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SPLENIC MARGINAL ZONE LYMPHOMA:
FROM GENETICS TO MANAGEMENT

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ABSTRACT

Splenic marginal zone lymphoma (SMZL) is a rare B-cell malignancy involving the spleen, bone marrow and, frequently, blood. SMZL lymphomagenesis involves antigen/super-antigen stimulation and molecular deregulation of genes (NOTCH2 and KLF2) regulating the physiological differentiation of spleen marginal zone B-cells. Diagnosis requires either spleen histology or, alternatively, the documentation of a typical cell morphology and immunophenotype on blood cells coupled with the detection of intrasinusoidal infiltration by CD20+ cells in the bone marrow. Among B-cell tumors, deletion of 7q and NOTCH2 mutations are almost specific lesions of SMZL, thus representing promising diagnostic biomarkers of this lymphoma. Although the majority of SMZL shows an indolent course with a median survival of approximately 10 years, ~30% of patients experience a poor outcome. No randomized trials are reported for SMZL and few prospective trials are available. A watch and wait approach is advisable for asymptomatic patients. Treatment options for symptomatic patients ranges from splenectomy to rituximab alone or combined to chemotherapy. In some geographic areas, a subset of SMZL associates with hepatitis C virus infection, prompting virus eradication as an effective lymphoma treatment. Deregulated cellular programs of SMZL are worthwhile to be explored as therapeutic targets in the next years; improved clinical and biological prognostication will be essential to identify patients who may benefit from novel approaches.
INTRODUCTION

Splenic marginal zone lymphoma (SMZL) is a rare indolent B-cell neoplasm involving spleen, bone marrow (BM) and, frequently, blood.

In many cases SMZL is diagnosed occasionally because of the detection of peripheral lymphocytosis; in advanced stage, symptomatic splenomegaly and cytopenia can be the presenting features. Approximately 20% of patients present with an autoimmune manifestation\(^1\), including autoimmune hemolytic anemia, immune thrombocytopenia, cold agglutinin disease, circulating anticoagulants, acquired von Willebrand disease or angioedema due to acquired C1-esterase inhibitor deficiency.\(^2,3\)

The diagnosis of SMZL and its distinction from similar indolent B-cell lymphoproliferative disorders may be challenging in some instances, especially if they rely on bone marrow morphology and phenotype without the support of spleen histology. Although the majority of cases show an indolent course with a median survival of approximately 8-10 years\(^4,5\), the clinical course of SMZL is heterogeneous. Indeed, ~30% of cases show worse outcome\(^4\), including 5-10% of cases undergoing transformation to diffuse large B-cell lymphoma (DLBCL).\(^6-8\) Therefore, prognostic scores and biomarkers are needed to sort out the fraction of SMZL patients who will undergo an aggressive clinical course. Within the last years, molecular genetics improved our knowledge of SMZL lymphomagenesis, allowed the identification of molecular lesions that are currently translated into diagnostic and prognostic markers, and pointed to deregulated cellular programs worthwhile exploring as therapeutic targets.

The rarity and indolent course of SMZL have limited the development of specific treatment options for this lymphoma. Consequently, no randomized trials are available for SMZL and few prospective trials are completed or ongoing. Evidences supporting the therapeutic options in SMZL are mostly based on retrospective series or translated from the experience in other indolent B-cell lymphomas. Treatment tailoring is largely limited by the lack of predictive factors. Dedicated approach with antiviral agents should be reserved to SMZL associated with hepatitis C virus (HCV) infection.\(^9\)
EPIDEMIOLOGY

SMZL generally accounts for <2% of all lymphoid malignancies.\textsuperscript{10,11} Of the 116,411 cases of non-Hodgkin lymphoma (NHL) in the Surveillance, Epidemiology and End Results (SEER) registries, 763 (0.6%) are SMZL. Median age at diagnosis is 69 years. The overall age-adjusted incidence of SMZL is 0.13 per 100,000 persons per year and the percent change in age-adjusted incidence is 4.81%, with increasing trends among white, male or aged \( \geq 70 \) years subjects.\textsuperscript{12}

The International Lymphoma Epidemiology Consortium NHL Subtypes Project, that pooled individual-level data from 20 case-control studies (17,471 NHL cases, 23,096 controls)\textsuperscript{13} points to an association with B-cell activating autoimmune conditions, asthma and hair dye use.\textsuperscript{14}

DIAGNOSIS

Definitive diagnosis of SMZL relies on spleen histology; if not available, it requires integration of BM histology with the cell morphology and immunophenotype in the blood and BM.\textsuperscript{11,15}

Cytology

Blood involvement is common and the typical cell morphology comprises lymphocytes with round nucleus with condensed chromatin and basophilic cytoplasm with polar, short villi, so-called “villous lymphocytes”. Heterogeneity in blood morphology is common, ranging from small lymphoid cells without specific features, to various degrees of monocytoid and plasmacytoid differentiation. Large cells, though rare, may suggest disease transformation into a large cell lymphoma.\textsuperscript{15}

Histology

The first description of SMZL by Schmid et al.\textsuperscript{16} relied on the recognition of a histological pattern recapitulating the marginal zone, as observed in the splenic white pulp. Grossly the spleen usually weights more than 400 g (and may exceed 2000 g) and the cut surface shows a typical multi-micronodular pattern.

SMZL develops in the white pulp with a biphasic picture. Medium-sized monocytoid B-cells are organized as pale ring around the follicle with a MZ pattern, while small centrocytes-like cells efface the mantle zone and colonize the germinal centers (Figure 1). A variable degree of plasmacytic differentiation may be present. Lymphoma cells may involve
the red pulp in patchy or diffuse fashion, with subsequent spread to the sinuses. Infiltration of the walls of the greater vessels is frequent. Large cells, mostly with immunoblastic cytology, are rare and their increase may suggest transformation into a more aggressive lymphoma. Epithelioid histiocytes may be observed, sometimes so numerous to obscure the neoplastic infiltrate. A predominant diffuse pattern mandates the exclusion of other lymphoma subtypes within the splenic B-cell lymphoma/leukemia unclassifiable provisional subgroup, including splenic diffuse red pulp small B-cell lymphoma (SDRPL) and hairy cell leukemia variant (HCL-v). Hilar lymph nodes are frequently involved, displaying a nodular proliferation with obliteration of the reactive germinal centers and engulfment of the sinuses.

In BM trephine biopsy a rather characteristic sinusoidal pattern of infiltration is often detectable, usually combined with an interstitial and nodular component. However pathologists must be aware that it may also be observed, though less frequently, in several low-grade B-cell lymphomas. A careful evaluation of cytology and immunophenotype, including the search for dendritic meshwork, more commonly present and disrupted in SMZL, is helpful in many instances.

**Immunophenotype**

SMZL does not harbor a specific immunophenotype, thus flow cytometry and immunohistochemistry antibody panels should be tailored to exclude other subtypes (Tables 1 and 2). The flow cytometry score of SMZL is low, ranging from 0 to 2, while a diagnosis of CLL requires a scores >3. SMZL consistently expresses CD20, CD79a, BCL2 and surface IgM, variably shows surface IgD and DBA44 and is typically negative for CD5, CD10, BCL6, CyclinD1/BCL1, CD43, Annexin A1, LEF1, CD103 and CD123. Monotypic expression of immunoglobulin light chains may represent a diagnostic clue. CD5-positive cases have been described and should be carefully distinguished from mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL). CD23 and CD21 may be positive in the tumor cells and are useful to delineate the residual follicular dendritic meshwork. Proliferation index (Mib1/Ki67) is low (usually <5%) and depicts a distinctive “targetoid” picture (Figure 1). IRTA1 positivity, reported on the neoplastic cells in cases of extranodal MZL (EMZL), is barely present in SMZL.
Differential diagnosis

A reactive follicular hyperplasia must be always considered; this pattern is frequent or even the rule in children, adolescents and young adults. A diagnosis of SMZL should not be proposed if spleen weighs <300-400 g and in absence of a monotypic cell population.

In most cases, the architectural and cytologic features with adequate immunophenotype allows to differentiate SMZL from other small B-cell lymphomas with micronodular pattern, particularly CLL, MCL and follicular lymphoma, which may occasionally mimic a MZ pattern. In the rare CD5+ cases, morphology, CyclinD1/BCL1 and SOX11 negativity and absence of t(11;14) rules out MCL.11

Among subtypes with privileged splenic involvement, hairy cell leukemia is distinguished for its characteristic morphology and phenotype. Differentiating SDRPL23 and HCL-v24 may be very difficult or even impossible only with blood or BM biopsy17, since they represent two recognized entities with ill-defined clinico-pathologic and immunophenotypic features, partially overlapping SMZL (Tables 1 and 2). Thus, a definite diagnosis may require detailed clinical information, a comprehensive phenotype and spleen histology, which usually shows a typical diffuse pattern of infiltration, with preserved or atresic white pulp follicles.23

In cases of splenic B-cell lymphomas not fulfilling the WHO 2008 criteria neither for better established nor provisional entities a diagnosis of splenic B-cell lymphoma/leukemia, unclassifiable should be preferred.11

Differentiating SMZL from lymphoplasmacytic lymphoma (LPL) may be challenging, particularly on BM biopsy, since SMZL may show a monoclonal serum component and plasmacytic morphology and both entities lack a distinct phenotype. LPL developing primarily in the spleen homogeneously infiltrates the white pulp without MZ pattern and without monocytoid B-cells. MYD88 L265P mutation, present in almost all cases of LPL and rare in SMZL, may be a useful diagnostic tool.25 A further diagnostic pitfall may be represented by detection of a BM clonal infiltrate in cases of non-CLL monoclonal B lymphocytosis.26

Finally, secondary splenic localization of EMZL presents a pattern that overlaps with SMZL but clinical dissemination is crucial to differentiate. Splenic involvement virtually excludes a diagnosis of nodal MZL; as well, apart the differential expression of IRTA1, which is negative in SMZL, clinical correlation is yet critical to reach a correct diagnosis, when dealing with a BM biopsy.
MOLECULAR PATHOGENESIS OF SMZL

Cell of origin and immunogenetics

The cellular origin of SMZL is still debated, and its identification is essential to correctly classify this lymphoma and to elucidate its pathobiology. According to the WHO Classification, the postulated normal counterpart of SMZL is a B-cell of ‘unknown differentiation stage’.\textsuperscript{11} Based on studies of immunoglobulin gene rearrangements, a derivation from antigen-experienced B-cells has been postulated in the vast majority of SMZL.\textsuperscript{27-29} Skewing of the immunoglobulin gene repertoire towards the usage of the $\textit{IGHV}1-2*04$ allele in SMZL suggests that they could derive from a progenitor population adapted in the spleen to particular antigenic challenges, though definitive answers to the issue of the cell of origin of SMZL will admittedly be provided only through multidisciplinary examination of the immune repertoire and transcriptome of normal B-cell populations of the spleen compartments.

The contribution of antigen stimulation to SMZL pathogenesis is suggested by the highly restricted immunoglobulin gene repertoire, including stereotyped configuration of the B-cell-receptor (BCR) in $\sim$10% of cases\textsuperscript{30} and selective usage of the immunoglobulin heavy chain variable ($\textit{IGHV}$) $1-2*04$ allele in $\sim$30%.\textsuperscript{31} Although the epitope recognized by $\textit{IGHV}1-2*04$ expressing BCR is unknown, the features of $\textit{IGHV}1-2*04$ rearrangements, including minimal somatic mutations and long complementarity determining region-3 sequence with common motifs, suggest a possible selection of T-cell independent MZ B-cells by superantigens, thus a role of antigenic drive in the lymphomagenesis.

Cytogenetic and genetic lesions

SMZL lacks recurrent chromosome translocations, including translocation that are typical of other lymphoma types as the $\text{t}(14;18)$ translocation affecting $\textit{BCL2}$ in FL, the $\text{t}(11;14)$ translocation affecting $\textit{CCND1}$ in MCL and the $\text{t}(11;18), \text{t}(14;18)$ and $\text{t}(1;14)$ translocations affecting the $\textit{BIRC3/MALT1}, \textit{MALT1}$ and $\textit{BCL10}$ genes, respectively, in EMZL. The lack of these abnormalities may assist the distinction of SMZL from pathologically mimicking tumors. Approximately 30% of SMZLs show hemizygous 7q deletion, which is also frequently seen in splenic B-cell lymphoma/leukemia unclassifiable, but rarely in other lymphoma subtypes.\textsuperscript{32,33} The gene(s) targeted by the 7q deletion remain obscure despite the combined investigation of genomic and transcriptomic profiles and mutation analysis of a number of candidate genes.\textsuperscript{33-36}
Unbiased genomic studies have unraveled the typical coding genome of SMZL.\textsuperscript{37-43} However, due to the limited number of SMZL genomes/exomes so far available the full spectrum of lesions that contribute to the malignant transformation of SMZL remains unknown. The notion that the most frequently mutated genes in SMZL (i.e. and NF-κB signaling genes, \textit{NOTCH2} and other NOTCH pathway genes, and \textit{KLF2}) are physiologically involved in proliferation and commitment of mature B-cells to the MZ, points to cell cycle and homing to the spleen compartment and MZ differentiation as the major programs deregulated in this lymphoma. Consistently, SMZL has an expression signature characterized by the upregulation of genes belonging to the MZ differentiation program, including NF-κB and NOTCH pathway genes.

Active NF-κB signaling is necessary for the generation and/or maintenance of normal MZ B-cells. Overall, mutations of positive and negative NF-κB regulators accounted for \( \approx 35\% \) of SMZL cases, implicating activation of NF-κB as a major contributor to the pathogenesis of this disease. The canonical NF-κB signaling is molecularly deregulated by a variety of mechanisms in 15\% of SMZL. \textit{IKBKB}, the central activating kinase of canonical NF-κB signaling, is constitutively activated by mutations in \( \approx 10\% \) of SMZL, while \textit{TNFAIP3}, the master negative regulator of NF-κB, is inactivated by mutations and/or deletions in \( \approx 5\% \) of cases. The TRAF3/MAP3K14-TRAF2/BIRC3 negative regulatory complex of non-canonical NF-κB signaling is disrupted by mutations in \( \approx 15\% \) of SMZL, allowing the cytoplasmic release, stabilization and constitutive activation of MAP3K14 (also known as NIK), the central activating kinase of non-canonical NF-κB signaling.\textsuperscript{44}

The NOTCH receptor genes encode a family of heterodimeric transmembrane proteins that function as ligand-activated transcription factors. Upon activation, the cleaved intracellular portion of the NOTCH receptors translocates into the nucleus recruits the MAML1 and MAML2 transcriptional co-factors to modify the expression of a number of target genes. The most prominent mechanism of NOTCH signal suppression is operated through its PEST domain that terminates signaling by directing the active intracellular portion of NOTCH towards proteasomal degradation. Other negative regulators of NOTCH signaling include SPEN and DTX1. SPEN represses NOTCH signaling by competing with the active intracellular NOTCH for binding to RBPJ. DTX1 represses NOTCH signaling by binding the NOTCH family proteins and inhibiting their recruitment of transcription coactivators. Genes of the NOTCH pathway are mutated in \( \approx 40\% \) of SMZL. \textit{NOTCH2} shows recurrent mutations in \( \approx 10-25\% \) SMZL, establishing \textit{NOTCH2} as one of the most frequently mutated gene in this lymphoma. \textit{NOTCH1}, a paralog of \textit{NOTCH2}, is also mutated in additional \( \approx 5\% \) SMZL.\textsuperscript{37-43}
NOTCH2 and NOTCH1 mutations in SMZL are selected to truncate the PEST domain of the protein, thus causing impaired degradation of the NOTCH2 and NOTCH1 proteins and, as a consequence, sustained NOTCH signaling. In addition to NOTCH2 and NOTCH1, other genes involved in NOTCH signaling (SPEN, DTX1 and MAML2) are affected by genomic lesions in SMZL, though at lower frequency.

Beside the pathogenic implications, NOTCH2 mutations may be of help to inform SMZL diagnosis and prognosis. From a diagnostic standpoint, NOTCH2 mutations are highly specific for SMZL among mature B-cell tumors, including conditions that look alike SMZL, thus representing a biomarker with positive predictive value for SMZL specification.\textsuperscript{37,38} From a prognostic standpoint, SMZL cases with NOTCH2 mutations have an inferior outcome.\textsuperscript{38}

KLF2 is a member of the KLF family of zinc-finger transcription factors that in normal lymphocytes physically binds the promoter and regulates the expression of genes involved in cell cycle/apoptosis, cell trafficking and NF-κB signaling. KLF2 is somatically mutated in 20-40% SMZL, thus representing one of the most frequently altered genes in this lymphoma along with NOTCH2.\textsuperscript{40,42,43} Most of KLF2 mutations disrupt its nuclear localization signal (NLS) causing the displacement of KLF2 from the nucleus to the cytoplasm, and/or affected codons required for the interaction between KLF2 and DNA. Consistently, deregulation of the transcriptional program orchestrated by mutant KLF2 leads to NF-κB activation. Indeed, mutations prevent the physiological ability of KLF2 to suppress NF-κB induction by upstream signaling pathways, including the B-cell receptor and the Toll-like receptor pathways.\textsuperscript{42} KLF4, a tumor suppressor that is highly homologous paralog of KLF2, is also frequently inactivated by aberrant promoter methylation in SMZL, suggesting a more general involvement of the Krüppel-like transcription factors in this lymphoma.\textsuperscript{45} Though not diagnostically useful because occurring also in other indolent B-cell tumors, KLF2 mutations are of prognostic relevance in SMZL because they mark cases with an inferior outcome.\textsuperscript{40,42,43}

As is the case for most cancer-associated genetic lesions, NOTCH2 upregulation or inactivation of KLF2 may not be sufficient, as single events, for malignant transformation. In fact, transgenic mice engineered to overexpress NOTCH2 or lack KLF2 in mature B-cells display an expansion of the MZ at the expense of the follicular compartment, but do not develop lymphoma.\textsuperscript{46,47} It is important to note, however, that lymphoma development may require longer times than those observed so far in mice, in line with the indolent course of SMZL and the elderly age of patients affected by this lymphoma. Consistent with a multistep
process of lymphomagenesis, *IGHV1-2*04 usage, *NOTCH2* mutations, *KLF2* mutations, *KLF4* aberrant methylation, and 7q deletion co-occur in SMZL, thus identifying a disease subset with a distinct genotype characterized by multi-genetic/epigenetic changes, and suggesting a possible cooperation between genetic/epigenetic abnormalities and B-cell receptor configuration in promoting transformation. Key molecular alterations of SMZL are summarized in Figure 2.

**HCV-associated SMZL**

Many epidemiological studies have investigated the association of HCV with NHL. In subtype-specific analyses, HCV is frequently associated with MZL and DLBCL. According to other models of lymphomagenesis (*Helicobacter pylori* in gastric EMZL, *Borrelia burgdorferi* for EMZL of the skin, *Chlamydophila psittaci* for EMZL of the ocular adnexa), also chronic stimulation by HCV may be a factor in development of a subgroup of SMZL cases. However, the role of HCV in SMZL can reflect geographic difference considering the relatively high seroprevalence in some series and the rarity of HCV-positive cases in others.

A clinical triad of SMZL, mixed cryoglobulinemia and HCV infection has been proposed as a model of infection-driven lymphomagenesis. Saadoun et al. reported SMZL associated with type II cryoglobulinemia and HCV infection: all 18 patients had type II mixed cryoglobulinemia (symptomatic in 13). Accordingly, in a large series from Italy, HCV serology was positive in 19% of cases with more frequent presence of nodal disease, cryoglobulinemia and serum monoclonal component.

Along with overlapping histological features between HCV-positive and HCV-negative cases, comparative genome hybridization array study did not find any difference in DNA copy number changes according to HCV status. On the other hand, supervised analysis of miRNA expression revealed differentially expressed miRNAs and miR-26b, a miRNA with tumor suppressive activity, was downregulated in HCV-positive SMZL.

**PROGNOSTIC FACTORS**

Several prognostic factors have been proposed for SMZL, including leukocytosis, lymphocytosis, lymphopenia, anemia, thrombocytopenia, use of chemotherapy, monoclonal component, β2-microglobulin, performance status ≥ 2, incomplete response, non-hematopoietic site involvement, advanced age, diffuse pattern of bone marrow infiltration, histological transformation.
The first score for the assessment of SMZL prognosis was proposed by Italian Lymphoma Intergroup (now Fondazione Italiana Linfomi, FIL). In this series of 309 SMZL patients, the 5-year cause-specific survival (CSS) rate was 76%. Using 3 laboratory variables (hemoglobin level less than 12 g/dL, elevated serum lactate dehydrogenase level and albumin level less than 3.5 g/dL), SMZL patients have been grouped into 3 prognostic categories: low-risk group (41%) with no adverse factors, intermediate-risk group (34%) with one adverse factor, and high-risk group (25%) with 2 or 3 adverse factors. The 5-year CSS rate was 88% for the low-risk group, 73% for the intermediate-risk group, and 50% for the high-risk group. This latter group accounted for 54% of all lymphoma-related deaths.

After that, an international study of 593 SMZL patients identified hemoglobin, platelet count, high lactate dehydrogenase level and extrahilar lymphadenopathy as parameters independently associated with lymphoma-specific survival (LSS). Three risk groups were identified with significantly different 5-year LSS (94%, 78% and 69%, respectively). In a subsequent study aimed to optimize the prognostication, clinically acceptable cut points were established: 9.5 g/dL for hemoglobin, and 80 x 10^9/L for platelet count. The patients were allocated into 3 groups: low-risk group (36%) with 0 points, intermediate-risk group (56%) with 1 or 2 factors, and high-risk group (8%) with 3 or 4 factors. The 3 groups had a 5-year LSS of 95%, 87% and 68%, respectively. This score has been validated in an independent series of SMZL patients.

The clinical scores are neither 100% sensitive nor 100% specific in identifying high risk patients. Molecular aspects of SMZL (i.e. immunoglobulin gene mutation status, NOTCH2 and KLF2 mutations, TP53 abnormalities and aberrant promoter methylation) represent promising prognostic biomarkers associated with inferior outcome, and their incorporation into the current available clinical prognostic models might improve risk stratification of patients.

**TREATMENT**

Consensus guidelines recommend only treating SMZL in presence of symptomatic splenomegaly, cytopenias, systemic symptoms, progressive nodal disease. Autoimmune cytopenias should be specifically treated. No randomized trials have been conducted in SMZL and, as consequence, there is no consensus on treatment for newly diagnosed and relapsed patients. The therapeutic options for SMZL widely range and include splenectomy, chemotherapy, rituximab alone or rituximab + chemotherapy. In addition, antiviral treatment should be
considered in patients with SMZL and concurrent chronic infection with HCV-related hepatitis who do not need immediately conventional treatment against the lymphoma.9,55

Staging and response criteria

According to Lugano classification88, SMZL is not fluorodeoxyglucose (FDG)-avid disease and must be staged by means of computed tomography. However, positron emission tomography (PET) can be considered if a transformation is suspected.66

In addition to Lugano principles88, considering the particular clinical presentation of SMZL, specific criteria for response assessment have been proposed.15 In particular a complete response is achieved in case of resolution of splenomegaly, normalization of blood cell counts, negative flow cytometry on blood and negative bone marrow histology with immunohistochemistry.

Splenectomy

As a therapeutic approach, the surgical removal of large spleens may eliminate a significant amount of disease, ameliorating abdominal discomfort and resolving cytopenias due to splenic sequestration.7 After surgery patients can remain free from treatment for many years.1 Because cytopenias due to marrow failure do not resolve after splenectomy, a bone marrow biopsy is advisable during the work-up to define the burden of bone marrow infiltration by the disease. One additional advantage of splenectomy is that it allows establishing a definitive diagnosis of SMZL.

Drawbacks of splenectomy are short-term (perioperative events) and long-term (immune suppression and infections) complications. In a recent series of 41 splenectomized patients89, peri-operative complications have been registered in one quarter of patients: pulmonary dysfunction (19.5%), deep venous thrombosis 1 (2.4%), portal vein thrombosis 1 (2.4%) and major bleeding 9 (21.9%). Infections caused by encapsulated bacteria are the major risk associated with splenectomy90 and vaccination against capsulated bacteria is mandatory at least 2 weeks prior to elective splenectomy.91 The risk of infections after splenectomy is low in lymphomas but still present after many years and potentially fatal.92 In 2 recent series from France7 and British Columbia62, around 5% of splenectomized patients died from infectious complications.

Physicians can be also reluctant to choose splenectomy in patients with comorbidities and/or advanced age; in these situations laparoscopic approach72,93 can extend the indication to splenectomy also in case of massive splenomegaly through the hand-assisted approach.94
Splenectomy should be contraindicated in cases with disseminated lymphoma with nodal involvement outside splenic hilum. On the other hand, a strict indication to splenectomy is present in cases with suspected transformation (for instance nodular lesion with augmented FDG uptake). Results of SMZL series of splenectomized patients are summarized in Table 3.

**Rituximab-based treatment**

The clinical scenario of systemic therapy in SMZL has been changed with the appearance of anti-CD20 monoclonal antibody rituximab and rituximab-based treatment has become a valid alternative to splenectomy.\(^95,96\)

Rituximab monotherapy allows to obtain at least similar results to splenectomy, avoiding toxicity of chemotherapy with potential capacity of eradicating the disease also at molecular level: Kalpadakis et al reported 58 patients treated with rituximab weekly for 6 weeks, followed by a maintenance phase every 2 months for 1 to 2 years: at the end of the induction phase CR rate was 45%, CRu 26% and PR 24%; the 5-year OS and PFS was 92% and 73%, respectively.\(^73\)

According to the European Society for Medical Oncology (ESMO) guidelines\(^66\) rituximab monotherapy is a reasonable first-line therapy and a less traumatic alternative to splenectomy. According to the Italian Society of Hematology guidelines,\(^67\) rituximab monotherapy is an option for patients in need of treatment without disseminated disease and for patients with contraindications to surgery.

Combination of rituximab with chemotherapy is considered a standard for symptomatic, indolent B-cell NHL.\(^66\) In SMZL this approach is indicated for fit patients with disseminated disease\(^66,67\) constitutional symptoms, and/or signs of high-grade transformation.\(^66\)

In FIL trial,\(^87\) 51 SMZL were treated with rituximab with cyclophosphamide, vincristine, non-pegylated liposomal doxorubicin and prednisone (R-COMP). The overall response rate was 84% and the 6-years PFS and OS were 54% and 72%, respectively. Toxicity was not negligible (grade >3 neutropenia 26%; grade >3 infections 8%, 2 deaths for infection).

The combination of rituximab with bendamustine (BR) is effective in indolent NHL, though it has never been tested in a dedicate trial of SMZL.\(^97,98\) In the Bright study\(^97\), the overall response rate to BR was 92% in 25 MZL of different histology. In the Stil trial\(^98\), the PFS of BR was not longer than that of R-CHOP among MZL, but the study was not powered to find difference in the MZL subset. The BRISMA (IELSG 36, EudraCT Number: 2011-
phase II trial using BR may provide in the near future specific information on the safety and activity of this regimen in SMZL.

Results of rituximab-based treatment in SMZL are summarized in Table 4.

**Novel agents**

No trial with novel agents was specifically dedicated to SMZL, though some data about activity in MZL of different histology can be derived from published studies conducted in indolent NHL (Table 5).

Ongoing clinical trials specifically dedicated to SMZL or including SMZL among other indolent NHL are evaluating new anti-CD20 monoclonal antibodies alone or in combination (EudraCT Number: 2013-004916-23, NCT01332968), ibrutinib (NCT01980628, NCT01974440) and PI3K inhibitors (NCT01282424, NCT01732926, NCT02369016, NCT02367040, NCT01732913).

**HCV infection and antiviral treatment**

The causal role of HCV in lymphomagenesis is strongly supported by the regression of lymphoma after eradicating the HCV infection. The first experience was reported by Hermine et al. in 9 SMZL patients with HCV infection treated with interferon (IFN): 7 patients obtained a complete hematological remission and HCV-RNA negativity. In a cohort of 704 consecutive HCV-positive patients with indolent NHL (comprising 137 SMZL) reported by FIL, 36 SMZL patients have been treated with IFN-based antiviral treatment as first line approach and 65% showed a response.

Although there is a clear association across the studies between the lymphoma regression and the clearance of HCV, the direct anti-lymphoma activity of interferon cannot be ruled out. Data on new IFN-free regimens with direct-acting antivirals (DAA) in HCV-associated lymphoproliferative disorders are based on clinical reports and described rapid response. Considering also the favorable impact of antiviral treatment on outcome of HCV-infected NHL patients, it should be considered the first option for HCV-associated SMZL if cytoreductive treatment is not immediately necessary.

**CONCLUSIONS**

Despite the relative rarity of SMZL, a huge effort has been made in last decade in defining diagnostic criteria, prognosis and molecular landscape of this neoplasm and of mimicking disorders. Though the understanding of disease genetics is significantly improved
over the last 5 years, the pathogenetic implications of the new discovered genetic lesions still remain to be formally documented. In addition, though *NOTCH2* mutations and *KLF2* mutations represent promising biomarkers have been proposed, their broad application in the clinical practice require diagnostic accuracy studies and their incorporation into the current available clinical prognostic models for SMZL.

Prospective studies dedicated to SMZL will clarify clinical benefit and toxicity profile of immunochemotherapy approach. Specific molecular targets seem to be reasonably actionable in SMZL but, also in the era of precision medicine, splenectomy and rituximab monotherapy are still effective options for the majority of the patients with SMZL.
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References


FIGURE LEGENDS

**Figure 1. Histopathology of SMZL.** In this typical case, at scanning magnification (A; HE 20x) a nodular lymphoid proliferation with a biphasic appearance effaces the white pulp, infiltrates the wall of a great vessel (arrow) and extends to the red pulp in a patchy distribution (B; CD79a 100x). Morphologic pictures show medium sized, monocytoid lymphocytes with only scattered large cells (C; HE 400x) and a variable degree of plasmacytic differentiation (D; Giemsa 400x). Anti-CD23 immunostain (E; 100X) depicts the CD23+ marginal zone cells as well as the residual dendritic meshwork within the colonizes follicles, also highlighted by Mib/Ki67 (F; 100x), which confers a targetoid appearance. A prototypical BM biopsy shows a small to medium sized lymphoid population (G; Giemsa 400x) with a nodular and sinusoidal distribution (H; CD20 400x); note the megaloblastoid features within the erythroblastic lineage (G), a common finding in cases associated to paraproteinemia and anemia.

**Figure 2. Key molecular alterations of SMZL.** Genes and pathways that are molecularly deregulated in SMZL are schematically represented. The prevalence of molecular alterations in SMZL is reported beside each gene or pathway.
Table 1 – Flow cytometry features of splenic marginal zone lymphoma and other leukemic B-cell lymphoproliferative disorders

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SMZL: splenic marginal zone lymphoma; CLL: chronic lymphocytic leukemia; MCL: mantle-cell lymphoma; FL: follicular lymphoma; HCL: hairy cell leukemia; HCL-v: hairy cell leukemia variant; -: < 10% of cases positive; +: 11–35% positive cases; ++: 36–75% positive cases; +++: >75% positive cases
**Table 2 – Immunohistochemistry features of splenic marginal zone lymphoma and other small B-cell lymphomas**

<table>
<thead>
<tr>
<th></th>
<th>SMZL</th>
<th>LPL</th>
<th>SDRPL</th>
<th>HCL-v</th>
<th>HCL</th>
<th>EMZL/NMZL</th>
<th>CLL</th>
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<th>FL</th>
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<tr>
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<td>+</td>
<td>+/-</td>
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</table>

SMZL: splenic marginal zone lymphoma; LPL: lymphoplasmacytic lymphoma; SDRPL: splenic diffuse red pulp lymphoma; HCL: hairy cell leukemia; HCL-v: hairy cell leukemia variant; EMZL/NMZL: extranodal/nodal marginal zone lymphoma; CLL: chronic lymphocytic leukemia; MCL: mantle cell lymphoma; FL: follicular lymphoma; -: <25% of cases; +/-: 25-50% of cases; +/-: 50-75% of cases; +: >75% of cases.*sporadic cases reported
Table 3 – Series of SMZL patients treated with splenectomy

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>N</th>
<th>ORR (%)</th>
<th>DOR/FFS/PFS</th>
<th>OS</th>
<th>Deaths due surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mulligan et al.⁶⁸</td>
<td>1991</td>
<td>20</td>
<td>95</td>
<td>median DOR 4 y</td>
<td>-</td>
<td>1</td>
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<tr>
<td>Troussard et al.⁵⁹</td>
<td>1996</td>
<td>28</td>
<td>75</td>
<td>-</td>
<td>71% at 5 y</td>
<td>1</td>
</tr>
<tr>
<td>Chacon et al.⁶¹</td>
<td>2002</td>
<td>60*</td>
<td>93,3</td>
<td>median FFS 40 mo</td>
<td>65% at 5 y</td>
<td>-</td>
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<tr>
<td>Thieblemont et al.¹</td>
<td>2002</td>
<td>48*</td>
<td>100</td>
<td>PFS 48% at 5 y</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Parry-Jones et al.⁶⁰</td>
<td>2003</td>
<td>33</td>
<td>-</td>
<td>-</td>
<td>LSS 95% at 10 y</td>
<td>-</td>
</tr>
<tr>
<td>Iannitto et al.⁶⁹</td>
<td>2004</td>
<td>21</td>
<td>91</td>
<td>median DOR 4 y</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tsimberidou et al.⁷⁰</td>
<td>2006</td>
<td>10</td>
<td>60</td>
<td>FFS 80% at 3 y</td>
<td>89% at 3 y</td>
<td>0</td>
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<tr>
<td>Olszewski et al.⁷¹</td>
<td>2012</td>
<td>652</td>
<td>-</td>
<td>-</td>
<td>67.8% at 5 y§</td>
<td>-</td>
</tr>
<tr>
<td>Kalpadakis et al.⁷³</td>
<td>2013</td>
<td>27</td>
<td>85</td>
<td>PFS 58% at 5 y</td>
<td>77% at 5 y</td>
<td>1</td>
</tr>
<tr>
<td>Lenglet et al.⁷</td>
<td>2014</td>
<td>100</td>
<td>97</td>
<td>PFS 61% at 5 y</td>
<td>84% at 5 y</td>
<td>0</td>
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<tr>
<td>Xing et al.⁶²</td>
<td>2015</td>
<td>52#</td>
<td>-</td>
<td>FFS 39% at 10 y</td>
<td>61% at 10 y</td>
<td>0</td>
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<tr>
<td>Pata et al.⁸⁹</td>
<td>2015</td>
<td>41</td>
<td>90</td>
<td>PFS 35% at 5 y</td>
<td>75% at 5 y</td>
<td>0</td>
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</tbody>
</table>

ORR: overall response rate; N: number of cases; DOR: duration of response; y: year; ORR: overall response rate; PFS: progression-free survival; OS: overall survival; FFS: failure-free survival; LSS: lymphoma-specific survival; y: year(s); mo: months *splenectomy alone in 29 patients; *splenectomy alone in 25 patients; §Survival of entire series of 1,251 patients with no impact of splenectomy on OS; #splenectomy alone in 42 patients
Table 4 – Series of SMZL patients treated with rituximab-based approach

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Study type</th>
<th>N</th>
<th>ORR</th>
<th>DOR/FFS/PFS</th>
<th>OS</th>
</tr>
</thead>
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<tr>
<td><strong>Rituximab monotherapy</strong></td>
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<tr>
<td>Bennett et al.(^82,102)</td>
<td>2005/2008</td>
<td>Retrospective - RR</td>
<td>11</td>
<td>91%</td>
<td>PFS 60% at 5 y</td>
<td>70% at 5 y</td>
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<tr>
<td>Tsimberidou et al.(^70)</td>
<td>2006</td>
<td>Retrospective – First line</td>
<td>25</td>
<td>88%</td>
<td>FFS 86% at 3 y</td>
<td>95% at 3 y</td>
</tr>
<tr>
<td>Kalpadakis et al.(^83,96)</td>
<td>2007/2014</td>
<td>Retrospective – First line</td>
<td>16</td>
<td>100%</td>
<td>PFS 92% at 2.4 y</td>
<td>100% at 2.1 y</td>
</tr>
<tr>
<td>Else et al.(^103)</td>
<td>2012</td>
<td>Retrospective – First line and RR</td>
<td>10</td>
<td>100%</td>
<td>DFS 89% at 3 y</td>
<td>-</td>
</tr>
<tr>
<td>Kalpadakis et al.(^73)</td>
<td>2013</td>
<td>Retrospective – First line</td>
<td>58</td>
<td>95%</td>
<td>PFS 73% at 5 y</td>
<td>92% at 5 y</td>
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<tr>
<td><strong>Rituximab + chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tsimberidou et al.(^70)</td>
<td>2006</td>
<td>Retrospective R-Chemo - First line</td>
<td>6</td>
<td>83%</td>
<td>FFS 100% at 3 y</td>
<td>100% at 3 y</td>
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<tr>
<td>Cervetti et al.(^85,86)</td>
<td>2010/2013</td>
<td>Retrospective R-2CDA - First line</td>
<td>47*</td>
<td>87%</td>
<td>PFS 80% at 5 y</td>
<td>86% at 5 y</td>
</tr>
<tr>
<td>Else et al.(^103)</td>
<td>2012</td>
<td>Retrospective R-chemo - First line</td>
<td>33</td>
<td>100%</td>
<td>DFS 71% at 3 y</td>
<td>-</td>
</tr>
<tr>
<td>Iannitto et al.(^87)</td>
<td>2015</td>
<td>Prospective R-COMP - First line</td>
<td>51</td>
<td>84%</td>
<td>PFS 54% at 6 y</td>
<td>72% at 6 y</td>
</tr>
</tbody>
</table>

RR: relapsed/refractory; ORR: overall response rate; Chemo: chemotherapy; 2CDA: 2-chlorodeoxyadenosine; y: year; FFS: failure-free survival; DFS: disease-free survival; * Rituximab in 32 pts
**Table 5** – Novel agents in marginal zone lymphomas

<table>
<thead>
<tr>
<th>Agent</th>
<th>Year</th>
<th>Study type</th>
<th>N</th>
<th>MZL</th>
<th>ORR</th>
<th>PFS/DOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat\textsuperscript{104}</td>
<td>2011</td>
<td>Phase I - RR 35</td>
<td>9</td>
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<td>22%</td>
<td>Median PFS 18.8 mo</td>
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<tr>
<td>Ibrutinib\textsuperscript{105}</td>
<td>2013</td>
<td>Phase I - RR 56</td>
<td>4</td>
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<td>25%</td>
<td>-</td>
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<tr>
<td>Idelalisib\textsuperscript{106}</td>
<td>2014*</td>
<td>Phase II – RR 125</td>
<td>15</td>
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<td>47%</td>
<td>Median PFS 6.6 mo, Median DOR 18.4 mo</td>
</tr>
<tr>
<td>Lenalidomide + Rituximab\textsuperscript{107}</td>
<td>2014</td>
<td>Phase II – First line 103</td>
<td>27</td>
<td></td>
<td>89%</td>
<td>Median PFS 53.8 mo</td>
</tr>
</tbody>
</table>

RR: relapsed/refractory; ORR: overall response rate; PFS: progression-free survival; DOR: duration of response

\*Updated at ASH 2014
**NOTCH** signalling mutations: 40%

**NF-κB** signalling mutations: 35%

**KLF2** mutations: 20-40%

Biased **IGHV1-2*04** usage: 30%

7q deletion: 30%
Splenic marginal zone lymphoma: from genetics to management

Luca Arcaini, Davide Rossi and Marco Paulli