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 erythroderma 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 (TCM) 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
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 (KIR). 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+ TCM 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/CTLA-4 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 EP3A4 (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, FTY, 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.

Izykowska et al. (80) focused on those changes that affect the expression of the same gene in more than a patient and identified two genes, NCO1 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 miR199a (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
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 miR342, miR31 and miR7 family members, may inhibit cancers by regulating genes involved in apoptosis. Moreover, several down-regulated miRNAs, such as miR223, miR203 and miR205, may inhibit cancer development by way of the STAT signaling (83,90). Dusilková 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

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**Table 1: Genomic and molecular biomarkers identified in SS by high-throughput methods**

<table>
<thead>
<tr>
<th>Gain</th>
<th>Loss</th>
<th>Gene</th>
<th>Chromosome</th>
<th>Up</th>
<th>Chromosome</th>
<th>Down</th>
<th>Chromosome</th>
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<tbody>
<tr>
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<td>ANK3</td>
<td>10q21.2</td>
<td>miR-21</td>
<td>17q23.1</td>
<td>miR-52a</td>
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<td>5p12.1</td>
<td>MRD10A</td>
<td>1p36.11</td>
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<td>5p32</td>
<td>miR-7175p</td>
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<tr>
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<td>2q22.2</td>
<td>ATM</td>
<td>11q22.3</td>
<td>miR-145</td>
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<td>miR-7175p</td>
<td>17q31.3</td>
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<tr>
<td>7p12.2</td>
<td>3q11-q12.2</td>
<td>BRAC2</td>
<td>13q13.1</td>
<td>miR-152</td>
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<td>CACNA1E</td>
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<td>2p12.2</td>
<td>miR-199a</td>
<td>19p13.2</td>
<td>miR-107</td>
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<tr>
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<td>10p15.1 (PRKCC)</td>
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<td>miR-223</td>
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<td>miR-200c</td>
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<td>LASS5</td>
<td>1p21.3</td>
<td>miR-200c</td>
<td>12p13.31</td>
<td>miR-146b</td>
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<tr>
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<td>17q11.2-24.1</td>
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<td>2p12.2</td>
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</table>

**Figure 2** Genomic and molecular biomarkers identified in SS by high-throughput methods. 1, recurrent regions affected by Copy Number variations in SS; 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.
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), NCOA1 (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 10p13.3-10p12.2. 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 PRKCB, 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, ITTP1, DSC1, and PKHD1L1. Moreover, the authors reported single somatic mutations in several genes such as PLCG1, STAT5B, GLI3, CARD11, NAV3, RIPK2, IL6, RAG2 and ITTPR2, 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 BCL, 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, 86 potential fusion transcripts were identified in 10 Sezary samples (69). Some of these fusion events were successfully validated, such as TYK2-UPF1, COL25A1-NFIKB2, FASN-SGMS1, SGMS1-ZEB1, SPATA21-RASA2, PTPRM1-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, MAPK3-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...
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-kB 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

<table>
<thead>
<tr>
<th>Clinical Trial</th>
<th>Therapeutic Agent</th>
<th>Phase Study</th>
<th>Molecular Target</th>
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<td>NCT01704015</td>
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<td>II</td>
<td>Anti CD44/HDAC inhibitor</td>
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<td>NCT01729805</td>
<td>Campath-1H</td>
<td>III</td>
<td>Anti CD52 before stem cell transplantation</td>
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<td>Pembrolizumab</td>
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<td>Anti PD-1</td>
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<td>I</td>
<td>Aurora A Kinase inhibitor/HDAC inhibitor</td>
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<td>I</td>
<td>HDAC inhibitor/HDAC inhibitor</td>
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<tr>
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<td>HDAC inhibitor plus chemotherapy</td>
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<tr>
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<td>NCT01661840</td>
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<td>Peptide anti-TPS inhibitors (MDMx-MDK2)</td>
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<td>Protein kinase inhibitor</td>
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Clinical trials.gov/T-Cell Leukemia Lymphoma Foundation
Drug categories were grouped and highlighted
NA: Not Available

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

Acknowledgements

This study was partially supported by Associazione Italiana Ricerca sul Cancro (AIRC Grant to G Russo and MG Narducci); Fondi Ricerca Corrente from Italian Ministry of Health.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Cristofoletti C, Narducci MG, Russo G. Sézary syndrome, recent biomarkers and new drugs. Chin Clin Oncol 2019;8(1):2. doi: 10.21037/cco.2018.11.02