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MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMAS: AN UPDATE

ARTIGO DE REVISÃO

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ÍNDICE

LISTA DE ABREVIATURAS USADAS NESTE TRABALHO

A

ABL1 – Ableson kinase 1

API2 – Apoptosis inhibitor 2

B

B. burgdorferi – Borrelia burgdorferi

ba-ISH – Break-apart *in situ* hybridization

BCL – B-cell chronic lymphocytic leukemia/lymphoma protein

BCL6 – B-cell chronic lymphocytic leukemia/lymphoma protein 6

BCL10 – B-cell chronic lymphocytic leukemia/lymphoma protein 10

BCR – B-cell receptor

C

C. jejuni – Campylobacter jejuni

C. psittaci – Chlamydophila (Chlamydia) psittaci

CagA – Cytotoxin-associated gene A

CARMA1 – Caspase recruitment domain-containing protein 11 (CARD11)

CCL – Chemokine (C-C motif) ligand (CCL17, CCL22)

CCR – Chemokine (C-C motif) receptor (CCR2)

CD - Cluster of differentiation (CD19, CD20, CD22, CD28, CD69, CD79a, CD86)

CGH – Comparative genomic hybridization

CHOP – Cyclophosphamide, doxorubicin, vincristine and prednisolone

CLL - Chronic lymphocytic leukemia

CML – Chronic myeloid leukemia

CR – Complete remission/Complete response

CT - Computerized tomography

CTD - Connective tissue disease

CVP – Cyclophosphamide, vincristine and prednisolone

D

DLBC – Diffuse large B-cell

DLBCL – Diffuse large B-cell lymphoma

DNA – Deoxyribonucleic acid

E

ECOG - Eastern Cooperative Oncology Group

EGILS - European Gastro-Intestinal Lymphoma Study Group

F

FISH – Fluorescence *in situ* hybridization

FLIPI – Follicular lymphoma international prognostic index

FM – Fludarabine, mitoxantrone

FOXP1 – Forkhead protein box subfamily P protein 1

H

H. pylori – Helicobacter pylori

H^+/K^+ ATPase – gastric proton pump (hydrogen potassium adenosine triphosphatase)

I

ICOS – Inducible T-cell costimulator

Ig – Immunoglobulin

IgG – Immunoglobulin G (γ heavy chain)

IGH - Immunoglobulin heavy chain

IGK - Immunoglobulin *kappa* light chain

IκB - Inhibitor of κB (NF-κB inhibitor)

IκK – Inhibitor of κB kinase

IL – Interleukin

IL-8 – Interleukin 8

IPI - International prognostic index

IPSID – Immunoproliferative small intestinal disease (α-chain disease)

L

LDH - Lactate dehydrogenase

M

MALT – Mucosa-associated lymphoid tissue

MALT1 – Mucosa-associated lymphoid tissue lymphoma translocation gene 1

MCL - Mantle-cell lymphoma

miR – microRNA (miR-34a, miR-203)

miRNA - microRNA

N

NF-κB – Nuclear factor kappa B

NHL – Non-Hodgkin lymphoma

NIK – NF-κB-inducing kinase

O

OS – Overall survival

ORR – Overall response rate/Objective response rate

P

PCR – Polymerase chain reaction

PET - Positron emission tomography

PFS – Progression-free survival

PI3K - Phosphoinositide 3-kinase

PPI – Proton-pump inhibitor

PR – Partial remission/Partial response

R

RANK – Receptor activator of nuclear factor κB

RF – Rheumatoid factor

RNA – Ribonucleic acid

S

SLL - Small lymphocytic lymphoma

T

T_h – T-helper cell

T_h2 – Type 2 T-helper cell

TLR - Toll-like receptor

TNFα - Tumor necrosis factor α

TNFAIP3 – Tumor necrosis factor α-induced protein 3 (A20)

TNFR – Tumor necrosis factor receptor

TNM - Tumor-node-metastasis classification system

T_{reg} – Regulatory T-cell

U

UPS – Ubiquitin-proteasome system

USA - United States of America

V

VEGF – Vascular endothelium growth factor

VEGF-A – Vascular endothelium growth factor A

W

WHO - World Health Organization

TITLE PAGE

Mucosa-associated lymphoid tissue lymphomas: an update

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ABSTRACT

Mucosa-associated lymphoid tissue (MALT) lymphoma is an indolent extranodal marginal zone B-cell lymphoma, originating in acquired MALT that is induced in mucosal barriers as part of a normal adaptive immune response to a chronic immunoinflammatory stimulus, including infections by *Helicobacter pylori*, *Borrelia burgdorferi* and *Chlamydophila psittaci* and autoimmune diseases. This antigenic stimulation initially leads to lymphoid hyperplasia; the acquisition of additional genetic aberrations culminates in the activation of intracellular survival pathways, with disease progression due to proliferation and resistance to apoptosis. While early-stage disease can frequently regress through the therapeutic reversal of the chronic immune stimulus, the presence of immortalizing genetic abnormalities or of advanced disease requires a more aggressive approach which is, presently, not consensual.

This lymphoma is a rare neoplasm, with a worldwide incidence of 1-1.5 cases per 10^5, per year. There are descriptions of MALT lymphomas affecting practically every organ and system, with a marked geographic variability partially attributable to the epidemiology of the underlying risk factors. The rarer locations, representing less than 1% of all cases, can have yearly incidences as low as 1 per 10^8, determining an inability to accrue representative series of patients for epidemiologic studies and robust clinical trials that could sustain informed evidence-based therapeutic decisions.

The present review article aims to update the state of the art of knowledge regarding the ethiopathogenesis and oncobiology of this rare malignancy, while summarizing the latest clinical results, to improve the evidence for a clinical decision and optimize the quality of patient care.

RESUMO

O linfoma do tecido linfóide associado às mucosas (*mucosa-associated lymphoid tissue*, MALT) é um linfoma de células B indolente, da zona marginal extraganglionar, que se origina em MALT adquirido induzido nas mucosas com função de barreira, como parte de uma resposta imunitária adaptativa normal a um estímulo imunoinflamatório crónico, incluindo infecções por *Helicobacter pylori*, *Borrelia burgdorferi* e *Chlamydophila psittaci* e doenças autoimunes. Esta estimulação antigénica leva a uma hiperplasia linfóide; a aquisição de aberrações genéticas adicionais culmina na activação de vias intracelulares de sobrevivência, com progressão da doença por proliferação e resistência à apoptose. Enquanto os estádios precoces frequentemente regridem através da reversão terapêutica do estímulo imunológico crónico, a presença de anomalias genéticas imortalizantes ou de doença avançada requer uma abordagem mais agressiva que, actualmente, não é consensual.

Este linfoma é uma neoplasia rara, com uma incidência mundial de 1-1.5 casos, por 10^5, por ano. Há descrições de linfomas MALT afectando praticamente todos os órgãos e sistemas, com uma variabilidade geográfica marcada, em parte atribuível à epidemiologia dos factores de risco subjacentes. As localizações mais raras, representando menos de 1% dos casos, podem ter incidências anuais tão baixas como 1 por 10^8, condicionando uma impossibilidade de recrutar séries de doentes representativas, para estudos epidemiológicos e ensaios clínicos robustos que sustentem decisões terapêuticas baseadas na evidência.

Este artigo de revisão pretende actualizar o estado da arte do conhecimento sobre a etiopatogénese e oncobiologia desta neoplasia rara, ao mesmo tempo que sumariza os resultados clínicos mais recentes, para melhorar a evidência para a decisão clínica e optimizar a qualidade do tratamento do doente.

KEYWORDS

Lymphoma, B-cell, marginal zone; mucosa-associated lymphoid tissue; lymphomagenesis; oncogenesis; *Helicobacter pylori*; inflammation

INTRODUCTION

Mucosa-associated lymphoid tissue (MALT) lymphomas, clinically and histopathologically described by the British pathologists Isaacson and Wright in the early 1980s, are the paradigm for the association between tumorigenesis and a chronic inflammatory stimulus. They are one of the best models of the relationship between specific genetic events and oncogenesis, tumor biology, clinical behavior and potential therapeutic targeting. [1–4] As such, they currently play an important role in basic and translational cancer research.

Although rare, these neoplasms are also clinically relevant due to two opposing characteristics. On the one hand, MALT lymphoma is a malignancy that, in many cases, can be cured with a short course of antibiotic therapy. On the other hand, those patients who do not or cannot respond to antibiotics are frequently treated with surgery, radiotherapy, chemotherapy, immunotherapy or any combination of the former, according to the medical team's experience and personal preference, due to the lack of clinical effectiveness data supporting evidence-based treatment choices.

While review articles are published on the topic on a regular basis (an average of about 12 reviews and systematic reviews in English were indexed in the United States National Library of Medicine database, per year, from the first description of MALT lymphoma to the end of 2011), the vast majority are short summaries of the literature sustaining specific clinical case reports, while others restrict themselves to the basic science aspects of the disease. Most of the remaining approaches focus on specific locations – often simultaneously looking at both MALT and non-MALT lymphomas –, or review specific treatment modalities. On the other hand, clinical trials in MALT lymphoma (mostly phase II) often overcome the paucity of available subjects by including several different locations in the same series. Considering

these contradictory characteristics, it can be difficult to consolidate the information into one coherent interpretation of the literature that can translate into a change in clinical practice.

To overcome some of these gaps, we propose to undertake a transversal review of the literature, from the bench to the clinic, and from the rarest to the most frequent locations, to integrate the available data into an updated and informed approach to the patient.

METHODS

We performed a search in the database of the United States National Library of Medicine (PubMed.org) by Medical Subject Header (MeSH) terms for "*Lymphoma, B-Cell, Marginal Zone*", which includes the entry terms "MALT Lymphoma", "MALT Lymphomas", "Lymphoma, MALT", "Lymphomas, MALT", "Lymphoma of Mucosa-Associated Lymphoid Tissue" and "Lymphoma of Mucosa Associated Lymphoid Tissue", "Mucosa-Associated Lymphoid Tissue Lymphoma" and "Mucosa Associated Lymphoid Tissue Lymphoma", "Lymphoma, Mucosa-Associated Lymphoid Tissue" and "Lymphoma, Mucosa Associated Lymphoid Tissue", and "Marginal Zone B-Cell Lymphoma" and "Marginal Zone B Cell Lymphoma". Restrictions were applied to select only journal articles where the MeSH term is a Major Topic. Filters and limits were applied as needed to optimize the quality of the search, including the restriction of results to Human studies. Since the MeSH term only retrieves articles from 1992 onwards, earlier reports were found using the Boolean equation "*MALT AND lymphoma*". To expand our findings, Stanford University's HighWire database and Elsevier's Science Direct were also queried with the search terms "*MALT AND lymphoma*". Additional articles were retrieved using the "related articles" function of these databases, as well as cited references of relevance. Articles with no available abstracts were rejected; articles in English, French, Spanish and Portuguese were read as full-text, whenever possible; articles in other languages were only read as abstracts.

RESULTS

On the final literature search performed on PubMed.org, the MeSH term "*Lymphoma, B-Cell, Marginal Zone*" retrieved 3146 articles (Table 1), which were reduced to 2598 when 548 non-Major Topic manuscripts were excluded. A further 643 articles did not have an indexed abstract and, as such, were also excluded.

Table 1: Results of the PubMed.org MeSH search for *"Lymphoma, B-Cell, Marginal Zone"*

	Total	**2010 2012**	**2005 2009**	**2000 2004**	**1995 1999**	**1990 1994**
MeSH	3146					
* *MeSH minor* [1]	*-548*					
MeSH Major	2598	371	898	740	562	27
* *No abstract* [1]	*-643*	*-97*	*-215*	*-158*	*-165*	*-8*
Abstract available	1955	274	683	582	397	19
Review	281	43	78	101	55	4
Trial	87	14	38	24	11	0

The data includes articles that were indexed as of May 24th 2012. In this table, "trial" refers to the simultaneous activation of the PubMed limits "*clinical trial*" and "*randomized controlled trial*", while "review" includes both the limits "*review*" and "*systematic review*". [1]Articles where the MeSH term was used as minor topic and those without available abstracts were not surveyed.

The MALT lymphomas

Classification

The World Health Organization (WHO) (Table 2) recognized this nosologic entity in 1992, classifying extranodal marginal zone lymphomas of the MALT type as mature B-cell non-Hodgkin lymphoid neoplasm, according to their cell of origin, and histopathologic, cytogenetic and molecular characteristics. [5]

Marginal zone lymphomas, the third most common type of lymphoma in humans, are indolent, low-grade, small B-cell non-Hodgkin lymphomas (NHL) whose biological behavior is unique among indolent lymphomas. [6–9] Histopathologically, marginal zone lymphomas can be divided into the nodal marginal zone lymphoma (monocytoid B-cell lymphoma) subtype and the two extranodal subtypes – splenic marginal zone lymphoma and MALT-type marginal zone lymphoma. [8,9] Extranodal locations predominate over nodal locations, and the MALT subtype is the most frequent finding. [7]

Table 2: Hierarchical classification of non-Hodgkin lymphomas (including MALT lymphoma), according to the WHO classification of tumors of hematopoietic and lymphoid tissues.

WHO Classification	Mature B-cell neoplasms: The non-Hodgkin lymphomas
Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB or FGFR1	Chronic lymphocytic leukemia/small lymphocytic lymphoma
Myeloproliferative neoplasms	Splenic marginal zone lymphoma
Myelodysplastic/myeloproliferative neoplasms	Extranodal marginal zone lymphoma of MALT
Myelodysplastic syndromes	Nodal marginal zone lymphoma
Acute myeloid leukemia and related precursor neoplasms	Lymphoplasmacytic lymphoma and Waldenström macroglobulinemia
Acute leukemias of ambiguous lineage	Heavy chain diseases
Precursor lymphoid neoplasms	Follicular lymphoma
Mature B-cell neoplasms	Primary cutaneous follicle center lymphoma
Mature T-cell and NK-cell neoplasms	Mantle-cell lymphoma
Hodgkin lymphoma	Diffuse large B-cell lymphomas and variants
Histiocytic and dendritic cell neoplasms	Lymphomatoid granulomatosis
Post-transplant lymphoproliferative disorders	Plasmablastic lymphoma
	Burkitt lymphoma

The "mature B-cell neoplasms" category includes B-cell prolymphocytic leukemia, hairy cell leukemia and variants, the plasma-cell neoplasms, and the mature B-cell non-Hodgkin lymphomas, which are listed on the right-hand column. *PDGFRA*: platelet-derived growth factor receptor type α; *PDGFRB*: type β; *FGFR1*: fibroblast growth factor receptor 1. Adapted from Swerdlow, *et al.*, 2008 [5]

Mucosa-associated lymphoid tissue

Primary lymphoid tissue can be found in the thymus and bone marrow, where lymphocytes differentiate from progenitor cells into functional, mature lymphoid cells. [10] Secondary lymphoid tissue is present in the lymph nodes, in the spleen and in mucosa-associated lymphoid tissue (MALT) that develops in relation with mucosal barriers that are in contact with the outside environment (gastrointestinal, respiratory and genitourinary tracts), where antigens accumulate and are processed and presented to lymphocytes, as part of a normal adaptive immune response. [11] The ileum and the cecal appendix, which are under constant stimulation by pathogenic microorganisms, develop structures composed of well-delimitated primary and secondary B-cell follicles, separated by T-cell rich zones, which are similar to nodal and splenic lymphoid tissue (Peyer's patches).[11] In all of these locations, MALT is found in the stroma under the epithelium, with numerous lymphocytes and antigen-presenting cells. [11]

MALT, like the other components of the immune system, can give rise to a lymphoproliferative disease – the MALT lymphoma. The immune cell of origin of this malignant proliferation appears to be a marginal zone (post germinal centre) B-cell present both in lymph nodes and in extranodal tissue, related to plasma cells. This hypothesis is supported by the findings that up to 30% of patients with MALT lymphoma have plasmacytic differentiation, and 30 to 40% have a detectable monoclonal immunoglobulin (Ig) pattern of identical subtype to the lymphoma cell surface-immunoglobulin. [12–14] Overt monoclonal gammopathy has been associated with bone marrow involvement, a marker of advanced-stage disease. [14]

Etiology

Despite their association with mucosa-associated lymphoid tissue, MALT lymphomas rarely arise in native physiologic MALT; rather, the majority of cases develop on extranodal acquired MALT infiltrates induced by an immune response to a chronic antigenic stimulus. [3,6] In fact, MALT lymphomas have been described in a causal association with several conditions that induce the development of acquired MALT and subsequent lymphomagenesis.

The best studied associations are with chronic infections, such as between cutaneous MALT and *Borrelia burgdorferi*, between *Chlamydophila psittaci* and ocular adnexal MALT, and between *Campylobacter jejuni* and immunoproliferative small intestinal disease (IPSID), with the highest levels of evidence being found for gastric MALT and *H. pylori* gastroduodenitis [3,6,15]. On the other hand, in most other locations, the underlying etiology remains cryptogenic.

Ethiopathogenesis

Chronic antigenic stimulation and the microenvironment

These etiologic associations have led to the hypothesis that chronic or repeated immune stimulation leads to a lymphoid expansion which, in the presence of environmental and microenvironmental factors and a genetic predisposition, can culminate in the emergence of a malignant clone. The mechanisms underlying the antigen-dependence of MALT lymphomas, and the impact of the inflammatory microenvironment, have gradually been elucidated over the three decades that have elapsed since the first description of this entity, with tumor progression now known to be driven by an interaction between B-cell receptor (BCR)-derived signals and T-helper (T_h) cell signals (Figure 1). [16]

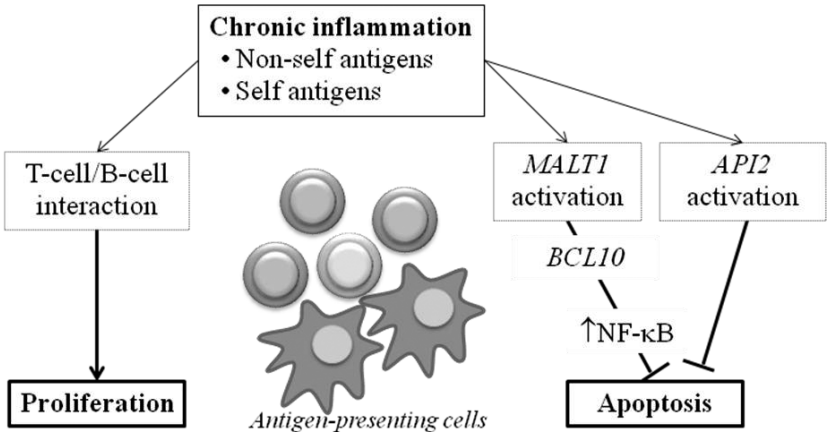

Figure 1: The role of a chronic inflammatory response on the pathogenesis of MALT lymphoma. Inflammation induced by self or non-self antigens leads to T-cell/B-cell/antigen-presenting cell interactions that underlie lymphoid proliferation. Mucosa-associated lymphoid tissue translocation protein 1 (MALT1) and apoptosis inhibitor 2 (API2) transcription is activated, culminating in apoptosis inhibition. Clonal selective pressures can result in the proliferation of monoclonal B-cells. *Non-self antigens* include chronic bacterial infections; *self-antigens* include auto-immune diseases. Adapted from Seto, 2004. [17]

MALT lymphoma BCR often have (Ig)V(H)-CDR3 homology to rheumatoid factors (RF), reflecting a selection to auto-IgG, which appears to be a specific characteristic of these lymphomas, not found in diffuse large B-cell (DLBC), follicular, Burkitt or mantle-cell lymphomas, chronic lymphocytic leukemia or multiple myeloma. [18,19]

It has been demonstrated that the lymphoma B-cells exhibit polyreactive surface BCR immunoglobulins, and that direct stimulation by the allo-antigens and auto-antigens recognized by these surface antibodies leads to the proliferation of the tumor cells; after this oligoclonal expansion, a dominant lymphoma clone can surface through selective pressure. [16,20–22] In fact, some series show that most MALT lymphomas express monoclonal antibodies, with the tumors evidencing intraclonal variation and positive and negative clonal selective pressure. [21] BCR polyreactivity has been shown to include simultaneous intermediate affinity to self-antigens (including stomach extract, DNA and IgG) and foreign antigens (including Helicobacter sonicate). [21] These findings, however, are not consensual, with some authors

suggesting that most MALT lymphoma antibodies are, in fact, monoreactive and of high-affinity, with polyreactivity being exclusive of tumors with t(11;18). [18]

This association between MALT lymphomas, the BCR and B-cell humoral immunity is reflected in the incidence of hypergammaglobulinemia in these tumors, including rare cases of secondary Waldenström's macroglobulinemia. [14,23,24]

It has also been shown that MALT lymphomas are infiltrated by type 2 T_h (T_h2)-polarized T-cells and that tumor proliferation is enhanced by intratumoral $CD4^+$ T-cells, while their depletion blocks tumor growth. [16] On the other hand, a large proportion of these $CD4^+$ T-cells are suppressive $CD25^+$ $FOXP3^+$ regulatory T-cells (T_{regs}) which are themselves recruited by tumor B-cells (through the secretion of chemokine (C-C motif) ligand 17 (CCL17) and CCL22); the depletion of $CD25^+$ cells is equally effective in blocking tumor growth. [16]

As is the case with other malignancies, the vascular microenvironment also seems to play an important role in MALT lymphomagenesis. In a mouse model of gastric MALT lymphoma, an extensive microvascular network is observed; vascular endothelium growth factor (VEGF)-C mediates tumor expansion, while anti-VEGF antibodies have a suppressive effect on tumor growth. [25]

Bacteria-induced lymphomagenesis

Helicobacter pylori

H. pylori infection, generally acquired in childhood, is a major cause of gastroduodenal disease, including chronic autoimmune gastritis, benign peptic ulcers, gastric carcinoma and gastric MALT lymphoma. [22,26,27] Nevertheless, only a very small proportion of *H. pylori*-infected subjects develop these complications, including MALT lymphomas. [26] In fact, in a population from Karachi, Pakistan, where an incidence of *H. pylori* infection of nearly 62% has been described, only 24 cases of gastric MALT lymphoma were observed out of

approximately 70 000 gastroscopies performed over a period of 18 years, of which only 54.2% were positive for *H. pylori*. [28,29] Likewise, the incidence of *H. pylori* infection in Portugal has been estimated at 50 to 90% (J.M. Romãozinho, personal oral communication, Sociedade Portuguesa de Endoscopia Digestiva), representing 5 to 9 million subjects at risk, only a small fraction of whom will be diagnosed with gastric MALT lymphoma, as described ahead.

The outcome of the infection depends on the host immune response mounted against *H. pylori*, especially the functionality of cytotoxic effector T-cells. [26] This has been demonstrated for chronic atrophic autoimmune gastritis, which is due to the infiltration and destruction of the gastric mucosa by cytotoxic T-cells specific for *H. pylori* epitopes that cross-react with the gastric proton-pump (H^+/K^+ ATPase). [26]

Arguments supporting the role of H. pylori

Chronic infection with *H. pylori* is significantly associated with the induction of gastric lymphoid follicles, representing the proposed first step in MALT lymphomagenesis of lymphoid expansion (Figure 1). [28]

In addition, *H. pylori* infection can be demonstrated serologically in most patients, and the bacterium can be histologically identified in the gastric mucosa of the majority of gastric MALT lymphomas, with some series describing incidences as high as 92%. [6,30,31] At the same time, the density and detectability of *H. pylori* decrease as the histology progresses from chronic gastritis to gastric MALT lymphoma. [32] These data suggest that bacterial colonization is important for early lymphomagenesis, but becomes less relevant as the disease progresses; in fact, a monoclonal B-cell clone can be identified in chronic gastritis, before the development of clinical lymphoma. [32]

H. pylori eradication through specific antibiotherapy (classic triple therapy with amoxicillin, clarithromycin and omeprazol, or one of its variations) leads to lymphoma regression in 75% of cases, in a few weeks to 18 months. [6] The odds of success associate with the clinical stage, being very high for early-stage lymphomas, lower for more advanced stages and practically nil once the serosa is breached. These observations also support the hypothesis that *H. pylori*-independence is a feature of lymphoma progression, associated with the acquisition of additional genetic alterations. [6] This aspect parallels the finding in gastric carcinoma (which also associates with *H. pylori* infection) that the absence of active infection by *H. pylori* is a significant adverse prognostic factor, with one series finding a decrease in 10-year overall survival (OS) in locally advanced disease, from 71.1% in *H. pylori*-positive patients to 21.3% in *H. pylori*-negativity. [33]

In vivo data has also shown that the experimental infection of BALB/c mice with Helicobacter *spp.* is able to reproduce most pathophysiological changes that take place during the early stages of MALT lymphomagenesis. [16]

The relationship between chronic infection with *H. pylori*, microenvironment and lymphomagenesis has been further elucidated *in vitro* by the fact that the tumor cells only proliferate in response to strain-specific *H. pylori* cell preparations when in the presence of tumor-infiltrating T-cells; on the other hand, the latter expand in response to *H. pylori* stimulation even when isolated from the tumor microenvironment. [34] The eradication of *H. pylori*, eliminating the stimulus to the T-cells expansion that sustains tumor-growth, will lead to tumor regression, as previously described. [34] The central role that tumor microenvironment T-cells play in MALT lymphomagenesis means that the modification or modulation of local T-cell immunity could be an attractive therapeutic approach. [35]

It has been suggested that lymphomagenesis and genetic aberrations are also facilitated by DNA-damage caused by reactive oxygen species produced by neutrophils as part of the immune response to an infection by *H. pylori* strains positive for the virulence factor cytotoxin-associated gene A (CagA). [6] In fact, CagA-positive strains associate with higher grades of mucosal inflammation, severe atrophic gastritis and gastric carcinogenesis, and activate the phosphoinositide 3-kinase (PI3K)/AKT pathway, an anti-apoptotic, pro-proliferative survival pathway, contrary to CagA-negative strains. [36,37]

Other H. pylori associations

A case-control study comparing *H. pylori* gastric infection rates in patients with extragastric ocular adnexal lymphoma and extragastric extra-ocular lymphoma at diagnosis found that the former had a significantly higher rate of infection (45% *vs* 25% *vs* 12% in normal controls), suggesting the possibility of an indirect mechanism connecting gastric antigenic stimulation and ectopic ocular lymphomagenesis. [38] Similarly, there are descriptions of rectal MALT lymphomas regressing after gastric *H. pylori* eradication therapy. [39] The extragastric immune-stimulating consequences of a chronically inflamed gastric mucosa due to *H. pylori* infection, and of self-*H. pylori* antigen cross-reactivity, are reflected in other known or putative associations, such as immune thrombocytopenia, primary biliary cirrhosis and celiac disease, all of which have been noted to improve with *H. pylori* eradication. [40,41]

Campylobacter jejuni

Immunoproliferative small intestinal disease (IPSID), also known as α-heavy chain disease due to the presence of pathologic plasma immunoglobulin molecules corresponding to

truncated α heavy chains, without the first constant domain or light chains, is a variant of MALT lymphoma. [42,43]

Based on published descriptions of regression of early-stage IPSID with antibiotic therapy, an association was proposed between this disorder and an unknown infectious agent, which was suggested to be *Campylobacter jejuni*, after the identification of this bacterium in biopsy samples of IPSID patients who respond to antibiotherapy. On the other hand, IPSID regression after antibiotic eradication of *Helicobacter pylori* (classed as *Campylobacter pylori* until the creation of the Helicobacter genus by Goodwin and colleagues in 1989 [44]) has also been described. [6,45,46] Nevertheless, the association between IPSID and *C. jejuni* is now included in the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissue, and is reflected in clinical practice by good responsiveness of early-stage disease to treatment with tetracycline. [5,43,47]

Chlamydophila (Chlamydia) psittaci

There is an association between MALT lymphomas of the ocular adnexa and ocular infection by *Chlamydophila psittaci*, which underlies a pathogenic model of antigen-driven lymphoproliferation similar to the one proposed for *H. pylori*. *C. psittaci* and other bacteria belonging to the Philus Chlamydiae are obligatory intracellular parasites with the ability to establish prolonged infections, and with mytogenic activity, inducing a policlonal cellular proliferation and resistance to apoptosis; it has been suggested that these characteristics can partially explain their tumorigenic action. [15] However, there appears to be a geographic variability in this association, which has led some authors to propose an interaction of additional ethiopathogenic agents. [48]

Other bacteria

The association between infection with *Borrelia burgdorferi*, the etiologic agent of Lyme disease, and cutaneous MALT lymphomas remains controversial. [49] While reports from Italy, Germany and Scotland support an association and relate lymphoma regression with antibiotherapy, results from the United States of America (USA) and from Asia suggest an absence of causality. [49] Taken together, these findings could point at a geographic effect. However, in a large series containing samples from the three continents, none of the cases were positive for the *B. burgdorferi* gene *hbb* using the polymerase chain reaction (PCR) technique. [49]

Furthermore, in the absence of a successful *H. pylori* eradication, there have been isolated case-reports of MALT regression after antibiotherapy. These include a large rectal MALT lymphoma with complete remission after three 10-day cycles of levofloxacin, despite persisting gastric *H. pylori* positivity, suggesting that rectal MALT lymphoma could be associated with chronic infection by another bacterium, as-yet unidentified. [39]

Additionally, in a series of 17 small-cell gastric lymphoma specimens, it was found that 53% of patients presented with non-*H. pylori* gastric flora (isolated or co-existing with *H. pylori*), giving rise to the possibility that *H. pylori* infection might not be the only etiologic agent behind the chronic antigenic stimulation driving lymphomagenesis. [50]

Autoimmune disease

MALT lymphoma is frequently associated with chronic immune stimulation in connective tissue disease (CTD) and other autoimmune diseases, which are characterized by a deregulated expression of the NF-κB pathway. [51,52]

As is the case with bacteria-induced lymphomas, different autoimmune disorders associate preferentially with distinct tissues of origin of the lymphoma. Sjögren's syndrome presents with an increased risk of MALT lymphoma of the parotid and other salivary glands, thymus and stomach, with descriptions of other rarer locations, such as the larynx or the lung. [53–55] In the salivary glands, which have no physiologic lymphoid tissue, the development of MALT lymphoma is preceded by lymphoepithelial (myoepithelial) sialadenitis, a Peyer patch-like accumulation of lymphoid tissue – containing germinal center, mantle and marginal zone – around salivary ducts, with intra-epithelial B-cells, which represents acquired MALT. [6] Hashimoto's thyroiditis also presents with acquired MALT and, predictably, carries an increased risk of thyroid MALT lymphoma. [6,56] It has also been described that over 80% of patients with MALT lymphoma of the thymus have a coexisting autoimmune disease or policlonal hypergammaglobulinemia, with persistence of the autoantibodies and serum immunoglobulins after thymectomy. [23]

Other cases have been described, such as the association between systemic lupus erithematosus and MALT lymphoma of the lacrimal gland, or between ocular adnexal lymphomas and IgG4-related systemic disease, a recently described fibroinflammatory disorder characterized by fibrosis and a lymphoplasmacytic infiltrate rich in IgG4-positive plasma cells. [57–59]

The genetics of MALT lymphoma

Lymphomas present with several genetic molecular aberrations, including translocations, mutations, gene amplifications and deletions of genes (including tumor suppressors), some of which have been shown to have diagnostic and prognostic value. [60] These molecular changes can be detected by conventional cytogenetics, fluorescence *in situ* hybridization (FISH), comparative genomic hybridization (CGH) arrays, DNA microarrays of gene expression

profiles and immunohistochemistry or flow cytometry for altered protein expression (including fusion proteins). [60]

Cytogenetics

Non-random chromosomal translocations are a feature of several neoplastic conditions, including hemato-oncologic diseases, with characteristic translocations (involving a limited group of genes) in both lymphoproliferative and myeloproliferative disorders (Table 3) [61]. In MALT lymphomas, likewise, characteristic cytogenetic alterations have been described, converging on the same intracellular pathways. [62,63]

Genes and signaling pathways involved

Immunoglobulin chain genes

The immunoglobulin heavy chain gene (*IGH*, located in chromosome 14q32) is frequently involved in translocations in MALT lymphomas and other lymphoproliferative diseases, as a consequence of the chronic antigenic stimulation which underlies the ethiopathogenesis of these neoplasms and the central role played by the BCR in lymphomagenesis. [6] The *kappa* light chain (*IGK*) and *lambda* light chain (*IGL*) genes can likewise be involved in lymphoid malignancies, through the same mechanism. In fact, B-lymphoid cells, as part of their normal immune response, undergo rearrangements of the Ig genes as part of somatic hypermutation and class-switch recombination. [64] These directed mutations originate a localized genetic instability which can lead to aberrant rearrangements, with the translocated juxtaposition of oncogenes to IGH enhancers. [64] The continued enhancer activation as a normal response to immune stimulation will, in turn, result in the overexpression of the activated oncogene, with inflammation driving oncogenesis.

Table 3: Cytogenetic aberrations with a significant association to lymphoproliferative and myeloproliferative malignancies.

Location	Gene	Genetics alterations	Involved Genes	Malignancy
2p12	*IGK*	t(2;8)(p12;q32)	*IGK-cMYC*	
8q32	*cMYC*	t(8;14)(q24;q32)	*IGH-cMYC*	Burkitt lymphoma
8q24	*cMYC*	t(8;22)(q24;q11)	*IGL-cMYC*	
14q32	*IGH*	t(14;19)(q32;p13)	*IGH-BCL3*	
22q11	*IGL*	t(5;14)(q31;q32)	*IL3-IGH*	B-CLL/SLL
5q31	*IL3*	t(7;14)(q21;q32)	*IGH-DK6*	
7q21	*DK6*	t(14;16)(q32;q23)	*IGH-MAF*	
16q23	*MAF*	t(4;14)(p16.3;q32)	*FGFR3-IGH*	Multiple myeloma
4p16.3	*FGFR3*	t(11;14)(q13;q32)	*CCND1-IGH*	
11q13	*CCND1*	t(v;3q27)	*BCL6*	DLBCL
3q27	*BCL6*	t(14;15)(q32;q11-13)	*IGH-BCL8*	
15q11-13	*BCL8*	t(14;18)(q32;q21)	*IGH-BCL2*	Follicular lymphoma
18q21	*BCL2*	t(3;14)(q27;q32)	*BCL6-IGH*	
9p13	*PAX5*	t(9;14)(p13;q32)	*PAX5-IGH*	Lymphoplasmacytic
11q21	*API2*	t(11;18)(q21;q21)	*API2-MALT1*	
18q21	*MALT1*	t(14;18)(q32;q21)	*IGH-MALT*	MALT lymphoma
1p22	*BCL10*	t(1;14)(p22;q32)	*BCL10-IGH*	
3p14	*FOXPIF*	t(3;14)(p14;q32)	*FOXPIF-IGH*	
9q34	*BCR*	t(11;14)(q13;q32)	*CCNDI-IGH*	MCL
22q11	*ABL*	t(9;22)(q34;q11)	*BCR-ABL*	CML

CLL: chronic lymphocytic leukemia; SLL: small lymphocytic lymphoma; DLBCL: diffuse large B-cell lymphoma; MCL: mantle-cell lymphoma; CML: chronic myeloid leukemia. Adapted from Najfeld, *et al.*, 2008, Swerdlow, *et al.*, 2008, and Kaushansky, *et al.*, 2010. [5,8,10]

Pathways converging on NF-κB

Normal lymphocyte function depends on the strict regulation of the transcriptional activity of NF-κB, and the deregulation of this signaling pathway is a contributor to lymphomagenesis. [65]

Nuclear factor kappa B (NF-κB) is a primary transcription factor normally sequestered in the cytoplasm by the inhibitor of κB (IκB) family proteins, which maintain it in an inactive form by the blockade of its nuclear localization signal. [66] As part of the innate immune response, NF-κB is a point of convergence of various pathways that originate on surface receptors such as the BCR, the receptor activator of nuclear factor κB (RANK), the tumor necrosis factor receptors (TNFR) and the toll-like receptors (TLR), in response to various stimuli (including bacterial endotoxins, viral replication, oxidative stress, lipid oxidation and ionizing radiation), leading to inducible modifications of gene expression. [66,67]

The set of genes whose transcription is modulated by NF-κB include cytokines, adhesion molecules, antiapoptotic factors and other transcription factors, which together modify the immune response, and other signaling pathways of cell survival, proliferation and apoptosis. [66,67] Deregulation of the pathways converging on NF-κB can thus lead to cellular immortalization and is frequent in immune, autoimmune and oncologic diseases, including MALT lymphoma, where their activation is fundamental for the continued development of the lymphoma after achieving *H. pylori*-independence. [3,52]

MALT1. Mucosa-associated lymphoid tissue translocation protein 1 (MALT1), is a protein with protease activity, encoded by the gene of the same name located in 18q21, which oligomerizes with the B-cell chronic lymphocytic leukemia/lymphoma protein 10 (BCL10), activating the IκB kinase (IκK). This kinase phosphorilates IκB, which dissociates from NF-κB, becoming a target to poliubiquitination and proteasome degradation, with exposure of the

nuclear localization signal and, consequently, activation of NF-κB. [6,66,68] This function of MALT1 as NF-κB activator has been described not only for B-lymphocyte BCR signaling, but also for receptors from other immune and even non-immune cells. [68]

In the absence of oligomerization, wild-type MALT1 is incapable of activating NF-κB, even if it is overexpressed. [6] However, when MALT1 undergoes oligomerization in the absence of BCL10, it acquires the NF-κB-activating ability of the hetero-oligomer; MALT1 activity and NF-κB activation are thus dissociated from upstream signaling originating in the surface BCR and, consequently, from antigenic stimulation. [6]

BCL10. BCL10 protein, encoded by the gene of the same name located in chromosome 1p22, and expressed in the cytoplasm of normal lymphoid tissue, links antigen receptor signaling to the NF-κB pathway, through its interaction with MALT1, as described above. [6,69] Antigen receptor stimulation recruits caspase recruitment domain-containing protein 11 (CARD11), a member of the membrane-associated guanylate kinase family, to the antigen-receptor complex. [6] BCL10 is then recruited, oligomerizes through CARD-CARD interactions, and induces MALT1 oligomerization and the canonical activation of NF-κB.

BCL10 acquires the ability to constitutively activate NF-κB, independently of antigenic stimulation, when it is overexpressed, such as when it is brought under the control of hyperactive promoter or enhancer regions, through chromosomal translocations. [6,70]

Nevertheless, these alterations, in isolation, are not sufficient for MALT lymphomagenesis, and the interaction with other immune, genetic and environmental factors is probably necessary for continued tumor growth. [62]

Recurrent translocations

Rearrangements of four of the genes described above – *MALT1*, *BCL10*, *IGH* and *IGK* – as well as *API2* and *FOXP1*, result from the 5 recurrent translocations that have been described in the literature for MALT lymphoma (Table 4).

Table 4: Recurrent chromosomal translocations described in MALT lymphomas.

Translocation	Fusion protein	Frequency
t(11;18)(q21;q21)	API2-MALT1	13-35%
t(1;14)(p22;q32)	BCL10-IGH	<4%
t(1;2)(p22;p12)	BCL10-IGK	
t(14;18)(q32;q21)	IGH-MALT1	0-20%
t(3;14)(p14;q32)	FOXP1-IGH	0-50%

Adapted from Isaacson, 2005; Tibiletti, *et al.*, 2007; Konoplev, *et al.*, 2010; Jaffe, *et al.*, 2008; Streubel, *et al.*, 2003; Streubel, *et al.*, 20035 Sanchez-Izquierdo, *et al.*, 2003 [6,7,20,71–74]

The three most common and characteristic translocations - t(11;18)(q21;q21), t(1;14)(p22;q32) and t(14;18)(q32;q21) – are present with variable frequency depending on the tissue of origin of the lymphoma. [6] They originate oncogenic fusion proteins that activate the NF-κB pathway, and lymphomas with these translocations present with an overexpression of NF-κB target genes, such as toll-like receptor (*TLR*) 6, chemokine (C-C motif) receptor 2 (*CCR2*), cluster of differentiation 69 (*CD69*) and *BCL2*. [62,75] On the other hand, lymphomas without these translocations have increased immune and inflammatory response markers such as interleukin 8 (IL-8), CD28, CD86 and inducible T-cell costimulator (ICOS). [75] The fact that the same oncogenic pathway is involved as a consequence of the various cytogenetic

alterations associated with MALT lymphoma, underlies the morphologic similarities between different tumors. [6]

t(11;18)(q21;q21)

This translocation results in the chimeric fusion of the apoptosis inhibitor 2 (*API2*) and *MALT1* genes; *API2-MALT1*, located in the derivative chromosome 11 and under the control of the *API2* promoter, is read in-frame, originating a transcript that codes a functional fusion protein with the ability of MALT1 to activate NF-κB. [6] Wildtype API2, encoded by the gene located on chromosome 11q21, cannot activate NF-κB directly; however, its promoter is stimulated by NF-κB. [6,70] Therefore, this fusion gene results in a positive feedback cycle whereby API2-MALT1 activates NF-κB which, in turn, stimulates the transcription of the former. While normally both MALT1 and API2 levels are strictly regulated in the cell, with a quick turnover, the chimeric protein is very stable and persists, unregulated, constitutively activating NF-κB. [66] Thus, this translocation has oncogenic properties and is an important driver of MALT lymphomagenesis. [6]

The API2-MALT1 fusion oncoprotein also leads to the proteolytic cleavage of the NF-κB inducing kinase (NIK), originating an N-terminal NIK fragment with kinase activity but resistance to degradation by the ubiquitin-proteasome system (UPS). NIK activates NF-κB through an alternative non-canonical pathway, resulting in constitutive NF-κB signaling, with increased B-cell adhesion and resistance to apoptosis. [65,66] The presence of the t(11;18) immortalizes the cell and releases it from BCR-antigen-dependence for its NF-κB activation and survival, a hypothesis that is supported by the finding that t(11;18) and BCR RF-homology are mutually exclusive. [19] These facts also agree with the clinical observations that the presence of t(11;18) correlates with resistance to a successful eradication of *H. pylori*, and that patients who respond to eradication therapy are generally negative for the fusion

transcript, suggesting that the latter tumors need chronic stimulation of their RF BCR by IgG in antigen-antibody complexes to survive. [6,19,75,76] The NF-κB-activating translocations t(1;14) and t(14;18) have also been noted to be associated with bacterial eradication-resistance. [75]

When present, t(11;18) is usually the only cytogenetic abnormality identified, while t(11;18)-negative lymphomas often present with multiple chromosomal abnormalities. [6]

This is the most common structural chromosomal abnormality described in MALT lymphomas, although its frequency varies with the tissue of origin, being particularly common in gastric MALT (with reports of frequencies ranging from 13-35% of cases) and pulmonary lymphomas. [20,71]

It has a very high specificity for MALT lymphomas, being the most specific translocation in these neoplasms. In fact, it appears to be exclusive or nearly-exclusive to this subtype of marginal zone lymphomas and of high diagnostic value, being absent from nodal and splenic lymphomas. These observations support their separation as distinct biological entities, as well as from transformed diffuse large B-cell lymphomas (DLBCL), primary DLBCL or T-cell lymphomas. [8,20,76,77] In a series of 93 marginal zone lymphomas, 31 of which were of non-MALT type and one which was unclassifiable, the t(11;18) was identified in 13%, corresponding to 11 gastric MALT lymphomas and the unclassifiable case. [77]

In primary gastrointestinal MALT lymphomas without high-grade transformation, API2-MALT1 transcript could be identified by reverse transcriptase polymerase chain reaction (RT-PCR) in 14% of Japanese patients, and by FISH in 22.7% of East Chinese patients, compared to 0% of those with areas of high-grade (DLBC) transformation, in both series. [76,78] On the other hand, though this translocation is rarely present in transformed MALT lymphomas (with some authors considering it exclusive of low-grade cases), it is associated with advanced

stages and, in this series, was more frequent in cases with submucosal involvement (31%), compared to lymphomas restricted to the mucosa (0%) [6,7,76]. The translocation also associated with a colonic location, where it was present in 67% of patients, compared to 8% of patients with gastric MALT lymphomas [76].

The translocation is absent from non-complicated *H. pylori*-positive gastritis but often present in gastric MALT lymphoma patients infected with CagA-positive *H. pylori*, although in the Nakamura series it was more frequent in *H. pylori*-negative patients (3 of 6), compared with *H. pylori*-positive (1 of 21). [6,76]

t(14;18)(q32;q21)

This translocation results in the fusion of the *IGH* gene with *MALT1*, resulting in the overexpression of MALT1, which oligomerizes and activates NF-κB. The overexpression of MALT1 also stabilizes BCL10 in the cytoplasm, increasing its expression. [6,7]

The incidence of t(14;18) can reach 80% of follicular lymphomas and up to 30% of DLCBL, where the breakpoint on chromosome 18 involves not *MALT1*, but the *BCL2* gene. [74] In MALT lymphomas, its incidence is different in gastric tumors (where it is virtually absent) and extragastric locations, with some series relating its presence in 100% of hepatic, 40% of ocular adnexal, 30% of skin and 20% of salivary gland tumors. [7,72,74]. When present, this translocation is frequently associated with other chromosomal abnormalities, such as trisomy 3, 8 and 12.

t(1;14)(p22;q32)

This translocation results in the juxtaposition of *BCL10* with the *IGH* gene enhancer region, with a resulting overexpression of BCL10 and activation of NF-κB. [70] The translocation t(1;2)(p22;p12) is a variant where *BCL10* is juxtaposed to the immunoglobulin *kappa* light chain (*IGK*) gene, resulting in an identical overexpression of BCL10. Together, the two

variants, though characteristic of MALT lymphomas, are found in under 4% of described cases and associate frequently with other cytogenetic aberrations, such as trisomy 3. [6]

t(3;14)(p14;q32)

This translocation apposes the forkhead protein box subfamily P protein 1 gene (*FOXP1*) with the *IGH* enhancer, resulting in its overexpression. [73] The FOX family proteins have been shown to be involved in signal transduction that mediates proliferation, differentiation and the immune response. [79] Though its precise mechanism of action remains to be described, in MALT lymphoma, as in DLBCL, FOXP1 overexpression has been described as an adverse prognostic factor. [73,79–81]

Like t(14;18), its frequency is vary variable among different anatomical locations, with the original series describing an incidence raging from 0% in gastric, lung and salivary gland tumors, to 10% of cutaneous locations, 20% of ocular adnexal masses and even 50% of thyroid samples. [73]

Other somatic alterations

Apart from the characteristic translocations, several other somatic genetic alterations can be identified in MALT lymphomas. In fact, t(11;18)-negative gastric MALT lymphomas frequently have trisomies (including chromosomes 3, 12 and 18) and allelic imbalances. [6] In a series of 218 patients with marginal zone lymphomas, MALT lymphomas were found to be more frequently associated with gains in 3p, 6p, 18p, and del(6q23) than the splenic and nodal types. [82] Frequent allelic imbalances include *p53* mutations or loss of heterozygosity, *p16* deletions and *MALT1* amplifications. [6] In a series of ocular adnexal MALT lymphomas, the most common genetic alterations identified by FISH were *MALT1* amplification (36.8% presented with three copies of *MALT1*) and *BCL6* amplification (29.8% had three copies), with 21.1% showing three copies of both genes, suggesting that these genes play an important

role in lymphomagenesis. [83] Tumor necrosis factor α-induced protein 3 (TNFAIP3, also known as A20), a global inhibitor of NFκB, is frequently inactivated through mutations or deletions in MALT lymphomas without the recurrent translocations. [62]

The specific frequencies of each genetic aberration vary in the literature; however, reports address distinct lymphoma locations and stages, use differing methodologies and focus on series from separate geographical locations. Nevertheless, it has been suggested that these geographical differences reflect a true heterogeneity in the distribution of genetic aberrations, and not just different sampling methods. [83] In fact, differences can be found even within the same country. In a series of 217 MALT lymphomas from North China, 21% had chromosomal translocations by FISH – 13% with t(11;18), 1% with t(1;14) and 1% with t(14;18), 2% with translocations involving *BCL6* and an unknown partner gene, and 4% with translocations between *IGH* and an unknown partner. [63] While t(11;18) was detected in several tissues, t(1;14) was only detected in the lung (12%) and stomach (1%), t(14;18) in the lung (6%) and ocular adnexa (2%) and *BCL6* translocations in the salivary glands (17%) and stomach (4%). [63] In another series of 57 ocular adnexal lymphomas from South China, 15.8% of lymphomas had chromosomal translocations by FISH; 7% had t(11;18), 1.8% had t(14;18), 1.8% had translocations involving *BCL-6* and 5.3% had translocations between the *IGH* gene and unidentified genes. [83] In a sample of 196 MALT lymphomas from East China, the frequency of API2-MALT1 by FISH was significantly different in distinct sites, ranging from 0% in skin and thyroid lymphomas, to 45% of MALT lymphomas with a pulmonary location, and including 12.8% of gastric lymphomas (Table 5), leading the authors to propose that the different locations are probably a reflection of distinct processes of lymphomagenesis. [78]

Table 5: API2-MALT1 transcript frequency in MALT lymphomas according to anatomical location, in patient series from different geographical areas.

Anatomical site	API2-MALT1 frequency (%)	Geographical location
Lung (n=20)	45.0%	East China [78]
Lung (n=17)	47.0%	North China [63]
Stomach (n=53)	12.8%	East China [78]
No DLCB component (n=44)	*22.7%*	East China [78]
DLBC component (n=9)	*0.0%*	East China [78]
Stomach (n=84)	14.0%	North China [63]
Salivary gland (n=20)	15.0%	East China [78]
Salivary gland (n=6)	17.0%	North China [63]
Intestinal tract (n=17)	11.8%	East China [78]
Small intestine (n=14)	29.0%	North China [63]
Ocular adnexa (n=50)	2.0%	East China [78]
Ocular adnexa (n=68)	6.0%	North China [63]
Ocular adnexa (n=57)	7.0%	South China [83]
Skin (n=17)	0.0%	East China [78]
Liver (n=8)	0.0%	East China [78]
Thyroid (n=5)	0.0%	East China [78]
Other sites (n=12)	0.0%	East China [78]

Adapted from Zhang, *et al.*, 2010 (patients from Liuzhou), Li, *et al.*, 2008 (patients from Shanghai) and Dong, *et al.*, 2009 (patients from Beijing).

Epigenetic modifications

It has been demonstrated that promoter hypermethylation of miR-203 in gastric MALT lymphoma downregulates the expression of this tumor suppressor miRNA, compared to adjacent normal tissue, with overexpression of its target Ableson kinase 1 (ABL1). The re-expression of miR-203 by demethylating agents or transfection, as well as the downregulation

of ABL1 by ABL tyrosine kinase inhibitors, are able to block MALT lymphoma growth and induce its regression. [84]

Epidemiology

MALT lymphoma represents 7% of newly-diagnosed lymphomas. [13] It is a rare malignancy, with a worldwide incidence estimated at 1 to 1.5 cases per 100 000, per year, which leads to an *a priori* mathematical prediction of 100 to 150 new cases in Portugal every year. [5] There are no systematic epidemiologic studies on the Portuguese population; however, it is known that in the 18 years between the WHO description of MALT lymphoma in 1992 and 2010, 44 cases of MALT lymphoma were treated in a University Hospital in Central Portugal (Hospitais da Universidade de Coimbra), with a direct area of influence comprising 3.3×10^5 inhabitants, and an area of influence and referral of 24×10^5 inhabitants (M.I. Pereira, personal communication [85]). Considering the estimated population of Europe of approximately 740 million subjects (United Nations, online data), about 7 000 to 10 000 new cases will develop in the European continent every year.

MALT lymphomas can affect practically all organs and systems; different anatomical locations have a large geographic variability, which has been partially attributed to a distinct epidemiological risk factor distribution. [86] Consequently, the incidence of the rarer subtypes (representing less than 1% of all cases) can be as low as 1 case per 10 million subjects, per year, which invalidates the accrual of adequate patient series for basic and clinical investigation. Therefore, while these rare anatomical locations can putatively have ethiopathogenic associations as strong as the one between *H. pylori* infection and gastric lymphoma, they remain undetected due to the lack of large epidemiologic case-control studies.

As with other indolent lymphomas, incidence increases with age, with the majority of patients being over 50 years old, and a median of 61. [5] In our Portuguese series, the average age was 58.5±15.1 years (ranging from 16.7 and 85.1), with a median of 59.7 and a modal class of 60 to 64. [85] Although some series suggest that the disease could be 2 to 3 times more frequent in males than in females, the worldwide data, as reported by WHO, indicates a female preponderance of 1.2 cases for each male patient. [29]

Anatomical location

Although human MALT lymphomas can be characterized by multiple locations, with clinical reports of cases developing in practically every organ, the digestive tract is the most frequently involved system, with the gastric MALT lymphoma accounting for the majority of cases, as mentioned. [6,7,87]

Considering the data from the Lyon series, and the estimated incidence of 10 000 new cases per year in Europe, there could be up to 3 500 new gastric lymphomas and 1 000 cases each of ocular adnexal, lung, head and neck, skin, and non-gastric gastrointestinal tumors, per year, in the European continent, with all other locations affecting less than 500 patients. Colo-rectal cancer, in comparison, with an incidence of 40 to 50 cases per 10^5 per year (United States Centers for Disease Control, online data), would affect 200 000 to 300 000 new patients in Europe every year.

Table 6: Most frequent anatomical locations for MALT lymphomas, comparing one large series from Lyon and a single-center series from Central Portugal

<table>
<tr><th colspan="6">Hospitais da Universidade de Coimbra, Portugal (n=44)
[1992-2010]</th><th colspan="2">Lyon (n=158)
[1987-1999]</th></tr>
<tr><td rowspan="6">Digestive</td><td rowspan="6">59.1%</td><td rowspan="4">Gastrintestinal tract</td><td rowspan="4">52.3 %</td><td colspan="2">Stomach</td><td>36.3%</td><td>33%</td><td rowspan="4">41.5%</td></tr>
<tr><td rowspan="3">Intestinal tract (16%)</td><td>Large intestine</td><td>11.4%</td><td rowspan="3">8.5%</td></tr>
<tr><td>Duodenum</td><td>2.3%</td></tr>
<tr><td>Cecal appendix</td><td>2.3%</td></tr>
<tr><td colspan="3">Gallbladder</td><td>2.3%</td><td>0%</td></tr>
<tr><td rowspan="2">Salivary glands</td><td rowspan="2">6.8%</td><td colspan="2">Parotid</td><td>4.5%</td><td rowspan="3">11%</td><td rowspan="3">Head and neck</td></tr>
<tr><td colspan="2">Other salivary</td><td>2.3%</td></tr>
<tr><td colspan="6">Tonsil</td><td>2.3%</td></tr>
<tr><td colspan="6">Lung</td><td>11.4%</td><td>9.5%</td></tr>
<tr><td colspan="6">Ocular adnexa</td><td>6.8%</td><td>10%</td></tr>
<tr><td colspan="6">Thyroid</td><td>2.3%</td><td>4%</td></tr>
<tr><td colspan="6">Skin</td><td>0%</td><td>10%</td></tr>
<tr><td colspan="6">Breast</td><td>0%</td><td>3%</td></tr>
<tr><td colspan="6">Unidentified primary mass [1]</td><td>15.9%</td><td>0%</td></tr>
<tr><td colspan="6">Multifocal (at diagnosis)</td><td>0%</td><td>11%</td></tr>
</table>

Lyon: 158-patient series described by Thieblemont, *et al.*, 2000. [87] Portugal: 18-year series (M.I. Pereira, 2010, personal communication). [85] [1]*Unidentified primary mass* refers to lymphomas diagnosed through medullary involvement or leukemization, with no identification of the primary solid mass at diagnosis.

Digestive and gastrointestinal tract

Gastrointestinal involvement is by far the most common location for MALT lymphomas, reflecting the tract's unique characteristics of contact with foreign antigens, mucosal permeability, large extension and intrinsic lymphoid system. Similarly, MALT lymphomas represent a large proportion of gastrointestinal lymphomas; in a revision of 194 cases of B-cell gastrointestinal lymphomas, one-fifth (20.6%) were pure MALT lymphomas and a further 8% were MALT lymphomas with a DLBC component, while 58% were pure DLBCL and the remaining 13.4% included follicular, mantle-cell, lymphoplasmacytic and lymphoblastic lymphomas. [88]

Gastrointestinal involvement by B-cell lymphomas is most common in the stomach (which accounts for 60% to 75% of gastrointestinal lymphomas), followed by the small intestine, ileum, colon and rectum (clinically simulating colorectal carcinoma). [3,87,89–91] Appendiceal involvement by MALT lymphoma can occasionally be found at appendectomy. [85,92] A variant of MALT lymphoma with well-defined and typical characteristics involves the small intestine and is known as immunoproliferative small intestinal disease (IPSID). [42]

The gastrointestinal tract adnexa can also be affected, with the salivary glands being a frequent location. There are also several descriptions of gall-bladder disease being identified histopathologically after cholecystectomy, which is usually curative when the lymphoma is localized, even if high-grade transformation has taken place. [85,93,94] It has been suggested that MALT could be induced in the gall-bladder by chronic lythiasic cholecystitis or bacterial infection. [93]

Though the liver can be often involved, the differential diagnosis of hepatic MALT lymphoma should include hepatic pseudolymphoma, composed of B-cell lymphoid follicles positive for CD10 and BCL-2. [95]

Ocular adnexa

NHL are the most frequent primary malignant tumors of the orbit and ocular adnexa, accounting for half of all cases of primary malignancies in these structures. [48,96] The involvement of the ocular adnexa by lymphomas has a high clinical relevance, both due to its incidence (1 to 2% of non-Hodgkin lymphomas and 8% of extranodal lymphomas, and presently increasing) and its functional and plastic complications. [48,97] These are a heterogeneous group of lymphomas, including 80% of MALT lymphomas and other types, such as mantle cell lymphomas [98].

All ocular or peri-ocular structures can be affected primarily or secondarily by ocular adnexal MALT lymphomas, including the conjunctiva (the most common location, representing 20 to 33% of cases of all epibulbar lymphomas, and up to 56.3% in one series), the orbit and periorbital fat, the lacrimal apparatus (15.6% in the same series), the palpebrae and intra-ocular structures, including the choroid; nevertheless, in over 75% of patients, a single lymphomatous lesion is identified [96,99,100]. When the conjunctiva is involved, macroscopy characteristically reveals a rosy-salmon, multinodular, pebbly appearance which, in rare cases of diffuse involvement, can simulate chronic conjuntivitis, or even panuveitis. [101,102]

Respiratory tract

The respiratory tract is another frequently affected system, and MALT lymphoma is the most common subtype of primitive or primary pulmonary lymphoma (defined as a clonal lymphoid proliferation affecting one or both lungs or bronchi, with no extrapulmonary involvement at diagnosis or the 3 subsequent months). It accounts for 58 to 87% of cases, with the remaining cases being diffuse large B-cell lymphomas (22% in one series) and lymphomatoid granulomatoses (11% in the same series) [51,103,104]. Although there are descriptions of MALT lymphoma of the lung coexisting with Sjögren's syndrome, pulmonary tuberculosis and even

fungal hyphomas, there are no robust association studies identifying an etiologic factor. [55,105–107]

Apart from the lungs or bronchi, other primary respiratory locations include the trachea and the larynx; nevertheless, lymphoproliferative diseases of the larynx are very rare, representing only 1% of laryngeal neoplasms. [53] Although the supraglottis is most frequently affected, as it is most often the location of acquired mucosa-associated lymphoid tissue, rare cases of a subglottic origin (with stenosis, stridor, dyspnea and hoarseness) have been described. [108]

Rarer locations

Other relevant, though rarer, locations include the thyroid (in some series accounting for approximately half of primary thyroid lymphomas), the mammary gland, the thymus and the genitourinary tract (bladder, kidney and prostate). [6,109]

There are some cases described of primary MALT lymphoma of the dura mater, which represent a very small fraction of primary central nervous system lymphomas (which are themselves a rare lymphoproliferative disease) with a good response to neurosurgery and external radiotherapy. [110] Central nervous system involvement is not restricted to the encephalon, but can also affect the spinal cord. [111]

Multifocal lymphoma

The involvement of various non-contiguous sites (including both different systems and discrete segments of the same system – such as different aspects of the gastrointestinal tract, separated by healthy tissue) is common in MALT lymphoma, both at diagnosis and throughout the evolution of the disease, and has been interpreted as recurrence, dissemination or independent development. [112] An analysis of the IGH V(D)J sequences of 4 patients with MALT lymphoma in two distinct sites in unrelated systems (metachronous gastric-nasopharyngeal, metachronous gastric-pulmonary, synchronous ocular-nasopharyngeal and

synchronous ocular-parotid), showed that only the latter case was a clonal disease, suggesting that multi-tissue involvement often develops independently as a result of chronic antigenic stimulation. [20] In contrast, a sequencing study of the *IGH* gene in 8 patients with synchronous or metachronous ocular adnexal lesions revealed clonality for all sites in 7 patients, while one patient with five-site disease was clonal for four samples (left orbital, right orbital, gastric and rectal) and had a fifth sample (buccal) differing by one nucleotide, suggesting that multi-tissue involvement is in fact a clonal event. [98]

There are descriptions of the concomitance of MALT lymphoma with other malignancies, such as multifocal gastrointestinal and pulmonary MALT, with the latter coexisting with areas of well differentiated adenocarcinoma, the coexistance of pulmonary MALT and tumorlet, of Epstein virus-associated gastric carcinoma and primary gastric MALT lymphoma, of colon adenocarcinoma and MALT, and of thyroid papillary carcinoma, thyroid MALT lymphoma and Hashimoto's thyroiditis. [113–117] It has been proposed that, in these circumstances, treatment decisions should prioritize the tumor with the worst prognosis at the moment of diagnosis, which is usually the carcinoma. [116]

Diagnosis

The diagnosis of MALT lymphoma rests on the clinical suspicion of lymphoproliferative disease or another malignancy, confirmed by histopathologic data; the latter must be complemented by the judicious use of immunohistochemistry (and eventually flow cytometry), cytogenetics and molecular biology, moreover considering that the histological differential diagnosis between severe gastritis and early stage lymphoma can be difficult. [4]

Histopathology

The histopathologic evaluation of a tissue biopsy sample remains fundamental for the diagnosis of MALT lymphoma. [118] MALT lymphoma is characterized by the presence of a

typical infiltrate located in the marginal zone of follicles with reactive germinal centers, with possible extension into the interfollicular region. [6,7] This infiltrate is made up of small, morphologically heterogeneous monoclonal B-cells, originating in post-germinative memory cells, and includes centrocyte-like marginal zone cells, monocytoid B-cells, immunoblasts and centroblast-like cells; plasmocytes can be seen in the sub-epithelial zones and are monoclonal in up to half of cases [6,7,11,22] Histologically, pathologic acquired MALT and MALT lymphoma are similar to physiological MALT. [22] Therefore, the principal diagnostic criterion for MALT lymphoma is the lymphoid tissue's invasion and destruction of the adjacent epithelium, originating typical lymphoepithelial lesions. [6,22] In some patients, the germinal centers can also be invaded and colonized by the neoplastic infiltrate, simulating a follicular lymphoma.[7] Immunohistochemistry can be a valuable aid in the differentiation between MALT lymphomas and other small cell lymphomas, including follicular lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and even mantle-cell lymphoma (MCL). [7,22]

The WNT signaling pathway, which is fundamental in the activation and self-renovation of stem cells, can be evaluated by the quantity of β-catenin reaching the nucleus; nuclear immunohistochemical marking for pβ-catenin-S552 is increased in extranodal marginal zone lymphoma and in atypical lymphoid hyperplasia, compared with normal and reactive lymphoid tissue, hinting at a potential role for this marker in the diagnosis and follow-up of MALT lymphoma. [119]

High-grade transformation

Although MALT lymphoma is an indolent disease, with large transformed cells being rare in the neoplastic infiltrate, it can undergo transformation to an aggressive diffuse large B-cell lymphoma (the most common histological type of primary gastric lymphoma, representing over half of cases), through poorly understood mechanisms. [7,81,120]

It has been noted that MYC overexpression can be detected in 80% of gastric DLBCL, but in only 20% of gastric MALT lymphomas. It downregulates at least 27 microRNAs (miR) with antiproliferative properties, of which miR-34a has been found to have the most marked antiproliferative effect, through its suppression of FOXP1. [81] Studies using small-interfering RNA-mediated *MYC* or FOXP1 knock-down demonstrated a block in lymphoma proliferation. [81]

Immunophenotype

Immunophenotyping can also contribute to the differential diagnosis between small cell lymphomas. [7] MALT lymphoma B-cells have an immunophenotype that is identical to the normal phenotype of a non-neoplastic marginal zone lymphocyte (Table 7), with positivity for the B-cell surface markers CD19, CD20 and CD22 and negativity for CD5 (unlike CLL/SLL) and for cyclin D1 (unlike MCL). [7,9,22] Malignant cells exhibit light-chain restriction, as a marker of clonality.

Flow cytometry can also be used to establish clonality, as has been described in a comparison of broncho-alveolar lavage from patients with CTD and MALT lymphoma, and from patients with CTD alone, where the analysis of clonality was able to identify the cases of MALT lymphoma. [51]

Table 7: Useful surface markers for the differential diagnosis of MALT lymphoma.

	Antigen	Notes
Positivity	sIg	BCR
	$CD19^+$	Pan-B-cell marker
	$CD20^+$	Pan-B-cell marker
	$CD22^+$	Pan-B-cell marker
	$CD79a^+$	Pan-B-cell marker
Negativity	$CD5^-$	Positive in CLL
	$CD10^-$	Positive in follicular lymphoma
	$CD23^-$	Positive in CLL
	Cyclin $D1^-$	Positive in MCL

CD: cluster of differentiation. BCR: B-cell receptor; CLL: chronic lymphocytic leukemia; MCL: mantle-cell lymphoma.

Cytogenetics and Molecular biology

The identification of the characteristic recurrent chromosomal translocations, by conventional cytogenetics, FISH or molecular biology, is informative and can contribute to the differential diagnosis of MALT lymphoma. However, these three methodologies can be technically demanding and are not readily available in all centers, and so are not routinely performed. [4] To address some of these limitations, an *in situ* break-apart (split-signal) hybridization technique (ba-ISH) for brightfield microscopy, to be used with MALT1 gene translocations, has been developed, overcoming the need for a fluorescence microscope as well as the lability of the fluorescent signal and its consequent loss over time. [61]

Medical Image

Imaging studies are fundamental not only for the diagnosis but also for the adequate staging of the lymphoma at presentation.

Gastrointestinal endoscopy

Esophagogastroscopy or esophagogastroduodenoscopy with multiple biopsies is the gold standard for the diagnosis of gastric MALT lymphoma. In a 24-patient series, the most common macroscopic endoscopic findings were mild hyperemia (67%), superficial erosions (17%) and superficial ulcers (17%). [29] Gastric ulcers, especially when unresponsive to conventional treatment, should be biopsied due to the risk of malignancy; though gastric carcinoma is the usual finding, some rare cases of gastric lymphoma can also present as an ulcer, including with local complications, such as perforation. [121] Push enteroscopy with serial biopsies, performed during esophagogastroscopy, is safe and easy, and can detect the synchronous involvement of the duodenum and jejunum by MALT lymphoma, a finding that was present in 11% of patients in a retrospective series. [112] Colonoscopy is also able to identify macroscopic changes in the mucosa (such as discoloration with a reduction of superficial vessels) in colorectal MALT lymphoma and, according to some authors, should also be part of the diagnostic workup of gastric MALT lymphoma do screen for metachronous involvement. [24]

Serial esophagogastroscopies with multiple biopsies are mandatory for the follow-up of post-remission gastric MALT lymphoma, especially in early-stage disease, where recurrence tends to be localized to the mucosa and undetectable by other imaging modalities. [122]

It has been suggested that the use of magnified endoscopy techniques for the evaluation of the microstructural pattern of the lesion and distribution of abnormal vessels could be useful both for diagnosis and follow-up. [123] In a series of 21 patients with localized gastric disease,

nonstructural areas with abnormal vessels were present at magnified esophagogastroscopy in 100% at diagnosis, disappearing with histopathologic remission; compared to histopathology, nonstructural areas had a sensitivity of 76.9% and a specificity of 87.5%, while the presence of abnormal vessels had both a sensitivity and specificity of 85.7%. [123]

Ultrasound

The addition of ultrasonography to gastrointestinal endosopy enables the endoscopist to evaluate the degree of organ involvement and infiltration of contiguous structures in a single procedure, which if fundamental for staging. [6] The presence of diffuse parietal thickening in endo-ultrasonography is suggestive of infiltration by lymphoma. [24] The ultrasonographic appearance of MALT lymphoma can be characteristic in some locations, as in the case of the submandibular gland, with a well-demarcated, hypoechoic, heterogeneous, solid mass, with linear echogenic strands and hypervascularity. [124] It has been suggested that when sonographic findings are this characteristic, surgical excisional biopsy can be replaced by ultrasound-guided core-needle biopsy. [124]

Computerized tomography

Abdominal computerized tomography (CT) can detect locally advanced gastric MALT lymphoma, presenting as a diffuse or localized parietal thickening, as well as lymphadenopathy, local complications (including perforation) and hepatosplenomegaly. [125] Gastrointestinal dissemination can manifest as circumferential parietal thickening of an intestinal segment, or localized polypoid masses with homogeneous and isoattenuating or hypoattenuating enhancement. [24,125]

Three-dimensional reconstruction in gastrointestinal lymphomas correlates with the underlying histopathology, with an increased likelihood of low-grade gastric MALT lymphoma in patients with normal scans, with minimal gastric wall thickening (5 to 10 mm)

or with small depressed lesions with vague margins. [126] On the other hand, a severe diffuse thickening of the gastric wall (> 10 mm), focal well-demarcated masses, or masses with homogeneous attenuation and mild contrast enhancement, suggest high-grade lesions; perigastric adenopathies are also more likely than in low-grade lesions. [126] In contrast, multiple lymphomatous polyposis is common in MCL, a bulky mass with uniform isoattenuation in the right lower quadrant suggests Burkitt lymphoma, and thickened nodular folds with multiple ulcerative lesions, perforations and obstruction are typical of T-cell enteropathy; DLCBL is multiform and often invasive. [126]

Pulmonary CT, on the other hand, is often not conclusive, as primitive pulmonary lymphomas present with a marked radiologic pleomorphism, including areas of alveolar condensation, diffuse ground glass and nodular opacities, solid lung masses, cystic lesions or bronchial involvement. [104,127,128]

Abdominal CT scanning does not appear to be useful for the follow-up of localized gastric MALT lymphoma. [122] In a series of 122 patients with early-stage gastric MALT lymphoma in complete remission, 5.7% of patients had recurrent disease, which was confined to the mucosa and without extragastric involvement and, thus, undetectable on CT. [122]

Positron emission tomography

Although positron emission tomography (PET) is insufficiently informative in many cases of indolent lymphoma, it appears to be useful in the diagnosis and orientation of ocular adnexal MALT lymphoma, particularly in atypical localizations. [118]

Staging

The staging of both Hodgkin and non-Hodgkin lymphoma is standardized through the Ann Arbor system, proposed by a committee in 1971, and updated in 1989 through the

introduction of the Costwolds modifications. [129,130] Musshoff, in 1977, had already proposed the modification of the Ann Arbor staging system through the subdivision of Stage II into II1 (regional nodal involvement) and II2 (non-regional nodal involvement) (Table 8), but it wasn't widely accepted into common clinical practice. [131]

Table 8: The Ann Arbor staging system for lymphomas

Stage	Involvement
I	1 nodal region *or* 1 organ
II	2 or more nodal regions on the same side of the diaphragm
III	2 or more nodal regions on opposing sides of the diaphragm
IV	1 or more organs (including the bone marrow)

In the case of extranodal lymphomas, the suffix "E" is appended to the stage. By definition, as extranodal lymphomas, stage IE does not include nodal involvement, while stage IVE refers to organs non-contiguous to the original site. The suffix "B" refers to the presence of B-type symptoms, while their absence is noted by the suffix "A".

Due to the intrinsic limitations of the application of the modified Ann Arbor system in primary extranodal lymphomas of the gastrointestinal tract, Radaszkiewicz *et al.*, in 1992, demonstrated the prognostic value of the separation of modified Ann Arbor stage IE gastric MALT lymphomas into stages IE1 (submucosa not transposed) and IE2 (extension beyond the submucosa) (Table 9). [132] An international workshop in Lugano, Switzerland, in 1993, led to a proposal to modify the Radaszkiewicz system by including in stage IIE not only infra-diaphragmatic regional and non-regional nodal involvement, but also intra-abdominal organ infiltration, and eliminating stage III, a proposal that became known as the Lugano Staging System, International Workshop Staging System or modified Blackledge System (Table 9).

[133] Some authors have suggested that the Lugano modification has mostly led to confusion and miscommunication, since it originated the only situation in lymphoproliferative disease in which a stage II classification does not imply nodal involvement. [134]

To overcome the perceived limitations of the various lymphoma staging system adaptations, both for the correct definition of the primary tumor extension and depth of infiltration and as a basis for therapeutic decisions, in 2003 the European Gastro-Intestinal Lymphoma Study Group (EGILS) proposed the Paris System or TNM-B. This is an adaptation of the existing tumor-node-metastasis (TNM) system in mainstream use for the classification of non-hematologic solid malignancies, for the staging of primary gastrointestinal lymphomas, defined as originating between the gastroesophageal junction and the anus (Table 9). [134]

The main benefit of the Ann Arbor-derived systems over the Paris system is their ease of application. The Ann Arbor modifications have also proved inadequate for the staging of ocular adnexal lymphomas, which led the American Joint Committee on Cancer to propose the use of a TNM system for these locations. [135] The utility of this system was demonstrated by the committee in 66 eyes (from 54 patients), where the progression-free survival (PFS) in stage T1N0M0 (isolated unilateral conjunctival involvement) was 24 months higher than in advanced stages or in bilateral infiltration (bT1N0M0). [135] Considering only those patients in Ann Arbor stage IE, the TNM system was able to differentiate a subset of patients with worse prognosis, with stage IE patients in stages bT1N0M0 or over T1N0M0 having a PFS at 24 months of 84.7%, compared to 100% for stage IE patients in stage T1N0M0. [135]

Table 9: Comparison of four currently available staging systems for primary gastrointestinal lymphomas.

Tissue Invasion	Ann Arbor	Radaszkiewicz	Lugano	Paris
Gastrointestinal tract	IE	IE	I	T*
Mucosa or submucosa	IE	IE1	I	T1
Mucosa	IE	IE1	I	T1m
Submucosa	IE	IE1	I	T1sm
***Muscularis propria* or subserosa**	IE	IE2	I	T2
Serosa	IE	IE2	I	T3
Intra-abdominal extension			II	
Adjacent tissues or organs	IE	IE	IIE	T4
Regional lymph nodes [1]	IIE	IIE	II1	T* N1
Infradiafragmatic distal lymph nodes [2]	IIE	IIE	II2	T* N2
Disseminated disease			IV	
Supradiafragmatic lymph nodes	IIIE	IIIE	IV	T* N3
Non-contiguous gastrointestinal [3]	IVE	IVE	IV	T* N* M1
Non-contiguous metastasis [4]	IVE	IVE	IV	T* N* M2
Marrow involvement	IVE	IVE	IV	T* N* M* B1

In the case of synchronous lesions originating in the gastrointestinal tract, staging refers to the characteristics of the most advanced lesion. [134] Note that the Lugano system does not include a stage III. *Any subtype of tumor extension (T1 to T4) or nodal (N0 to N3) or metastatic (M0 to M2) involvement. [1]The lymph nodes that can be considered "regional", according to the location of the primary tumor, are listed in Table 11. [2]The remaining nodes are considered "distal". [3]Non-contiguous gastrointestinal involvement refers to the presence of lymphoma in more than one gastrointestinal site with segments of discontinuity that are free of disease (such as the involvement of the stomach and rectum, with a free small intestine and bowel). [4]Including the non-contiguous involvement of the peritoneum.

Table 10: TNM system qualifiers

	Stage	Definition
Tumor	Tx	Extension of lymphoma not established
	T0	No evidence of primary lymphoma
Nodes	Nx	Nodal involvement not evaluated
	N0	No evidence of nodal involvement
Metastasis	Mx	Dissemination of lymphoma not evaluated
	M0	No evidence of lymphoma dissemination
Bone marrow	Bx	Bone marrow infiltration not evaluated
	B0	No evidence of bone marrow infiltration

Table 11: Regional lymph nodes (EGILS definition)

Primary site	Regional lymph nodes	
Gastric	Perigastric	
	Along the arteries	Left gastric
		Common hepatic
		Splenic
Duodenal	Pancreaticoduodenal	
	Pyloric	
	Hepatic	
	Superior mesenteric	
Jejuno-ileal	Mesenteric	
	Ileocolic	Distal ileum
	Posterior cecal	
Colorrectal	Pericolic	
	Perirrectal	
	Along the arteries	Ileocolic
		Colic (right, middle and left)
		Inferior mesenteric
		Superior rectal
		Internal iliac

Regional lymph nodes, according to the site of the primary tumor, as defined by the EGILS in their proposal of the Paris Staging System for the classification of gastrointestinal lymphomas. [134]

Most MALT lymphomas are at diagnosis characterized by non-disseminated disease, with both marrow and distal nodal involvement being rare; on the other hand, regional lymph node infiltration (with a monocytoid B-cell lymphoma appearance) is relatively frequent in the case of gastric or salivary gland MALT lymphomas. [7] Isolated nodal involvement can also be observed in this extranodal lymphoma, primarily in cases of nodal relapse after remission of the primary lymphoma with treatment. [136]

Prognosis

Staging alone is not sufficiently predictive of disease outcome in lymphoproliferative diseases, and a patient's survival is influenced by several concurrent prognostic factors. To adequately integrate all these factors into the clinical decision, prognosis can be quantified in B-cell lymphomas through the use of internationally validated scales or indices, such as the International Prognostic Index (IPI), which was in developed in 1993 for aggressive B-cell lymphomas, and its subsequent adaptations for specific lymphoma subtypes, such as the Follicular Lymphoma IPI (FLIPI), published in 2004. [137,138]

There is no consensual prognostic index that is specific for MALT lymphomas. However, it has been demonstrated that IPI scores correlate significantly with time to relapse in MALT lymphomas, differentiating low, low-intermediate and high risk groups. [139] The FLIPI, on the other hand, while dividing patients into three risk groups, was unable to separate the clinical evolution of the low and intermediate risk groups. [139]

Indicators of poor prognosis include the presence of a large-cell component at diagnosis, B symptoms, high serum β2-microglobulin or serum lactate dehydrogenase (LDH), low serum albumin, advanced age (over 60 years) or poor performance status (2 or above on the WHO/Eastern Cooperative Oncology Group (ECOG) scale), and the presence of a bulky tumor. [137] The absence of complete remission with first-line treatment is a further *a posteriori*

indicator of poor prognosis. We have discussed above how genetic aberrations, such as the presence of the t(11;18), correlate with resistance to treatment. Rearrangements of the *BCL6* locus, or BCL6 protein overexpression, appear to associate with large-cell transformation of MALT; on the other hand, it has been reported that overall survival in gastric DLCBL, with or without a MALT component, correlates strongly with BCL6 overexpression. [140,141] In univariate analysis, a diagnosis of autoimmune disease and a multifocal distribution of MALT lymphoma were predictors of relapse of gastric lymphoma, while on multivariate analysis only the presence of extragastric disease had prognostic value. [139]

It has been suggested that the molecular characterization of MALT lymphomas will not only facilitate diagnosis, but also increase prognostic accuracy, optimizing the selection of therapeutic strategies. [142]

Treatment

Current guidelines are consensual in indicating *H. pylori* eradication therapy as the first line approach in gastric MALT lymphoma. However, due to the paucity in the medical literature of extensive series of patients with MALT lymphomas and, more importantly, of prospective clinical studies, in conjunction with the different antigenic stimuli underlying lymphomagenesis in the different sites, the optimal treatment of most non-gastric locations or of *H. pylori*-negative and eradication-resistant *H. pylori*-positive gastric lymphomas has not been convincingly established. Treatment decisions are often made on a case by case basis, with different centers reporting a variety of approaches which (with the exception of watchful waiting and antibiotherapy) have relevant side-effects. [13,91,111] These aspects are particularly relevant for MALT locations with a still-cryptogenic etiology which are, simultaneously, the rarest cases.

These lymphomas follow an indolent clinical course with prolonged overall survival (80% at 5 years) and disease-free survival, on par with other low-grade lymphomas and, in early stage disease, tend to respond to a wide variety of treatment approaches; however they are characterized by a high recurrence rate, with most patients relapsing within 5 years, often in organs with acquired MALT that are distant from the original location. [7,9,111,143] Second remissions can be regained with retreatment; however, the disease-free interval tends to decrease after each subsequent remission. [143]

These aspects of the disease underlie the need for the availability of multiple treatment modalities and, within chemotherapeutics, of multiple drug classes, to control the lymphoma over a long-term period. [143] These characteristics also justify the importance of opting for well-tolerated approaches with low rates and severity of chronic and acute toxicity. In our Portuguese series – and despite a median age at diagnosis of nearly 60 years old – we observed a 15-year survival of 60% (and a 5-year survival of 80%), highlighting the importance of ensuring that treatment choices will give the patient long-term quality of life. [85]

Early-stage disease tends to remain localized for a long time, and responds satisfactory to local treatment approaches, such as surgery or radiotherapy. [111] However, survival correlates inversely with the stage at diagnosis (90-95% at 5 years for stage I, 75% for stage II and as low as 30% for stage IV), with about one-third of patients presenting with advanced disseminated disease at diagnosis and requiring systemic treatment. [111]

Watchful waiting

Since MALT lymphomas are indolent neoplasms, in selected patients with asymptomatic or minimally-symptomatic non-gastric MALT lymphoma without a large-cell component, a strategy of expectant active surveillance of the patient with repeated imaging studies and hematological monitoring can be the most adequate approach at diagnosis. A period of

watchful waiting with repeated esophagogastroscopic biopsies has also been proposed as a valid option after a successful eradication of *H. pylori* in gastric MALT lymphoma. [144]

The possibility of spontaneous regression of MALT lymphomas also validates the watchful waiting approach. Regression can occur even when there is histological confirmation of the lesion, as in a case of MALT lymphoma of the lung which was identified by computerized tomography scanning and diagnosed histopathologically in a biopsy sample, which spontaneously regressed 16 days after the biopsy, and persisted in complete regression at 20 months of follow-up. [145] Regression has even been reported in cases with a transformed high-grade component. [146]

The presence of symptoms which interfere with the patient's quality of life is a good indication to suspend watchful waiting and introduce treatment. [48]

Antibiotherapy

H. pylori eradication

The antibiotic eradication of *H. pylori* infection is the first line treatment for gastric MALT lymphomas in Ann Arbour Stage IE (the majority of tumors at diagnosis), leading to a complete endoscopic and histopathologic remission with an excellent prognosis and the possibility of cure in approximately 80% of patients (most patients in Stage IE1 and smaller proportion of patients in stage IE2), while lymphomas in Stage IIE and above usually don't respond; regression of Stage I gastric DLBCL has also been described following *H. pylori* eradication therapy in adult and pediatric patients [6,142,147,148,149].

Although the probability of MALT lymphoma regression in response to a successful *H. pylori* eradication is influenced by the patient's cytogenetics, through the mechanisms described above, it has been suggested that introducing empiric eradication therapy in the absence of molecular testing is clinically justified, due to the high remission rates that can be achieved. [4]

The EGILS published its updated recommendations for the treatment of gastric MALT lymphoma in 2011. [150]

H. pylori status. Testing for *H. pylori* infection should be performed in all patients with gastric MALT lymphoma, through esophagogastroscopy with biopsy and a rapid urease test, culture or histopathology, through a urea breath test, or through the use of a fecal antigen test. [151] Comparing the available tests for *H. pylori*, the antigen test has been noted to have a higher sensitivity and negative predictive value (both 100%) than the rapid urease test, while the latter was found to have a higher specificity and positive predictive value. [152] Proton pump inhibitors (PPI) should be suspended at least one week before testing. [151]

Eradication therapy. The first line therapy for eradication is the triple association between a PPI, clarithromycin and either metronidazole or amoxicillin, over 7 days. [151] In special select cases, the antibiotics may have to be selected through an antibiotic sensitivity test, as in the reported case of a patient with a known penicillin-allergy, who was found to be infected with a clarithromycin- and metronidazole-resistant strain, and was successfully eradicated with minomycin and levofloxacin, in association with the PPI. [153]

Successful eradication should be confirmed by repeat testing for *H. pylori* four weeks or more after completion of therapy. [151] We have described a success rate of eradication in the Portuguese population of 80.6% with an amoxicillin/clarithromycin/PPI association, despite *in vitro* sensitivity of all strains to the antibiotics used. [154] In the case of treatment failure, eradication should be re-attempted with a quadruple association of a PPI, tetracyclin, metronidazole and bismuth salicylate [151].

With the loss of efficacy of anti-*H. pylori* antibiotics due to increasing resistances, the development of an effective anti-Helicobacter vaccine is crucial. [155] In a mouse model of chronic *H. pylori* infection-induced gastric carcinoma, a nasally administered multi-epitope

vaccine was able to induce a broad immune response with increased interferon gamma production and a significant reduction in *H. pylori* colonization, but with non-significant gastric histological-change scores. [155]

Lymphoma regression. While over 80% of patients can achieve a complete remission with *H. pylori* eradication, there are no clear predictive factors for response to eradication therapy, and primary refractoriness to *H. pylori* eradication can be found in 10 to 20% of low-grade gastric MALT lymphomas [142,156]. In a series of 95 Ann Arbor Stage IE1 patients, there were 7.4% of non-responders; while there were no differences in response according to sex, age, endoscopic appearance or large-cell component. Complete remissions were achieved in 98.5% of distal tumors, but only in 69.2% of proximal tumors, a difference that was statistically significant. [148]

The fact that gastric MALT lymphoma regression, in response to *H. pylori* eradication, can take up to 18 months, means that refractoriness should not be assumed prematurely, and determines a compulsory extended follow-up period, with regular esophagogastroscopies and repeat biopsies, although the optimal frequency of endoscopic evaluation has not been definitely established. [4,6] Additionally, in a series of patients with gastric MALT lymphoma in complete remission, 5% eventually developed local early-stage metachronous gastric carcinoma, diagnosed by long-term endoscopic follow-up, which also underlines the importance of close endoscopic follow-up. [156] Nevertheless, the ideal follow-up interval length after initial eradication treatment remains to be defined, with some authors suggesting immediate treatment after a successful eradication without lymphoma remission, while other propose continued watchful waiting. [142] On the other hand, the identification of resistance-associated genetic aberrations, such as t(11;18), could be an indication of true refractoriness to eradication, guiding therapeutic decisions. Likewise, the presence of a large-cell component

should help inform a choice to opt for alternative therapies if eradication fails to induce regression.

However, complete remissions achieved through *H. pylori* eradication are prolonged. In a series of 122 stage IE1 patients in complete remission, after a median of 35 months of follow-up only 7 (5.7%) showed lymphoma recurrence, which was limited to the mucosa and only detectable on endoscopic biopsies. In 4 of these patients, recurrence was associated with re-infection with *H. pylori*, and regressed after re-eradication; the tumors in the 3 remaining patients were *H. pylori*-negative and regressed spontaneously [122].

The presence of persistent minimal histological residuals after *H. pylori* eradication with endoscopic normalization can be managed through a watchful waiting approach with regular endoscopic biopsies, as was demonstrated in a series of 108 patients at 12 months post-eradication; 32% of patients went on to achieve a complete remission, 62% maintained stable minimal histological residuals, and only 5% had local progression of the disease, with one patient evolving to a high-grade lymphoma. [144,147]

There have been some descriptions of regression of *H. pylori*-negative MALT lymphomas after eradication therapy, which have been interpreted by the authors as being causally related, though its physiopathologic basis needs to be further explained. [157]

Non-H. pylori antibiotherapy

In MALT lymphomas with a putative association to a chronic non-Helicobacter bacterial infection, specific antibiotherapy can be a valid and effective therapy, as been demonstrated by the regression of IPSID with tetracycline or a case of complete remission of rectal MALT lymphoma with levofloxacin, despite the persistence of gastric *H. pylori* colonization. [39,43] The eradication of *Chlamydophila psittaci* through the use of oral doxicycline has been

described for ocular adnexal MALT lymphomas, both as single-therapy, and in conjunction with specific chemotherapy, with good results. [100,111]

Surgery

In gastric MALT lymphoma, the current view is towards stomach-conserving conservative treatment, avoiding first-line surgical resection. [147] Nevertheless, there are several cases of a curative surgical approach, especially when non-gastric MALT lymphoma was an unexpected finding after resection, such as cholecystectomy or appendectomy with right hemicolectomy and partial resection of the right ureter, for a large appendicular mass. [92,94] Surgery has also been found to be curative in cases where the lymphoma collocates with a more aggressive carcinoma that is completely ressected, as related in a case of curative hemicolectomy for a large, hemorrhagic, ulcero-proliferative colonic mass including both carcinoma and lymphoma components. [117] As such, surgery can play a role in non-gastric MALT lymphoma in combination with either chemotherapy or radiotherapy. [90,91]

Regardless of curative intent, an invasive approach can be indicated for the control of local complications of the tumor, such as airway obstruction in the case of bronchial MALT lymphomas [127].

Radiotherapy

Radiotherapy has a high curative potential in the stomach-conserving treatment of gastric MALT lymphoma, in *H. pylori*-negative patients or in lack of response to eradication in *H. pylori*-positive cases, with 80% of eradication-refractory patients achieving a complete remission with radiotherapy [147]; a dose of 30 to 40 Gy in 15 to 20 fractions has been proposed [89,156].

In ocular adnexal MALT lymphomas, isolated radiotherapy is also a valid option when there is a curative intent. In a series of 30 patients with stage IE and IIE, doses of 28,8 to 45,8 Gy in

15 to 26 fractions resulted in a complete remission rate of 100%, a 5-year overall survival of 100% and a 5-year progression-free survival of 96%. [158]

One of main limitations of radiotherapy is its local complications which, in ocular adnexal lymphomas, can compromise the quality of vision. In the 30-patient series described, 17% of patients developed grade 2 cataracts 8 to 45 months after irradiation, with no other ocular complications and recovery of visual acuity after cataract surgery. [158] Others have described radiation retinopathy with macular edema and reduced visual acuity, two years after a dose of 35 Gy, with an improvement in edema with intravitreal anti-VEGF-A (bevacizumab) but only a mild improvement of low-vision (comparable with the results obtained in radiation retinopathy in coroidal melanoma [160]); it has been suggested that improvement in visual acuity with anti-VEGF-A can be achieved in newly-diagnosed patients, but not in established radiation maculopathy [159,161].

There is also an important role for radiotherapy in non-gastric non-ocular MALT lymphomas, as described for a solitary rectal tumors treated with external radiotherapy or resection and local irradiation. [90,162]

Chemotherapy

Eradication-refractory gastric MALT lymphomas have high rates of response to chemotherapy, and it is a valid approach after confirmed failure of first-line eradication. Likewise, it is justified in systemic disease with *a priori* dissemination and in selected cases of extra-gastric lymphoma; on the other hand, the use of chemotherapy after a successful response to *H. pylori* eradication, in localized MALT lymphoma, proposed by some authors to prevent recurrence, is still controversial [4,13,163].

Alkylators

A phase II study of oral chlorambucil as single-agent treatment of ocular adnexal MALT reported 79% of complete remissions with minimal side-effects, although 15% of complete responders (12% of patients) eventually relapsed at a median follow-up of 32 months, suggesting that it can be a valid choice when systemic therapy is warranted. [164] In contrast, the LY03 clinical trial found no differences in either recurrence rate or the 5-year progression-free survival between watchful waiting after a successful eradication in localized gastric MALT lymphoma, and chlorambucil maintenance, suggesting that there is currently no indication for single-agent chemotherapy with this agent as for the prevention of recurrence. [163]

Bendamustine has also demonstrated clinical effectiveness as a single-agent in MALT lymphoma, including in multiple-relapsed rituximab-resistant patients, where an ORR of 86%, with 43% of CR and acceptable toxicity, has been reported, demonstrating that it is a valid approach for refractory disease. [143,165]

Nucleoside analogues

It has been noted that tumor microenvironment T-cells play an important role in lymphomagenesis induced by chronic antigenic stimulation, underlying the potential utility of chemotherapeutic agent that simultaneously target malignant B-cells and microenvironment T-cells, such as nucleoside analogues. [35] In a series of 14 patients with gastric MALT lymphoma treated with fludarabine, there was a significant reduction (compared to eradication alone) in peripheral blood T-cells, but not in biopsy samples, where there was an increase in $CD3^+$, $CD4^+$ and $CD8^+$ cells and $FOXP3^+$ T_{regs}. [35] A phase II study of single-agent cladribine as first-line therapy, with a prolonged 6-year follow-up, reported a global CR rate of 84% (which was as high as 100% in primary gastric lymphomas, but only 43% in

extragastric disease), with an 84% survival at 80 months, supporting a first-line approach with systemic chemotherapy. [166] On the other hand, the T-cell modulation associated with nucleoside analogues such as fludarabine and cladribine can lead to long-term immunosuppression and increased infectious risk. [111] Gemcytabine, a less T-suppressive deoxycytidine analogue with proven anti-tumor activity in several solid tumors, as well as Hodgkin's and advanced non-Hodgkin's lymphoma, is well-tolerated. [111] However, a phase II trial in 16 patients with advanced marginal zone lymphoma, 7 of whom had extranodal MALT lymphoma (2 gastric and 5 extra-gastric), was discontinued due to disappointing results, with a very low overall response rate of 16.7%, suggesting an absence of clinically significant antitumor activity in these patients. [111]

Combination chemotherapy

A controlled prospective clinical trial of early-stage (IE and IIE) gastric MALT lymphoma comparing surgery, radiotherapy, and chemotherapy with cyclophosphamide, vincristine and prednisolone, with or without doxorubicin (CVP/CHOP), showed a significantly higher event-free survival at a 7.5 year median follow-up with combination chemotherapy (87%), compared to either surgery or radiotherapy (52% in either arm), but with identical OS in all three arms. [167]

A phase III trial comparing first-line fludarabine-mitoxantrone (FM) with CVP in stage IE non-gastrointestinal MALT lymphoma, with a 3-year median follow-up, related a CR rate of 100% in both arms, with four patients treated with CVP relapsing and achieving a second CR with FM, suggesting that the latter might be more effective than the former. [168]

New agents

Thalidomide. Thalidomide is an antiangiogenic and immunomodulatory drug with anti-tumor necrosis factor α (TNFα) and anti-NF-κB activity, which justifies its potential utility in MALT lymphomas. [52] It has been used as a salvage therapy in a series of 10 *H. pylori*

eradication-refractory chemo-resistant gastric MALT lymphoma, with an overall response rate of 50% (20% CR and 30% PR), with an ORR of 0% in patients with the API2-MALT1 transcript and of 86% in patients without the transcript; the latter (but not the former) showed a significant downregulation of the expression of NF-κB in residual neoplastic cells and tumor microenvironment. [52] These data suggest that the presence of t(11;18)(q21;q21) is predictive of no-response to thalidomide. [52]

Proteasome inhibitors. The role of the UPS in the regulation of the NF-κB pathway has been described above, and serves as the rationale behind the use of proteasome inhibitors in the treatment of MALT lymphomas. [169] Previous basic and clinical experience with these agents has demonstrated that it disrupts multiple UPS-dependent cellular pathways, with apoptosis as the final event. [13] Bortezomib, the first proteasome inhibitor approved for clinical use, is now used as first-line treatment in multiple myeloma; the described relationship between the malignant marginal zone B-cell and plasma cells, and the drug's demonstrated efficacy in other B-cell lymphomas, also give support to the use of bortezomib in MALT lymphoma. [13]

A single-arm, phase II study in eradication-refractory gastric and extragastric MALT lymphoma patients, using 1.5 mg/m2 of single-agent bortezomib on Day 1 (D1), D4, D8 and D11 of 21-day cycles, achieved an objective response rate of 80% (43% of CR and 37% of PR) with an OS of 100% at 23 months. [13] However, toxicities were higher than expected, with 94% of patients requiring dose-reductions during therapy. [13] A subsequent phase II trial at a lower dose of 1.3 mg/m^2 of bortezomib monotherapy in relapsed or refractory MALT lymphoma (of varying stage and location) also presented a high rate of treatment-related complications, while producing an overall response rate of just 48% in the assessable subjects (31% CR and 17% PR) and a progression rate of 20%. [169] Given the indolence of MALT lymphoma, acute and long-term toxicity is still an important issue to consider when

comparing risk-benefit ratios of different treatment approaches, and should be taken into account when interpreting these results. [13]

Histone deacetylase inhibitors. Histone deacetylase inhibitors (HDACi) are epigenetic modulators that modify gene expression, and consequently, the levels of proteins involved in several intracellular pathways associated with tumorigenesis and tumor progression, including angiogenesis, apoptosis and the cell cycle, as well as tumor immunology and the production of pro-inflammatory cytokines. [143] Vorinostat, a HDACi with activity against class I and class II histone deacetylases approved for advanced cutaneous T-cell lymphoma, has demonstrated single-agent efficacy at a dose of 200 mg, b.i.d. in a phase II trial in heavily pretreated relapsed or chemotherapy-refractory marginal zone lymphoma (subtypes not revealed by the authors), producing an overall response rate of 22% (one CR and one PR in a 9-patient series), a 6-month PFS of 86% and a prolonged median PFS of 18.8 months. [143] An objective response was usually preceded by a long period (of up to 2 years) of disease stabilization, over multiple cycles, as seen with hypomethylating agents in myelodysplastic syndromes. [143] These results suggest that HDACi exert their benefit by slowing disease progression, even in the absence of overt response criteria. [143]

Other new approaches. The demonstration of the role of miR-203 promoter hypermethylation and ABL1 overexpression serve as the rationale both for the use of demethylating agents and of ABL tirosine kinase inhibitors. [84] In a mouse-model, it has been shown that imatinib is able to induce MALT lymphoma regression. [84] Considering the role of MALT1 as an NF-κB activator, it has been suggested that the targeting of its protease activity could be a useful treatment approach for conditions associated with NF-κB signaling deregulation, including MALT lymphomas. [68]

Immunotherapy

Monotherapy with anti-CD20 monoclonal antibody (rituximab) can induce sustained complete remissions of MALT lymphoma, with descriptions of success in ocular adnexal lymphoma in both localized disease (lacrimal gland, with a weekly dose of 375 mg/m^2, over four weeks, every 6 months, over two years) and systemic disease (bilateral periorbital, lacrimal, subconjuntival and intra-ocular infiltration with systemic lymph node involvement). [57,170] In the latter case, although the patient had a lymphoma recurrence at 6 months, retreatment with rituximab led to reinduction of complete remission. [170]

Rituximab monotherapy has also induced complete remissions in other locations, such as a stage I rectal MALT lymphoma with trisomies 3 an 18, unresponsive to *H. pylori* eradication, or in primary bronchial MALT lymphoma with bone marrow involvement. [127,171]

In MALT lymphoma, as in other B-cell lymphomas, rituximab has been used as part of combination immuno-chemotherapy and radiotherapy, with good results, and an improvement in the responses to single-agent chemotherapy. [24,143] A phase II trial of rituximab plus CVP in untreated advanced stage disease (stage III and over) related an ORR of 88%, with 60% of CR, and a 3-year OS of 95%, with tolerable side-effects. [172] A retrospective analysis of 31 patients receiving chemotherapy-based regimens, and 31 patients receiving chemotherapy-based regimens plus rituximab, found an increase in the ORR with rituximab from 54.8% to 83.9%, but with no differences in time to progression. [173]

A single-arm phase II trial of fludarabine plus rituximab in gastric and extragastric MALT lymphoma obtained an ORR of 100% (90% CR), with a progression-free survival at 2 years of 100% in gastric lymphoma and 89% in extragastric locations. [174] In contrast, a study of cladribine versus cladribine with rituximab did not find an improvement in ORR with the

combination in MALT lymphoma, although there was an improvement in patients with splenic or nodal marginal zone lymphomas. [12]

These results highlight the importance of starting well-designed phase III studies that can clarify the role of the various treatment approaches and of combination modalities.

Radioimmunotherapy

Radio-immunotherapy with ^{90}Y-ibritumomab tiuxetan, an anti-CD20 monoclonal antibody containing a radioactive isotope was able to induce complete remissions (of up to 24 months) in 67% of patients with highly treated refractory gastric and extra-gastric MALT lymphoma at the third relapse (or over), in a small phase II uncontrolled single-arm study, with 17% further partial remissions and 17% stable disease. [175] A prospective single-arm open-label phase II trial of rituximab followed by ^{90}Y-ibritumomab tiuxetan as first-line treatment of early-stage (IE) ocular adnexal disease produced 83% of overall complete remissions and 17% of partial remissions; although this series included 25% of follicular lymphomas, the authors opted to present the overall aggregate results. [176] The authors estimated an absorbed radiation dose to the orbital soft tissues was under 3 Gy, which is under a tenth of the 30 to 40 Gy proposed for radiotherapy, potentially overcoming some of the local complications of the latter. [176]

Future directions

It has been proposed that miRNA underexpression (such as miR-34a downregulation by *MYC* overexpression in high-grade disease) could in the future be approached by gene replacement therapy. [81] This approach, if effective, could be extended to any of the dozens of miRNAs that are known to be deregulated in lymphoprolipherative diseases.

DISCUSSION AND CONCLUSIONS

MALT lymphomas are rare and heterogeneous malignancies that occupy a unique position in the spectrum of oncologic disease, as they can potentially be cured with a simple course of antibiotics.

Nevertheless, as indolent lymphomas, they present to the clinician the singular challenge of having to identify the optimum balance between effective therapy and minimal toxicity for a neoplastic disease that can have a decades-long course of remission and relapse, often in the absence of robust data and representative series on which to base an evidence-based practice of medicine.

The known association with chronic immune stimulation has offered invaluable insights into lymphomagenesis and, by extension, the mechanisms of neoplastic transformation in general. The knowledge thus acquired has, in turn, exposed key molecules of cell-cycle regulation, survival, apoptosis and proliferation, which can be manipulated as specific therapy targets. Such findings can often be reciprocally translated between MALT lymphomas and other lymphoproliferative and plasma cell diseases, which share common pathways of malignization.

The clinical translation of these findings must, necessarily, rely on strengthened long-term multicentric international collaborations to enable the accrual of representative numbers of patients for epidemiologic studies and prospective, randomized, blinded clinical trials. Only then can we hope to move towards the truly targeted, personalized treatment approach that these patients require.

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Instruções editoriais:

(As instruções editoriais completas – nos seus aspectos específicos para artigos de Revisão – da revista Haematologica são apresentadas no final deste trabalho, em Anexo)

- **Artigos de Revisão**: "No particular format is required for these articles. However, they should have an informative, unstructured abstract of about 200 words, and ideally should not exceed 8 printed pages." Não é estabelecido limite do número de palavras.
- **Título** de uma frase apenas com capitalização da primeira palavra; frase subordinada separada por dois pontos ou ponto-e-vírgula
- **Running head** com título curto, à direita, com abreviaturas
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- **Resumo** com um máximo de 250 palavras

- Títulos das **secções** e **subsecções** de primeiro, segundo e terceiro nível numa linha diferente, sem abreviaturas. Subsecções de quarto nível representadas como primeira linha do parágrafo respectivo.
- **Tabelas** identificadas no texto, entre parêntesis, apenas com a expressão "(Table *número*)"
- **Figuras** identificadas no texto, entre parêntesis, apenas com a expressão "(Figure *número*)"
- **Referências** seguindo as normas de Vancouver, numeradas segundo a ordem de aparecimento no texto

ANEXO

Excertos dos aspectos aplicáveis aos **artigos de revisão** das normas editoriais e de estilo da revista Haematologica, na sua versão mais recente (12 de Agosto de 2009).

Haematologica – Style sheet latest revision 12 ago. 09

Manuscripts

Review Articles are typically solicited by the Editors, but the journal may also consider reviews submitted on authors' own initiative: pre-submission inquiries are welcome. No particular format is required for these articles. However, they should have an informative, unstructured abstract of about 200 words, and ideally should not exceed 8 printed pages.

3.
Review articles should not simply go over or summarize general information which is already known. They should be introduced by a general summary of content in the form of an Abstract of no more than 250 words. A similar Abstract should be used to introduce Decision Making and Problem Solving and Progress in Hematology papers

The use of commercial names of drugs should be avoided. Drugs should only be referred to under their generic names unless different products are being compared, e.g.

Use deferiprone, not Ferriprox.

7.1.
American English.
Only American English spellings should be used, e.g.

randomized
harboring
labeled

1.Title

should consist of a phrase or a sentence. Question forms should be avoided. The title may be made up of one sentence or one sentence with a subclause using a colon or semi-colon, e.g.

> The Italian AICE-Genetics hemophilia A database: results and correlation with clinical phenotype

or two sentences e.g.

> Follow-up of healthy donors receiving granulocyte colony-stimulating factor for peripheral blood progenitor cell mobilization and collection. Results of the Spanish Donor Registry

In titles please capitalize the first letter of the sentence only, e.g.

> An update on multiple myeloma
> Not: An Update on Multiple Myeloma

Use of abbreviations	this is to be avoided in titles, headings and subheadings, e.g. use "acute myeloid leukemia" instead of AML and "myelodysplastic syndromes" instead of MDS. Abbreviations such as "RT-PCR" are acceptable.

Running heads

In the case in which the manuscript is the work of 3 or more authors, the first author (initial of first name, full stop, a space, full surname) should be presented as a left running head followed by "et al.", e.g.

A shortened title should be presented as a right running head, e.g.

> Deregulated miRNA in polycythemia vera

It is advisable to keep the running title as short as possible: you can use acronyms and abbreviations, e.g.

> An update on treatment of PTT

Main text

Headings

Headings should be presented on a separate line and should consist of a sentence without the use of abbreviations or acronyms (i.e. do not use CLL, PMF, GvHD, etc.).

Subheadings

The principal sections of text can be subdivided under subheadings in the form which the authors consider to be the most appropriate. These will be presented on a separate line.

Second- and third-level headings

These will also be presented on a separate line, however, fourth-level headings will consist of the first sentence of the related paragraph followed by a full stop. The next sentence of the paragraph will follow on immediately after a space.

Tables and Figures

The presentation of Tables and Figures (please do not use abbreviations such as "Fig." or "Tab.") should always follow the same order in which they are presented in the main text. All references to Tables and Figures should be presented in brackets and should only specify "Table" or "Figure" and the relevant identification number. Table and Figure titles and legends should not be used. Only include other information when absolutely essential, e.g.

(Figure 1)
(see Table 1 for a description of the process)

Tables

Title — A short descriptive title should be provided for each table, e.g.

Table 1. Distribution of IGHV families in HIV-NHL.

Structure — Tables should consist of a minimum three columns and three rows. These may include row headings.

Headings

Column headings
- each column should have a single column heading.
- all columns must have a heading on the first row although presentation of a column heading in the first column containing row headings is at the discretion of the author.
- if used, the column heading in the first column should be flush. All other column headings should be centered.
- column headings which span two or more column headings should be in the form of a brief title and are not to be grammatically linked to the related subheadings.

Row headings
- row headings should remain within the space provided in the first column.
- all row headings should remain within the space allocated for each row.
- row subheadings should be indented under the relative row heading.

All column and row headings should specify the units used in that column or row using brackets, e.g.

Age at diagnosis (years) or WBC ($x10^9$/L) or Hematocrit (%)

Footnotes

Footnotes should be presented according to the following order:

1. footnotes concerning general information
2. footnotes concerning abbreviations
3. footnotes with callouts

The full definition of all abbreviations used should be explained in the order in which they appear in the table:

1. column headings – left to right
2. row headings – top to bottom
3. cell data items – left to right from top to bottom

If the same abbreviations are later used in other tables, footnotes should carry a reference to the footnotes of the table in which the abbreviations concerned are first used, e.g.

Abbreviations are explained in Table 2.

Always use superscripted numbers for callouts of general, column, row or cell data. Capitalize first word of sentence and first word after a full stop.

[1]Representing all patients within each agents clinical trials; [2]possibly resulting from ventricular repolarization.

References

Please use the Vancouver style (http://www.icmje.org) for the formulation of the references; e.g.

should be numbered according to the order in which they are presented in the main text.