and high SATB1 expression (5-10) were considered SATB1-positive. To minimize variations in staining intensity among different experiments, several steps were taken: i) a positive (thymus, LyP) and negative (SS) control were routinely included to check staining procedure; ii) as smooth muscle cells of vessels, fibroblast and epithelial cells are weakly reactive, these cells were applied as internal controls; iii) the same batch of antibody was used for all slides. Immunostaining of SATB1 was independently evaluated by three investigators, and two of them had no previous knowledge of the clinical data. In case of different intensity estimation, the lower score was adopted. Variability between observers was examined among all patients, and was <5%.

Cell culture and cell death induction. Jurkat E6.1 cells (ATTC) were maintained in RPMI-1640 medium (Lonza Ltd., Basel, Switzerland) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, Life Technologies), containing 50 μg/ml gentamycin. The cells were cultured at 37˚C in a humidified CO_2 incubator under 5% CO_2 and 95% air. For induction of apoptosis, the cell were cultured on 10 μg/ml UCHT-1 CD3 monoclonal antibody (mAb)-coated plates (BD Pharmingen), treated with 2.5 μg/50 μl DX2 CD95 monoclonal antibody (BD Pharmingen) and 0.5 μg/50 μl recombinant Protein G (Sigma-Aldrich), or in the presence of 10 ng/ml recombinant human interleukin-2 (IL-2) (Sigma-Aldrich) for 3 days and treated with 100 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) and 1 μg/ml ionomycin (Io) for 16 h. The control cells were cultured in the same conditions without addition of mAb or PMA/Io.

Downregulation of SATB1. SATB1 was downregulated in Jurkat E6.1 cells (ATCC) using $siRNA_{SATB1}$ (corresponding to sequence 5'-CCCTGTCAGTAGGTCTATGAA-3') obtained from Qiagen. The cells were transfected with $siRNA_{SATB1}$ or non-targeting siRNA by electroporation technique using SE Cell Line 4D Nucleofector kit (Lonza) and 4D-Nucleofector Unit (Lonza) according to the manufacturer's instructions. Briefly, the cells were seeded out 2 days before electroporation to a density of $1x10^5$/ml. Then, total of $1x10^6$ cells were resuspended in 100 μl of SE Nucleofector solution, together with 30 nM of $siRNA_{SATB1}$ Qiagen or 2 μg pmaxGFP™ Control Vector (Lonza). The mixture was then transferred into a cuvette provided in the kit and the cells were electroporated using 4D-Nucleofector device (Lonza) with program CL-120. Transfection efficiency was analyzed at the day of the experiments by GFP fluorescence intensity analysis using Tali® Image-based cytometer (Invitrogen, Life Technologies) in cells transfected with pmaxGFP Control Vector (Lonza). Downregulation of SATB1 was confirmed using western blot analysis as previously described (25).

Cell death analysis. The analysis of cell death was performed using Tali Image-based cytometer and Tali Apoptosis kit (both from Invitrogen, Life Technologies) according to the manufacturer's instructions. Briefly, the cells were resuspended in Annexin binding buffer at a concentration of $1x10^6$ cell/ml. Then, 5 μl of Annexin V Alexa Fluor 488 was added to each 100 μl of sample, mixed and incubated at room temperature in the dark for 20 min. After centrifugation (5 min 300 x g), the cells were resuspended in 100 μl of Annexin V binding buffer. Then, the cells were incubated with addition of 1 μl of propidium iodide (PI) at room temperature in the dark for 3 min. Subsequently, 25 μl of stained cells were loaded into a Tali Cellular Analysis Slide (Invitrogen, Life Technologies). The data were analyzed using Flowing software (ver2.5.0; Turku University, Finland) on the assumption that viable cells are both Annexin V Alexa Fluor 488- and PI-negative

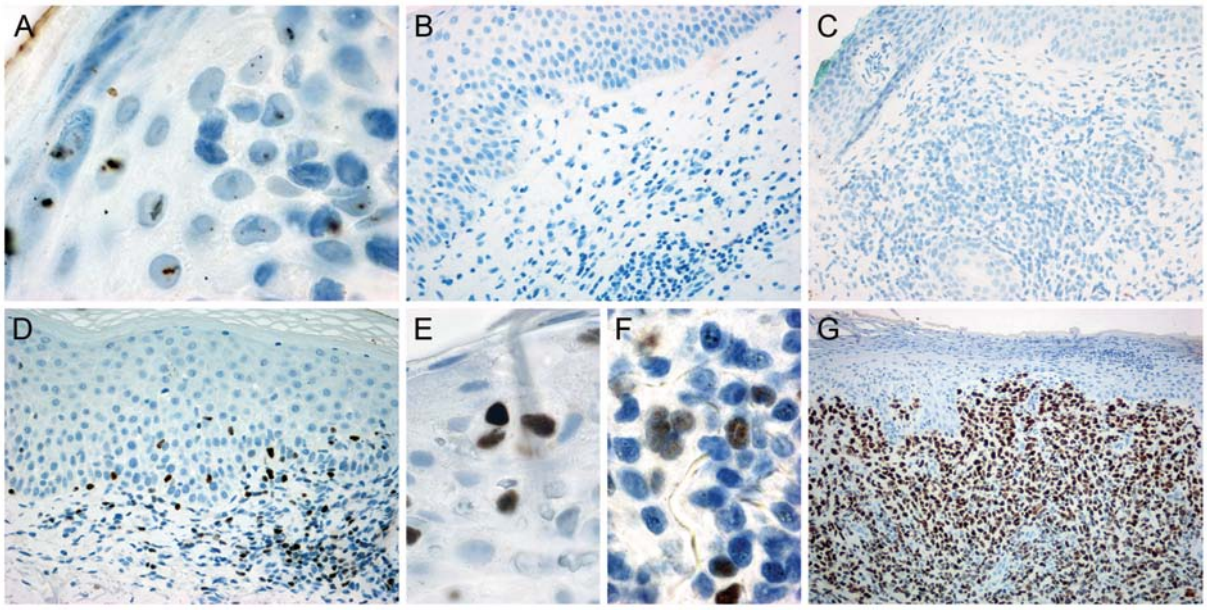

Figure 1. Light microscopic images of immunohistochemical staining for special AT-rich sequence-binding protein-1 (SATB1) in skin tissue specimens of cutaneous T-cell lymphoma (CTCL) patients. (A) Sézary syndrome, negative reaction with SATB1 antibody, cerebriform lymphocytes within epidermis. (B) Mycosis fungoides (MF) (patch stage), negative reaction with SATB1 antibody. (C) MF (plaque stage), negative reaction with SATB1 antibody. (D) MF (patch stage), positive reaction with SATB1 antibody, medium- and small-size lymphocytes. (E) MF positive reaction with SATB1 antibody, collections of mycosis lymphocytes located within the epidermis. (F) MF positive reaction with SATB1 antibody, cerebriform lymphocytes (G) Lymphomatoid papulosis, positive reaction with SATB1 antibody.

cells, apoptotic cells are Annexin V Alexa Fluor 488-positive, whereas necrotic cells are Annexin V Alexa Fluor 488-negative and PI-positive.

Statistical analysis. Jurkat E6.1 cell death data are shown as mean values ± SEM. Comparisons between different groups of cell death data were performed using a two-tailed Mann-Whitney U test. In the life span study, the data underwent Kaplan-Meier survival analysis, which included use of Gehan-Breslow-Wilcoxon, log-rank (Mentel-Cox) and log-rank for trend tests. GraphPad Prism 5.0 (GraphPad Software) was used for statistical analyses and a P-value <0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. Clinical characteristics, stage, and mean/median survival are summarized in Table I. The median age at diagnosis was 51 years (range, 28-75 years). Specifically, at the time of diagnosis, 21.67% of patients were aged <40 years, 26.67% were 40-50 years old, 28.33% were 51-60 years old, and 23.33% were aged >60 years. The male to female ratio was 2.33:1. Disease subset was diagnosed as MF (95%), SS (3.33%) and LyP (1.67%). Additionally, clinical and histological variants included folliculotropic MF (1.67%). The majority of patients (83.33%) had T1 (limited patches, papules and/or plaques covering <10% of the skin surface) or T2 (covering ≥10% of the skin surface) stage at diagnosis and only 11.67% had T4 stage (confluence of erythema covering ≥80% of body-surface area). There were no patients diagnosed with T3. According to extended ISCL/EORTC classification, at the time of diagnosis, 56.67% of total patients had only patches (T1a), whereas 5% had patches and plaques (T1b). Similarly, 21.65% patients were diagnosed in T2a (patch only) and 5% in T2b (plaque ± patch).

Patients were also classified according to the expression of SATB1. Thirty-five percent of total patients were deficient in SATB1 or presented low expression, whereas the majority of patients were characterized by moderate or high SATB1 expression (65%) and were considered as SATB1-positive.

Overall and disease-specific survival by demographic factors. Results of analysis of changes in survival with regard to demographic factors are shown in Tables I-III, with Kaplan-Meier survival curves in Fig. 2. Median survival was 20.08 years and mean survival varied according to the age at diagnosis and gender. Disease-specific mean survival was the highest for patients aged >60 years (20.42 years) and the lowest for patients diagnosed at the range of 40-50 years (18.08 years). Although patients diagnosed at the age of <40 years were characterized by the best 20-year overall survival (OS) (92.31%), disease-specific survival (DSS) was lower in this patient group than in the group with an age range of 51-60 years (94.12%) and >60 years (100%) (Table I). Moreover, pairwise comparison revealed that patients aged >60 years had extremely higher hazard ratio (HR) of DSS (HR=11.26, P=0.0456), as compared to the group diagnosed at the range of 40-50 years (Table III).

Furthermore, DSS was 20.04 years for women and only 19.21 years for men. Similarly, females had better 10- and 20-year OS/DSS (100 and 94.44% for both OS and DSS, respectively) than men (Table I). Women were more likely to survive, but this was not statistically significant (HR=1.77, P=0.1035 for OS and HR=1.13, P=0.2030 for DSS) (Table II).

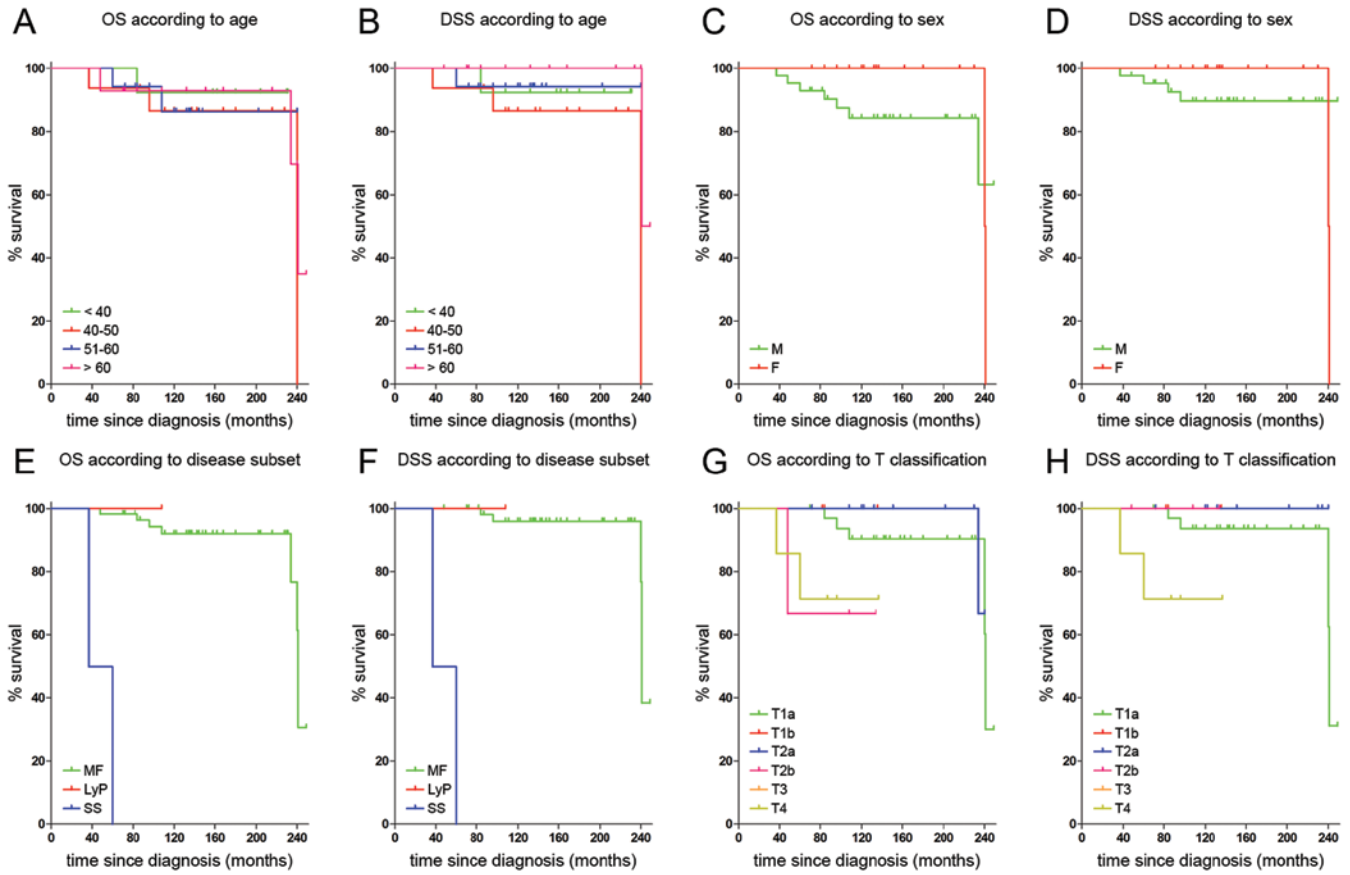

Figure 2. Kaplan-Meier analysis of overall survival (OS) and disease-specific survival (DSS) by age, gender, disease subset and T-classification. (A) OS by age. (B) DSS by age. (C) OS by gender. (D) DSS by gender. (E) OS by disease subset. (F) DSS by disease subset. (G) OS by T-classification. (H) DSS by T-classification.

Overall and disease-specific survival by disease subset and T-classification. Changes in OS and DSS are shown in Tables I-III, with Kaplan-Meier survival curves in Fig. 2. Patients with MF and LyP were characterized by much higher OS and DSS than patients with SS. Moreover, statistically significant differences were noted in HR as compared to MF and LyP or SS (HR=2.78 and HR=2.9^{-13}, P<0,0001, respectively) (Table II).

In addition, mean survival was decreased together with T stage (19.15 years for patients diagnosed at T1, 18.84 years for T2, and 9.31 years for T3 stage). Similarly, 20-year OS was decreased according to T stage (89.19% for T1, 87.50% for T2, and 71.43% for T4; Table I); however, the HR analysis showed a statistically significant difference only when comparing T1 and T4 stages (HR=0.06, P=0.0224; Table III). Pairwise comparison of extended T-classification showed statistically significantly lower OS in patients diagnosed in T2a vs. T2b (HR=4.8^{-3}, P=0.0374) and T2a vs. T4 (HR=0.05, P=0.0455), and in T1a vs. T4 (HR=0.07, P=0.0348; Table III). DSS was the highest for patients with T2 (100%) and the lowest for T4 stage (71.43%) (Table I). Furthermore, pairwise comparison showed statistically significantly lower DSS in patients with T4, as compared to T1 (HR=0.04, P=0.0199) and T2 (HR=0.03, P=0.0292) (Table III). Extended T-classification confirmed the above results, but indicated statistically significant differences only between patch stages (T1a or T2a) and T4 (HR=0.05, P=0.0287 and HR=0.05, P=0.0455, respectively).

Overall and disease-specific survival by SATB1 expression. Changes in OS and DSS in patients according to SATB1 expression are shown in Tables I-III, with Kaplan-Meier survival curves in Fig. 3. Analysis indicated that both mean survival and disease-specific mean survival were higher in patients characterized with moderate or high expression of SATB1 (increase from 16.35 to 20.02 years and from 17.00 to 20.56 years, respectively; Table I). Similar results were obtained after excluding SS and LyP from SATB1-positive and -negative groups. Moreover, the SATB1-positive patients had increased OS and DSS, as compared to patients with a lack or low SATB1 expression. There was a statistically significant increase in the likelihood of survival in both groups with included (HR=6.40, P=0.0033 for OS and HR=11.08, P=0.0033 for DSS) and excluded SS and LyP (HR=4.38, P=0.0303 for OS and HR=7.99, P=0.0286) (Table II). However, analysis of trend measured in patients classified by SATB1 labeling intensity indicated statistically significant increase of OS (HR=4.83 for low expression, HR=21.45 for medium expression, HR=4.52 for high expression, P=0.0211) and DSS (HR=3.84 for low expression, HR=34.62 for medium expression, HR=5.93 for high expression, P=0.0148) only in groups with included SS and LyP. Furthermore, pairwise comparison showed that patients characterized by moderate expression of SATB1 were more likely to survive than patients without its expression (HR=21.45, P=0.0011), and patients with high expression 4.52 times (P=0.0104), as compared to group without any SATB1

Table I. Summary of demographic and clinical staging characteristics according to ISCL/EORTC classification.

Characteristics	No.	%	Mean survival (years)	Disease-specific mean survival (years)	Median survival (years)	Disease-specific median survival (years)	Overall survival (%)			Disease-specific survival (%)		
							5 years	10 years	20 years	5 years	10 years	20 years
Total patients	60		18.68	19.30	20.08	20.08	95.00	90.00	86.67	96.67	93.33	91.67
Age (years)												
<40	13	21.67	18.31	18.31	N/A	N/A	N/A	92.31	92.31	N/A	92.31	92.31
40-50	16	26.67	18.08	18.08	20.00	20.00	93.75	87.50	81.25	93.75	87.50	81.25
51-60	17	28.33	18.25	19.12	N/A	N/A	94.12	88.24	88.24	94.12	94.12	94.12
>60	14	23.33	19.03	20.42	20.08	20.42	92.86	92.86	85.71	100.00	100.00	100.00
Gender												
Male	42	70.00	18.21	19.21	N/A	N/A	92.86	85.71	83.33	95.24	90.48	90.48
Female	18	30.00	20.04	20.04	20.04	20.04	N/A	100.00	94.44	N/A	100.00	94.44
Disease subset												
MF	57	95.00	19.16	19.82	20.08	20.08	98.25	92.98	89.47	100.00	96.49	94.74
LyP	1	1.67	N/A	N/A	N/A	N/A	N/A	100.00	N/A	N/A	100.00	N/A
SS	2	3.33	4.04	4.04	4.04	4.04	0.00	N/A	N/A	0.00	N/A	N/A
Clinical/histologic variants												
MF	56	93.33	19.14	19.81	20.08	20.08	98.21	92.86	89.29	100.00	96.43	94.64
Foll. MF	1	1.67	N/A	N/A	N/A	N/A	N/A	N/A	100.00	N/A	N/A	100.00
T-classification												
T1												
Total	37	61.67	19.15	19.51	20.08	20.08	N/A	91.89	89.19	N/A	94.59	91.89
T1a	34	56.67	19.10	19.48	20.08	20.08	N/A	91.18	88.24	N/A	94.12	91.18
T1b	3	5.00	N/A	N/A	N/A	N/A	N/A	100.00	100.00	N/A	100.00	100.00
T2												
Total	16	26.67	18.84	N/A	N/A	N/A	93.75	93.75	87.50	100.00	100.00	100.00
T2a	13	21.67	19.83	N/A	N/A	N/A	N/A	100.00	92.31	N/A	100.00	100.00
T2b	3	5.00	8.78	N/A	N/A	N/A	66.67	100.00	100.00	100.00	100.00	100.00
T3	0	0.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
T4	7	11.67	9.31	9.31	N/A	N/A	71.43	71.43	71.43	71.43	71.43	71.43
SATB1^{-}												
Included SS												
Total	21	35.00	16.35	17.00	20.08	20.08	85.71	76.19	76.19	90.48	80.95	80.95

Table I. Continued.

Characteristics	No.	%	Mean survival (years)	Disease-specific mean survival (years)	median survival (years)	Disease-specific Median survival (years)	Overall survival (%)			Disease-specific survival (%)		
							5 years	10 years	20 years	5 years	10 years	20 years
Intensity (IHC)												
0	9	15.00	13.61	15.00	20.08	20.08	77.78	55.56	55.56	88.89	66.67	66.67
1-2	12	20.00	12.49	12.49	N/A	N/A	91.67	91.67	91.67	91.67	91.67	91.67
Excluded SS												
Total	19	31.67	17.65	18.41	20.08	20.08	94.74	84.21	84.21	100.00	89.47	89.47
Intensity (IHC)												
0	8	13.33	14.93	16.49	20.08	20.08	87.50	62.50	62.50	100.00	75.00	75.00
1-2	11	18.33	N/A	N/A	N/A	N/A	100.00	100.00	100.00	100.00	100.00	100.00
SATB1^{+}												
Included LyP												
Total	39	65.00	20.02	20.56	N/A	N/A	N/A	97.44	92.31	N/A	100.00	97.44
Intensity (IHC)												
3-4	25	41.67	20.19	N/A	N/A	N/A	N/A	96.00	96.00	N/A	100.00	100.00
5-10	14	23.33	19.83	20.00	20.38	20.38	N/A	100.00	85.71	N/A	100.00	92.86
Excluded LyP												
Total	38	63.33	20.01	20.56	N/A	N/A	N/A	97.37	92.11	N/A	100.00	97.37
Intensity (IHC)												
3-4	25	41.67	20.19	N/A	N/A	N/A	N/A	96.00	96.00	N/A	100.00	100.00
5-10	13	21.67	19.83	20.00	20.00	N/A	N/A	100.00	84.62	N/A	100.00	92.31

M, male; F, female; IHC, immunohistochemistry; MF, mycosis fungoides; SS, Sézary syndrome; LyP, lymphomatoid papulosis; N/A, not available.

Table II. Analysis of demographic and clinical staging factors with regard to changes in overall and disease-specific survival.

	Overall survival					Disease-specific survival				
Factors	HR	95% CI	P-value[a]	P-value[b]	P-value[c]	HR	95% CI	P-value[a]	P-value[b]	P-value[c]
Age (years)										
<40	1.00			0.8565	0.7915	1.00			0.3517	0.2226
40-50	0.59	0.06-5.72				0.59	0.06-5.72			
51-60	0.61	0.06-5.91				1.19	0.07-19.24			
>60	0.95	0.06-15.35				6.34	0.12-323.70			
Gender										
Male	1.00		0.1035	0.4172		1.00		0.2030	0.8876	
Female	1.77	0.44-7.04				1.13	0.21-6.08			
Disease subset										
MF	1.00			**<0.0001**	**<0.0001**	1.00			**<0.0001**	**<0.0001**
LyP	2.78	0.25E-2-3020.00				2.774	0.13E-3-57323.00			
SS	0.29E-12	0.15E-15-0.57E-9				0.12E-16	0.18E-20-0.75E-13			
T-classification										
Simplified classification										
T1	1.00			0.1089	0.0909	1.00			**0.0192**	0.0869
T2	1.06	0.19-5.84				4.47	0.39-51.21			
T4	0.06	0.35E-2-0.91				0.04	0.24E-2-0.76			
Extended classification										
T1a	1.00			0.1214	0.0991	1.00			0.0896	0.1688
T1b	2.85	0.95E-2-847.90				2.86	0.34E-2-2368.00			
T2a	1.92	0.29-12.68				4.36	0.36-52.13			
T2b	0.05	0.10E-2-2.68				2.90	0.84E-2-1000.00			
T4	0.07	0.45E-2-1.036				0.05	0.32E-2-0.88			
Progression										
T1a to T2a	1.00			0.8137	0.5064	1.00			0.78	0.4102
T1b to T2a	2.91	0.02-343.10				2.92	0.99E-2-859.00			
T2a to T2b	2.86	0.01-591.00				2.85	0.35E-2-2342.00			
T2 to T3	0.38	0.06-2.51				0.29	0.04-2.15			
SATB1 expression (IHC)										
Included LyP and SS										
No	1.00		**0.0033**	**0.0129**		1.00		**0.0033**	**0.0068**	
Yes	6.40	1.48-27.62				11.08	1.94-63.18			
Excluded LyP and SS										
No	1.00		**0.0303**	0.0857		1.00		**0.0286**	3.562	
Yes	4.38	0.81-23.60				7.99	0.92-69.23			
SATB1 intensity (IHC)										
Included LyP and SS										
0	1.00			**0.0260**	**0.0211**	1.00			**0.0276**	**0.0148**
1-2	4.83	0.81-28.67				3.84	0.53-28.08			
3-4	21.45	3.33-138.20				34.62	3.81-314.80			
5-10	4.52	0.82-24.85				5.93	0.75-46.62			
Excluded LyP and SS										
0	1.00			0.0695	0.0984	1.00			6.481	0.0897
1-2	9.80	1.00-96.07				9.37	0.58-152.40			
3-4	18.42	2.36-143.50				32.07	2.52-408.10			
5-10	3.593	0.5472-23.59				4.16	0.3841-45.01			

HR, hazard ratio; CI, confidence interval; IHC, immunohistochemistry; SS, Sézary syndrome; LyP, lymphomatoid papulosis. Bold, statistically significant differences. [a]Gehan-Breslow-Wilcoxon test; [b]Log-rank (Mantel-Cox) test; [c]log-rank test for trend.

Table III. Pairwise comparison of demographic and clinical staging factors with regard to changes in overall and disease-specific survival.

	Overall survival				Disease-specific survival			
Factor	HR	95% CI	P-value[a]	P-value[b]	HR	95% CI	P-value[a]	P-value[b]
Age (years)								
<40 vs. 40-50	0.59	0.06-5.72	0.6723	0.6494	0.59	0.06-5.72	0.6723	0.6494
<40 vs. 51-60	0.61	0.06-5.91	0.6939	0.6683	1.19	0.07-19.24	0.9603	0.9037
<40 vs. >60	0.95	0.06-15.35	0.9117	0.9738	6.34	0.12-323.70	0.3576	0.3576
40-50 vs. 51-60	1.58	0.27-9.11	0.8621	0.6111	2.85	0.40-20.26	0.5445	0.2951
40-50 vs. >60	2.35	0.36-15.35	0.6453	0.3713	11.26	1.05-120.80	0.1530	**0.0456**
51-60 vs. >60	1.17	0.15- 9.20	0.8359	0.8832	5.84	0.11-304.90	0.3819	0.3819
T-classification								
Simplified classification								
T1 vs. T2	1.06	0.19-5.84	0.9814	0.9461	4.47	0.39-51.21	0.3408	0.2291
T1 vs. T4	0.06	0.35E-2-0.91	**0.0244**	**0.0426**	0.04	0.24E-2-0.76	**0.0199**	**0.0319**
T2 vs. T4	0.16	0.013-1.91	0.1476	0.1467	0.03	0.16E-2-0.70	**0.0292**	**0.0288**
Extended classification								
T1a vs. T1b	2.85	0.95E-2-847.9	0.7194	0.7188	2.86	0.34E-2-2368	0.7595	0.7592
T1a vs. T2a	1.92	0.29-12.68	0.3154	0.4996	4.36	0.36-52.13	0.3640	0.2450
T1a vs. T2b	0.05	0.10E-2-2.68	0.0966	0.1422	2.90	0.84E-2-1000	0.7216	0.7213
T1a vs. T4	0.07	0.45E-2-1.036	**0.0348**	0.0531	0.05	0.32E-2-0.8804	**0.0287**	**0.0405**
T1b vs. T2a	N/A	N/A	1.0000	1.0000	N/A	N/A	1.0000	1.0000
T1b vs. T2b	0.14	0.27E-2-6.82	0.3173	0.3173	N/A	N/A	1.0000	1.0000
T1b vs. T4	0.23	0.01-4.55	0.3367	0.3354	0.23	0.01-4.55	0.3367	0.3354
T2a vs. T2b	0.48E-2	0.32e-4-0.73	**0.0374**	**0.0374**	N/A	N/A	1.0000	1.0000
T2a vs. T4	0.05	0.26E-2-0.94	**0.0455**	**0.0451**	0.05	0.26E-2-0.94	**0.0455**	**0.0451**
T2b vs. T4	1.21	0.10-14.56	0.8886	0.8822	0.25	0.01-5.61	0.3841	0.383
Progression								
T1a-T2a vs. T1b-T2a	2.91	0.02-343.1	0.6625	0.6609	2.92	0.99E-2-859.0	0.7119	0.7114
T1a-T2a vs. T2a-T2b	2.86	0.01-591.0	0.7009	0.6995	2.85	0.35E-2-2342	0.7604	0.7598
T1a-T2a vs. T2-T3	0.38	0.06-2.51	0.2022	0.3152	0.29	0.04-2.15	0.1370	0.2252
T1b-T2a vs. T2a-T2b	N/A	N/A	1.0000	1.0000	N/A	N/A	1.0000	1.0000
T1b-T2a vs. T2-T3	0.28	0.90E-2-8.62	0.4669	0.4658	0.28	0.90E-2-8.62	0.4669	0.4658
T2a-T2b vs. T2-T3	0.31	0.66E-2-14.15	0.5465	0.5449	0.31	0.66E-2-14.15	0.5465	0.5449
SATB1 intensity (IHC)								
Included LyP and SS								
0 vs. 1-2	4.83	0.81-28.67	0.0956	0.0830	3.84	0.53-28.08	**0.2222**	**0.1845**
0 vs. 3-4	21.45	3.33-138.2	**0.0011**	**0.0013**	34.62	3.81-314.8	**0.0017**	**0.0016**
0 vs. 5-10	4.52	0.82-24.85	**0.0104**	0.0827	5.93	0.75-46.62	**0.0236**	0.0905
1-2 vs. 3-4	3.64	0.15-89.46	0.3203	0.4296	21.83	0.33-1437.00	0.1489	0.1489
1-2 vs. 5-10	8.73	0.17-445.10	0.2801	0.2801	8.73	0.17-445.10	0.2801	0.2801
3-4 vs. 5-10	0.49	0.05-4.89	0.5927	0.5435	0.14	0.27E-2-6.82	0.3173	0.3173
Excluded LyP and SS								
0 vs. 1-2	9.80	1.00-96.07	0.0513	**0.0499**	9.37	0.58-152.40	0.1167	0.1159
0 vs. 3-4	18.42	2.36-143.50	**0.0050**	**0.0054**	32.07	2.52-408.10	**0.0068**	**0.0075**
0 vs. 5-10	3.59	0.55-23.59	**0.0246**	0.1829	4.16	0.38-45.01	0.0673	0.2410
1-2 vs. 3-4	0.29	0.20E-2-41.89	0.6256	0.6256	N/A	N/A	1.0000	1.0000
1-2 vs. 5-10	N/A	N/A	1.0000	1.0000	N/A	N/A	1.0000	1.0000
3-4 vs. 5-10	0.50	0.05-4.96	0.5839	0.5533	0.14	0.27E-2-6.82	0.3173	0.3173

HR, hazard ratio; CI, confidence interval; IHC, immunohistochemistry; SS, Sézary syndrome; LyP, lymphomatoid papulosis; N/A, not available. Bold, statistically significant differences. [a]Gehan-Breslow-Wilcoxon test; [b]Log-rank (Mantel-Cox) test.

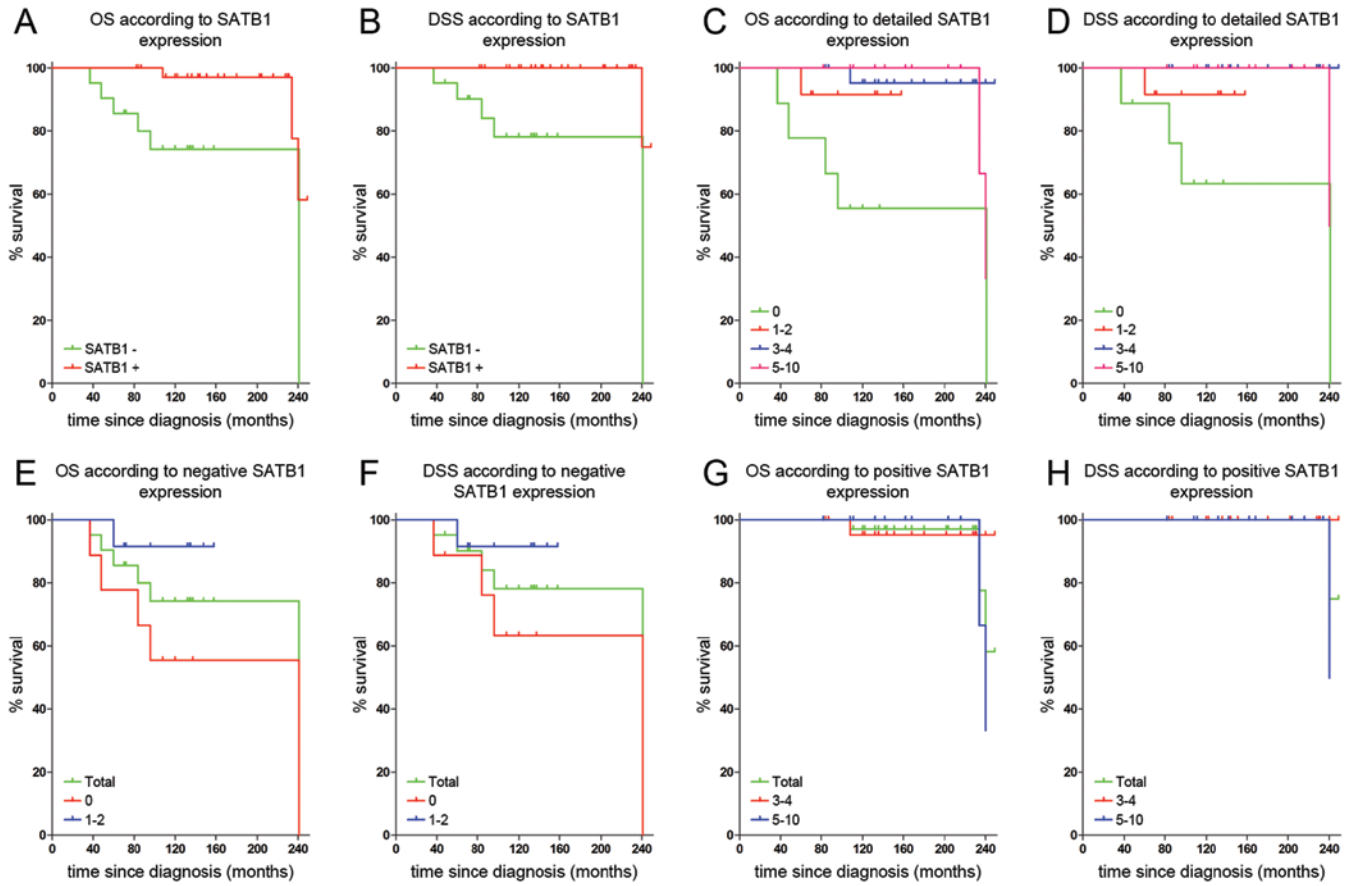

Figure 3. Kaplan-Meier analysis of overall survival (OS) and disease-specific survival (DSS) by special AT-rich sequence-binding protein-1 (SATB1) expression. (A) OS by positive and negative SATB1 expression. (B) DSS by positive and negative SATB1 expression. (C) OS by numerical SATB1 expression. (D) DSS by numerical SATB1 expression. (E) OS by numerical SATB1 expression (negative). (F) DSS by numerical SATB1 expression (negative). (G) OS by numerical SATB1 expression (positive). (H) DSS by numerical SATB1 expression (positive).

labeling (Table III). Exclusion of SS and LyP showed similar results; however, statistically significant differences in distribution of survival curve were observed after comparison of groups of patients that were characterized as SATB1-negative (HR=9.80, P=0.0499). According to pairwise comparison of DSS after exclusion of SS and LyP, statistically significant changes in the likelihood of survival were observed only when comparing patients with moderate expression of SATB1 to patients without any SATB1 labeling (HR=32.07, P=0.0068; Table III).

Risk of disease progression by demographic factors, disease subset and T-classification. Disease progression was noted in 50% (without T1a to T1b and T2a to T2b) or 51.67% (with T1a to T1b and T2a to T2b), and is shown in Tables IV-VI, with Kaplan-Meier time-to-event curves in Fig. 4. Risk of disease progression (RDP) was considered in patients divided according to age, gender, disease subset as well as T-classification. The analysis of total patients showed that RDP increased with time and independently of T1a to T1b and T2a to T2b progression (1.67% for 5 years RDP, 21.67% for 10 years RDP, and 48.33 or 50% for 20 years RDP without and with T1a to T1b and T2a to T2b, respectively; Table IV).

According to the age at the time of diagnosis, the highest 20-year RDP was observed in patients at the age range of 51-60 years (58.82%), whereas the lowest was in patients classified in the group aged 40-50 years (37.50%) (Table IV). However, pairwise comparison of age groups did not show statistically significant changes (Table VI). Similarly, analysis of RDP, both without and with progression from T1a to T1b and T2a to T2b, showed higher risk in women (4.76% for 10 years RDP and 16.67 or 19.05% for 20 years RDP, respectively) than in men (16.67% for 10 years RDP and 44.44% for 20 years RDP) but was not statistically significant (Table IV).

Pairwise comparison showed statistically significant lower risk of progression for patients diagnosed in both LyP and SS (HR=7.6^{-3} and HR=5.4^{-13}, respectively), as compared to MF (P<0.0001). Moreover, analysis of patients grouped according to T-classification showed the highest 20-year RDP in patients diagnosed at T2 (75% for RDP both without or with progression from T1a to T1b and T2a to T2b) and the lowest at T1 stage (37.84 or 40.54% for RDP without or with progression from T1a to T1b and T2a to T2b, respectively; Table IV). Furthermore, analysis of trend showed a statistically significant decrease of RDP calculated without or with T1a to T1b and T2a to T2b progression (HR=0.48 for T2 and HR=0.08 for T4, P=0.0157 or HR=0.52 for T2 and HR=0.10 for T4, P=0.0224, respectively; Table V). However, pairwise comparison revealed statistically significant changes between T1 and T4 stages (HR=0.08, P=0.0125 or HR=0.10,

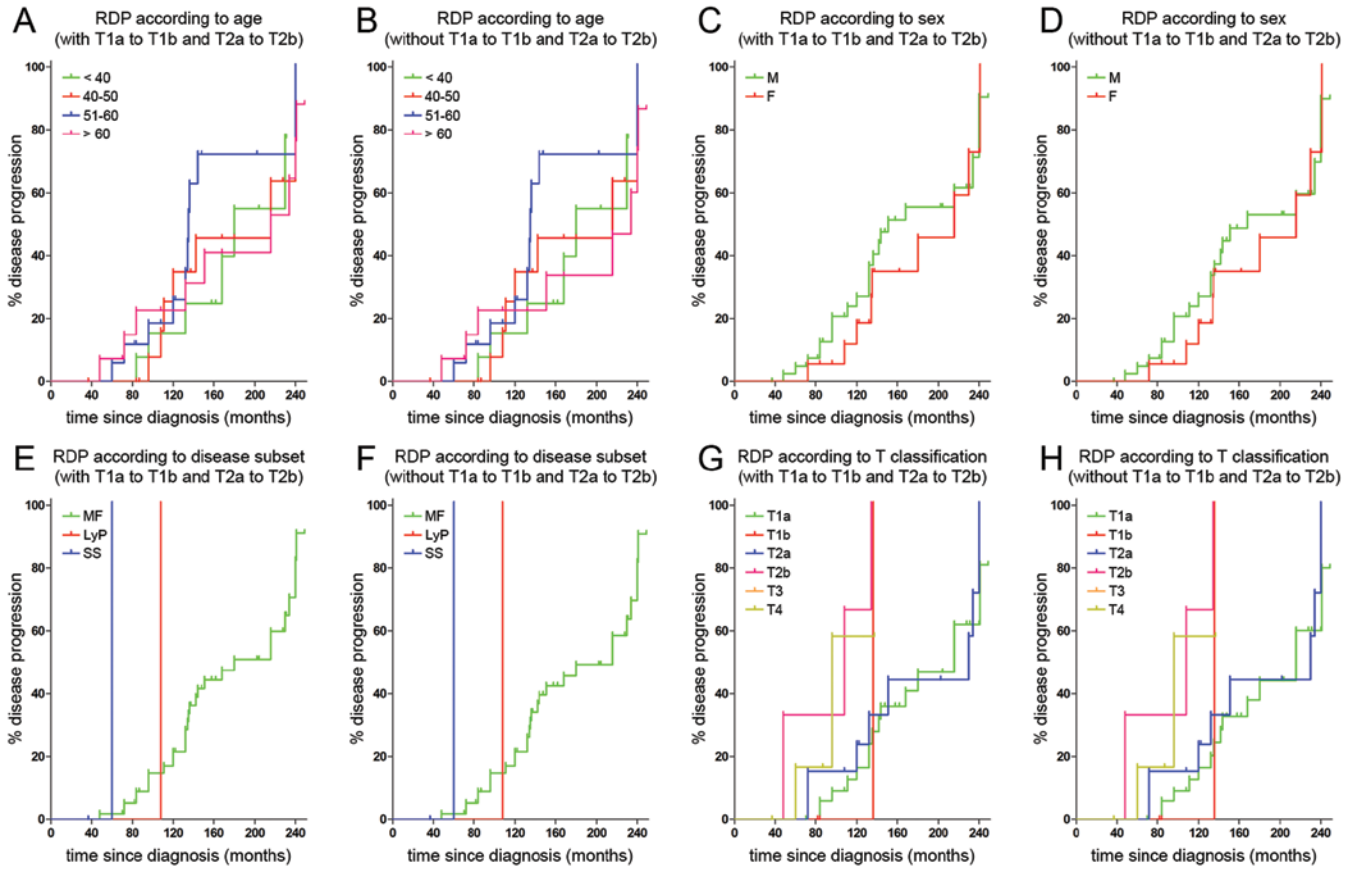

Figure 4. Kaplan-Meier analysis of risk of disease progression (RDP) by age, gender, disease subset and T-classification. (A) RDP with T1a to T1b and T2a to T2b by age. (B) RDP without T1a to T1b and T2a to T2b by age. (C) RDP with T1a to T1b and T2a to T2b by gender. (D) RDP without T1a to T1b and T2a to T2b by gender. (E) RDP with T1a to T1b and T2a to T2b by disease subset. (F) RDP without T1a to T1b and T2a to T2b by disease subset. (G) RDP with T1a to T1b and T2a to T2b by T-classification. (H) RDP without T1a to T1b and T2a to T2b by T-classification.

P=0.0146 for RDP without or with progression from T1a to T1b and T2a to T2b, respectively; Table VI). Similarly, comparison of RDP according to extended T-classification showed statistically significant decrease with stage (P=0.0042 or P=0.0072; Table V). However, pairwise analysis showed statistically significant differences between patients diagnosed at T1a and T2b (HR=4.6^{-3}, P=0.0006 or HR=7.5^{-3}, P=0.0009, respectively), T1a and T4 (HR=0.08, P=0.0138 or HR=0.09, P=0.0162, respectively), as well as between patients diagnosed at T2a and T2b (HR=0.08, P=0.0376) (Table VI).

Risk of disease progression by SATB1 expression. Disease progression was also analyzed according to SATB1 expression and is shown in Tables III-VI, with Kaplan-Meier time-to-event curves in Fig. 5. According to patients grouped as SATB1-negative, the 20-year RDP was higher in patients characterized without any SATB1 labeling (55.56%) than in patients with low SATB1 expression (50%). Additionally, SATB1-positive patients were characterized by lower 20-year RDP (46.15%), as compared to SATB-negative patients (52.38%). Furthermore, analysis of trend showed a statistically significant increase of RDP calculated without or with T1a to T1b and T2a to T2b progression in SATB1-positive patients (HR=3.39, P=0.0005 or HR=3.85, P=0.0002, respectively), even if SS and LyP were excluded from experimental groups (HR=3.35, P=0.0008 or HR=3.86, P=0.0003, respectively; Table V). However, pairwise comparison revealed statistically significant changes between patients without SATB1 labeling and its moderate expression (HR=3.22, P=0.0051), without SATB1 labeling and its high expression (HR=2.54, P=0.0196), low and moderate SATB1 expression (HR=4.14, P=0.0165 or HR=5.23, P=0.0064), as well as between low and high SATB1 expression (HR=4.86, P=0.0255 or HR=5.72, P=0.0110). Similar results were obtained following HR analysis without SS and LyP patients (Table VI).

It is also of note that SATB1-positive patients stayed longer in each T stage (8.64 years in T1, 8.36 years in T2, 3.5 years in T3, and 7.25 years in T4) than SATB1-negative patients (4.58 years in T1, 5.67 years in T2, 2.08 years in T3. and 7.25 years in T4), which correlated with enhanced survival of these patients.

Apoptosis induction in Jurkat cells with downregulated SATB1. To investigate the possible mechanism by which patients deficient in SATB1 or characterized by low SATB1 expression have poorer prognosis, we subjected downregulated Jurkat cells to apoptosis analysis after activation with CD3 mAb, CD95 mAb and PMA/Io after a 3-day stimulation with IL-2 (Fig. 6). The results showed that downregulation of SATB1 using siRNA caused statistically significant resistance to activation-induced cell death (AICD) in Jurkat cells, in all cases of treatment (from 49.22 to 25.07%, P=0.0294; from

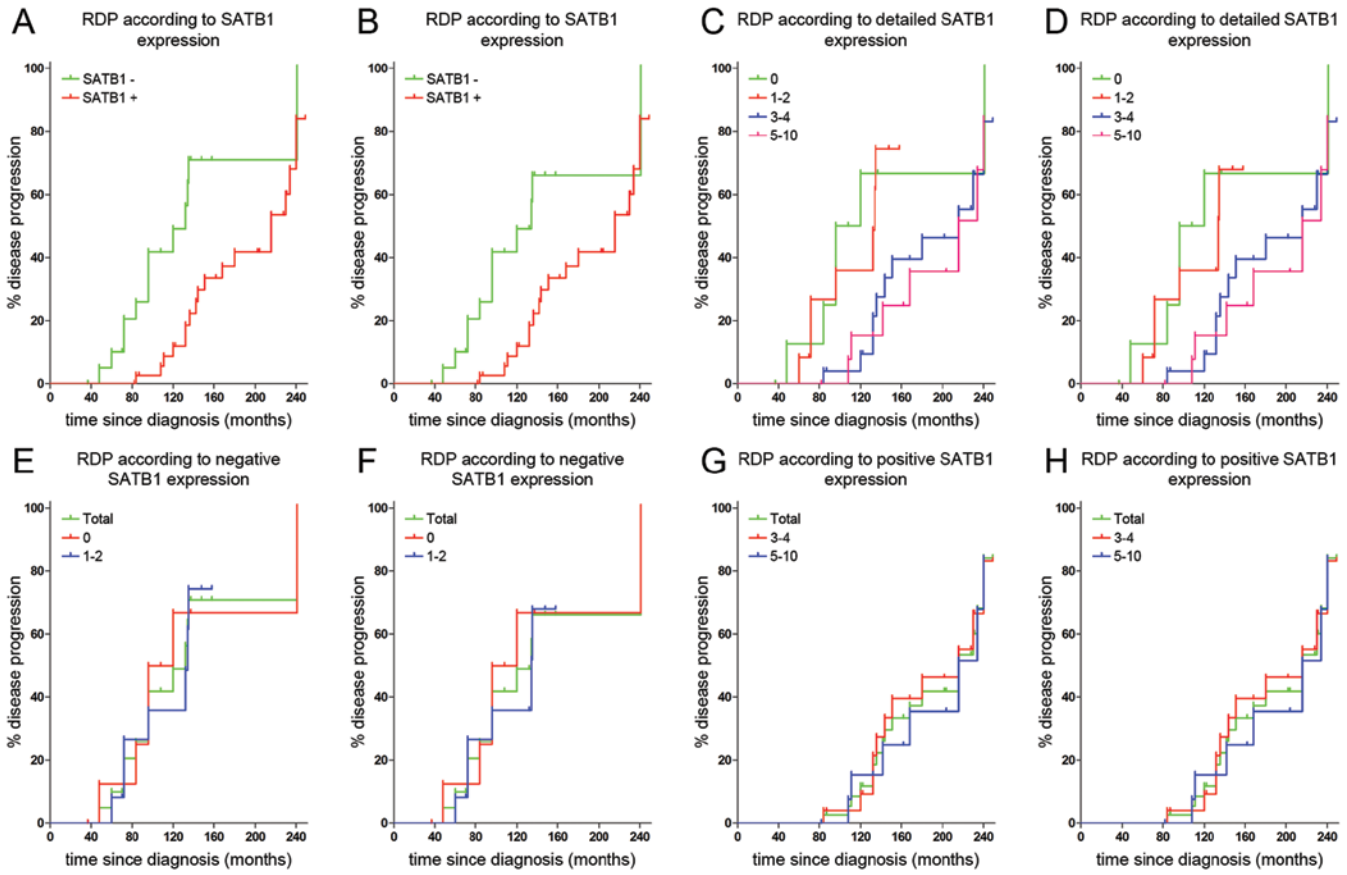

Figure 5. Kaplan-Meier analysis of risk of disease progression (RDP) by special AT-rich sequence-binding protein-1 (SATB1) expression. (A) RDP with T1a to T1b and T2a to T2b by positive and negative SATB1 expression. (B) RDP without T1a to T1b and T2a to T2b by positive and negative SATB1 expression. (C) RDP with T1a to T1b and T2a to T2b by numerical SATB1 expression. (D) RDP without T1a to T1b and T2a to T2b by numerical SATB1 expression. (E) RDP with T1a to T1b and T2a to T2b by numerical SATB1 expression (negative). (F) RDP without T1a to T1b and T2a to T2b by numerical SATB1 expression (negative). (G) RDP with T1a to T1b and T2a to T2b by numerical SATB1 expression (positive). (H) RDP without T1a to T1b and T2a to T2b by numerical SATB1 expression (positive).

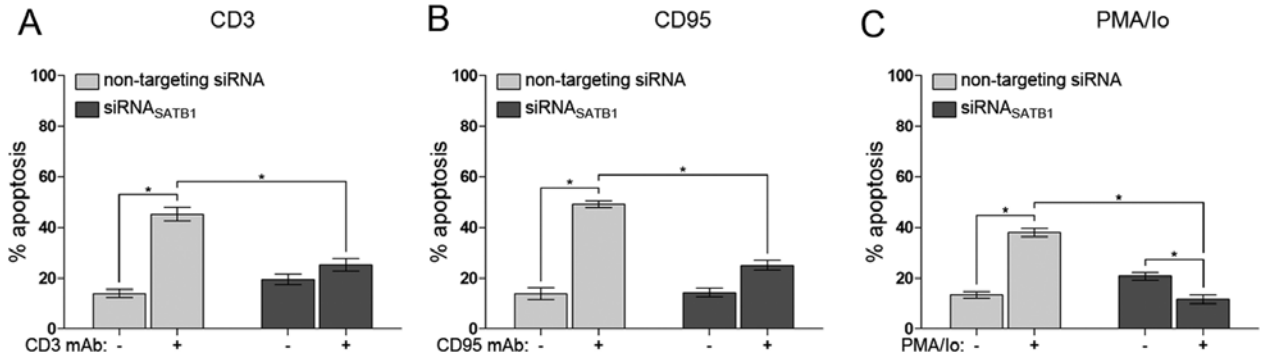

Figure 6. Effect of downregulation of special AT-rich sequence-binding protein-1 (SATB1) on activation-induced cell death in Jurkat cells. (A) Percentage of apoptotic cells after 16 h culture on 10 μg/ml UCHT-1 CD3 monoclonal antibody-coated plates. (B) Percentage of apoptotic cells after 16-h treatment with 2.5 μg/50 μl DX2 CD95 monoclonal antibody. (C) Percentage of apoptotic cells after a 3-day culture in the presence of 10 ng/ml recombinant human IL-2 and 16-h treatment with 100 ng/ml phorbol 12-myristate 13-acetate and 1 μg/ml ionomycin.

45.27 to 25.28, P=0.0159; and from 38.03 to 11.67%, P=0.0357; after treatment of cells transfected with non-targeting siRNA and siRNA$_{SATB1}$ with CD3 mAb, CD95 mAb and PMA/Io, respectively). Moreover, the analysis did not show any statistically significant differences between appropriate controls (cells transfected with non-targeting siRNA and siRNA$_{SATB1}$).

Discussion

MF is a type of epidermotropic primary CTCL characterized by a slow clinical course and proliferation of small and medium-sized T lymphocytes with cerebriform nuclei (26-29). MF is the most common form of CTCL and accounts for

Table IV. Summary of demographic and clinical staging characteristics according to ISCL/EORTC classification.

			Risk of disease progression w/o T1a to T1b and T2a-T2b (%)			Risk of disease progression with T1a to T1b and T2a-T2b (%)		
Characteristics	No	%	5 years	10 years	20 years	5 years	10 years	20 years
Total patients	60		1.67	21.67	48.33	1.67	21.67	50.00
Age (years)								
<40	13	21.67	N/A	15.38	46.15	N/A	15.38	46.15
40-50	16	26.67	0.00	25.00	37.50	0.00	25.00	37.50
51-60	17	28.33	5.88	23.53	58.82	5.88	23.53	58.82
>60	14	23.33	7.14	21.43	50.00	7.14	21.43	57.14
Gender								
Male	42	70.00	4.76	4.76	16.67	4.76	7.14	19.05
Female	18	30.00	N/A	16.67	44.44	N/A	16.67	44.44
Disease subset								
MF	57	95.00	1.75	19.30	47.37	1.75	19.30	49.12
LyP	1	1.67	N/A	100.00	N/A	N/A	100.00	N/A
SS	2	3.33	0.00	50.00	N/A	0.00	50.00	N/A
Clinical/histologic variants								
MF	56	93.33	1.79	19.64	46.43	1.79	19.64	48.21
Foll. MF	1	1.67	N/A	N/A	100.00	N/A	N/A	100.00
T-classification								
T1								
Total	37	61.67	N/A	13.51	37.84	N/A	13.51	40.54
T1a	34	56.67	N/A	14.71	38.24	N/A	14.71	41.18
T1b	3	5.00	N/A	0.00	33.33	N/A	0.00	33.33
T2								
Total	16	26.67	6.25	31.25	75.00	6.25	31.25	75.00
T2a	13	21.67	N/A	23.08	69.23	N/A	23.08	69.23
T2b	3	5.00	33.33	33.33	33.33	33.33	33.33	33.33
T3	0	0.00	N/A	N/A	N/A	N/A	N/A	N/A
T4	7	11.67	14.29	42.86	42.86	14.29	42.86	42.86
SATB1^{-}								
Included SS								
Total	21	35.00	9.52	42.86	52.38	9.52	42.86	57.14
Intensity (IHC)								
0	9	15.00	11.11	55.56	55.56	11.11	55.56	55.56
1-2	12	20.00	8.33	33.33	50.00	8.33	33.33	58.33
Excluded SS								
Total	19	31.67	5.26	42.11	52.63	5.26	42.11	57.89
Intensity (IHC)								
0	8	13.33	12.50	62.50	62.50	12.50	62.50	62.50
1-2	11	18.33	0.00	27.27	45.45	0.00	27.27	54.55
SATB1^{+}								
Included LyP								
Total	39	65.00	N/A	10.26	46.15	N/A	10.26	46.15
Intensity (IHC)								
3-4	25	41.67	N/A	8.00	44.00	N/A	8.00	44.00
5-10	14	23.33	N/A	0.00	50.00	N/A	0.00	50.00
Excluded LyP								
Total	38	63.33	N/A	7.89	44.74	N/A	7.89	44.74
Intensity (IHC)								
3-4	25	41.67	N/A	8.00	44.00	N/A	8.00	44.00
5-10	13	21.67	N/A	0.00	46.15	N/A	0.00	46.15

IHC, immunohistochemistry; MF, mycosis fungoides; SS, Sézary syndrome; LyP, lymphomatoid papulosis. N/A, not available.

Table V. Analysis of demographic and clinical staging factors with regard to changes in risk of disease progression.

	Risk of disease progression w/o T1a to T1b and T2a to T2b					Risk of disease progression with T1a to T1b and T2a to T2b				
Factors	HR	95% CI	P-value[a]	P-value[b]	P-value[c]	HR	95% CI	P-value[a]	P-value[b]	P-value[c]
Age (years)										
<40	1.00			0.4388	0.9992	1.00			0.5252	0.8179
40-50	0.97	0.31-3.04				0.97	0.31-3.04			
51-60	0.55	0.19-1.55				0.55	0.19-1.55			
>60	1.38	0.41-4.61				1.13	0.35-3.60			
Gender										
Male	1.00		0.3744	0.5946		1.00		0.3192	0.5136	
Female	1.23	0.57-2.66				1.29	0.60-2.75			
Disease subset										
MF	1.00			**<0.0001**	**<0.0001**	1.00			**<0.0001**	**<0.0001**
LyP	0.76E-2	0.65E-4-0.90				0.76E-2	0.65E-4-0.90			
SS	0.54E-12	0.13E-16-0.22E-7				0.54E-12	0.13E-16-0.22E-7			
T-classification										
Simplified classification										
T1	1.00			0.0541	**0.0157**	1.00			0.0732	**0.0224**
T2	0.48	0.21-1.14				0.52	0.22-1.21			
T4	0.08	0.96E-2-0.72				0.10	0.01-0.80			
Extended classification										
T1a	1.00			**0.0042**	**0.0057**	1.00			**0.0072**	**0.0090**
T1b	0.17	0.60E-2-4.55				0.22	0.95E-2-5.14			
T2a	0.62	0.24-1.58				0.67	0.27-1.68			
T2b	0.46E-2	0.26E-3-0.08				0.75E-2	0.47E-3 -0.12			
T4	0.08	0.88E-2-0.69				0.09	0.01-0.77			
Progression										
T1a to T2a	1.00			0.7314	0.9971	1.00			0.8041	0.9056
T1b to T2a	0.14	0.44E-2-4.29				0.22	0.92E-2-5.20			
T2a to T2b	0.72	0.06-7.97				0.80	0.08-8.08			
T2 to T3	1.00	0.20-5.00				1.11	0.24-5.17			
SATB1 expression (IHC)										
Included LyP and SS										
No	1.00		**0.0005**	**0.0089**		1.00		**0.0002**	**0.0033**	
Yes	3.39	1.36-8.45				3.85	1.57-9.48			
Excluded LyP and SS										
No	1.00		**0.0008**	**0.0129**		1.00		**0.0003**	**0.0048**	
Yes	3.35	1.29-8.70				3.86	1.51-9.85			
SATB1 intensity (IHC)										
Included LyP and SS										
0	1.00			0.0935	**0.0391**	1.00			**0.0386**	**0.0251**
1-2	1.25	0.36-4.31				1.10	0.33-3.63			
3-4	3.22	0.90-11.46				3.22	0.90-11.46			
5-10	2.54	0.66-9.68				2.54	0.66-9.68			
Excluded LyP and SS										
0	1.00			0.1261	**0.0321**	1.00			0.0574	**0.0202**
1-2	1.48	0.40-5.46				1.28	0.37-4.45			
3-4	3.22	0.90-11.46				3.22	0.90-11.46			
5-10	2.97	0.74-11.95				2.97	0.74-11.95			

HR, hazard ratio; CI, confidence interval; IHC, immunohistochemistry; SS, Sézary syndrome; LyP, lymphomatoid papulosis. Bold, statistically significant differences. [a]Gehan-Breslow-Wilcoxon test; [b]log-rank (Mantel-Cox) test; [c]log-rank test for trend.

Table VI. Pairwise comparison of demographic and clinical staging factors with regard to changes in risk of disease progression.

	Risk of disease progression w/o T1a to T1b and T2a to T2b				Risk of disease progression with T1a to T1b and T2a to T2b			
Factors	HR	95% CI	P-value[a]	P-value[b]	HR	95% CI	P-value[a]	P-value[b]
Age (years)								
<40 vs. 40-50	0.97	0.31-3.04	0.8946	0.9546	0.97	0.31-3.04	0.8946	0.9546
<40 vs. 51-60	0.55	0.19-1.55	0.2255	0.2570	0.55	0.19-1.55	0.2255	0.2570
<40 vs. >60	1.38	0.41-4.61	0.9216	0.6004	1.13	0.35-3.60	0.7045	0.8354
40-50 vs. 51-60	0.56	0.21-1.53	0.3856	0.2586	0.56	0.21-1.53	0.3856	0.2586
40-50 vs. >60	1.07	0.34-3.34	0.8778	0.9020	0.93	0.31-2.78	0.7200	0.9002
51-60 vs. >60	2.13	0.76-5.95	0.4031	0.1477	1.81	0.67-4.91	0.5392	0.2432
T-classification								
Simplified classification								
T1 vs. T2	0.48	0.21-1.14	0.0973	0.0956	0.52	0.22-1.21	0.1309	0.1288
T1 vs. T4	0.08	0.96E-2-0.72	**0.0125**	**0.0238**	0.10	0.01-0.80	**0.0146**	**0.0302**
T2 vs. T4	0.47	0.09-2.40	0.3304	0.3627	0.47	0.09-2.40	0.3304	0.3627
Extended classification								
T1a vs. T1b	0.17	0.60E-2-4.55	0.5812	0.2879	0.22	0.95E-2-5.14	0.6362	0.3473
T1a vs. T2a	0.62	0.24-1.58	0.4703	0.3209	0.67	0.27-1.68	0.5649	0.3956
T1a vs. T2b	0.46E-2	0.26E-3-0.08	**0.0006**	**0.0002**	0.75E-2	0.47E-3-0.12	**0.0009**	**0.0005**
T1a vs. T4	0.08	0.88E-2-0.69	**0.0138**	**0.0217**	0.09	0.01-0.77	**0.0162**	**0.0281**
T1b vs. T2a	1.61	0.12-20.73	0.8389	0.7149	1.61	0.12-20.73	0.8389	0.7149
T1b vs. T2b	0.16	0.01-1.58	0.1489	0.1167	0.16	0.01-1.58	0.1489	0.1167
T1b vs. T4	0.72	0.07-7.35	0.4237	0.7822	0.72	0.07-7.35	0.4237	0.7822
T2a vs. T2b	0.08	0.87E-2-0.69	**0.0376**	**0.0222**	0.08	0.87E-2-0.69	**0.0376**	**0.0222**
T2a vs. T4	0.29	0.05-1.85	0.1843	0.1916	0.29	0.05-1.85	0.1843	0.1916
T2b vs. T4	1.60	0.27-9.33	0.7676	0.6000	1.60	0.27-9.33	0.7676	0.6000
Progression								
T1a-T2a vs. T1b-T2a	0.14	0.44E-2-4.29	0.6031	0.2577	0.22	0.92E-2-5.20	0.6812	0.347
T1a-T2a vs. T2a-T2b	0.72	0.06-7.97	0.7003	0.7862	0.80	0.08-8.08	0.6521	0.8521
T1a-T2a vs. T2-T3	1.00	0.20-5.00	0.5965	0.9983	1.11	0.24-5.17	0.6868	0.8973
T1b-T2a vs. T2a-T2b	7.39	0.15-372.40	0.3173	0.3173	7.39	0.15-372.40	0.3173	0.3173
T1b-T2a vs. T2-T3	1.91	0.12-29.45	1.0000	0.6419	1.91	0.12-29.45	1.0000	0.6419
T2a-T2b vs. T2-T3	2.67	0.14-49.75	0.7893	0.5109	2.67	0.14-49.75	0.7893	0.5109
SATB1 intensity (IHC)								
Included LyP and SS								
0 vs. 1-2	1.25	0.36-4.31	0.6604	0.7289	1.10	0.33-3.63	0.7277	0.8735
0 vs. 3-4	3.22	0.90-11.46	**0.0051**	0.0712	3.22	0.90-11.46	**0.0051**	0.0712
0 vs. 5-10	2.54	0.66-9.68	**0.0196**	0.1727	2.54	0.66-9.68	**0.0196**	0.1727
1-2 vs. 3-4	4.14	1.10-15.67	**0.0165**	**0.0362**	5.23	1.44-18.95	**0.0064**	**0.0118**
1-2 vs. 5-10	4.86	1.17-20.07	**0.0255**	**0.0291**	5.72	1.49-21.97	**0.0110**	**0.0111**
3-4 vs. 5-10	1.16	0.44-3.06	0.7493	0.7645	1.16	0.44-3.06	0.7493	0.7645
Excluded LyP and SS								
0 vs. 1-2	1.48	0.40-5.46	0.4383	0.5522	1.28	0.37-4.45	0.5026	0.6983
0 vs. 3-4	3.22	0.90-11.46	**0.0051**	0.0712	3.22	0.90-11.46	**0.0051**	0.0712
0 vs. 5-10	2.97	0.74-11.95	**0.0109**	0.1249	2.97	0.74-11.95	**0.0109**	0.1249
1-2 vs. 3-4	3.44	0.84-13.99	**0.0498**	0.0844	4.51	1.16-17.43	**0.0197**	**0.0292**
1-2 vs. 5-10	6.29	1.25-31.76	**0.0261**	**0.0259**	7.49	1.65-33.96	**0.0098**	**0.0090**
3-4 vs. 5-10	1.37	0.50-3.71	0.3984	0.5388	1.37	0.50-3.71	0.3984	0.5388

HR, hazard ratio; CI, confidence interval; IHC, immunohistochemistry; SS, Sézary syndrome; LyP, lymphomatoid papulosis. Bold, statistically significant differences. [a]Gehan-Breslow-Wilcoxon test; [b]log-rank (Mantel-Cox) test.

54-72% of all CTCL cases. Nevertheless, MF is rare with an IR of 4.1-7.7/1,000,000 person-years, with a male to female IR of 1.66-1.72 (1,2). The OS of MF patients is poorer than the predicted survival of the age-, gender- and race-matched control population without MF, with the exception of patients with stage IA and classified to T1a and T1b (limited patch and/or plaque MF) (4,30-35). Kim *et al* (34) reported that male patients are associated with significantly poorer prognosis than female patients. By contrast, Hess Schmid *et al* (36) reported a significantly better prognosis in males than in females with diagnosed CTCL. Our results did not support an association between gender and survival, which is in agreement with another study performed on a large population (37). Although the results presented here showed that females had better OS and DSS than men, pairwise comparison did not show statistically significant results.

Furthermore, the data presented here indicated that older patients had better DSS, but only when comparing patients aged 40-50 years and >60 years at the time of diagnosis. However, in several large studies, advanced age at the time of diagnosis was found to be an independent negative prognostic factor (34,35,37).

Multiple large population studies have also sought to identify clinical factors predictive of survival in patients with MF and SS. These risk factors include basic demographics, skin T stage, the presence of extra-cutaneous disease, lymphadenopathy and peripheral blood involvement (4,30-35,38-42). Other factors have also been proposed as potentially prognostic, and include: large-cell transformation, levels of serum lactate dehydrogenase, β2-microglobulin, eosinophilia and serum IL-2 receptor (43-46). There are currently 20 TNMB categories: 6 skin stages (T1a, T1b, T2a, T2b, T3 and T4), 7 nodal stages (N0, N1a, N1b, N2a, N2b, N3, Nx), 2 metastatic stages (M0, M1), 5 blood stages (B0a, B0b, B1a, B1b and B2) which are then used to record 9 stages from IA to IVB (35,47,48). In the present study, we concentrated only on T-classification. Our results indicated statistically lower OS and DSS in patients with diagnosed T4 stage than T1. Moreover, DSS was also statistically less frequent in T4 patients as compared to patients with T2 stage. The results of extended T-classification analysis are in accordance with the results of Agar *et al* (35) and showed that the presence of cutaneous plaques (T1a) are characterized by considerably poorer OS as compared with patients with patches only (T2a). Although our study did not reveal statistically significant differences between T1a and T1b patients, it may prove difficult to consistently distinguish thick from thin plaques on the basis of histologic criteria. However, Martí *et al* (49) and Zackheim (4) proved that thick plaques are associated with a poor prognosis. Similarly, results presented here indicated poorer OS and DSS in patients with T4 as compared to both T1a and T2a. As was shown by Kim *et al* (34), the RDP deteriorated with more advanced T-classification, with a greater risk in patients with T2 compared with T1 patients and in T3 or T4 compared with T2 patients. In our study, the analysis of pairwise comparison of simplified T-classification also showed greater RDP in T2 (only 10 and 20 years) and T4 (only 5 and 10 years) patients as compared with patients diagnosed at T1 stage. Furthermore, we showed statistically significant poorer likelihood of RDP with T stage, with indication that patients with diagnosed T2b had lower risk of progression. Additionally, Kim *et al* (34) published data that patients with T3 and T4 disease had a similar RDP. Although our results of disease progression are comparable to those reported by the Dutch group of 309 patients, American group of 525 patients, and UK group of 1,502 patients, the analysis presented by van Doorn *et al* (33) did not define disease progression in patients with progression from T1 to T2, T1 or T2 to T4 stages and research by Kim *et al* (34) and Agar *et al* (35) involved a relatively larger population. In contrast to the results presented by Kim *et al* (34), our study presented much longer median time from diagnosis to disease progression (or end of observation) by T-classification in T1, T2 and T4: 8.0 years for T1 stage, 6.0 years for T2 stage, and 8.0 years for T4 stage. For T3 disease, the median was similar (2.0 years).

SATB1 was the first matrix associated region of DNA (MAR)-binding protein (MARBP) restricted to cell type and is expressed predominantly in thymocytes (12,16). It has been shown that SATB1 is organized into a cage-like network anchoring loops of heterochromatin and tethering specialized DNA sequences and serves as a global platform for the assembly of chromatin remodeling and/or modifying complexes with the anchored genomic loci (50). It has also been noted that depending on its post-translational modifications, SATB1 has the ability to activate or suppress multiple genes (51). Furthermore, SATB1 forms a functional architecture within the cell nucleus, referred to as the SATB1 network, and functions as a regulatory network of gene expression (16,52,53). Moreover, it has been suggested that SATB1 binds to the minor groove of DNA specifically recognizing a unique group of AT-rich DNA sequences (12,16). Yasui *et al* (52) showed that SATB1 acts as a docking site for chromatin remodeling/modifying factors such as ISWI, ASF1 and NURD complex containing HDAC1. Our previous study showed the colocalization of SATB1 and F-actin in the transcriptional active regions of the cell nucleus after apoptotic cell death induction and that this functional interaction was observed between SATB1 and more densely organized nuclear F-actin structures at the border between condensed and decondensed chromatin (25). This contributes to the hypothesis that nuclear SATB1 is involved in chromatin remodeling associated with transcriptional processes during active cell death. The new concept of active organization of cell nucleus states that the chromatin enables coordinated regulation of expression simultaneously in many genes (11).

Several studies have shown that the SATB1 protein is expressed in cells changing their function, e.g. in differentiating progenitor cells (54-56). The typical example of this process is the maturation of thymocytes into T lymphocytes (16). In the present study, we expanded our previous research on the role of SATB1 in the clinical course of CTCLs (11). We showed here that both mean survival and disease-specific mean survival were higher in patients characterized with moderate or high expression of SATB1. Furthermore, a similar correlation was observed after excluding SS and LyP from SATB1-positive and -negative groups. Moreover, the SATB1-positive patients had increased OS and DSS accomplished with increase in the likelihood of survival, as compared to patients with a lack or low SATB1 expression. Additionally, the present study demonstrated that patients characterized by even moderate

expression of SATB1 survived longer than patients without its expression. Our results also indicated that SATB1-positive patients, in contrast to SATB1-negative patients, were characterized by lower RDP and SATB1-positive patients stayed longer in each T stage. This contributes to the results obtained by Wang *et al* (7), which state that deficiency in SATB1 expression causes apoptosis resistance.

Various levels of SATB1 expression have been found in different types of tumors and many studies underline its important role in pathogenesis but also as a prognostic factor (11,50,57-62), revealing that the role of SATB1 in tumors is complicated and tumor-specific (63). To confirm the results obtained by Wang *et al* (7) and to examine the possible mechanism by which patients with SS have poorer prognosis, we analyzed the changes in AICD of Jurkat cells after SATB1 downregulation. As we showed, the SATB1-downregulated cells were characterized by increased resistance to apoptosis. Bayer *et al* (64) demonstrated that FoxP3 negatively regulates SATB1 in regulatory T cells (Treg) and that suppression of SATB1 is required for their suppressive function and inhibition of effector differentiation. As has been shown, FoxP3 suppresses transcription of SATB1 by directly attaching to SATB1 locus. It has also been demonstrated that SATB1 is involved in the negative regulation of IL-2Rα (51,52). Features of Treg cells suggest their role in the immunopathology of CTCL and may be strong candidates for the explanation of the immunosuppression that accompanies the evolution of the disease (65). The *in vitro* study by Berger *et al* (65) revealed that CTCL cells adopt a Treg phenotype ($CD25^+$/$CTLA4^+$ and $FoxP3^+$) after interaction with dendritic cells loaded with apoptotic cells. Another study indicated a poorer prognosis for Sézary patients with the expression of FoxP3 (66). By contrast, Heid *et al* (67) showed a better prognosis for the group of patients with high FoxP3 expression. However, the groups were too small for a statistical comparison. We suggest here that clinical relevance of the correlation of FoxP3 and SATB1 expression needs to be confirmed in a larger cohort of CTCL patients, including large numbers of well-characterized Sézary patients.

In conclusion, the present study revealed that positive expression of SATB1 correlates with better prognosis of CTCL patients. Since SATB1 is strongly up- or downregulated in various types of cancer, it is a suitable candidate as a prognostic tool or an immunotherapeutic target.

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