Review Article

Sézary syndrome, recent biomarkers and new drugs

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Abstract: Sézary syndrome (SS) is a primary cutaneous T-cell lymphoma (CTCL) characterized by erythroderma, lymphadenopathy and leukemic involvement of the peripheral blood. The high relapse rates and a poor prognosis complicate its clinical course and treatment. The phenotypic characterization and genomic/transcriptomic approaches revealed high heterogeneity of Sezary cells, identifying a wide spectrum of biomarkers implicated in the development of this lymphoma. In this context, we discuss the major malignancy-related biomarkers reported in the literature for the diagnosis, prognosis and staging of SS. The hope for a single reliable diagnostic marker appears increasingly unrealistic, but the discovery of multiple potential biomarkers, with pathogenetic implications, paves the road to promising personalized therapies in SS.

Keywords: Biomarkers; clinical trials; cutaneous T-cell lymphoma (CTCL); Sézary syndrome (SS); targeted therapies

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Introduction

Sézary syndrome (SS) is a rare aggressive leukemic variant of cutaneous T-cell lymphomas (CTCL), a spectrum of the malignant clonal proliferation of T lymphocytes with a predilection for skin involvement. CTCL presents commonly as mycosis fungoides (MF) (1,2). Most patients with SS are older adults, prevalent white males, with erythrodermia present in more than 80% of body surface area. Skin erythema that is often exfoliative, accompanied by severe pruritus, is present for months to years prior to diagnosis (*Figure 1A,B*) (4,5). Palmoplantar keratoderma, nail abnormalities, and alopecia are other commonly associated findings (5,6). Lymphadenopathy (≥1.5 cm in size) is common. Sezary cells (SS-cells) in blood, skin and lymph nodes, are the hallmark of SS (*Figure 1C*) and are usually $CD4^+$ $CD45RO^+$ (7). SS represents 3% of the CTCL with an increasing incidence rate (0.1–0.3/ 1,000,000) (1,8) and a medium age presentation of 60 years (9). Despite current treatments, the prognosis for patients is bad (10). Early diagnosis of SS is therefore key to achieve enhanced therapeutic responses (11). The diagnostic criteria are clonal T-cell receptor (TCR) rearrangement, peripheral Sezary cell counts of ≥1 G/L; CD4/CD8 ratio of ≥10; $CD4^+CD7^-$ cells of ≥40%, or $CD4^+CD26^-$ cells of ≥30%. The availability of anti-TCRVβ antibodies and of molecular techniques provides a suitable tool for SS diagnosis (12,13). Moreover, SS-cells express a phenotype of central memory T-cells (T_{CM}) cells and are positive for cutaneous lymphocyte-associated antigen (CLA), and CC chemokine receptors (CCRs) (14). There are no specific biomarkers for neoplastic cells in SS patients and this represents a serious obstacle in evaluating early diagnosis and/or progression in this lymphoma (15). Biomarkers or biological markers, defined by Hulka and Wilcosky (16) as "cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids", include tools and technologies that can aid in

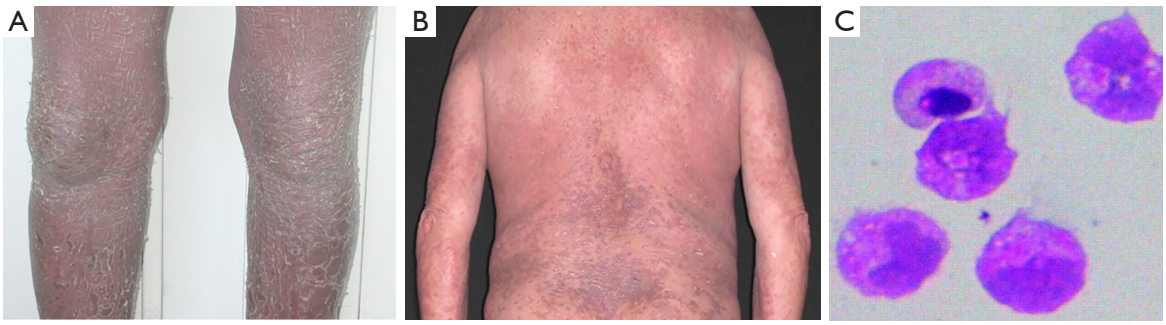

Figure 1 Skin of patient with Sézary syndrome shows (A) exfoliative (B) erythrodermia in more than 80% of body surface. (C) Morphological features of SS-cells immuno-stained with hematoxylin/eosin. Original enlargement ×40 (3).

understanding the diagnosis, progression, regression or outcome of clinical treatment of disease. Ideal diagnostic biomarkers are consistently and highly over-expressed in the malignant cells while absent or nearly absent in the normal cell population (17). While no single biomarker can be used to reliably diagnose SS, the combination of multiple markers has great potential and should be investigated prospectively.

In this review, we provide an overview of diagnostic, prognostic and therapeutic biomarkers in SS, discovered by high-throughput technologies over time. Finally, we discuss the most therapeutic agent available for the cure of this lymphoma and the ongoing interesting clinical trials.

Cell surface markers

The CLA and CCR4 expression are linked with the ability of SS-cells to localize to the skin, while the CCR7 expression is connected with SS entry into lymph nodes (7,18-20). TCR-Vβ detected either by molecularly or with specific monoclonal antibodies is so far a very good marker to assess the clonality of SS cells and also to follow tumor burden (13,21,22). Since as TCR-Vβ analysis is dependent on specific antibodies availability that is limited only to 70% of TCR-Vβ repertoire, the recent introduction of next-generation sequencing (NGS), for sequencing CD3/TCR, has improved the identification of malignant T cells (23,24). Furthermore, molecules such as the Natural Cytotoxicity Receptor (NCR) NKp46/NCR1 (25), CD85j/Ig-like transcript 2 (ILT2)-receptor (26), CD158k/KIR3DL2 receptor (27,28) and PD-1 (29) originally identified on natural killer (NK) cells; Ganglioside GD3(CD60), Integrin CD49d (30) and Syndecan 4 (SD-4) (31), present on activated normal T cells, were also found to be expressed at elevated levels in mainly high tumor burden SS patients. Cell-activation marker down/up-regulated in CD4 T cells from SS patients were also CD26, CD7 (3,20) and CD164, FCRL3 (32), CTLA-4 (33) Vimentin (34), respectively.

The intracellular protein T-Plastin, peculiarly expressed in malignant Sezary cells, remains so far usefulness as a diagnostic marker, because of its intracellular expression and the lacking of antibodies for flow cytometry (35,36). Gene expression does not always correlate with a presence on the cell surface because cell surface markers can be up- or down-regulated at the transcription or translation level.

NKp46/NCR1

The Natural Cytotoxicity Receptor NKp46 is an activating receptor principally expressed by NK cells. Bensussan *et al.* (25) showed that NKp46 is a cell surface marker, expressed in peripheral $CD4^+$ lymphocytes of SS patients. The functional consequences of the engagement of NKp46 will be a severe inhibition of signals on proliferation CD3 dependent in Sezary cells. Their results revealed that NKp46, in combination with KIR3DL2/CD158k, could represent an available marker able to monitor the clinical course of this lymphoma (25).

CD85j/ILT2

CD85j/immunoglobulin (Ig)-like transcript 2 (ILT2) molecule is a member of the leukocyte immunoglobulin-like receptor (LIR) family (37). It transduces a negative signal able to inhibit stimulation of an immune response when it binds to MHC class I (MHC-I) molecules. Nikolova and colleagues (26) reported that CD85j is over-expressed Sezary cell lines and in circulating Sezary cells, with respect

to $CD4^+$ lymphocytes from healthy individuals. They also demonstrated that the triggering of CD85j leads to the recruitment of Src homology 2 domain-containing tyrosine phosphatase (SHP-1) and to the inhibition of proliferation induced by CD3/T-cell receptor stimulation in malignant CTCL cells. Finally, the authors demonstrated a lower susceptibility of Sezary cells expressing $CD4^+ILT2^+$ to anti-CD3 monoclonal antibody-induced cell death than the $CD4^+ILT2^-$ lymphocytes.

KIR3DL2/CD158k

Another receptor found expressed in SS-cells is represented by KIR3DL2 who is part of the members of the killer-cell immunoglobulin-like receptors (*KIRs)*. It was initially identified as expressed on NK cells and on rare circulating $CD8^+$T cells (38). They have been named according to their two (KIR2D) or three (KIR3D) extracellular Ig-like domains. The KIRs have a long cytoplasmic tail (KIR-L) containing immune-receptor tyrosine-based inhibitory motifs or a short cytoplasmic tail (KIR-S) able to associates with molecules involved in activating signal (39). The KIR3DL2 expression is up-regulated upon activation of NK and T cells. In particular, KIR3DL2 ligation on activated T cells results in an anti-apoptotic effect and the production of IL-17 (40,41). KIR3DL2 has been identified as a cell surface marker in SS-cells in 2001 and its sensitivity for the diagnosis of Sézary syndrome was confirmed in several studies (42-44). Indeed, the peripheral $CD4^+KIR3DL2^+$ T cells corresponded to the clonal malignant T-cell population identified by its unique TCR-Vβ rearrangement (45,46). Hurabielle *et al.* (47) demonstrated that KIR3DL2 expression is the most sensitive diagnostic SS-cells marker, and represents the best independent prognostic factor for the death of Sezary cells. Moreover, KIR3DL2 is relevant during the follow-up of this lymphoma (47). Recently, Roelens *et al.* (12) optimized a flow cytometry strategy demonstrating the heterogeneity of peripheral $CD158k^+$ Sezary cells, being not exclusively of $CD62L^+$ $CCR7^+$ T_{CM} phenotype. The authors characterized the phenotypic diversity of $CD158k^+$T SS-cells in a large group of Sezary patients, including markers of T-cell subsets, like stem-cell and resident memory cells, elucidating a remarkable disparity between blood- and skin-derived $CD158k^+$ T cells. Finally, skin-derived SS-cells show a more advanced maturation pattern with respect to circulating ones. The expression of CD69 and CD103 activation markers on the majority of skin-derived Sezary confirmed this data (12).

PD-1

Programmed death-1 (PD-1/CD279) belongs to the CD28/CTLA4 super-family and is expressed in a subset of activated T lymphocytes, monocytes dendritic cells, B-cells, and NK (29). The PD-1 exerts a key role in regulating immune response (48); it inhibits the TCR-mediated T-cell proliferation and cytokine secretion, by its ligands PD-L1, and PD-L2 (49). PD-1 is a part of a novel group of immune checkpoint able to promote apoptosis of antigen-specific T cells or to inhibit apoptosis in regulatory T cells (48) A new class of drugs, the PD-1 inhibitors, being developed for the treatment of several types of cancer, including CTCL (50).

Several studies reported the over-expression of PD-1/CD279 in various types of T-cell lymphomas, including SS (51-54). Samimi *et al.* (53) reported an increased expression of PD-1 in SS-cells than MF patients and healthy donor. A differential expression of PD-1 between SS and MF was observed in a study performed in skin biopsy by Cetinözman *et al.* (54); the authors provided a further support of the different entities of this two CTCL.

CD60 and CD49d

CD60 is a 9-O-acetylated form of the GD3 ganglioside expressed only by a part of T lymphocytes, the T memory effector cells ($CD45RO^+$) (55). The ganglioside is an intracellular second lipid messenger able to induce apoptosis in lymphoid and myeloid cells. These effects are exerted by the way of FAS and FAS ligand, tumor necrosis factor (TNF-α) and/or amyloid signaling (56) and can be enriched through its de-O-acetylation. This mechanism could be useful for the development of new drugs therapies (30).

Another molecule involved in the migration of lymphocytes throughout the vessel wall is Integrin CD49d, also known as α4β1 or very late antigen-4 (VLA4): CD49d exerts this effect after it binds to vascular cell adhesion molecule-1 (VCAM-1), a cell adhesion molecule expressed on the activated endothelium. Scala *et al.* (30) evaluated the diagnostic and prognostic relevance of CD60 and CD49d expression on circulating $CD4^+$T cells in 62 patients with SS. Over-expression of CD60 and down-regulation of CD49d predict the survival rate in these patients.

Syndecan-4

Syndecan-4 (SD-4) is a transmembrane heparan sulfate (HS) receptor expressed in a varies structure of HS in different

cell types (57). Over-expression of specific epitopes of this receptor was found in Sezary cells (58). The constitutive over-expression of SD-4 in peripheral SS-cells distinguishes it from other leukemia or from other inflammatory cutaneous diseases (31).

CD26

CD26/dipeptidyl peptidase IV (DPPIV) is a proteolytic enzyme constitutively expressed in healthy peripheral T lymphocytes (59). This molecule is a cell activation marker up-regulated inside of mitogenic signals and down-regulated after the interplay with insulin-like growth factor II receptor (60). The expression of CD26 is losses in the cell surface of malignant T Sezary cells. CD26/DPPIV modulates the skin-homing chemotactic activity of many molecules, e.g., interferon-inducible T cell-α chemoattractant (I-TAC/CXCL11), a chemokine able to chemo-attractant T lymphocytes by CXCR3 (61). This mechanism could explain the ability of SS-cells $CD26^-$ for skin recruitment or for its intensification in peripheral blood (62). Narducci and colleagues confirmed the data on the abundance of skin-infiltrating Sezary T cells $CD26^-$, analogous to peripheral Sezary cells (3). The authors demonstrated that the addition of soluble CD26 reduces the CXCR4-SDF-1 chemotaxis activity, abundantly signaling functionally active in skin homing of Sezary cells. Sokolowska-Wojdylo *et al.* reported that the CD26 is a very sensitive and specific marker, useful both in early detection and in therapeutic monitoring of SS disease (20).

CD164 and FCRL3

CD164/Sialomucin core protein 24, also known as endolyn functions as a cell adhesion molecule, is involved in the regulation of the adhesion, migration and proliferation, of hematopoietic progenitor cells (63). Sialomucin promotes myogenesis by enhancing CXCR4-dependent cell motility. FCRL3 or Fc Receptor-Like 3 is an immunoglobulin receptor which plays a role in the regulation of the immune system (64). Wysocka *et al.* demonstrated, for the first time, an over-expression of both FCRL3 and CD164 in Sezary T lymphocytes (32). The same authors demonstrated an inverse correlation between CD164 and CD26 expression and proposed the CD164 as a specific and sensitive marker for diagnosis, prognosis and staging of SS. Benoit B.M. and colleagues (15) demonstrated the high expression of CD164 in both peripheral and skin resident malignant $CD4^+$ cells in CTCL patients. Additionally, the authors demonstrated an over-expression of KIR3DL2 and CD164 in 82% of Sezary patients.

CTLA-4/CD152

Cytotoxic T-lymphocyte antigen 4 (CTLA-4, CD152) is a T cell surface protein involved in immuno-regulation, inhibiting T cells activation or proliferation. A low expression of CTLA-4 is observed in un-stimulated cells whereas a higher expression is observed in stimulated cells (65-68). Gene fusion between CTLA-4 and CD28 molecule accompanied by over-expression was observed T Sezary cells (66,69,70). Gibson and colleagues (67) propose a mechanism via GATA3, a T-cell transcription factor, able to regulate Th2 differentiation, responsible for the over-expression of CTLA-4 in Sezary lymphocytes. The authors observed an up-regulation of CTLA-4 and GATA3 in normal T cell upon Bortezomib treatment, a proteasome inhibitor. The findings of CTLA4-CD28 gene fusion will be important for the therapeutic targeted strategy in SS (70).

Vimentin

Huet *et al.* (34) identified a monoclonal antibody termed SC5 that specifically recognizes vimentin (VIM), a type III intermediate cytoskeletal filament. It is expressed selectively by T lymphocytes isolated from healthy individuals upon activation, and by circulating Sézary syndrome lymphocytes. Ortonne and colleagues (46) identified VIM at the surface membranes of Sezary cells and normally activated lymphocytes, conversely, in some patients the authors detected the presence of a nonmalignant circulating clone expressing high amounts of VIM and lacking CD158k.

Gene expression markers

The numerous expression studies conducted in SS identified a multitude of genes up- or down-regulated useful for differential diagnosis and progression in SS. Gene transcription studies have been performed extensively using blood samples or skin biopsies with quantitative reverse transcriptase PCR (qRT-PCR), microarray platform and transcriptome sequencing (RNA-Seq) (24,71). Nebozhyn *et al.* (72) developed a method, by qRT-PCR, to evaluate a set of five genes, concurrently: PLS3, STAT4 GATA3, TRAIL and CD1D. They demonstrated an efficiency of this set of genes of 90% in 49 SS patients and 65 healthy

controls. Michel *et al.* (73) tested another panel of genes, KIR3DL2, NKp46 and PLS3, by qRT-PCR, obtaining an accuracy of 100% in 81 Sezary patients. Because KIR3DL2 and NKp46 were also expressed in NK cells of healthy controls, the authors proposed a flow cytometry analysis in combination with qRT-PCR, for this two markers. Litvinov *et al.* identified a set of 17 genes containing CCR4, IL2RA, TOX and STAT5A able to discriminate MF from SS patients (74). Boonk *et al.* (75) compared $CD4^+$ T lymphocytes from 59 SS versus 19 erythrodermic inflammatory dermatosis patients analyzing copy number alterations data, gene expression and flow cytometry analysis. Malignant T SS-cells showed MYC gain (40%) and MNT loss (66%); up-regulation of EPHA4 (66%), DNM3 (75%), PLS3 (66%), and TWIST1 (69%); and down-regulation of STAT4 (91%). Loss of the CD26 (≥80% $CD4^+$ T lymphocytes) and/or of the CD7 (≥40% $CD4^+$ T lymphocytes) in combination with the aberrant expression of TWIST1, STAT4, PLS3 and DNM3 will be useful in the differential diagnosis between SS and patients affected by erythrodermic inflammatory dermatoses (100% specificity) (75). Recently, the same authors demonstrated, in SS patients, a direct correlation between over-expression of PLS3 and a favorable disease outcome; on the other hand, evaluated an inverse correlation between a higher leukocyte count and survival rate (76). Caprini *et al.* (77) were able to characterize 113 deregulated genes in lesioned chromosomal regions combining mapping and transcriptional data. The authors found important cancer-related genes such as members of the NF-kappaB pathway (TRAF2, BAG4, NKIRAS2, BTRC and PSMD3) that might explain its constitutive activation in CTCL (77). Moreover, they identified some genes like PIP5K1B and BUB3 that might exert critical roles in Sezary disease. Litvinov *et al.* (78) reviewed over 400 different studies to select 284 genes that were reported to play an important role in CTCL pathogenesis and progression. Their results underscore significant molecular heterogeneity with respect to gene expression between different patients and even within the same patients over time. Authors demonstrated that CCL18, CCL26, CCR4, FYB, T3JAM, MMP12, LEF1, LCK, ITK, GNLY, IL2RA, IL-26, IL-22, IL1F, BLK, CDO1, GTSF1, EED, THAP11, SYCP1, cTAGE1, POU5F1, POU2AF, STAT4, STAT5 and TOX could jointly be used as diagnostic and poor prognostic markers in CTCL patients, while SERPINB13, PSORS1C2, WIF1 and BCL7A were preferentially up-regulated in benign skin conditions and/or in indolent CTCL.

High-throughput technologies such as RNA sequencing, are an attractive approach for clinical diagnostic or staging of this lymphoma, allowing deeper characterization of targeted cancer genes that are frequently altered, at an affordable cost and in an efficient time frame.

Lefrançois *et al.* (79) compared RNA-Seq gene expression data obtained from FFPE samples collected earlier of 2008 with respect to those collected after 2009. Both analyses produced nearly identical trends and findings. Furthermore, the authors characterized a significant novel set of differentially expressed genes up-regulated in CTCL such as SMAD1, BIRC5, CASP1, BCL11A, IRF1, MAX and SELL and down-regulated such as THBS4, SERPINB3 and MDM4.

Iżykowska *et al.* (80) focused on those changes that affect the expression of the same gene in more than a patient and identified two genes, NCOR1 and CTBP1, which although the genomic alterations were over-expressed at mRNA level, in more SS patients. These genes are both involved in histone deacetylases (HDAC) complexes, able to regulate the HDAC activity. The destabilization of this complex alters gene expression in numerous human diseases (81), including SS. These results deserve attention because of the approved treatment of HDAC inhibitors in SS since 2006 (82).

MicroRNA and other regulatory RNA

MicroRNAs (miRNAs) are short non-coding RNA molecules involved in critical biological processes, such as proliferation, apoptosis, immune function, development, and the stress response through negative regulation of the stability and translation of target messenger RNAs (83,84). miRNAs are tissue/specific biomarkers with clinical relevance for tumor diagnosis and prognosis (84). MiRNAs microarray studies, conducted in SS identified, an aberrant expression of a significant number of miRNAs (85). Of note, several miRNA genes (>50%) are mapped in cancer-related regions or in fragile sites in SS patients (86), as for example miR21, miR486 and miR31 (87). Some of these miRNAs, which are over-expressed in SS, negatively modulate PTEN expression directly, such as miR214,miR21, miR106b and miR486, whereas others modulate PTEN expression indirectly, such as mi199a (88,89). Furthermore, Benoit *et al.* (15) evaluated as malignancy marker, in $CD164^+$and $CD164^-$ SS cells, the expression level of FCRL3, TOX, miR21 and miR214. Worthy of note is the role played by the over-expression of miRNA155 in CTCL. Several studies elucidated its contribution to promoting inflammation and

CNVs[1]		Somatic Mutations[2]		MicroRNAs[3]			
Gain	Loss	Gene	Chromosome	Up	Chromosome	Down	Chromosome
1p36.13	1p22.1	ANK3	10q21.2	miR-21	17q23.1	miR-15a	13q14.2
4q12	**1p36.11 (ARID1A)**	ARID1A	1p36.11	miR-143	5q32	miR-16	13q14.2
5p15.33	**2p23.3 (DNMT3A)**	ATM	11q22.3	miR-145	5q32	miR-17-5p	13q31.3
6p25.3	2q37.3	BRAC2	13q13.1	miR-152	17q21.32	miR-22	17p13.3
7p22.2	3q11.2-q13.2	CACNA1E	1q32.1	miR-155	21q21.3	miR-31	9p21.3
7q36.1	4p13-p12	CADPS	3p14.2	miR-199a	19p13.2	miR-107	10q23.31
8q12.1 (TOX)	4q31.3	CARD11	7p22.2	miR-200c	12p13.31	miR-146b	10q24.32
8q24.21 (MYC)	5q33.1	CREBBP	16p13.3	miR-214	1q24.3	miR-203	14q32.33
9p24.2	6p21.1	DNAH9	17p12	miR-223	Xq12	miR-205	1q32.2
10p15.1 (PRKCQ)	**6q23.3**-25.2 **(A20/TNFAIP3)**	DNMT3A	2p23.3	miR-486	8p11.21	miR-342	14q32.2
10p15.1-p12.33	7p15.3-q21.2	ENPP2	8q24.12	miR-574-5p	4p14		
10p12.33	8p23.1-p11.21	FAM47A	Xp21.1				
17q21.2 (STAT5B)	8q12.3-q22.1	FAS	10q23.3	Fusion Transcripts[4]			
17q11.2-25.1	**9p21.2 (CAAP)**	FAT3	11q14.3	BMP2K	**INPP4B**	chr4	chr4
20q13.2	**9p21.3 (CDKN2A-CDKN2B)**	FAT4	4q28.1	CARD11	PIK3R3	chr7	chr1
21q22.3-q11.23	**10p11.22 (PTEN,FAS)**	GPR158	10p12.1	CBLB	**ZEB1**	chr3	chr10
	10q21.2-23.31	JAK3	19p13.11	**CD28**	CTLA4	chr2	chr2
	10q23.31 (ZEB1)	KMT2C	7q36.1	CENPE	**INPP4B**	chr4	chr4
	10q24.32-q26.11 **(NFKB2)**	MLL2	12q13.2	COL25A1	**NFKB2**	chr4	chr10
	11p13-q23.2	NuRD/MTA1	14q32.33	**DHFR**	ARHGAP18	chr5	chr6
	11q22.3 (ATM)	PDCD11	10q24.33	**DHFR**	RASGRF2	chr5	chr5
	11q23.2 (USP28)	PLCG1	20q12	FASN	**SGMS1**	chr17	chr10
	12p13.2-q22	POLD1	19p13.33	ICOS	**CD28**	chr2	chr2
	13q12.2-**14.2 (RB1)**	POT1	7q31.33	**IKZF2**	**GLI2**	chr2	chr2
	16q22.1-q24.3	PREX2	8q13.2	**IKZF2**	BACH2	chr2	chr6
	17p11.2 (NCOR1)	PRKCQ	10p15.1	**IKZF2**	SPAG1	chr2	chr8
	17p13.1 (TP53)	PTPRK	6q22.33	**INPP4B**	ATAD1	chr4	chr10
	19p13.3 (E2A)	RAD51C	17q22	IWS1	**IKZF2**	chr2	chr2
	22q11.23	SOCS7	17q12	MAP3K7	**MYB**	chr6	chr6
		STAT3	17q21.2	**MBNL1**	KIAA2018	chr8	chr3
		STAT5A	17q21.2	**MYB**	**MBNL1**	chr3	chr6
		STAT5B	17q21.2	PPFIA1	**NFKB2**	chr11	chr10
		TET1	10q21.3	RBMS1	**GLI2**	chr2	chr2
		TET2	4q24	**SGMS1**	**ZEB1**	chr10	ch10
		TP53	17p13.1	TOX	MYBL1	chr8	chr8
		UNC13C	15q21.3	TYK2	UPF1	chr19	chr19
		ZEB1	10p11.22	**USP32**	RAD51B	chr17	chr14
				USP32	TTC18	chr17	chr10

Figure 2 Genomic and molecular biomarkers identified in SS by high-throughput methods. [1], recurrent regions affected by Copy Number variations in SS: in bold focal gene lesions; [2], most frequent somatic mutations in SS by NGS; [3], up- or down-regulated microRNAs identified in SS; [4], most common fusion transcripts found in (69,80,93,94): in bold are indicated genes involved in multiple fusion events.

cancer development by way of the STAT signaling (83,90). On the other hand, several down-regulated miRNAs, such as miR342, miR31 and miR7 family members, may inhibit cancers by regulating genes involved in apoptosis, such as CDKN2A-B and TNFSF11 (85). Ballabio and colleagues (85) described also an increased level of miR223 in SS-cells with 91% specificity and 90% sensitivity compared with healthy controls. Ralfkiaer *et al.* (83) distinguished CTCL from benign skin diseases with a miRNA panel (miR155, miR203,miR205) with an accuracy of 95%.

Dusílková *et al.* (91) studied a system for detecting miRNAs in the plasma of patients affected by CTCL. MiRNAs levels measured in serum or plasma were very stable and resistant to RNA degradation and their detection is becoming promising for clinical monitoring. Based on the miR155 up-regulation and miR203/miR205 down-regulation (with 100% specificity and 94% sensitivity) their data provide evidence of a very efficient method able to evaluate the CTCL progression and to distinguish it from benign lesions.

Finally, Lee *et al.* (92) identified 12 long non-coding RNAs in three SS patient with miRNA-Seq demonstrating the presence of long non-coding RNAs in this lymphoma (*Figure 2*, microRNAs).

Copy number variation and somatic mutation markers

The genetic alterations in CTCL, in particular in SS, have been studied using cytogenetic and array-based methods for a long time. These studies identified extensive genetic instability with complex karyotypes but no highly recurrent translocations (95,96). A series of genomic studies identified large chromosomal regions affected by recurrent copy

number alterations (CNAs). SS patients display significant CNAs (gains) in regions of already known and described oncogenes, like MYC (8q24.21), TOX (8q12.1) (95,97) and deletion in regions of suppressor genes like *TP53* (17p13.1) (95,98), PTEN, FAS (10p11.22), RB1 (12p13.1), ZEB1 (10q23.31) (88,93,97,99), CDKN2A-CDKN2B (9p21.3) (100), E2A (19p13.3) *DNMT3A (2p23.3)* (80,101), USP28 (11q23.2), CAAP (9p21.2), NCOR1 (17p11.2) (80) and A20 (6q23.3) (102). In addition to the genes already reported, Vermeer and colleagues (95), observed losses of RPA1, HIC1, TCF8/ZEB1, DUSP5 and gain of STAT3/STAT5 and IL-2 (receptor) genes. Caprini *et al.* (77) using single nucleotide polymorphism and comparative genomic hybridization array confirmed previous studies with recurrent losses and gain regions, whereas identified novel genomic lesions recurring in >30% of tumors: loss of 9q13-q21.33 and gain of 10p15.3-10p12.2. Noteworthy, the same authors, observed that more than three recurrent chromosomal alterations (gain or loss) had shown a significant correlation with SS prognosis (77) (*Figure 2*, CNVs).

NGS is particularly suitable for the discovery of different types of genetic alterations ranging from pathogenic single nucleotide substitutions to large chromosomal aberrations in cancer genomes. In recent years, genome-wide studies using NGS have also provided a more comprehensive picture of the genetic landscape of CTCL (*Figure 2*, somatic mutations).

The majority of somatic single nucleotide substitutions observed in SS were C>T transitions (40–75%) (94,103), which were much less common in other hematological cancers. This mutational signature is regarded as caused by ultraviolet (UV) light exposure when occurring at NpCpG sites (104) or aging when occurring at NpCpC sites. In SS, both aging (43%) and UV radiation (30%) contribute to C>T transitions (94,105). At present, it is unclear whether exposure to UV light plays a causative role in the onset of the disease (106). Woollard and colleagues (107) described the molecular heterogeneity of CTCL tumor cells identifying more than 800 somatic non-synonymous mutations, including indels, stop-gain/loss, splice variants, and recurrent gene variants. The authors identified a new candidate driver gene in CTCL like POT1, TP53 and DNMT3A and confirmed previous literature data of alterations of TP53 and DNMT3A. Novel somatic mutations were identified in PLCG1 and BRCA2 detected in 11% and in 14 of SS patients, respectively. Chromosomal aberrations of PRKCQ, a gene involved in activation of TCR and NF-κB signaling, occurred in 20% of SS patients (107). Prasad *et al.* (69) identified four genes with recurrent somatic mutations, including stop-gain mutation, missense mutations, damaging somatic mutations, in a cohort of 15 SS patients: TP53, ITPR1, DSC1, and PKHD1L1. Moreover, the authors reported single somatic mutations in several genes such as PLCG1, STAT5B, GLI3, CARD11, NAV3, RIPK2, IL6, RAG2 and ITPR2, some of which previously described in CTCL: PLCG1, *STAT5B*, *GLI3*, *CARD11* and *NAV3* (93,94,104,108-111). Other genes with somatic point mutations were *IL6*, *RIPK2*, *RAG2* (69). More recently, all CTCL samples with publicly available sequencing data have been reanalyzed applying uniform methods and metrics to generate a more representative overview of point mutation frequencies in putative driver genes (112). Park *et al.* (112) identified 55 putative driver genes in a genomic study conducted in 220 CTCLs. The authors discovered novel mutations involved in chromatin remodelling, such as BCOR, KDM6A, SMARCB1 and TRRAP; in immune surveillance, like CD58 and RFXAP; in signaling of MAPK kinase, like MAP2K1 and NF1; in signaling of NF-κB, such as PRKCB and CSNK1A1; in PI-3-Kinase signaling, like PIK3R1and VAV1;in RHOA/cytoskeleton remodeling (ARHGEF3); in RNA-splicing (U2AF1); in TCR signaling, such as PTPRN2and RLTPR and in T cell differentiation (RARA). Point mutations either predicted or confirmed to be gain-of-function were reported in JAK1 (0.9% of cases), JAK3 (2.7%), STAT3 (0.9%), and STAT5B (3.6%) (112). Recurrent mutations were also reported in four genes not previously identified in cancer: CK1α, PTPRN2, RARA, and RLTPR. Finally, they validate two putative oncogene CSNK1A1 and RLTPR recently described as involved in the TCR signaling (112).

Fusion transcript

Fusion genes are hybrid genes composed by parts of two or more genes, which origin from aberrant chromosomal or transcription rearrangements (113). Fusion genes might be responsible for inactivation of either tumor suppressor genes or activation for oncogenes. They could act as driver genes of tumor initiation and progression. Fusion genes will be useful as diagnostic and prognostic markers and represent an important class of targetable events to improve patient outcomes.

A well described gene-fusion in SS was CTLA4/CD28 transcript (70,94,108). Expressed in normal T cells, the

CD28 is a molecule involved in a cascade of activation signals including cell proliferation and transcription of CTLA4. CTLA4, in turns, inhibits proliferation by opposing the effects of CD28 (114). In the fusion gene, the inhibitory cytoplasmic tail of CTLA4 was replaced by the activating tail of CD28. This fusion could provide a novel aberrant stimulatory mechanism contributing to oncogenic proliferation and survival (114). Sekulic *et al.* reported one SS-patient trial with CTLA4-blocking antibody Ipilimumab produced dramatic initial responses for first 2 months, but the response was limited and the patient died 3 months after the last dose (70). Wang *et al.* identified 41 in-frame fusion transcripts, by RNA-seq (94), 29 of them were successfully validated. In addition to CARD11-PIK3R3 fusion, they observed CD28-CTLA4/ICOS and IKZF2-GLI2 fusions. Prasad and colleagues, performing RNA-Seq in 15 SS, identified putative driver gene mutations implicated in the progression of this lymphoma (69). In addition, 86 potential fusion transcripts were identified in 10 Sezary samples (69). Some of these fusion events were successfully validated, such as TYK2-UPF1, COL25A1-NFKB2, FASN-SGMS1, SGMS1-ZEB1, SPATA21-RASA2, PITRM1-HK1, and BCR-NDUFAF6. Iżykowska *et al.* (80) identified 9 new fusion events in frame (EHD1-CAPN12, TMEM66-BAIAP2, MBD4-PTPRC, PTPRC-CPN2, MYB-MBNL1, TFG-GPR128, MAP4K3-FIGLA, DCP1A-CCL27, MBNL1-KIAA2018) in nine SS patients; 5 fusion transcripts were described as ectopic expression of fragments genes not expressed in normal T lymphocytes (BAIAP2, CPN2, GPR128, CAPN12, FIGLA). Those transcripts, except for one (TFG-GPR128) (115), have never been reported in the literature in SS (*Figure 2*, fusion transcripts).

Current therapy in Sézary syndrome

The treatment of SS is primarily determined by disease extent and the impact on quality of life, prognostic factors and patient age/comorbidities an important principle in SS treatment is the preference for treatments that preserve, rather than suppress, the patient's immune system. SS is associated with dysfunction of cellular immunity resulting in impaired response to infections and tumors, therefore the use of immunosuppressing therapies may lead to overwhelming infection. As infection is a common cause of death, another principle in SS treatment is vigilant surveillance for microbial colonization and infection (116).

First-line therapies for SS include extracorporeal photopheresis (ECP); subcutaneous interferon-α (IFN-α); oral bexarotene; and low-dose oral, subcutaneous, or intramuscular methotrexate. Many combination regimens are possible: bexarotene and IFN-α; bexarotene and ECP; IFN-α and ECP; bexarotene, IFN-α, and ECP; IFN-α and low-dose MTX; and IFN-α, low-dose MTX, and ECP. Chlorambucil and prednisone are also recommended (117). Adjuvant systemic agents comprehend antihistamines, doxepin, and gabapentin (6). Emollients and topical corticosteroids may be a helpful presence of pruritus.

Systemic therapy may also be combined with skin-directed therapy: psoralen plus ultraviolet A (PUVA) with bexarotene, IFN-α, and ECP; low-dose MTX and topical nitrogen mustard; PUVA and bexarotene; and total skin electron beam therapy (TSEBT) may be combined with ECP, IFN-α, and bexarotene. Because it is a radiosensitizer, MTX is not administered at the same time as TSEBT. Topical corticosteroids may be used in combination with any systemic therapy (6,118,119).

Second-line therapies for SS include single-agent chemotherapy (liposomal doxorubicin, gemcitabine, low-dose pralatrexate, pentostatin, chlorambucil, etoposide, cyclophosphamide, temozolomide, and high-dose MTX), HDACi (oral vorinostat and intravenous romidepsin), multiagent chemotherapy [fludarabine and cyclophosphamide; cyclophosphamide, doxorubicine, vincristine, prednisone (CHOP)], or targeted immunotherapy including brentuximab (anti-CD30), alemtuzumab (anti-CD52), and mogalumizumab (anti-CCR4) (117,119).

Although many treatments are available, most responses are partial and not durable, therefore the course of therapy in SS is that of successive treatments either alone or in combination. Furthermore, the effect of treatment on overall survival is unknown (6). An exception may be allogeneic bone marrow transplantation (BMT), which has provided durable complete remissions in SS, however, it is associated with high morbidity and mortality and is currently only considered in young, relatively healthy patients with advanced disease (120).

Current clinical trials with new drugs

The newly identified biomarkers and especially the genetic analysis combined with the availability in the cancer therapeutics field of new targeted therapies has opened the road to new therapeutic possibilities in these patients Several new agents for the treatment

Clinical Trial	Therapeutic Agent	Phase Study	Molecular Target
NCT01626664	KW-0761	II	Anti CCR4
NCT01728805	KW-0761 (Mogamulizumab) vs Vorinosta	III	Anti CCR4/ HDAC inhibitor
NCT01352520	SGN-35 (Brentuximab Vedotin)	II	Anti CD30
NCT01703949	Brentuximab Vedotin	NA	Anti CD30
NCT02588651	Brentuximab Vedotin	II	Anti CD30
NCT01777152	ECHELON-2 -Brentuximab Vedotin/CHP	III	Anti CD30 plus chemotherpy
NCT01805037	Brentuximab Vedotin + Rituximab	I/II	Anti CD30/ anti CD20
NCT01578499	SGN-35 vs Methotrexate or Bexarotene	III	Anti CD30/ Folate antagonist/ Synthetic nuclear retinoid
NCT01678443	Monoclonal Antibody	I	Anti CD45 before stem cell transplantation
NCT00047060	Campath-1H (Alentuzumab)	I/II	Anti CD-52 before stem cell transplantation
NCT02535247	Pembrolizumab	II	Anti PD-1
NCT01482962	Alisertib (MLN8237)	III	Aurora A Kinase inhibitor
NCT01567709	Alisertib + Vorinostat	I	Aurora A Kinase inhibitor/ HDAC inhibitor
NCT01897012	Alisertib + Romidepsin	I	Aurora A Kinase inhibitor/ HDAC inhibitor
NCT01998035	5-azacitidine + Romidepsin	I/II	Chemotherapic/ HDAC inhibitor
NCT01871727	E7777 (ONTAK)	III	Engineered protein combining Interleukin-2 and Diphtheria toxin
NCT02464228	Tipifarnib	I	Farnesil transferase inhibitor
NCT02061449	Poly ICLC + Radiation + and Romidepsin	I	Focal radiation with or without the Toll-like receptor agonist Poly ICLC/ Romidepsin
NCT02594267	Pralatrexate + CHOP	I	Folate antagonist
NCT02158975	MLN9708 (Ixazomib)	II	GATA3 ihibitor
NCT02576496	EDO-S101	I	Alkylating Histone Deacetylase Inhibition (HDACi) Fusion Molecule
NCT01590732	Romidepsin plus ice	I	HDAC inhibitor
NCT01261247	Panobinostat	II	HDAC inhibitor
NCT02232516	Romidepsin + Lenalidomide	II	HDAC inhibitor / Immunomodulatory drug
NCT01796002	Romidepsin CHOP vs CHOP	III	HDAC inhibitor plus chemotherapy
NCT01902225	Romidepsin + Doxil	I	HDAC inhibitor/Doxorubicin HCl liposome
NCT01947140	Romidepsin + Pralatrexate	I/II	HDAC inhibitor/Folate antagonist
NCT02520791	MEDI-570	I	ICOS- Inducibile T-cell costimulator (CD278)
NCT02561273	Lenalidomide + Chemotherapy	I/II	Immunomodulatory/Chemotherapy agent
NCT01431209	Ruxolitinib Phosphate	II	Jak inhibitor
NCT01431209	Ruxolitinib phosphate	II	Jak inhibitor
NCT02192021	Doxorubicin low dose	I	Micro neddle applicator of chemo-immunotherapy
NCT02168140	CPI-613 + Bendamustine hydrochloride	I	Mithocondrial inhibitor/ Chemotherapy agent
NCT02495415	Fenretinide	II	N-(4-hydroxyphenyl) Retinamide, 4-HPR
NCT02264613	ALRN-6924	I/II	Peptide anti TP53 inhibitors (MDMX-MDM2)
NCT02448381	SGX301 (Soligenix)	III	Photosentitizer, synthetic hypericin, topically applied
NCT01660451	BAY80-6946 (Capanlisib)	II	Pan class I PI3K inhibitor
NCT02783625	Duvelisib with Romidepsin or Bortezomi	I	Dual inhibitor PI3K Delta/Gamma /HDAC inhibitor/Proteosoma inhibitor
NCT02567656	Dual PI3K Delta/Gamma Inhibitor	I	Dual inhibitor PI3K Delta/Gamma Inhibitor
NCT02309580	Ibrutinib	I	Protein kinase inhibitor
NCT01336920	Carfilzomib	I	Proteosoma inhibitor
NCT01738594	Carfilzomib With and Without Romideps	I	Proteosoma inhibitor/HDAC inhibitor
NCT01738594	Carfilzomib with or without Romidepsin	I	Proteosoma inhibitor/HDAC inhibitor
NCT02142530	Carfilzomib + Belinostat	I	Proteosoma inhibitor/HDAC inhibitor
NCT02341014	Carfilzomib + Lenalidomide + Romidepsi	I/II	Proteosoma inhibitor/Immunomodulatory/ HDAC inhibitor
NCT02342782	yttrium Y 90 + Basiliximab	NA	Radio immuno-conjugate anti IL-2R
NCT02580552	MRG-106	I	Syntetic miRNA antagonist of miR-155

Clinical trials.gov/T-Cell Leukemia Lymphoma Foundation
Drug cathegories were grouped and highlighted
NA: Not Available

Figure 3 Current clinical trials in T-cell leukemia/lymphoma.

of SS are in development, including those that target KIR3DL2 (28), CD3 (a pan-T-cell marker), CD25 (IL-2 receptor, the target of denileukin diftitox), PD-1 receptor (an immune checkpoint targeted by pembrolizumab), and PI-3 kinase(a signal transducer inhibited by duvelisib). Second-line systemic agents include aprepitant, mirtazapine, and selective serotonin reuptake inhibitors (6). Food and Drug Administration (FDA)-approved JAK inhibitors such as Tofacitinib and Ruxolitinib make the idea of targeting malignant T cells driven by hyperactive JAK kinases attractive. Pharmacological inhibition of complex NF-κB by Bortezomib decreased its DNA-binding ability and induced cell death of SS cells *in vitro* (106,121-123). Two systemic HDAC inhibitors (HDACis), romidepsin (class I-specific HDACi) and vorinostat (pan-HDACi), are FDA approved for the treatment of CTCL. Trials with (novel) HDACis as single agents or in combinations are now underway in diverse cancer types, including hematologic and solid tumors, but have not yet reached the clinical (124). More details of the current clinical trials are indicated in *Figure 3*.

Conclusions

High-throughput methods provide invaluable insights into the molecular mechanisms or progression of this disease. The rare cancers are moving toward personalized

medicine; the large scale of clinical trials are expanding and the identification of somatic alterations are driving the treatment decision. News insight molecular biomarkers allowed a partial stratification of SS patients implementing accurate diagnostic methods. Individual therapies are still deficient and the progress of the disease, with the manifestation of resistant clones, is very frequent and gradual. Patients with an advanced stage of disease should be inserted in multicenter clinical trials. Therapeutic strategies should maintain a quality of life that should be considered alongside response rates in clinical research. The characterization of new targetable biomarkers and the development of monoclonal antibodies seems to be increasingly promising for the cure of this lymphoma.

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Footnote

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