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REVIEW

# Gastrointestinal B-cell lymphomas: From understanding B-cell physiology to classification and molecular pathology

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## Abstract

The gut is the most common extranodal site where lymphomas arise. Although all histological lymphoma types may develop in the gut, small and large B-cell lymphomas predominate. The sometimes unexpected finding of a lymphoid lesion in an endoscopic biopsy of the gut may challenge both the clinician (who is not always familiar with lymphoma pathogenesis) and the pathologist (who will often be hampered in his/her diagnostic skill by the limited amount of available tissue). Moreover, the past 2 decades have spawned an avalanche of new data that encompasses both the function of the reactive B-cell as well as the pathogenic pathways that lead to its neoplastic counterpart, the B-cell lymphoma. Therefore, this review aims to offer clinicians an overview of B-cell lymphomas in the gut, and their pertinent molecular features that have led to new insights regarding lymphomagenesis. It addresses the question as how to incorporate all presently available information on normal and neoplastic B-cell differentiation, and how this knowledge can be applied in daily clinical practice (e.g., diagnostic tools, prognostic biomarkers or therapeutic targets) to optimalise the managment of this heterogeneous group of neoplasms.

## INTRODUCTION

The gastrointestinal (GI) tract is the most common site of extra-nodal lymphoma, accounting for 40% of cases. This is not surprising since the GI tract contains more native lymphoid tissue than that collectively present in all of the lymph nodes and spleen. Virtually all B-cell lymphoma-subtypes that arise in peripheral lymphoid organs may also occur as primary tumors of the GI tract. In contrast, GI Hodgkin's disease is quite rare, and although certain extranodal T-cell and NK-cell lymphomas can occur primarily in the GI tract, most nodal peripheral T-cell lymphomas rarely if ever present as primary GI lymphomas. Accurate diagnosis/classification of lymphomas based upon limited biopsy material obtained at endoscopy may be problematic for several reasons: the small size of endoscopic tissue-samples often precludes evaluation of lymphoid nodule architecture; hyperplastic mucosa-associated lymphoid tissue (MALT) may show reactive changes that simulate low-grade lymphoma, especially in fragmented specimens; and some immune-mediated inflammatory disorders, such as *Helicobacter pylori* (*H. pylori*)-related gastritis and celiac disease, evolve

*via* poorly defined transitional stages to lymphoma. Thus, molecular analysis has assumed an increasingly important role in the evaluation of GI lymphoid lesions and provides an important adjunct to careful clinical evaluation and histologic assessment of mucosal biopsy samples.

## NORMAL B-CELL MATURATION

B-lymphocytes elaborate a multitude of antibodies with diverse antigen-binding specificities, and are produced during two successive stages: a primary B-cell repertoire is first generated in the bone marrow, while antigen encounter in the peripheral lymphoid organs (spleen, lymph nodes and MALT) will lead to a secondary B-cell repertoire (Figure 1)[1].

The primary B-cell repertoire emerges from committed hematopoietic stem-cells and is determined by transcriptional events involving numerous factors, including PU.1, Ikaros, E-box-factor-2A, early-B-cell-factor (also known as OLF1), paired-box-protein-5 (PAX5, also known as BSAP) and Forkhead-box-protein-P1 (FOXP1)[2,3]. It requires a highly regulated series of genetic events, so called immunoglobulin (Ig) gene-rearrangements, that result in the membranous expression of a functional B-cell receptor[4]. The variable regions of the Ig heavy-chain are assembled from 123 variable (V), 27 diversity (D) and 6 junctional (J) genes present on chromosome 14 at the Ig heavy-chain gene locus. The variable regions of the Ig light-chains are assembled from V and J elements at either the *Igκ* or *Igλ* gene locus on chromosome 2 and 22, respectively. As there are many different V-, D- and J-segments in the germline, each B-cell has the potential to generate a unique antibody that is expressed on subsequent progeny. The earliest B-cell found in bone marrow, so called pro-B-cells, are transformed into early pre-B-cells following recombination of the $D_H$ and $J_H$ gene-segments in the Ig heavy-chain locus. Subsequent rearrangements attach one of the $V_H$ gene-segments to the $D_H$-$J_H$-segment and give rise to late pre-B-cells, which express a functionally rearranged $V_H$-$D_H$-$J_H$-Cμ-chain on the cell-surface. Subsequent rearrangement of the Ig light-chain gene-locus leads to surface expression of a complete IgM-molecule, at which time the cell is designated an immature B-cell. Immature B-cells undergo alternative splicing of Ig heavy-chain mRNA to become mature B-cells that express both IgM and IgD.

These mature B-cells, considered naïve because they have not yet encountered an antigen, migrate to secondary lymphoid organs, including the GI MALT, where they form primary follicles. Here they transform into large B-cells following antigenic stimulation, after which most proliferate and differentiate into short-lived, IgM-producing plasma-cells[5]. A few B-cell blasts migrate into the centre of the follicle where they proliferate and differentiate into germinal centre (GC) B-cells[6]. Naïve B-cells that are not triggered to differentiate by antigen exposure are pushed aside and form the mantle surrounding the GC. Follicles containing a GC and mantle are called secondary B-follicles.

GC B-cells undergo randomized introduction of mutations in the Ig-gene that encode the antigen-binding site and this process is termed somatic hypermutation (SHM)[7]. B-cells with unfavourable mutations will not bind with high affinity to antigens and consequently, will not appropriately interact with nor receive survival signals from the GC T-cells. In fact, more than 90% of the GC B-cells die as a result of apoptosis. Positively selected GC B-cells that do survive activate T-cells to express CD40-ligand (CD40L) and secrete interleukin (IL)-4 and IL-10. These molecules induce a class switch recombination (CSR) from IgM and/or IgD to IgG, IgA, or, less commonly, IgE[8]. B-cells in the gut will preferentially switch to IgA. Although SHM and CSR display distinct molecular features, they are mediated by a shared pathway that is controlled by a B-cell-specific enzyme: activation-induced cytidine deaminase (AID)[9].

Selected GC B-cells may either undergo subsequent round(s) of SHM, CSR and positive selection, or differentiate into post-GC B-cells (plasma-cells or memory B-cells). Plasma-cell differentiation requires the continued presence of B-lymphocyte-induced-maturation-protein-1 and X-box-binding-protein-1 in the absence of B-cell-lymphoma-6 (BCL6), PAX5 and metastasis-associated-1-family-member-3[10]. Alterations in these transcription factors results in plasma-cell death or dedifferentiation. The CD40/CD40L-mediated interaction with GC T-cells in an IL-4-rich microenvironment is important for directing GC B-cells toward the memory B-cell pathway[11]. Memory B-cells reside in a particular zone of the B-follicle, the marginal zone, which is the outer part of the follicular mantle.

## CLASSIFICATION OF GI B-CELL LYMPHOMAS

Lymphoma classification has evolved over the past decades and now incorporates a combination of morphology, immunophenotype and genetic aberrations that correlates well with clinical presentation, disease course and overall prognosis. The 2008 WHO classification is now widely accepted and has enabled clincians to better understand GI lymphomas[12].

### *MALT-lymphoma*

MALT-lymphoma is a low-grade B-cell lymphoma that preferentially affects the stomach. It accounts for 5%-10% of all GI malignancies and at least 50% of gastric lymphomas, making it the most frequent GI lymphom[13]. MALT-lymphomas show differentiation and immunophenotypic features similar to those of marginal zone B-cells. Gastric MALT-lymphoma is etiologically linked to chronic *H. pylori* infection. Presumably, sustained antigenic stimulation by *H. pylori* triggers a polyclonal B-cell proliferation and attracts neutrophils to the site of inflammation, with subsequent release of reactive oxygen species. These molecules are genotoxic and cause a wide range of genetic abnormalities that accumulate as lym-

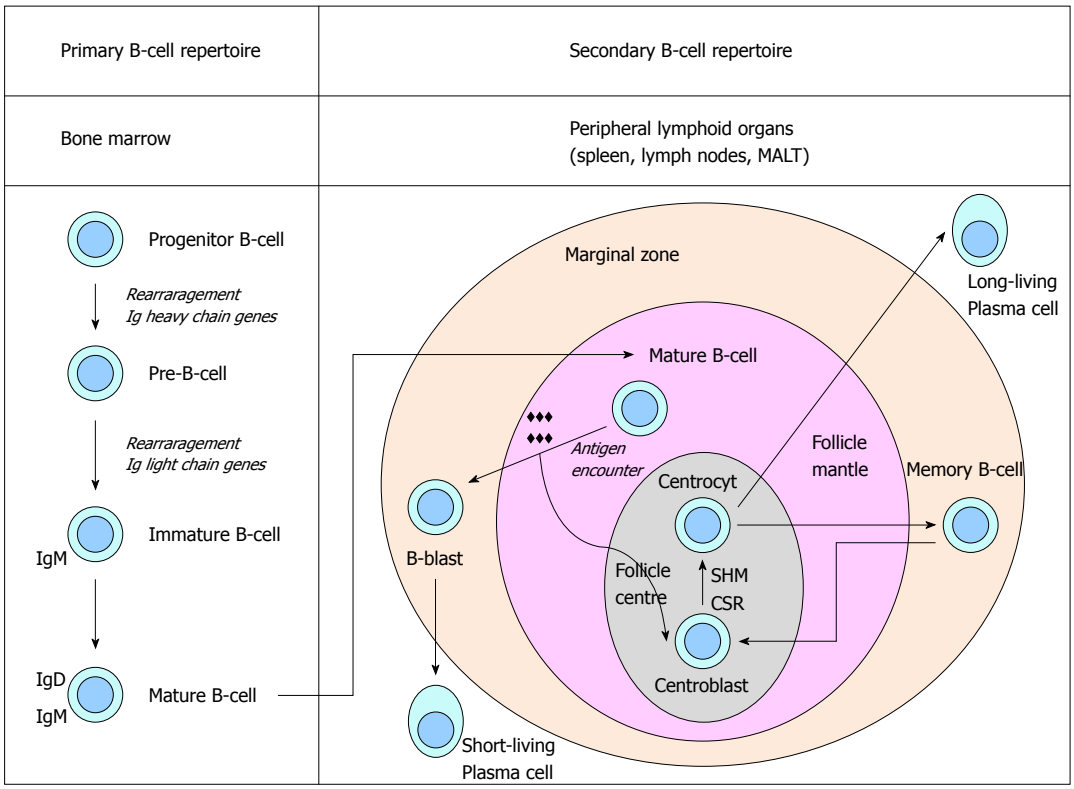


**Figure 1 B-cell development**. Schematic representation of the events in the development of primary and secondary B-cell repertoire. Ig: Immunoglobulin; CSR: Class switch recombination; SHM: Somatic hypermutation.

phocytes proliferate[14]. Prolonged B-cell proliferation also increases the risk of various types of DNA-damage, such as double-strand DNA-breaks, due to intrinsic genetic instability of B-cells during SHM and CSR[15]. Characteristic genetic changes of MALT-lymphomas include translocations t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21) and t(3;14)(p13;q32), which result in *BIRC3-MALT1, IGH-BCL10, IGH-MALT1* and *IGH-FOXP1* rearrangements respectively[16-18]. Although the t(11;18)(q21;q21) translocation is the most frequent molecular anomaly encountered in gastric MALT-lymphomas, the genes targeted by three of these translocations (*BCL10* and *MALT1*) participate in the same signalling pathway that drives activation of nuclear factor κB (NF-κB)[19,20]. This molecule is a key transcription factor in immune responses. It regulates expression of several survival- and proliferation-related genes in B-cells. Thus, its constitutive activation results in uncontrolled B-cell proliferation and lymphomagenesis.

Demonstration of a monoclonal rearrangement of the Ig heavy- and/or light-chain gene(s) by molecular analysis is an important adjunct to the diagnosis of MALT-lymphoma because morphologically, these tumors often contain a polymorphous mixture of centrocyte-like cells, small lymphocytes, plasma-cells, and scattered large blasts that mask the presence of neoplastic marginal zone B-cells (Figure 2A). The tumor cells frequently surround reactive GC, as well as invade and destroy the overlying GI epithelium, resulting in lymphoepithelial lesions (Figure 2B). The immunophenotype of MALT-lymphoma is similar to that of normal marginal zone B-cells, with positive immunostains for surface Ig (strongly IgM positive), pan B-cell markers (CD20, CD19, CD79a), and complement receptors (CD21, CD35). However, there are no specific biomarkers for MALT-lymphomas, with the exception of t(11;18)(q21;q21) which can be demonstrated in either fresh-frozen or paraffin-embedded tumor tissue by molecular techniques. Detection of t(11;18)(q21;q21) may facilitate and/or confirm the diagnosis of MALT-lymphoma, although current guidelines do not recommend routine screening for t(11;18)(q21;q21) once the diagnosis of gastric MALT-lymphoma is established since its identification has no bearing on treatment or outcome. All patients with *H. pylori*-positive gastric MALT-lymphoma should undergo eradication therapy, regardless of their t(11;18)(q21;q21)-status[19-21]. Importantly, the presence of a t(11;18)(q21;q21) in a MALT-lymphoma does not exclude the possibility of progression to diffuse large B-cell lymphoma (DLBCL)[22], despite earlier literature claiming the contrary. In general, MALT-lymphomas are indolent lymphomas with a 5-year survival rate of approximately 95%. Once progression to DLBCL occurs, data on survival are conflicting: in the early 1990s, two retrospective studies reported 5-year survival of 42% and 56%, respectively[23,24], while prospective studies conducted 10 years later showed no or only a marginal difference in overall survival between gastric MALT-lymphoma and DLBCL[25,26]. The mechanism by which low-grade MALT -lymphomas transform to DLBCLs are unknown, although some studies have implicated *FOXP1,* a transcription factor, as a key molecule in this process[27].

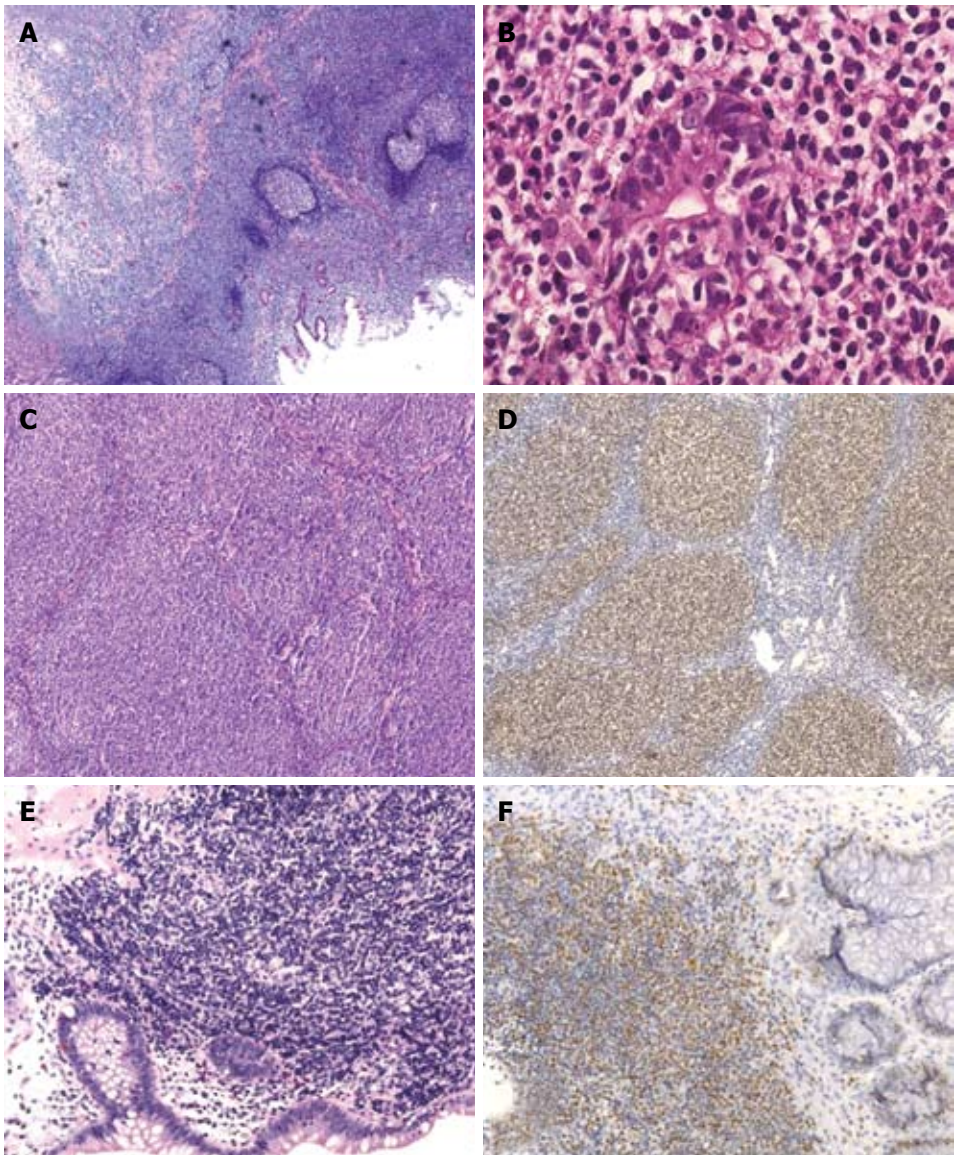


**Figure 2 Histology of gastrointestinal mucosa-associated lymphoid tissue, follicular and mantle-cell lymphomas.** A: Hematoxylin eosin (HE) staining of a gastric mucosa-associated lymphoid tissue lymphoma demonstrates presence of reactive follicles surrouned by a neoplastic lymphoid infiltrate (magnification 50 ×); B: Destruction of a gastric gland by the neoplastic B-cellss: lymphoepithelial lesion (magnification 400 ×); C: HE staining of a duodenal follicular lymphoma highlights the presence of aberrant follicular growth pattern (magnification 100 ×); D: Aberrant B-cell-lymphoma-2 expression by a duodenal follicular lymphoma (magnification 100 ×); E: HE staining of a mantle-cell lymphoma in the colon (magnification 200 ×); F: Aberrant cyclin D1 expression by an intestinal mantle-cell lymphoma (magnification 100 ×).

### *Follicular lymphoma*

Follicular lymphoma is the most common subtype of non-Hodgkin's lymphoma in the Western world[6]. Most cases are initially detected in the lymph nodes. Involvement of extranodal sites, including the GI tract, usually occurs following dissemination of nodal disease[28]. Primary GI follicular lymphoma accounts for less than 3% of GI lymphomas, but it is the second most common lymphoma subtype of the small intestine and the most common type of duodenal lymphoma[29,30]. Follicular lymphomas represent the neoplastic counterpart of GC B-cells, so they display a follicular architecture (Figure 2C) and contain variable numbers of centroblasts and centrocytes. Distinguishing these tumors from MALT-lymphoma may be difficult, especially in cases of MALT-lymphoma with colonization of GC by tumour cells. In contrast to colonized reactive GCs, however, the neoplastic GC B-cells of follicular lymphomas aberrantly express BCL2 (Figure 2D) as well as CD10 and BCL6. BCL2 is an anti-apoptotic and oncogenic protein and its overexpression often reflects the presence of a *BCL2*-gene-rearrangement that occurs *via* a t(14;18)(q32;q21)-translocation. These *BCL2*-rearrangements are detected in 85%-90% of all follicular lymphomas and, thus, detection of abnormal BCL2 immunoexpression or *BCL2-IGH* demonstration by molecular pathology distinguishes follicular from MALT-lymphoma[31]. Of interest and in contrast to systemic follicular lymphomas, primary duodenal follicular lymphomas frequently express IgA as well as the $\alpha 4\beta 7$ mucosal homing receptor (suggesting an origin from local antigen-driven B-cells) and lack AID expression[32,33]. Clinically, primary GI follicular lymphoma behaves remarkably indolent, which, even left untreated,

does not develop tumorous growth, only rarely disseminates and does not transform into high grade disease. As such, in the absence of documented disease progression, a watch and wait approach appears to be the most sensible strategy[32-35].

### Mantle-cell lymphoma

Mantle-cell lymphoma is an intermediate-grade B-cell neoplasm that predominantly occurs in older male patients who present with systemic disease involving the peripheral lymph nodes, spleen and bone marrow[36]. It shows a striking tendency to affect the lower GI tract (30% of patients) but only represents the primary disease site in a minority of patients. Mantle-cell lymphoma accounts for no more than 10% of all GI lymphomas. It may produce multiple intestinal polyps, termed lymphomatous polyposis[37]. This type of B-cell lymphoma features aberrant CD5 expression, simulating the phenotype of naïve B-cells in the follicle mantle (1). Nearly 90% of GI mantle-cell lymphomas display cyclin-D1 overexpression due to a t(11;14)(q13;q32) which juxtaposes the heavy-chain Ig-encoding region on chromosome 14q32 next to the *CCND1*-oncogene on chromosome region 11q13[38]. *CCND1* encodes cyclin-D1, which is an important regulator of the $G_1$-S transition phase in the cell cycle. Uncontrolled overexpression of cyclin-D1 leads to B-cell growth and mantle-cell lymphomagenesis[39]. Morphologically, mantle-cell lymphomas are more monomorphic than MALT-lymphomas, are devoid of immunoblasts and centroblasts, and lack plasmacytic differentiation (Figure 2E). Recognition of mantle-cell lymphoma is straightforward in most cases of lymphomatous polyposis, but early tumors show subtle endoscopic findings, in which case a histologic diagnosis may prove difficult. Most mantle-cell lymphomas demonstrate strong nuclear staining for cyclin D1 (Figure 2F), although this feature is not entirely specific as it is also present in 2% of DLBCLs. Molecular tests that investigate the presence of t(11;14)(q13;q32) in biopsy material are useful in challenging cases. Primary GI tract mantle-cell lymphomas have a poor prognosis with a median survival of 3-4 years[37]. Chemotherapy is the treatment of choice for these patients, although historic data showed its limited efficacy. Newer approaches, including addition of anti-CD20 (Rituximab) to conventional chemotherapy and myeloablative therapy with stem-cell transplantation support, have significantly improved outcome, but it still remains an incurable lymphoma[40,41].

### DLBCLs

DLBCLs frequently arise at extranodal sites, including the gut, and are composed of a diffuse infiltrate of large transformed blasts with the morphology of centroblasts and/or immunoblasts (Figure 3A and B). Uncommon morphologic variants include T-cell/histiocyte rich and anaplastic B-cell lymphomas[42]. These tumors develop *via* a variety of mechanisms. Some represent transformed small B-cell non-Hodgkin's lymphomas (MALT-, follicular or mantle-cell lymphomas) but most arise *de novo* as a result of various genetic events, including *BCL6*-gene rearrangements and mutations[43,44]. Gastric DLBCLs may contain a low-grade MALT lymphoma in the adjacent mucosa and may feature prominent lymphoepithelial lesions. Some of these cases have been previously classified as "high grade" or "blastic" MALT lymphomas, but the current WHO classification discourages the use of these terms and recommends labeling tumors comprised of solid sheets of B-cell blasts as a DLBCL[12]. Microarray analyses have revealed biological subtypes of DLBCL[45,46]. One category is characterized by a gene expression signature resembling that of GC B-cells. Another has a genotype similar to that of activated post-GC B-cells (ABC). Distinguishing between these subtypes of DLBCL may be important because GC-type DLBCLs have a better prognosis than ABC-type tumors[45,46], but also because the latter may benefit from therapies targeting the NF-κB pathway[47]. Because gene expression profiling cannot be routinely used to sub-classify these tumors, immunohistochemistry seems the best option due to practical/economic reasons. Various immunohistochemical algorithms have been developed to replicate the microarray results and/or stratify patients according to survival. The most helpful panel of markers that may be used for these purposes includes BCL6, CD10, FOXP1, multiple myeloma oncogene 1, interferon regulatory factor 4 and germinal center B cell-expressed transcript-1[48]. However, use of these markers to stratify DLBCL into prognostic groups remains controversial[48]. An optimal treatment strategy specific for primary GI DLBCL has not been established yet and remains controversial. Various treatment protocols have been tried, such as systemic chemotherapy similar to that used to treat nodal DLBCL[49,50]. Recently, it was shown that these tumours may benefit from surgical resection followed by chemotherapy (with an acceptable quality of life deterioration because of the invasive surgical procedure)[51].

### Burkitt lymphomas

Burkitt lymphomas are among the most proliferative of all human malignancies, and are endemic in regions with high rates of neonatal Epstein-Barr virus (EBV) infection and malaria, such as equatorial Africa, where they clinically present as jaw tumours[52]. Systemic disease may also occur among AIDS patients, but patients who lack immunodeficiency may rarely develop tumors as well[53,54]. Sporadic Burkitt lymphomas tend to affect young patients and cause symptoms of small bowel obstruction due to tumour development in the ileocaecal region. These tumours grow as sheets of medium-sized lymphoblasts with a neoplastic "starry sky" appearance reflecting the presence of numerous pale macrophages removing apoptotic debris[55] (Figure 3C and D). The tumour-cell nuclei are medium-sized with thick rims of chromatin and multiple prominent nucleoli. Their contours are round to ovoid without cleaves or folds. The cytoplasm of the tumour-cells is basophilic reflecting an abundance

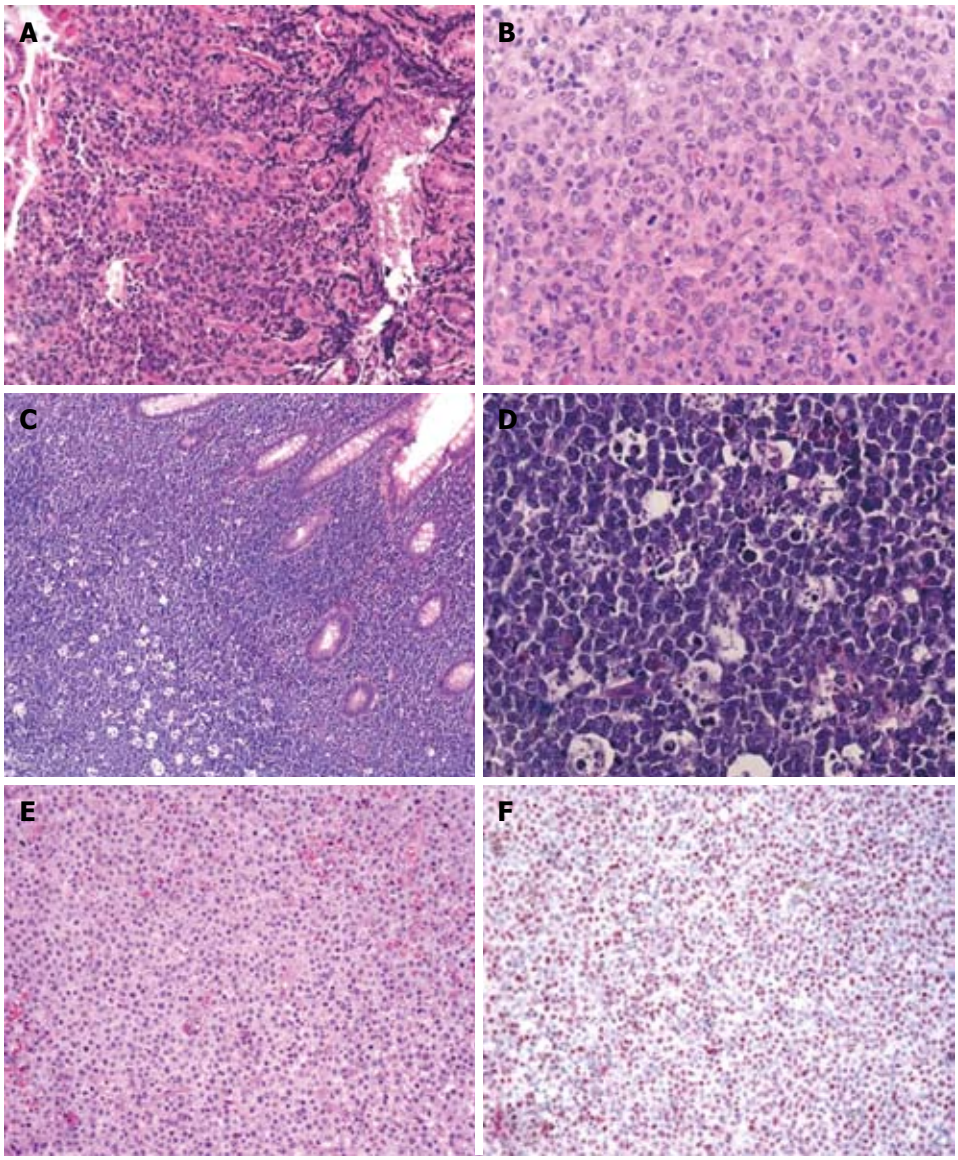


**Figure 3 Histology of other gastrointestinal B-cell lymphomas.** A: Hematoxylin eosin (HE) staining of a gastric diffuse large B-cell lymphoma (magnification 200 ×); B: Polymorphic appearance of the large tumor B-cells in a gastric diffuse large B-cell lymphoma (magnification 400 ×); C: HE staining of an intestinal Burkitt lymphoma with the typical "starry sky" appearance (magnification 100 ×); D: Presence of multiple pale macrophages filled with apoptotic debris in an intestinal Burkitt lymphoma (magnification 400 ×); E: HE staining of an intestinal monomorphic post-transplantation lymphoproliferative disorders (PTLD) (magnification 200 ×); F: *In situ* hybridization demonstrates presence of EBV-encoded RNA (= red colored nuclei) in the neoplastic cells of an intestinal monomorphic PTLD (magnification 100 ×).

of polyribosome with clear vesicles that represent lipid droplets. Classical Burkitt lymphoma is genetically characterized by a *C-MYC* translocation, usually t(8;14)(q24;q32), which places *C-MYC* under control of the Ig heavy-chain gene enhancer. Rarer variants, including three-way translocations, t(8;14;18), t(2;8)(p12;q24) and t(8;22)(q24;q11), have been described, all of which involve *C-MYC* and the *Igκ* or *Igλ* light-chain genes respectively[56]. Demonstration of *C-MYC* rearrangement by molecular genetics is an important tool in distinguishing Burkitt lymphoma from DLBCL since adults require different treatment regimens for these entities[57,58]. Some B-cell lymphomas used to be classified as 'Burkitt-like' lymphoma in the Revised European-American Lymphoma classification of 1994. However, the 2008 WHO classification abandoned the term Burkitt-like because it was used inconsistently for a variety of lymphomas, including atypical Burkitt lymphomas and DLBCLs with high proliferation rate[12]. Burkitt lymphomas remain a highly curable condition when treated with high doses of systemic chemo- and immunotherapy.

### *Post-transplantation lymphoproliferative disorders*

Post-transplantation lymphoproliferative disorders (PTLDs) represent explosive B-cell proliferations that develop as a result of immunosuppression and immunomodulation among transplant recipients. They often arise in extranodal sites, particularly in the transplanted organs themselves and in the GI tract[59]. Risk factors for development of PTLD include mismatched EBV serologic status, such as transplant of a donor organ from a seropositive donor into a seronegative patient, and intensive drug-induced immunosuppression[60,61]. The prevalence of PTLD de-

pends on the type of organ transplantation performed. It occurs in 0.5% of bone marrow recipients, 1%-2% of liver transplant patients, 0.7%-4% of renal transplant patients, 2%-10% of patients with heart transplants, and up to 30% of patients who undergo small bowel transplantation[59]. PTLDs are heterogeneous, ranging from reactive hyperplasia over polymorphic B-cell proliferations to monomorphic lymphomas (Figure 3E), usually of B-cell type. This heterogeneity is reflected in the current WHO classification of PTLDs[12]. Early forms of PTLD and polymorphic PTLD may respond to reduction of immunosuppression, but only 10% of monomorphic proliferations respond to this mode of treatment[62]. For this reason, recognition and classification of these lesions is very important. Clues to the diagnosis include awareness of the clinical history and histologic detection of nuclear polymorphism, scattered apoptotic cells, variable plasmacytic maturation, multilobated immunoblasts reminiscent of those seen in acute infectious mononucleosis, and geographic necrosis[60,61]. Most PTLD proliferations are EBV driven, so demonstration of EBV viral proteins using immunostains (e.g., latent membrane protein) or *in situ* hybridization for EBV-encoded RNA (Figure 3F) may aid the diagnosis, even in small endoscopic biopsy samples[59,63]. While cases of polymorphic PTLD are usually clonal at the molecular level, clonality studies are useful in distinguishing polymorphic B-cell lymphoma from reactive lymphoid proliferations. Similar lymphoproliferative disorders are observed in HIV patients, congenital immunodeficiency syndromes, or after administration of immunomodulatory drugs in autoimmune disorders like rheumatoid arthritis.

## MOLECULAR PATHOLOGY OF GI B-CELL LYMPHOMAS

A variety of molecular changes in GI lymphomas can be investigated using routine molecular techniques, namely polymerase chain reaction (PCR), real-time PCR (RT-PCR), karyotyping, and fluorescence-*in-situ* hybridization (FISH). Evaluation of the presence or absence of Ig-gene-rearrangements is most commonly performed using a PCR-based assay followed by the demonstration of lymphoma-specific translocations using (RT-)PCR, karyotyping, or FISH.

Detection of the same Ig heavy- and light-gene rearrangements in a B-cell population by PCR confirms the clonal nature of the B-cells, which does not always correspond to a neoplastic lesion. These assays rely on amplification of the rearranged variable region of the Ig heavy-(or light-)chain gene in tissue samples, followed by analysis of product size by a capillary electrophoresis[64,65]. Reactive or polyclonal B-cell populations produce numerous antibodies marked by different V(D)J-segments. Amplification of the rearranged Ig heavy-(or light-)chain gene variable region results in a range of products of different sizes, which appear as a broad smear or ladder on a gel. In contrast, monoclonal B-cells produce the same antibody marked by the same V(D)J-segments, and, thus, give rise to one or two dominant PCR-products (Figure 4A). These days, most labs use multiplex PCR protocols to detect B-cell clonality: the rearranged Ig heavy-(or light-)chain gene variable regions are amplified using multiplex Biomed-primers and analysed by a Genescan or heteroduplex analysis, which will result in a Gaussian curve or a peak in the case of a polyclonal or monoclonal B-cell population respectively (Figure 4A and B).

Most molecular changes in (GI) lymphomas involve chromosomal translocations or inversions and lead to juxtaposition of genes that are normally separate. These translocations may have either a qualitative or quantitative effect on gene expression. Translocations that cause gene disruption and recombination generate novel gene-sequences that encode chimeric oncoproteins. An example of these qualitative changes includes the *BIRC3-MALT* fusion/t(11;18)(q21;q21), which occurs in approximately 25% of all gastric MALT-lymphomas[13] (Figure 5A). A translocation that juxtaposes enhancers, or promoters, of constitutionally highly expressed genes results in inappropriate expression of an intact gene. Examples of such quantitative changes include overexpression of *BCL2*, *CCND1* and *CMYC* as a consequence of translocation of these genes to the adjacent Ig heavy-chain gene enhancer. These changes occur in t(14;18)(q32;q21), t(11;14)(q13;q32) and t(8;14)(q24;q32) associated with follicular, mantle-cell and Burkitt lymphoma, respectively (31;38;54), and may be demonstrated in tissue samples by either (RT-)PCR, karyotyping or FISH.

### *(RT-)PCR*

The PCR-based approach to molecular analysis is useful because it can demonstrate B-cell monoclonality as well as detect lymphoma-associated translocations by amplifying the fusion gene[66]. RT-PCR not only amplifies target gene-sequences, but may be used to quantify gene expression[67]. Amplified DNA is detected as the reaction progresses in real time, whereas the product is detected at the end of standard PCR. Labeled probes bind to their target DNA-sequences which are not detected as long as the probe is intact. Upon initiation of PCR, however, the probe is destroyed and the label is released and measured. The suitability of (RT-)PCR analysis for detection of lymphoma-associated translocations is limited when chromosomal breakpoints are spread over a large genomic region, as is the case for t(11;14)(q13;q32) involving the *BCL1*-gene and t(11;18)(q21;q21) involving the *MALT1*-gene[68,69] (Figure 5A). Anomalies such as t(14;18)(q32;q21) may also be found in healthy individuals, so the high sensitivity of RT-PCR can yield false-positive results[70].

### *Karyoytyping*

Conventional karyotyping describes the number and appearance of chromosomes under a light microscope. Features assessed include chromosome length, position of centromeres, differences between sex chromosomes, and any other physical characteristic. Freshly prelevated

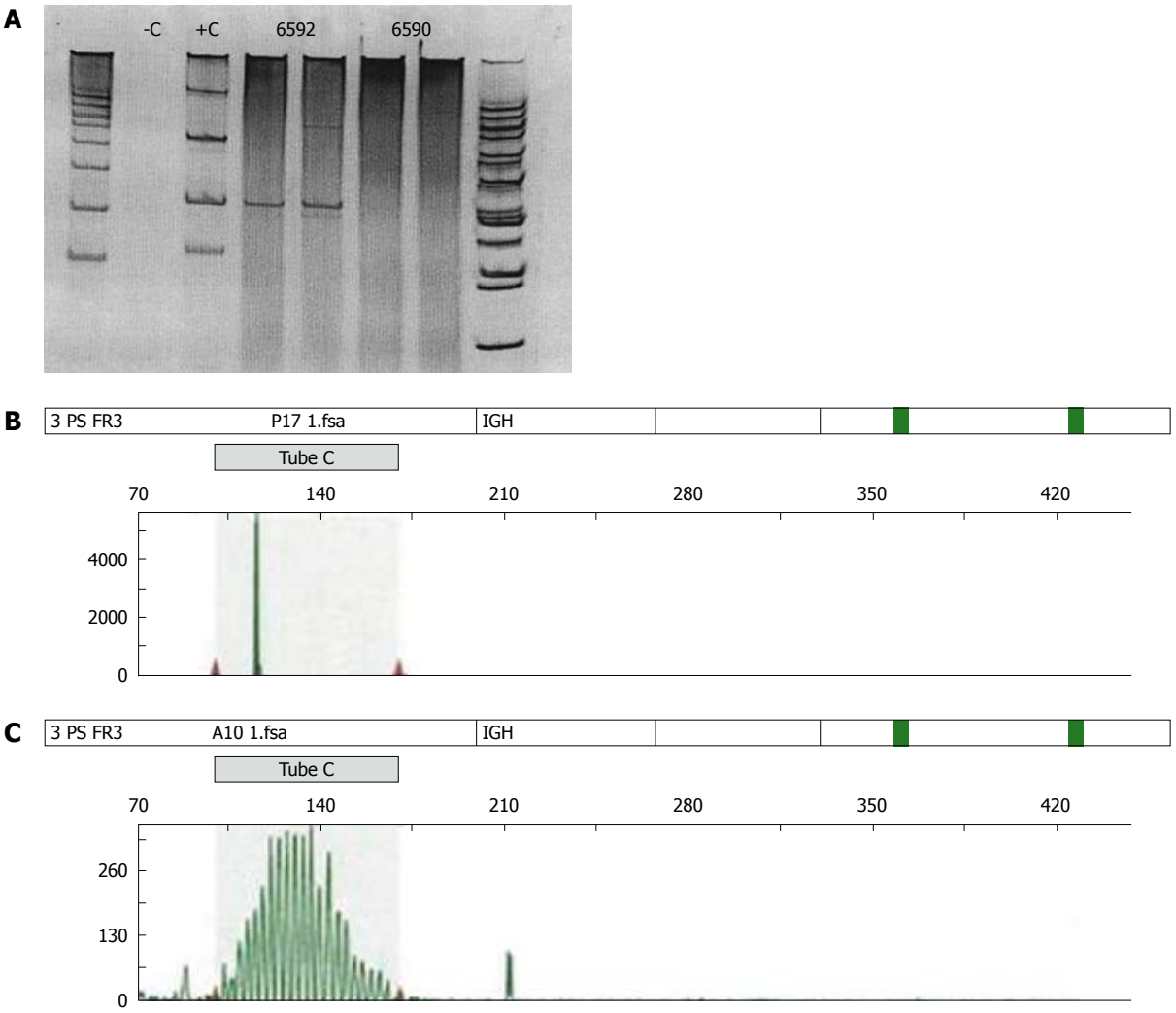


**Figure 4 Polymerase chain reaction.** A: Polyclonal B-cell pathology results in a broad smear of the polymerase chain reaction (PCR) product on a gel, while a monoclonal pathology (lymphoma) will give rise to a sharp band on a gel, hereby reflecting PCR products that have the same size. +C: Positive control; -C: Negative control; 6592: Tumor case; 6590: Reactive case; B: Gaussian curve in a polyclonal B-cell population examined by multiplex PCR; C: Peak in a neoplastic B-cell clone examined by multiplex PCR.

tumor cells are brought into culture, stimulated to proliferate by addition of growth factors, and forced to enter metaphase, which is the mitotic stage during which highly coiled chromosomes align in the middle of the cell before being separated into each of two daughter cells. Mitosis is arrested using a colchicine solution, the preparation is squashed on a glass slide, and the chromosomes are histochemically stained using a Giemsa or similar stain resulting in a characteristic banding pattern. The chromosomes are then photomicrographed and arranged by trained technicians in a karyogram that facilitates evaluation for structural abnormalities. Recently, the ability of conventional metaphase cytogenetics to detect genome-wide genetic changes has been expanded by the introduction of multicolour painting of the chromosomes by using mixtures of different fluorochromes, which allows easier detection of structural chromosomal aberrations[71,72]. However, the time-consuming nature of this type of analysis prohibits its use for routine diagnosis in non-specialized centers. Also, karyotyping is useless when dividing tumor cells cannot be obtained as a consequence of lack of sufficient, fresh material or limited tumor load in the biopsy (e.g., endoscopic GI biopsies). This disadvantage of conventional cytogenetics has led to the creation of genome-wide methods that do not need dividing tumor cells, such as FISH, conventional comparative genetic hybridization (CGH) and its successor array CGH[73,74]. The latter 2 are out of scope of this review as they are only applied in specialized academic centres.

### *FISH*

FISH is a cytogenetic technique that detects specific DNA-sequences with (in)directly fluorescent-labeled DNA-probes that bind only to chromosomal regions with which they share a high degree of sequence similarity. Two probe categories are used for detection of translocations: dual-fusion probes and break-apart pobes[75]. These probes differ in terms of the type of information they yield, their sensitivity, and ease of interpretation. Dual-fusion probes consist of pairs of probes labeled with different

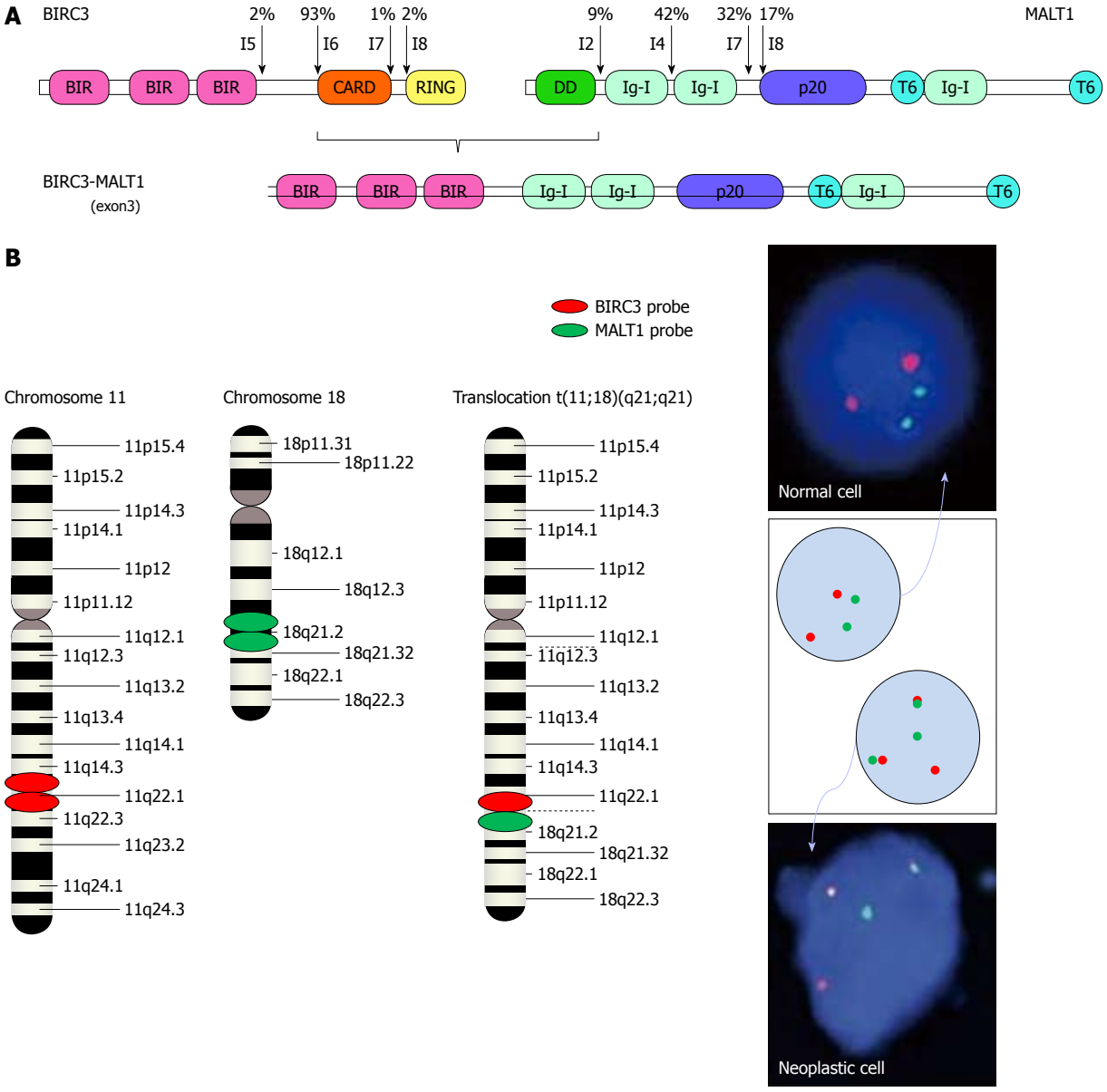


**Figure 5 Fusion protein BIRC3-MALT1 in mucosa-associated lymphoid tissue lymphomas.** A: Known break points (arrows) in *BIRC3* and *MALT1* are shown with their frequencies. The break points within *BIRC3* almost always occur in I6 (according to Ensembl Gene ENSG00000023445), whereas those within *MALT1* are located in I2, I4, I7 and I8, which result in four possible versions of the *BIRC3-MALT1* fusion gene: *BIRC3*(I6)-*MALT1*(I2), *BIRC3*(I6)-*MALT1*(I4), *BIRC3*(I6)-*MALT1*(I7) and *BIRC3*(I6)-*MALT1*(I8). The fusion gene depicted is the *BIRC3*(I6)-*MALT1*(I4) version. BIR: Baculovirus inhibitor of apoptosis repeat; CARD: Caspase recruitment domain; DD: Death domain; I: Intron; Ig: Immunoglobulin-like; p20: Caspase-like p20 domain; RING: Really interesting new gene; T6: Tumor necrosis factor receptor associated factor 6 binding site; B: Interphase FISH with dual-fusion probes results in 2 separate red and 2 separate green signals in normal lymphocytes, whereas t(11;18)(q21;q21)-positive MALT lymphoma cells will display 1 red and 1 green signal (that represent the normal loci) accompanied by 2 pathological fused red/green signals.

colors, each of which binds to a different chromosome. Dual-fusion probes are designed to span the breakpoint-region in the two genes involved in a reciprocal translocation. For example, differently colored probes binding to the *BIRC3* (red) and *MALT1* (green) genes are used to detect t(11;18)(q21;q21) in gastric MALT-lymphomas[76] (Figure 5B). Two separate red and two separate green signals should be visible in normal cells, whereas a t(11;18)(q21;q21)-positive MALT-lymphoma will generate two fused red/green signals that may appear as a single yellow signal, accompanied by one red and one green signal that represent normal loci. Break-apart probes also consist of pairs of two differently colored individual probes. Each probe binds to sequences flanking the known breakpoint-region in a locus of interest. Two sets bicolored fused signals will be visible in a normal diploid cell and represent the two alleles. Abnormal diploid cells in which one allele has been split by a translocation will show a separated signal in addition to the normal fused signal. FISH is now a firmly established technique in the diagnosis and assessment of lymphoid malignancies[69,77,78]. In contrast to karyotyping, FISH does not require fresh material and dividing cells, labor and time-intensive manual preparation, or analysis of slides by a technolo-

gist and it can be used on paraffin-embedded material. Its main disadvantage is the limited number of commercially available probes. This assay will only provide information about the probe being tested, but other aberrations will not be detected.

## CONCLUSION

The adequate diagnosis and classification of a lymphoid lesion in an endoscopic biopsy of the gut may challenge clinicians, mainly because of the small size of endoscopic tissue samples, but also because of reactive conditions that may mimic lymphomas. By understanding normal B-cell physiology and the pathways that lead to B-cell lymphoma development, one may have insight how this knowledge was translated into the application of morphological, immunohistochemical and especially molecular-genetic tests (PCR, cytogenetics, FISH). These tests have assumed an increasingly important role in the evaluation of GI lymphoid lesions and provides an important adjunct to classical histological assessment of mucosal biopsy samples.

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