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Nodal aggressive non-Hodgkin’s lymphoma in the adult: a review article

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ABSTRACT

Background. The term aggressive lymphoma is a clinical definition encompassing different lymphoid tumors which are potentially curable, but run a rapidly fatal course if not properly treated. Under this definition, no longer quoted in the REAL/WHO Classification, several histotypes are included that differ remarkably in cell size and morphology, phenotypic and molecular characteristics, kinetics and clinical presentation. Tailored approaches have been designed aiming to adapt therapy to each specific histotype.

Aim and design. Since aggressive lymphomas account for the majority of lymphoid neoplasms, and because some variation exists as to their categorization and treatment, the authors will discuss the more recent and consolidated findings in the etiology, pathogenesis, histopathology, diagnostics and therapy of such tumors based on both the evidence in the literature and their own expertise. In particular, each tumor type is individually discussed, as are the most critical areas in patient management.

Conclusions. An updated review of more recent biological and therapeutic aspects is intended to assist the hematologist, pathologist and practitioner in the optimal management of patients with aggressive lymphoma.

Key words: aggressive non-Hodgkin lymphoma, chemotherapy, chemoimmunotherapy, supportive therapy.

Aggressive non-Hodgkin lymphomas (NHL) can be of B or, less frequently, T-cell origin and are characterized by large lymph node masses and/or disseminated disease. In contrast to indolent neoplasms, a sizable fraction of aggressive lymphomas can be cured, but without treatment the disease is rapidly fatal.

In the present report, the reviewers’ panel will discuss the more recent and consolidated findings in the etiology, pathogenesis, histopathology, diagnostics, and therapy of the following disease entities diffuse large B-cell lymphoma (DLBCL), follicular lymphoma grade IIIb (FCLIIIb), Burkitt’s lymphoma (BL), lymphoblastic lymphoma (Lb-L), mantle cell lymphoma (MCL), transformed lymphoma originating from a low grade disease, peripheral T-cell lymphomas (PTCL), and the post-transplant lymphoproliferative disorders (PTLD).

Epidemiology

During the last five decades, the mortality from NHL has increased significantly from a rate of 3.2 deaths/100,000 person-years, to a rate of 7/100,000 person-years.1-5 Because mortality rates are sensitive to improvements in survival, but are less sensitive to improvements in diagnostic strategies, the increase of the mortality rate for NHL provides solid evidence for the increase in incidence of these neoplasms. At present, in Europe and in the USA, the annual incidence of all NHL is estimated to be 15-20 cases/100,000.1-5 Based on the data of the International Non-Hodgkin’s Lymphoma Classification study, aggressive NHL in western countries account for approximately 50% of all NHL. In particular, the entity known as DLBCL alone accounts for approximately 30% of all NHL. The median age of aggressive NHL considered as a whole falls between the sixth and seventh decade, although some specific types of aggressive NHL present at a lower median age, as exemplified by BL (median age 30 years). The distribution of aggressive NHL is overall similar between the two sexes (male:female ratio=1.5). One exception is MCL, which shows a marked predilection for the male sex (>70% of cases).
Etiology and pathogenesis

Causes and predisposing factors

Epidemiological studies have not documented environmental or occupational risk factors, with the possible exception of exposure to pesticides. A relevant etiological factor that is significantly correlated to the development of aggressive B-cell NHL is host immunodeficiency, both congenital and acquired in its many forms. The risk of aggressive NHL is most evident in patients affected by T-cell immunodeficiency. Notably, the relative risk of aggressive NHL is 100-200 in congenital immunodeficiency (ataxia-teleangiectasia and Wiskott-Aldrich syndrome), 30-40 in common variable immunodeficiency, 250-400 in the course of HIV infection and 20-100 in post-transplant iatrogenic immunosuppression. In addition, some systemic autoimmune disorders, namely rheumatoid arthritis and systemic lupus erythematosus, are associated with an increased relative risk of DLBCL. In these conditions, however, the relative risk is approximately 3-8 and therefore markedly lower that that observed in immunodeficiency conditions. The host’s infection by several viruses may also predispose to or cause aggressive NHL, and the pathogenic role of these viruses will be discussed in detail later in this review.

Molecular histogenesis

The histogenesis of lymphoma can be assessed by identifying the precise cellular subset from which a given lymphoma category derives. This is achieved by defining the lineage and the precise differentiation stage of the various types of lymphoma and by comparing them with characteristic features of the different maturation stages of normal lymphocytes. To date, the histogenesis of lymphoma has been clarified reasonably well in the case of lymphomas derived from B cells, whereas is still poorly understood in the case of lymphomas originating from T cells. Precursor B cells in the bone marrow attempt to perform immunoglobulin (Ig) gene rearrangement and, if successful, they are positively selected into the peripheral B-cell pool comprising naïve B-cells. For many B cells, the subsequent maturation steps are linked to the histological structure of the germinal center (GC). Within the GC, antigen-activated B cells accumulate somatic point mutations within their rearranged Ig heavy and light chain genes (a phenomenon known as somatic hypermutation) which modify the affinity of their B-cell receptor to the antigen. Only B cells which have acquired mutations leading to high affinity binding are positively selected and differentiate into memory B-cells or plasmablasts, while the majority of B cells are eliminated by apoptosis within the GC.

Lb-L stems from lymphoblasts, i.e. from B- and T-cell precursors undergoing the maturation process that leads to the development of effective, non-autoreactive lymphocytes apt to migrate from the bone marrow or thymus to the peripheral organs of the immune system. Within aggressive B-cell NHL deriving from mature B cells, the use of somatic hypermutation as a specific marker of B-cell transition through the GC allows the definition of two broad histogenetic categories of these disorders: lymphomas devoid of somatic Ig hypermutation, deriving from pre-GC B cells including MCL; lymphomas associated with somatic Ig hypermutation and thus putatively derived from GC or post-GC B-cells. Among aggressive NHL, these include DLBCL, BL, and most PTLD (Figure 1).

General pathogenetic mechanisms of aggressive NHL

Molecular lesions of aggressive NHL include i) proto-oncogene activation by chromosomal translocation; ii) inactivation of tumor suppressor genes; and iii) virus-related alterations. Chromosomal translocations are the dominant mechanism of proto-oncogene activation in aggressive NHL. A common feature of translocations of aggressive mature B-cell NHL is the placement of the proto-oncogene in the proximity of regulatory regions derived from Ig genes or other loci that are expressed at high levels in mature B cells, thus leading to deregulated expression of the proto-oncogene. A different translocation mechanism is the formation of a fusion transcript, as exemplified by the t(2,5) translocation. Proto-oncogenes deregulated in aggressive NHL include transcription factors, namely c-MYC and BCL-6; cell cycle regulators, namely cyclin D1; inhibitors of apoptosis, namely BCL-2; and signal transducers, namely ALK.

Tumor suppressor genes involved in aggressive NHL undergo biallelic inactivation and include cell cycle regulators, such as p15 and p16; inducers of apoptosis, namely Death-associated protein kinase; and regulators of genomic integrity, such as p53, FHIT (Fragile Histidine Triad) and MGMT (O6-Methylguanine DNA Methyltransferase).

Viruses associated with aggressive NHL may favor transformation by a direct mechanism through introduction of exogenous genes into target cells (EBV and HHV8), or by an indirect mechanism (HIV and HCV), through modifications of the microenvironment or host immunity or via chronic antigen stimulation. EBV infection associates with BL (30% of sporadic and epidemic cases and 100% of endemic cases) and a fraction of PTLD. The EBV-encoded proteins LMP-1 and EBNA-2 are capable of inducing B-cell transformation in vitro and in vivo. HHV8 is a lymphotropic virus associated with primary effusion lymphoma, whose genome
codifies for several viral oncogenes, including a viral cyclin homologous to cyclin D.\textsuperscript{10} Several epidemiological studies indicate that HCV infection is significantly over-represented in B-cell NHL.\textsuperscript{11-14} A recent meta-analysis of 48 studies has definitively established that the prevalence of HCV is higher in patients with B-cell NHL than in the general population (17\% vs 1.5\%; OR 10.8; 95\% CI: 7.4-16) or in other hematologic malignancies (13\% vs 2.9\%; OR 4.2; 95\% CI: 2.5-7).\textsuperscript{15} Such differences are particularly striking in Italian and Japanese populations, where the infection is endemic.\textsuperscript{11,14} In general, the relationship between HCV and B-cell NHL is stronger for specific sub-types of indolent B-cell NHL.\textsuperscript{14}

However, a significantly higher incidence of HCV infection has also recently been observed within aggressive cell B-NHL.\textsuperscript{11,14,15} These forms are frequently characterized by extranodal involvement and usually have no previous history of indolent B-cell malignancy.

How HCV may provoke B-cell lymphoproliferative disorders is still unclear. In animal models, viral core and NS3 proteins induce a \textit{mutator phenotype} causing double-stranded DNA breaks and enhancing the mutation frequency of proto-oncogenes (i.e. \textit{BCL-6}, \textit{p53}, \textit{β-catenin}) and immunoglobulin genes with \textit{hit-and-run}-like mechanisms.\textsuperscript{16} However, the predominant hypothesis suggests that the virus is a putative exogenous stimulus triggering activation of target B-lymphocytes and possibly selecting clonal cell populations and leading to uncontrolled, HCV-antigen driven neoplastic clonal B-cell proliferation.
Molecular pathology

Diffuse large B-cell lymphoma

The molecular pathogenesis of DLBCL is complex and includes more than one disease variant. Chromosomal translocations of band 3q27 are detected in 30–40% of cases and cause the rearrangement of BCL–6, a transcriptional repressor selectively expressed by GC B cells and controlling GC formation. The translocation inhibits the down-regulation of BCL–6 which is required for further differentiation of GC B cells, and creates a DNA error-prone GC microenvironment by functionally inactivating p53. Translocations of BCL–2 occur in approximately 20% of DLBCL arising de novo and in most cases transformed from a prior follicular phase. Other molecular lesions occasionally involved in the pathogenesis of DLBCL include translocations of BCL–8 and MUC–1, amplification of REL and inactivation of the p53 tumor suppressor gene. Despite the molecular heterogeneity of DLBCL, most cases (70%) are affected by aberrant somatic hypermutation (SHM), which introduces multiple nucleotide substitutions in the coding and regulatory regions of several proto-oncogenes, namely c–MYC, Pax–5, RhoH/TTF and PIM–1. Aberrant SHM is thought to be due to malfunctioning of the physiological process that is normally active in GC B cells. The precise pathogenetic role of aberrant SHM is still under investigation.

Gene profiling studies have revealed at least two variants of DLBCL, defined as germinal center and activated B-cell like DLBCL, with prognostic significance that is discussed later in this review.

Follicular lymphoma grade IIIB

The molecular pathogenesis of FCLIIIB is heterogeneous. Rearrangements of BCL–6, resembling those in DLBCL, are detectable in approximately 50% of cases, whereas BCL–2 translocations are restricted to a minority of cases. On these bases, FCLIIIB resembles DLBCL and apparently does not evolve from a follicular lymphoma of lower grade.

Burkitt’s lymphoma

All cases of BL display chromosomal breaks at 8q24, on one side, and of one Ig gene, on the other. The functional consequence is the deregulation of the c–MYC proto-oncogene through repositioning of the Ig regulatory regions. c–MYC codes for a transcription factor that is ubiquitously expressed and involved in regulation of proliferation, differentiation and apoptosis. In addition, c–MYC positively regulates the transcription of several genes, including TERT (Telomerase Reverse Transcriptase), whose product modulates telomerase activity. The stimulation of telomerase induced by TERT increases the number of proliferative cycles that a cell can perform. Other genetic lesions associated with a fraction of BL are inactivation of p53, FHIT and p16. EBV infection is restricted to 30% of sporadic BL, whereas it is consistently present in endemic BL.

Mantle cell lymphoma

MCL is typically associated with t(11;14)(q13;q32). The translocation re-positions the BCL–1 locus at 11q13 with ligh and leads to homotypic deregulation of cyclin D, a gene located in proximity to the breakpoint and encoding a member of the D-type cyclins which regulates the early phases of cell cycle. As for other D-type cyclins, cyclin D1 is thought to act primarily as a growth factor sensor integrating extracellular signals with the cell cycle clock. The pathogenetic role of BCL–1 activation in human neoplasia is suggested by the ability of cyclin D1 overexpression to transform cells in vitro and contribute to B cell lymphomagenesis in transgenic mice.

Peripheral T-cell lymphoma

Few categories of peripheral T-cell lymphomas (PTCL) have been investigated at the molecular level. Anaplastic large cell lymphomas (ALCL) expressing the ALK (Anaplastic Lymphoma Kinase) protein associate with molecular derangements of the ALK gene. Eighty-five per cent of ALK+ ALCL bear the t(2;5)[p23;q35] translocation, involving the genes ALK on chromosome 2 and NPM (nucleophosmin or numatrin) on chromosome 5. The translocation gives rise to the fusion protein NPM/ALK. The ALK gene is not expressed in normal lymphoid cells, whereas NPM is ubiquitously expressed. Because of the t(2;5) translocation, expression of ALK is regulated by the NPM promoter and, therefore, is deregulated in neoplastic cells. In addition, because NPM/ALK can homodimerize, the translocation causes the constitutive activation of the catalytic domain of the ALK tyrosine kinase. In the remaining 15% of ALK+ ALCL, ALK fuses to a partner gene other than NPM to produce variant ALK proteins, which gain the ability to homodimerize and, therefore, display constitutive kinase activity.

Post-transplant lymphoproliferative disorders

The clinical and morphological heterogeneity of Post-Transplant Lymphoproliferative Disorder (PTLD) correlates with the molecular heterogeneity of these disorders. EBV infection associates with 100% of early PTLD (<1 year post-transplant), whereas it is restricted to 40–50% of late PTLD (>1 year post-transplant).

Current concepts for a lymphoma classification

Lymphoid neoplasms are currently categorized
according to the REAL/WHO Classification, which consists of a list of distinct diseases defined by an amalgamation of information on cell morphology, phenotype, genotype, clinical characteristics and identification of a normal counterpart if possible. Notably, the Classification does not provide grades of malignancy, in contrast to other approaches. In fact, it was felt that the clinical behavior and therapeutic response of malignant lymphomas are not influenced by the cell size, number of mitotic figures and differentiation stage (as usually postulated for most, if not all, non-hematopoietic neoplasms), but rather depend on the category to which the tumor belongs, and, within each category, on a series of biological mechanisms which vary somewhat from patient to patient. Such a view is supported by the results of gene expression profiling techniques that allow the simultaneous evaluation of thousands of genes through m-RNA hybridization on micro-arrays containing DNA spots or oligonucleotides corresponding to the investigated genes. The main limitations of these techniques are that they require optimally preserved m-RNA and complex statistical analysis; they are also very expensive. In addition, the gene data need confirmation at the protein level, as the analysis; they are also very expensive. The former presentation is more(idly improving.

**Morphology**

This is not predictive of either the (B or T) cell origin or the maturation stage within the specific cell lineage. Neoplastic cells display high nuclear/cytoplasmic ratio, dense chromatin, hardly visible nucleoli, and a narrow rim of moderately basophilic cytoplasm. The nuclear contours are irregular, at times convoluted. In the past, the latter finding was regarded as pathognomonic of T-cell derivation, but it can also occur in B-Lb-L. Mitotic figures are numerous. Histiocytes phagocytosing nuclear debris are encountered, which may produce a starry-sky pattern.

**Phenotype**

The phenotype can be adequately assessed in routine sections thanks to the currently available antigen retrieval techniques. This permits the accurate subclassification of Lb-L as summarized in the foot note. Notably, BSAP (i.e. the PAX5 gene product) represents the first lineage marker during B-cell ontogeny, although it can be expressed in a proportion of acute myeloid leukemias carrying t(9;21). Some molecules that are usually associated with the B-cell lineage (e.g. CD79a, CD10 and Bcl-6) can actually be detected in a proportion of T-Lb-L, since they are transiently expressed by immature T-cells. Both B- and T-Lb-L lack myelo-monocytic, erythroid and megakaryocytic markers. The negativity for cyclin D1 contributes to the distinction of B-Lb-L with a mature phenotype from MCL with blastoid morphology. The search for cytokeratins represents a useful tool for the differential diagnosis between T-Lb-L and thymoma, which can contain huge amounts of T-lymphoblasts, but conversely to T-Lb-L shows a regular frame of cytokeratin epithelial cells. Immunohistochemistry also allows the distinction of Lb-L from other small blue cell tumors, including Ewing's sarcoma (vimentin+/CD99+), PNET (vimentin+/CD99+/S100+/chromogranin+), neuroblastoma (NSE+/chromogranin+/synaptophysin+), chondrosarcoma (vimentin+/S100+), and rhabdomyosarcoma (vimentin+/desmin+/striated-muscle actin+).

**Mantle cell lymphoma**

This lymphoma is usually composed of small cells, characterized by cleaved nuclei with moderately dispersed chromatin and a minute central nucleolus. The cytoplasm is narrow and grayish following Giemsa staining. The number of mitotic figures is usually low–moderate. Some epithelioid macrophages and flat hyaline venules are included in the lymphomatous growth. Three morphologic variants of the tumor (small cell, blastoid and polymorphic) are recognized that should be differentiated from B-cell chronic lymphocytic leukemia (B-CLL), Lb-L and DLBCL. At the lymph node level, MCL gives rise to different growth...
patterns: mantle zone-like, nodular and diffuse.\textsuperscript{12,66} The tumor frequently involves the bone marrow (with variable location)\textsuperscript{30,68} and the intestine, where it may produce the so-called lymphomatous polyposis.\textsuperscript{69} It can present with a leukemic picture or prominent splenomegaly. The latter condition should be differentiated from splenic marginal-zone lymphoma (S-MZL).\textsuperscript{30}

**Phenotype**

Besides positivity for CD19, CD20, CD79a, and BSAP, MCL shows almost regular expression of CD5 and cyclin D1\textsuperscript{1,2,66,71} The latter reflects the occurrence of t(11;14).\textsuperscript{12,66} Notably, although characteristic, cyclin D1 positivity is not pathognomonic of the process, as it can be detected in other lymphoid neoplasms such as hairy cell leukemia, multiple myeloma and – more rarely and controversially – B-CLL.\textsuperscript{22} In addition, it is absent in about 10% of MCL cases.\textsuperscript{22} The recent production of a highly specific rabbit monoclonal antibody has markedly improved the detection of this marker, whose preservation – like that of CD5 – is critical and requires optimal fixation.\textsuperscript{22} Staining for CD21 reveals irregular meshworks of follicular dendritic cells often connected with the hyaline vessels.\textsuperscript{1,2,66} The search for CD23 (with a few exceptions),\textsuperscript{24} CD10 and IRTA1 is negative, thus contributing to the distinction of MCL from B-CLL, follicular lymphoma (FL) and MZL.\textsuperscript{25} Bcl-6 protein has recently been found in a proportion of MCL, throwing into doubt its relevance in differentiating FL and DLBCL.\textsuperscript{66} Neoplastic cells are usually IgM+/IgD+, a finding that may be relevant for the differential diagnosis between MCL and S-MZL (IgM+/IgD+) especially in cases with medullary intrasinusoidal diffusion.\textsuperscript{68} Cases with higher proliferation rates (Ki-67 rate >30%) have a more aggressive clinical course.\textsuperscript{77} They are often characterized by expression of nuclear survivin, p53, and MDM2 and lack of p27\textsuperscript{KIP}1.\textsuperscript{78}

**Follicular lymphoma grade IIIIB**

**Morphology**

FLIIB is almost exclusively composed of centroblasts and is included among the aggressive lymphomas because of its clinical behavior and peculiar molecular characteristics.\textsuperscript{1,2,26,55,56} In particular, the diagnosis of FLIIB is acceptable only when the tumor arises de novo and the follicular pattern predominates over a possibly associated diffuse large B-cell component (otherwise the tumor is a DLBCL secondary to FL).\textsuperscript{1,2,55,56}

**Phenotype**

FLIIB is characterized by positivity for CD20, BSAP and – sometimes to a lower extent – CD79a. CD10 and Bcl-6 are commonly expressed, although one of them (rarely both) can be lost.\textsuperscript{1,2,55,68} Interestingly, c-MYC aberrations may produce a Burkitt-like morphology.\textsuperscript{40} Finally, occasional expression of CD5 has been reported in the absence of cyclin D1 positivity, a fact that allows differentiation from MCL.\textsuperscript{81,82} Bcl-2 protein negativity is found in the majority of FLIIB cases, due to the absence of t(14;18) or unproductive BCL-2 gene rearrangement.\textsuperscript{1,2,55,56,68} Cases lacking t(14;18) usually carry a different chromosomal aberration.\textsuperscript{96}

**Diffuse large B-cell lymphoma**

**Morphology**

DLBCL consists of large cells (mean diameter ≥20 μm), often characterized by pronounced nuclear polymorphism, prominent nucleoli and a rim of basophilic cytoplasm.\textsuperscript{1,2,49} At the tissue level, the neoplasm grows diffusely, sometimes spreading through residual sinuses in the lymph node. Mitotic figures are always numerous. In a variable percentage of patients, DLBCL evokes a fibrotic reaction, which may become prominent in the retroperitoneum and mediastinum: in the latter site, the process reveals peculiar clinico-pathologic features that justify its identification as a distinct subtype of DLBCL.\textsuperscript{81,85} In 20–25% of cases, one cytotype predominates over the others, thus allowing the identification of morphologic variants: centroblastic, with large multilobed nuclei, immunoblastic (with and without plasma cell differentiation), plasmablastic, and anaplastic.\textsuperscript{1,2,49,56,68} A further cytological variant of DLBCL is the so-called T-cell-rich/histiocyte-rich B-cell lymphoma (T/HRBCL).\textsuperscript{45,67-69} This is characterized by the presence of scattered large tumor cells intermingled with a huge amount of reactive T-lymphocytes and/or histiocytes: its borders with lymphocyte predominant or lymphocyte-rich Hodgkin’s lymphoma are not always sharp and transition from one tumor type to the other is possible.\textsuperscript{97,98} In rare cases, DLBCL consists of epithelial-like or spindle-shaped elements\textsuperscript{91} or is provided with prominent interlacing cytoplasmic projections, which produce a neuroblastoma-like appearance.\textsuperscript{92,93} There is no consensus on the usefulness of a cytological classification of DLBCL.\textsuperscript{49,93}

**Phenotype**

The neoplastic cells express a series of B-cell associated antigens, such as CD19, CD20, CD79a, and BSAP.\textsuperscript{1,2,49,55,56} In approximately 30% of DLBCL of the immunoblastic and CD30- anaplastic types, the leukocyte common antigen/CD45 is absent.\textsuperscript{94,95} The PU1 and LSP1 molecules have been found to be useful for the differential diagnosis between T/HRBCL and I lymphocyte predominant Hodgkin’s lymphoma by showing opposite patterns (PU1+/LSP1- vs. PU1-/ LSP1+).\textsuperscript{96} Recently, attention has been paid to CD5 positivity.\textsuperscript{96} This occurs in the absence of cyclin D1 over-expression, a fact that allows the easy differentiation of CD5- DLBCL from polymorphic MCL.\textsuperscript{49} The adverse prognostic rel-
evance of CD5 expression originally reported by Yamaguchi et al. has not been confirmed by others. Following the seminal paper of Alizadeh et al. in 2000, further gene expression profiling studies based on larger series of cases have confirmed that DLBCL can be subdivided into subtypes depending on their gene signature: germinal-centre B-cell (GCB)-like, activated blood B-cell (ABC)-like, and type 3 or unclassified. These subgroups have significantly different clinical behaviors and responses to therapy. After two initial unsuccessful studies, some groups have recently identified possible immunohistochemical gene expression profiling surrogates applicable at low cost to formalin-fixed, paraffin-embedded samples. By adopting the tissue micro-array strategy, Säez et al. have detected eight biological markers (cyclin E, CDK1, SKP2, EBER, MUM1, CDK2, Bcl-6, and Rb-P) that can improve the capacity for predicting failure and survival in combination with the International Prognostic Index. In another study, also based on tissue micro-arrays, Hans et al. have sub-classified 152 DLBCL, 142 of which had been successfully evaluated by gene expression profiling, into GC (n=64) and non-GC B-cell derived (n=88). This sub-classification was based on the low cost of fluorescence ISH (FISH) or chromogen-ISH (CISH) reveals an alteration of the corresponding gene(s), which represents a second hit in the natural history of the process. This produces a kinetic advantage for one of the sub-clones, which assumes blastic morphology and rapidly predominates over the others, giving rise to a secondary DLBCL that is significantly more aggressive and chemo-resistant than de novo forms.

Burkitt’s lymphoma

Morphology

The endemic and sporadic forms of BL are characterized by the same morphology: they consist of homogenously medium-sized cells with a narrow rim of deeply basophilic vacuolated cytoplasm and a round nucleus with granular or reticulated chromatin and 2-6 nucleoli, usually at distance from the nuclear membrane. The tumor tends to grow cohesively, has exceedingly high mitotic and apoptotic rates and contains numerous macrophages phagocytosing nuclear debris, which produce the typical starry-sky pattern.

Phenotype

Neoplastic cells express B-cell markers, CD10 and Bcl-6, but lack Bcl-2. The Ki-67 rate is very high, but exceeds 100%.

Burkitt-like or atypical Burkitt’s lymphoma

This is a controversial entity that was included in the REAL/WHO Classification because of the belief that it should be treated more aggressively than DLBCL, although no definite proof exists as to its morphologic and biological homogeneity. Unlike to true BL, neoplastic cells are variably sized, have a large cytoplasmic rim (at times with a paranuclear clear dot) and prominent nucleoli. The morphologic similarities with BL might be due to the common C-MYC gene rearrangement. In immunohistochemistry, they are CD10, Bcl-6 and Bcl-2. The Ki-67 rate is very high, but less than 100%.

Peripheral T-cell lymphoma

PTCL represents 10-15% of all lymphoid tumors in western countries, being endemic in some geographic areas such as the southern part of Japan, where is related to HTLV1 infection (so-called adult T-cell lymphoma/leukemia). The family of PTCL includes neoplasms derived from peripheral T-lymphocytes and NK cells.
cells.\textsuperscript{1,2} With only three exceptions (mycosis fungoides, CD30+ lymphoproliferative disorders of the skin and large granular lymphocyte leukemia), all PTCL behave aggressively.\textsuperscript{56,111–114} They can be roughly subdivided into specified and unspecified forms.\textsuperscript{1,2} The former correspond to a series of rare, but distinct clinico-pathologic entities more often recorded at extra-nodal sites, while the latter refer to an array of morphologically heterogeneous neoplasms irrespective of their presentation.\textsuperscript{1,3}

**Morphology**
PTCL consist of more or less polymorphic elements of variable size, provided with irregularly shaped nuclei and a rather wide rim of cytoplasm, more frequently clear in Giemsa staining. Neoplastic cells can be intermixed with reactive elements, such as follicular dendritic cell, histiocytes, eosinophils, plasma cells and epithelioid elements.\textsuperscript{1,2,56,111–113} The latter can at times obscure the neoplastic population (so-called Lennert’s lymphoma).\textsuperscript{1,2,56,111–113} High-endothelium venules are comprised within the tumor, being prominent in the angioimmunoblastic/AILD-type.\textsuperscript{1,2,56,111–113} In the lymph node, the lymphomatous growth selectively involves the paracortex, at times sparing reactive follicles (so-called T-zone pattern).\textsuperscript{1,2,56,111–113} Hemophagocytosis by histiocytes may accompany the lymphomatous growth.\textsuperscript{15}

**Phenotype**
PTCL show phenotypic aberrations consisting in CD4/CD8 restriction, loss or co-expression and/or loss of one or more of the T-cell associated antigens (CD2, CD3, CD5, CD6, and CD7).\textsuperscript{1,2,56,111–113} They can express CD30 irrespective of anaplastic morphology as well as CD15 and cytotoxic markers, including TIA-1, granzyme B, perforin, CD56 and CD57.\textsuperscript{1,2,56,111–113} CD15 and cytotoxic markers, including TIA-1, granzyme B, perforin, CD56 and CD57.\textsuperscript{1,2,56,111–113} The former correlate with an angioimmunoblastic/AILD-type.\textsuperscript{1,2,56,111–113} In the lymph node, the lymphomatous growth selectively involves the paracortex, at times sparing reactive follicles (so-called T-zone pattern).\textsuperscript{1,2,56,111–113} Hemophagocytosis by histiocytes may accompany the lymphomatous growth.\textsuperscript{15}

**AILD**
This is characterized by diffuse effacement of the lymph node structure, low cellular density, abundant arborizing epithelioid venules and possible burnt-out (i.e. sclero-hyalinotic) residual follicles.\textsuperscript{1,2,56,111–113} Lymphomatous cells are variably sized and have irregularly shaped nuclei and a wide rim of clear cytoplasm. They are admixed with EBV-infected B-immunoblasts, eosinophils, plasma cells and histiocytes.\textsuperscript{1,2,56,111–113} In immuno-histochemistry, neoplastic cells show usual CD4 restriction, frequent CD10 expression and moderate Ki-67 marking.\textsuperscript{111} Within this context, there is an abundant follicular dendritic cell component (CD21+/CD23+/CD35+) that contributes to producing the low cellular density.\textsuperscript{1,2,56,111–113} B-immuno-blasts may undergo neoplastic transformation and give rise to an unrelated DLBCL.\textsuperscript{118}

**ALCL**
This is the most frequent type of specified PTCL. The present review will focus on systemic ALCL, omitting the primary cutaneous lesions that are now included among CD30+ lymphoproliferative disorders of the skin (see above).\textsuperscript{1,2,115}

**Morphology**
Systemic ALCL is mostly composed of very large cells (so-called hallmark cells) with an eccentric kidney-shaped nucleus, a rod-shaped nucleolus and a wide rim of basophilic cytoplasm with a clear paranuclear area.\textsuperscript{120–124} The tumor tends to grow cohesively and to spread through sinuses, a fact that in the past led to a misdiagnosis of metastatic undifferentiated carcinoma or malignant histiocytosis.\textsuperscript{120–127} Morphologic variants of the process have been described: giant-cell rich, lympho-histiocytic, small cell, mixed, Hodgkin’s-like and, more rarely, sarcomatoid, signet-ring cell-like, and neutrophil, eosinophil or epithelioid cell-rich.\textsuperscript{120–127} In reality, ALCL is always characterized by a morphologic spectrum: from small cells to hallmark cells.\textsuperscript{120–124} However, one cytotype tends to predominate over the others, thus producing different patterns.\textsuperscript{120–124} Notably, divergent cytotypes may be observed in biopsies taken from the same patient at different anatomic sites, either simultaneously or sequentially (e.g. at presentation and relapse).\textsuperscript{120–124}

**Phenotype**
The tumor shows regular CD30 expression, common epithelial membrane antigen (EMA) positivity, frequent presentation of cytotoxic markers (TIA-1, granzyme B, and perforin), possible lack of the leukocyte common

**Foot note:**
\textsuperscript{1}t(2;5)(p23;q35)=fusion gene and chimeric protein NPM/ALK (with cytoplasmic location);
\textsuperscript{2}t(1;2)(q25;p23)=fusion gene and chimeric protein TMP3/ALK (with cytoplasmic location);
\textsuperscript{3}t(1;2)(q25;p23)=fusion gene and chimeric protein TGF/ALK (with cytoplasmic location);
\textsuperscript{4}t(2;11;2)(p23;p15;q31)=hybrid gene and chimeric protein CAR/TALK (with cytoplasmic location);
\textsuperscript{5}t(2;12)(q23;p21)=fusion gene and chimeric protein TGF/ALK (with cytoplasmic location);
\textsuperscript{6}t(2;22)(p11.2;q11.2)=fusion gene and chimeric protein MYH9/ALK (with cytoplasmic location);
\textsuperscript{7}t(2;20)(q13;p13.3)=fusion gene and chimeric protein NPM/ALK (with cytoplasmic location);
\textsuperscript{8}t(2;10)(p13;q11)=fusion gene and chimeric protein BCL2/ALK (with cytoplasmic location);
\textsuperscript{9}t(2;23)(q11.2;q12)=fusion gene and chimeric protein TPH1/ALK (with cytoplasmic location);
\textsuperscript{10}t(2;11;2)(p23;p15;q31)=hybrid gene and chimeric protein CAR/TALK (with cytoplasmic location);
\textsuperscript{11}t(2;12)(q23;p21)=fusion gene and chimeric protein TGF/ALK (with cytoplasmic location);
\textsuperscript{12}t(2;22)(p11.2;q11.2)=fusion gene and chimeric protein MYH9/ALK (with cytoplasmic location);
\textsuperscript{13}t(2;20)(q13;p13.3)=fusion gene and chimeric protein NPM/ALK (with cytoplasmic location);
\textsuperscript{14}t(2;10)(p13;q11)=fusion gene and chimeric protein BCL2/ALK (with cytoplasmic location);
\textsuperscript{15}t(2;23)(q11.2;q12)=fusion gene and chimeric protein TPH1/ALK (with cytoplasmic location);

\textsuperscript{1}t(2;5)(p23;q35)=fusion gene and chimeric protein NPM/ALK (with nuclear and cytoplasmic location);
\textsuperscript{1}t(1;2)(q25;p23)=fusion gene and chimeric protein TMP3/ALK (with cytoplasmic location);
\textsuperscript{1}inv(2)=hybrid gene and chimeric protein ATIC/ALK (with cytoplasmic location);
\textsuperscript{1}t(2;3)(p23;q21)=fusion gene and chimeric protein TGF/ALK (with cytoplasmic location);
\textsuperscript{1}t(2;12)(q23;p21)=fusion gene and chimeric protein TGF/ALK (with cytoplasmic location);
\textsuperscript{1}t(2;22)(p11.2;q11.2)=fusion gene and chimeric protein MYH9/ALK (with cytoplasmic location);
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\textsuperscript{1}t(2;23)(q11.2;q12)=fusion gene and chimeric protein TPH1/ALK (with cytoplasmic location);
antigen/CD45, occasional CD15 staining, and T- or null-phenotype.\textsuperscript{58,120-124,128} This profile might make the distinction between ALCL and classic Hodgkin’s lymphoma (CHL) difficult. In this respect, the search for BSAP is of paramount importance: in fact, this molecule is regularly lacking in ALCL, while it is usually expressed in the Hodgkin and Reed–Sternberg cells of CHL.\textsuperscript{58} Recently, survivin positivity has been reported in a proportion of cases: it seems to correlate with a more aggressive clinical behavior.\textsuperscript{129} Sixty to ninety per cent of systemic ALCL express ALH.\textsuperscript{58,121-124,130,131} This molecule – which is not detected in normal lymphocytes, cHL and DLBCL with anaplastic morphology – is thought to be involved in the pathogenesis of the process\textsuperscript{132-136} and can be located in the nucleus and/or cytoplasm.\textsuperscript{58,121-124,130,131} It corresponds to translocations involving the ALK gene and different partners, leading to the formation of hybrid genes that encode for the chimeric proteins summarized in the foot note.\textsuperscript{58,121-124,130,131,136,137} Notably, the cases carrying these translocations show an excellent response to therapy\textsuperscript{58,121-124,130} with the exception of the leukemic cases,\textsuperscript{138} and preferentially occur in the first two decades of life. By contrast, the ALK- anaplastic tumors have a much worse prognosis (5-year overall survival 30% vs. 85%) and occur in the elderly.\textsuperscript{58,121-124,130} This has led to question of whether or not the term ALCL should be applied to large cell tumors with anaplastic morphology and T/null phenotype, but lacking the ALK protein.\textsuperscript{129} Besides its prognostic impact, ALK positivity contributes – along with BSAP negativity (see above) – to identifying the rare forms of ALCL with Hodgkin’s-like features and to differentiate them from cytologically aggressive cHL.\textsuperscript{58,124,129}

**Cytogenetic and molecular diagnosis**

DLBCL, a histologically well-defined subset of NHL, is genetically heterogeneous. By G-banding, most cases show a complex hyperdiploid karyotype and diverse cytogenetic abnormalities that include recurring and non-recurring translocations, deletions, duplications and marker chromosomes. Conventional cytogenetic analysis performed on tumor biopsies allows the detection of clonal chromosomal abnormalities in more than 85% of cases.\textsuperscript{140} Breakpoints are clustered at several sites including 14q32, 18q21, 1q21, 3q27, 1p36, 8q24, 3p21, 6q21, 1p22, and 22q11. Molecular cytogenetic techniques such as multicolor fluorescence in situ hybridization (M-FISH), spectral karyotyping (SKY), and comparative genomic hybridization (CGH) are providing a more comprehensive view of the genetic alterations in DLBCL.\textsuperscript{141} CGH has recently allowed researchers to demonstrate that 18q gains or amplification and 17p losses are associated with a particularly aggressive clinical behavior.\textsuperscript{142} In up to 30% of cases DLBCL arise from the transformation of a low grade follicular NHL. During the clonal evolution, the neoplastic cells acquire additional cytogenetic and molecular abnormalities, but retain the t(14;18) rearrangement which can be used as a molecular marker for the detection and follow-up of these patients. Excluding known transformed FL, the translocation has been demonstrated in 18–20% of patients with de novo DLBCL. The BCL–6 proto-oncogene was first identified at breakpoints on 3q27 involved in t(3;14) (q27;q32) and t(3;22) (q727;q11) and t(3;2) (q27;2p11) and subsequently, other translocations have been reported. In some DLBCL patients BCL-6 deregulation is largely independent of chromosome 3q27 rearrangement and is due to somatic point mutations at the 5′-untranslated regulatory region.\textsuperscript{143} Although several studies have been performed to investigate the relationship between BCL–6 gene rearrangement or mutations and clinical outcome, such analyses are no longer clinically attractive.

Gene expression profile studies have recently revealed the existence of at least two molecularly distinct types of DLBCL. As mentioned earlier, the first is recognized as GC B-cell-like (GCB), strongly resembles normal GC B-cells, and is associated with a relatively good prognosis. The second subtype is the ABC-like which expresses a subset of genes characteristic of mitogenically activated B cells, more mature steps of B-cell differentiation and has a poor clinical outcome.\textsuperscript{140,144} In BL, the translocation t(8;14)(q24.1;q32) is present in 75–80% of all cases. The t(2;8)(p12;q24.1) and t(8;22)(q24.1;q11) variants make up the remainder of cases. The juxtaposition of the c-MYC oncogene mapped to 8q24.1 with the immunoglobulin chain is thought to lead to inappropriate expression of the oncogene thus inducing molecular transformation. The molecular detection of c-MYC rearrangement is easy by Southern blotting but not by DNA polymerase chain reaction (PCR) since the distribution of breakpoints spans over a very large genomic region. Therefore, although long distance PCR methods for the detection and monitoring of BL have been reported,\textsuperscript{145,146} PCR analysis and other molecular diagnostic procedures are not routinely performed.

In the case of ALCL, the t(2;5) (p23;q35) is detected in about 50% of cases.\textsuperscript{146} This translocation fuses the ALK gene (2p23) and the NPM gene (5q35), so that the ALK kinase becomes constitutively activated and triggers malignant transformation. The NPM–ALK fusion gene is found more frequently in children and young adults, and its presence is associated with a relatively good prognosis.\textsuperscript{147} In about 10–20% of ALK- NHL, variant ALK fusions have been detected, among which the
**ATIC–ALK** rearrangement resulting from the inv(2)(p23q35) is probably the most recurrent. Other variants are TFG–ALK, CLTC–ALK (previously designated CLTCL–ALK), TMP3–ALK and MSN–ALK. In the majority of these cases a molecular demonstration of these rearrangements can be made by reverse transcriptase–PCR and more rarely by DNA PCR. Nonetheless, the relatively low number of these cases accounts for the negligible application of a routine molecular diagnostic investigation which does not add anything relevant to the immunohistochemical evaluation of ALK overexpression. Gene expression profile analysis revealed that ALK+ NHL and Hodgkin’s disease share the differential expression of several genes. Further studies are needed to clarify the biological and clinical significance of these gene expression patterns.

Mantle cell lymphoma is characterized by the presence of t(11;14)(q13;q32). As a consequence of this translocation the **PRAD1/BCL1** gene is repositioned near an immunoglobulin enhancer leading to overexpression of cyclin D1, a crucial cell cycle regulator. Conventional cytogenetics and FISH approaches have been established for a more accurate detection of t(11;14) and together allow a cytogenetic diagnosis in more than 90% of MCL patients. In addition, CGH studies recently revealed gain or loss of genes located on chromosomes 10p13, 11q13, 11q22–11q23 and 13q14 which could be important for understanding the heterogeneous clinical behavior of this disease. More than 80% of breakpoints are clustered in a region of 80–100 bp of the **BCL1** gene, termed the major translocation cluster (MTC), but many other breakpoints are scattered over a region of more than 120 kb so that the investigation of genomic DNA by molecular techniques such as DNA PCR is often limited. As a consequence, genomic PCR fails to detect those patients carrying breakpoints falling outside the MTC region and allows the detection of only 50% of positive cases. Southern blot analysis reveals a higher number of positive cases but due to its complexity and time consuming nature is not routinely employed. Despite the relatively limited number of patients for whom DNA PCR provides informative results, this approach has been widely used for diagnostic purposes and to evaluate minimal residual disease. In this latter application, mixed results have been reported. Indeed, the achievement of a molecular remission was not predictive of a better clinical outcome when obtained after chemo-immunotherapy. In contrast, improvement of event–free survival and overall survival were recorded when PCR-negative results were obtained after high dose chemotherapy, rituximab and hematopoietic stem cell transplantation. The use of quantitative reverse transcriptase–PCR for cyclin D1 has also been proposed, but does not seem to offer better information than that afforded by to immunohistochemical protein evaluation which remains the gold standard for the evaluation of MCL. Differential gene expression analysis allowed the precise measurement of tumor cell proliferation and the identification of subsets of patients whose median survival differed by more than 5 years.

### Pre- and post-treatment evaluation

A diagnosis of aggressive NHL cannot be established without the examination of tissue obtained at biopsy. On the basis of the lymphoma presentation, the biopsy can be taken from enlarged superficial lymph nodes (in the neck, axillary or inguinal regions), lymphoid tissue in Waldeyer’s ring, mediastinal nodes (by core needle biopsy or mediastinoscopy or mediastinotomy) or retroperitoneal lymph nodes (by ultrasound-guided core needle biopsy or laparoscopy or laparotomy). Once the diagnosis has been established the first critical step is the pre-treatment evaluation and staging to identify prognostic factors.

### History and physical examination

A careful history and physical examination are the most important factors in evaluating a patient. The physical examination includes evaluation of all lymphic sites of involvement. An assessment of Karnovsky performance status is important in all patients, and especially for those entering clinical research trials.

### Lymph node evaluation

Precise measurement and recording of the two-dimensional size and location of enlarged lymph nodes minimizes the likelihood of inter– and intra–observer variability in physical examination. At the time of diagnosis, a lymph node that is larger than 1 cm in its short transverse diameter should be considered compatible with involvement by NHL.

### Liver and spleen assessment

Detection of an enlarged spleen or liver upon physical examination should be considered suspicious for involvement by lymphoma. Abdomen ultrasound and computed tomography scans are useful for measuring organ size and identifying the presence of tumor masses.
Laboratory studies

Blood counts
Laboratory studies that should be routinely performed in patients with NHL include a complete blood count to assess bone marrow reserves, and a white blood cell differential with careful examination of the peripheral blood to look for the presence of circulating lymphoma cells.

Serum chemistry and serology
Serum chemistry should include a mineral panel and an assessment of hepatic function and liver enzymes. Lactic dehydrogenase (LDH) is a strong indicator of tumor activity and is included in the International Prognostic Index. The uric acid level may predict patients at increased risk of urate nephropathy. For patients with renal dysfunction, urine cytology has been recommended as a useful means of assessing involvement by lymphoma.

Bone marrow assessment
A bone marrow aspirate and biopsy should be performed in all patients. Bilateral bone marrow biopsies have been recommended because they increase the sensitivity of detection of NHL involvement by 10-20%. However, an adequate (>2 cm) unilateral bone marrow specimen is generally sufficient.

Lumbar puncture
A lumbar puncture with examination of cerebrospinal fluid is recommended only in patients with signs or symptoms suggestive of central nervous system disease, or for those at high risk for involvement, for example, with massive head and neck disease, nasal and paranasal sinus involvement, spinal compression (with/without epidural space involvement), lymphoblastic or Burkitt’s/Burkitt’s-like lymphoma or mantle-cell lymphoma, disseminated disease with testicular involvement and possibly bone marrow involvement.

Endoscopy
This is recommended for certain histologic types (T-cell or Burkitt’s lymphoma). In mantle-cell NHL, the bowel is often involved, characteristically as polyposis.

Imaging studies
Standard radiography assessment includes a chest X-ray and CT scans of the chest, abdomen and pelvis, as well as other apparently involved sites (e.g. neck, orbit, central nervous system). Magnetic resonance imaging (MRI) may assist in the assessment of bone disease that is equivocal on plain radiographs.

Gallium scan
Single photon emission computed tomography (SPECT) with gallium scanning should be included as part of response assessment at centers with expertise in performing the study in clinically relevant situations (e.g. to distinguish masses with viable tumor from fibrosis). Evaluation of supradiaphragmatic disease is more accurate than that of subdiaphragmatic involvement.

Position emission tomography (PET)
PET scanning is a non-invasive metabolic imaging technique. It detects fluoro-2-deoxy-D-glucose (FDG) labeled with fluorine 18 taken up by tumor cells, as well as inflammatory tissue. PET scanning has a better resolution than gallium scanning, as well as a greater sensitivity. It also takes less time to perform. Because of its lack of specificity, PET scanning is not useful in the diagnosis of NHL. On the other hand, several recent studies have suggested a potential role for PET scanning in monitoring response to therapy by distinguishing active tumor from fibrotic tissue.

Special lymphoma sites
Nodal lymphoma that also occurs in extranodal sites requires further staging studies that vary somewhat by site.

Central nervous system lymphoma
An MRI scan of the CNS and lumbar puncture should be performed. Assessment should also include an ocular slit lamp examination.

Testicular lymphoma
Involvement tends to be bilateral both at presentation and at the time of recurrence, so the contralateral testis should be carefully evaluated to plan optimal treatment. Patients with disseminated disease also involving the testicle have an increased risk of CNS involvement, about 20% in some series, and therefore a lumbar puncture and CT or MRI of the head are indicated.

Gastric lymphoma
Although most of these tumors are confined to the stomach, extragastric involvement may occur either at diagnosis or as a manifestation of progression of disease. An endoscopic ultrasound may be helpful in assessing the degree of invasion of the gastric wall, establishing whether lymph nodes adjacent to the stomach are involved and identifying patients at high risk of bleeding or perforation without performing laparotomy.
Breast lymphoma

Primary lymphoma of the breast accounts for fewer than 1% of cases of NHL. These tumors are often detected on routine mammography and mistakenly diagnosed as carcinoma of the breast. Once an ultrasound excludes the presence of a cystic lesion, a core needle biopsy or excisional biopsy is needed to make the diagnosis. There is also an increased risk of contralateral involvement.

Bone lymphoma

A patient with bone pain and swelling should undergo a bone scan. A bone scan should also be considered in patients with elevated levels of alkaline phosphatase. Radionuclide bone scans are highly sensitive – more sensitive than conventional radiographs – as an indicator of bone involvement. However, bone scans are not as specific as conventional radiographs, and areas positive on the bone scan should be confirmed by plain radiographs or, if more complicated bone structures such as the facial bones are shown to be positive, by CT scans (and biopsied if need be). Bone involvement is found in less than 10% of patients without specific symptoms and a bone scan is not recommended as a routine staging procedure.

Staging and prognostic factors

Ann Arbor staging system

Once the evaluation procedures have been completed, patients are assigned an Ann Arbor stage for prognosis and treatment planning purposes. This staging system reflects the number of sites involved and their relation to the diaphragm, the existence of B symptoms and the presence of extralymphatic involvement. Lymphatic structures are lymph nodes, spleen, thymus, Waldeyer’s ring, appendix and Peyer’s patches. Involvement of other structures is regarded as extralymphatic disease. Extralymphatic disease may be either localized or diffuse. Localized involvement of an extra-lymphatic site, a so-called E-lesion, is usually interpreted as a lesion which, although extralymphatic, can be sensibly contained in a curative radiation field, as initially described by Musshoff.

The International Prognostic Index

In 1990, there was a consensus that an international classification system based on clinically relevant prognostic factors in large-cell lymphoma should be developed and utilized in this disease. For this reason, 16 single institutions and cooperative groups in the United States, Europe and Canada participated in the The International Prognostic Index (IPI) project. In 2031 patients of all ages, this model, based on age (<60 years vs. ≥60 years), tumor stage (I–II vs. III–IV), serum LDH concentration (normal vs. elevated), performance status (0–1 vs. ≥2) and number of extranodal disease sites (0–1 vs. ≥2), led to the identification of four risk groups with different predicted 5-year survival rates. (Table 1). In 1274 patients aged 60 or younger, an age-adjusted model based on tumor stage, serum LDH level and performance status identified four risk groups. These two indexes, the IPI and the age-adjusted international index, have been shown to be significantly more accurate than the Ann Arbor classification in predicting long-term survival.

Other prognostic factors

Most of the other prognostic factors are provided by further analysis of the lymphoma specimen. However, large studies in which the predictive value of these new parameters is analyzed should be performed; such studies could, for example, provide new insights into the influence of biological parameters on clinical outcome. The potential new biological prognostic indicators include parameters related to the proliferation rate of lymphoma cells, parameters related to lymphoma cells and their environment, cytokine production, the drug resistance of tumor cells, and, more recently, genetic profiling with specific prognostic evidence.

Therapy of aggressive non-Hodgkin’s lymphoma

Introduction

Aggressive lymphomas, once considered uniformly and rapidly fatal cancers, are today among the most successfully treated malignant diseases. In fact, following administration of doxorubicin-containing regimens, the five-year survival rate for the most common histotype (i.e. DLBCL) ranges between 26 and 75 percent, according to the prognostic variables. The treatment mainstay is aggressive, dose-intensive combination-chemotherapy. The negative impact of dose reductions has been well documented. In a study, a relative dose intensity for doxorubicin of over 75 percent was the single most important predictor of survival.

A second important general principle that applies to aggressive lymphoma management is their response to second-line chemotherapy. A sizable proportion of patients with good performance status who fail to respond to conventional regimens do achieve durable second remissions and possibly cures with salvage programs. Salvage options include newer non-cross resistant drugs, high-dose chemotherapy programs with autologous hematopoietic stem cell support, as well as hematopoietic stem cell allografting following either standard or reduced-intensity pre-transplant conditioning.
Over the years, a variety of different chemotherapy regimens have been developed and tested. This multiplicity of choices applies mainly to advanced-stage disease. In fact, for the therapy of localized lymphomas there is a nearly complete consensus in favor of a combined modality approach, consisting of a limited number of cycles of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy followed by localized irradiation. Conversely, for advanced-stage aggressive lymphomas, CHOP or equivalent chemotherapy is a reasonably effective and appropriate treatment only for good prognosis patients. For patients in the worst prognostic subgroups, both second generation (adding non-cross resistant agents) and third generation regimens (adding more active agents in a shorter period of time) failed to show any significant improvement in remission rate, disease-free survival and overall survival over standard first generation CHOP. For these latter patients there is a clear need for novel treatment approaches, including high dose chemotherapy and various forms of immunotherapy. Autografting of hematopoietic stem cells permits the use of drugs with predominant bone marrow toxicity at several-fold larger dosages and represents an effective means to overcome drug resistance. More recently, the addition of rituximab to CHOP was able to improve the results over standard CHOP with no increase in toxicity in at least two independent studies, while radio-immunotherapy has shown remarkable activity in relapsed/refractory aggressive lymphomas. Finally, several studies are underway to assess the role and the applicability of allogeneic stem cell transplantation as a means to exploit its associated graft-versus-lymphoma reaction. The treatment of peripheral T-cell lymphoma, Burkitt’s and Burkitt-like lymphoma, lymphoblastic lymphoma, mantlecell lymphoma and post-transplant lymphomas will be considered separately. All are uncommon but aggressive disorders that respond to specialized, usually complex regimens.

In conclusion, the appropriate treatment of aggressive lymphomas is far from being a settled issue. However, by taking into account the patient’s age and clinical fitness, the histological classification, a definition of early versus advanced stage, and by paying attention to prognostic variables such as the IPI score and disease status (onset versus relapse/refractoriness), it is possible to individuate the most appropriate treatment among the many now available.

**Diffuse large B cell lymphoma and follicular lymphoma grade III B**

**First line therapy of advanced stage**

Since the introduction of anthracycline-containing combination chemotherapy regimens in the 1970s, aggressive NHL has been considered a curable disease. CHOP chemotherapy was considered the gold standard treatment for aggressive NHL on the basis of a randomized trial showing no benefit on time to treatment failure and overall survival compared to those achieved with more intensified regimens. However CHOP is curative in only approximately 40% of patients and numerous clinical studies have been carried out to find new treatment strategies that can increase the cure rate. Three main strategies have been evaluated: intensification of chemotherapy through addition of higher doses of chemotherapy or more frequent doses of existing agents; early high dose therapy (HDT) and autologous stem cell transplantation (ASCT) during first line therapy; combination of monoclonal antibodies with chemotherapy.

**Intensified chemotherapy.** Recent studies suggest the possible benefit of dose intensification strategies. The SWOG conducted a pilot study evaluating dose-intensified CHOP (CHOP-DI) with filgrastim support every 14 days for six planned courses. Treatment with CHOP-DI was safely administered and resulted in a 14% better survival than that of historical controls. Moreover, two prospective randomized trials of a German cooperative group compared six cycles of CHOP-21, CHOP-14 (bi-weekly), CHOEP-21 (CHOP plus etoposide 100 mg/m² days 1-3) and CHOEP-14 (bi-weekly) in a 2x2 factorial study design in patients older than 60 years (NHL-B2 protocol) and in patients younger than 60 years (NHL B1 protocol). All patients also received radiotherapy (36 Gy) to sites of bulky or extranodal disease. In patients aged less than 60 years (all with low risk aggressive NHL), complete response and overall survival were significantly improved.
with CHOEP-14 and CHOEP-21, but not with CHOP-14. In contrast, in patients aged over 60 years of age (76% with low or low intermediate risk) CHOP-14 significantly improved time-to-treatment failure and overall survival. Recently the Groupe d’ Etude des Lymphomes de l’Adulte (GELA) conducted a multicenter randomized trial of ACVBP (consisting of four cycles of an intensified CHOP-like regimen followed by sequential methotrexate, etoposide and cytarabine consolidation) demonstrating that this provided a survival advantage over eight cycles of standard CHOP. Therefore it has become clear in recent years that, it is possible for some patients with aggressive NHL, to improve survival by intensification of chemotherapy. However this approach is still not recommended outside of the setting of clinical trials.

**High dose therapy and autologous SCT during first line therapy.** The use of HDT and autologous SCT in previously untreated patients is still controversial. A possible benefit for patients in partial response at the end of their first induction therapy has been postulated in some phase II studies. The use of HDT and SCT was expected to ameliorate chemotherapy outcomes in high-risk DLBCL patients. Indeed, in some clinical trials the survival curve after early HDT/SCT (in first CR) or after employing a high dose sequential (HDS) therapy upfront proved superior to conventional chemotherapy, indicating the potential of this treatment to eradicate the disease. These observational studies in contrast with two meta-analyses of up to 11 randomized trials, which showed a similar overall survival in patients receiving first-line HDT/SCT or standard chemotherapy. However in some studies HDT/SCT, when employed as a complete standard course of induction (rather than an abbreviated course) or where the HDT/SCT arm achieved less than 25% drop-outs, provided a significant increase of survival. Furthermore, in all trials, no real benefit of HDT and ASCT was described for low risk IPI patients. At the present time the results of these comparative studies are conflicting, and thus the routine use of HDT and ASCT as consolidative therapy for newly diagnosed large cell lymphoma is not recommended outside of clinical trial. Recently chemoimmunotherapy has been shown to greatly improve the long-term outcomes of DLBCL and ongoing randomized studies should compare chemoimmunotherapy to HDT/SCT in first-line therapy for DLBCL.

**Combination of chemotherapy and monoclonal antibodies.** The most consistent improvement over CHOP chemotherapy in the treatment of aggressive NHL has come with the addition of the chimeric anti CD20 monoclonal antibody rituximab. A phase II study of CHOEP plus rituximab as first line therapy has yielded an high response rate, 5 year-PFS and overall survival both in high and low risk patients. Subsequently a phase III study of GELA compared 8 cycles Rituximab plus CHOP (R-CHOP) with 8 cycles of CHOP alone in the treatment of 399 elderly previously untreated patients with DLBCL. Complete response rates, 2-year overall survival and 2-year EFS were significantly better in the R-CHOP arm without increase of adverse events. The survival advantage obtained with R-CHOP suggests that this regimen should replace CHOP alone as the gold standard treatment. Subsequent 5-year overall survival and EFS follow up confirmed that superiority of R-CHOP was maintained over time and the benefit was not restricted to a subgroup of patients. In a retrospective analysis of the same group of patients R-CHOP appeared significantly more effective in Bcl-2 positive than CHOP, suggesting that the benefit of rituximab might overcome the Bcl-2 associated chemo-resistance. Also, a large retrospective study at British Columbia University confirmed a significant improvement of overall survival and EFS in patients of age <60 and age >60 years when rituximab was combined with CHOP compared to an historical CHOP chemotherapy. Moreover an intergroup US study randomized a population of elderly patients to receive CHOP or CHOP plus rituximab with a different schedule to the GELA trial, and all responding patients were then randomized to receive either rituximab maintenance therapy or not. The study is ongoing and definitive results are not yet available. Recently a phase III International study denominated MInT, was undertaken to address the valuation.

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<tr>
<td></td>
<td>R-CHOP</td>
<td>76 p&lt;0.005</td>
<td>57 p&lt;0.001</td>
<td>70 p=0.007</td>
</tr>
<tr>
<td>Sehn et al.,198</td>
<td>CHOP</td>
<td>nr*</td>
<td>52</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>R-CHOP</td>
<td>nr</td>
<td>71 p=0.0009</td>
<td>77 p=0.0001</td>
</tr>
<tr>
<td>Pfrendschuh et al.,199</td>
<td>chemo</td>
<td>68</td>
<td>61</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>R-chemo</td>
<td>86 p&lt;0.0001</td>
<td>80 p&lt;0.001</td>
<td>95 p&lt;0.001</td>
</tr>
</tbody>
</table>

*nr= not reported; **5-year time-to-treatment failure, 5-year overall survival.
ue of rituximab in 758 patients younger than 60 years with low or intermediate-low risk DLBCL. Rituximab was combined with CHOP or a CHOP-like regimen in the same schedule as that used in the GELA trial. Patients receiving rituximab with chemotherapy had significantly better complete remission rate, 2-year time-to-treatment failure and overall survival.178 This is the first randomized trial supporting the use of rituximab in younger patients with low risk IPI, albeit in a selected subset of patients most of whom had limited stage disease (Table 2).

First line therapy of limited stage lymphoma
Limited stage, also referred to as early or localized stage disease, accounts for approximately 15–20% of DLBCL. There is extreme heterogeneity of patients within the group described as having limited-stage lymphoma, making comparison of outcomes across this population difficult. Modern therapy for limited-stage NHL was developed when some studies focused on the concept of a brief course of chemotherapy (three cycles of CHOP) followed by involved field radiation therapy (IFRT) for patients in stage I-II.174 Subsequently the SWOG compared, in 401 patients with localized disease, three cycles of CHOP followed by IFRT to eight cycles of CHOP and found that the brief chemotherapy plus IFRT was superior to the eight cycles of CHOP.201 In this study, bulky stage II disease was excluded because it is known to have a prognosis similar to that of advanced stage disease. The GELA group has recently reported the results of a randomized trial, in localized stage I-II including bulky disease, comparing three cycles of CHOP IFRT (329 patients) to ACVBP209 an aggressive combination therapy used for advanced disease. With a median follow-up of 7.7 years, EFS and overall survival rates were significantly higher in the group given ACVBP chemotherapy than in the group given CHOP plus radiotherapy (p<0.001 and p=0.001, respectively). This difference remains significant also in patients without bulky disease.202

A score incorporating four risk factors (stage II vs I, age >60 vs <60, increased vs normal serum LDH and PS>1 vs<1) defined as the modified IPI score for limited disease, was tested in two different series of patients with limited-stage disease. This four risk score can be assessed for each patient, and the prognosis determined based on the number of adverse risk factors. Patients without any risk factors (IPI score=0) defined very limited disease have an outstanding prognosis with 5 and 10-year overall survival exceeding 90%. Patients with stage I or II without bulky and at least one adverse risk factor (IPI score=1) should be placed in a group defined limited disease with a 5–10 years overall survival of 70%. For these patients a brief chemotherapy plus IFRT should be considered the standard therapy. Although dose-dense chemotherapy in the GELA trial showed to be effective in this group of patients, the risk of toxicity due to dose-dense chemotherapy should be accurately balanced against the potential benefits. Patients with stage II with more than one risk factor (IPI score >1) or stage II bulky disease were defined as advanced disease, with a 5–10 years overall survival of approximately 50% for whom standard chemotherapy or dose dense regimens should be considered203,204 (Table 3).

Therapy of recurrent and refractory disease
Conventional treatment. At least 40% to 50% of patients with advanced-stage DLBCL do not attain a remission with initial therapy or relapse after achieving a remission. Selected patients may occasionally experience prolonged remissions with IFRT. A variety of drugs are active as single agents in patients with relapsed/refractory DLBCL. The most commonly used agents include etoposide,204 cytarabine,205 cisplatin,206 mitoxantrone,207 and ifosfamide.208 Single-agent activity rates as high as 20% to 30% have been reported, although response durations are brief, and most patients are treated with combination chemotherapy regimens. Results of several commonly used salvage-chemotherapy regimens are presented in Table 4. Prognostic factors for patients treated with these salvage regimens are similar to the factors that are important for upfront treatment of newly diagnosed patients. These regimens are commonly used as short-duration treatments to reduce tumor burden before treatment with high-dose therapy and autologous hematopoietic SCT.209-217 Although response rates exceeding 50% are commonly observed with these regimens, prior treatment frequently makes it difficult to administer repeat-

Table 3. Clinical stage, IPI risk factors and outcome for patients with limited disease.

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>IPI modified risk</th>
<th>Treatment</th>
<th>5-year median overall survival</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, IE</td>
<td>0</td>
<td>CHOP x 3+IFRT</td>
<td>&gt;90%</td>
<td>Very limited</td>
</tr>
<tr>
<td>I,IE,II IE (no bulky)</td>
<td>=1</td>
<td>CHOP x 3+IFRT</td>
<td>70%</td>
<td>Limited</td>
</tr>
<tr>
<td>II-IE bulky</td>
<td>&gt;1</td>
<td>CHOP x 8</td>
<td>50%</td>
<td>Advanced</td>
</tr>
</tbody>
</table>

Reprinted by permission of Miller. Haematologica 2004 CHOPx3: three cycles of CHOP; CHOPx8: eight cycles of CHOP; IFRT: involved field radiotherapy.
ed cycles of therapy, and few patients experience long-term disease-free survival with these regimens alone. Approximately one third of patients with relapsed/refractory DLBCL respond to rituximab,\textsuperscript{218} and rituximab is commonly added to the standard regimens. There is relatively little experience with the use of radiolabeled antibodies for DLBCL, although results appear to be poorer than those seen for indolent lymphomas.\textsuperscript{219,220}

**High-dose therapy.** The poor results of treatment with conventional-dose salvage-chemotherapy regimens have led to the increasing use of high-dose therapy followed by autologous SCT for patients with relapsed or refractory lymphoma. A large number of phase II trials of autologous SCT for patients with relapsed/refractory DLBCL have been published\textsuperscript{188,221-229} with a median 3-yr disease-free survival rate of 35% (range, 11%-71%), and virtually all demonstrate the prognostic importance of chemotherapy sensitivity before transplantation. These trials demonstrate that substantial numbers of patients with relapsed/refractory disease can experience long-term disease-free survival after autologous SCT. Other prognostic factors usually associated with favourable outcomes after transplantation include good performance status, younger age, absence of tumor bulk, and absence of extensive prior therapy. Despite the widespread use of autologous SCT for DLBCL, the value of this approach was not validated until the results of the International PARMA trial were published.\textsuperscript{230,231} Consensus panel recommendations indicate that high-dose therapy followed by autologous SCT is now considered standard therapy for patients with chemotherapy-sensitive relapsed DLBCL.\textsuperscript{222,223} There is less agreement on the use of autologous SCT for patients with resistant relapsed or refractory disease. There is a quite limited experience with the use of allogeneic transplantation as salvage treatment for relapsed or refractory disease.

**Transformed lymphomas**

Histopathological transformation of follicular lymphoma to large-cell aggressive NHL is an event occurring in 15-32% of patients.\textsuperscript{234,235} The incidence appears to reach a plateau by 6 years after diagnosis and the median time to transformation is 66 months.\textsuperscript{235,236} High β2-microglobulin serum levels at diagnosis and failure to achieve complete remission after first-line therapy predict a higher risk of transformation for follicular lymphoma.\textsuperscript{234,235} The prognosis for transformed lymphoma is generally poor, the median survival from transformation being about 10 months. This accounts for a large part of mortality in patients with follicular lymphoma. Young patients with limited disease that is chemosensitive may experience prolonged survival and durable disease-free survival in transformed lymphoma can be obtained with standard chemotherapy as it can for de novo diffuse large-cell lymphoma. Therefore, data do not show a relevantly different behavior than that of de novo B–DLCL.\textsuperscript{235-237} Age, response to salvage therapy, B symptoms, LDH values, bone marrow involvement, stage, no prior chemotherapy and early transformation are all factors predictive of poor survival after transformation\textsuperscript{235-238} while response to CHOP-like regimens is a positive predictor of survival.\textsuperscript{235,236} No evidence supports a net benefit from delaying autologous SCT at the moment of lymphoma transformation. A median overall survival of 40-60% at 4-5 years after autologous SCT has been reported, climbing to 69% in chemosensitive patients while the 5-year disease-free survival is about 30% in the over 200 patients included in published series. Survival after autologous SCT was not dissimilar to that reported for patients with non-transformed indolent NHL and primarily aggressive NHL undergoing autologous SCT.\textsuperscript{103,237-239}

Recently high response rates, ranging from 50% to 80%, with an acceptable safety, were reported in transformed patients with less than 25% bone marrow involvement treated with ibritumomab and tositumomab and also with rituximab therapy.\textsuperscript{240-243}

**Mantle cell lymphoma**

From a clinical viewpoint, MCL brings together the worst characteristics of indolent and aggressive lym-

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**Table 4. Chemotherapy salvage regimens for DLBCL.**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Drugs</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>CR + PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMVP-16</td>
<td>Ifosfamide, methotrexate, etoposide</td>
<td>37</td>
<td>25</td>
<td>62</td>
</tr>
<tr>
<td>DHAP\textsuperscript{218}</td>
<td>Dexamethasone, cytarabine, cisplatin</td>
<td>28</td>
<td>26</td>
<td>54</td>
</tr>
<tr>
<td>ESHAP\textsuperscript{211}</td>
<td>Etoposide, methylprednisolone, cytarabine, cisplatin</td>
<td>26</td>
<td>22</td>
<td>48</td>
</tr>
<tr>
<td>DICEP\textsuperscript{212}</td>
<td>Cyclophosphamide, etoposide, cisplatin</td>
<td>52</td>
<td>26</td>
<td>78</td>
</tr>
<tr>
<td>DICE\textsuperscript{213}</td>
<td>Dexamethasone, ifosfamide, cisplatin, etoposide</td>
<td>23</td>
<td>44</td>
<td>67</td>
</tr>
<tr>
<td>ICE\textsuperscript{214}</td>
<td>Ifofosamide, carboplatin, etoposide</td>
<td>28</td>
<td>44</td>
<td>72</td>
</tr>
<tr>
<td>IEV\textsuperscript{215}</td>
<td>Ifosfamide, epirubicin, etoposide</td>
<td>32</td>
<td>45</td>
<td>77</td>
</tr>
<tr>
<td>I-CHOPE\textsuperscript{216}</td>
<td>Doxorubicin, vincristine, etoposide, cyclophosphamide, prednisone</td>
<td>17</td>
<td>31</td>
<td>48</td>
</tr>
<tr>
<td>EPOCH\textsuperscript{217}</td>
<td>Etoposide, vincristine, doxorubicin, cyclophosphamide, prednisone</td>
<td>36</td>
<td>34</td>
<td>70</td>
</tr>
</tbody>
</table>

CR: complete remission, PR: partial remission.
phomas. Like indolent lymphomas, it is rarely if ever curable with standard chemotherapy, but unlike the former the median survival is approximately 3 to 4 years only.\textsuperscript{244-247}

Given the common involvement of extranodal sites, including the gastrointestinal tract, Waldeyer's ring and CNS, staging should always include upper and lower gastrointestinal tract endoscopy and lumbar puncture.

Various adverse prognostic factors have been identified for MCL, including a high International Prognostic Index score,\textsuperscript{248} an increased mitotic index, p53 gene mutations, and a blastoid morphology. However, their practical use in stratifying patients for different therapies is of little value in a disease in which even the favorable risk patients have a median overall survival of only 5 years,\textsuperscript{249} and no evidence of plateau in the survival curve. Only occasional patients present with isolated peripheral blood and marrow lymphocytosis, and may have an indolent course, similar to that of patients with chronic lymphocytic leukemia. These rare forms cannot be easily distinguished from atypical chronic lymphocytic leukemia carrying the bcl1 translocation.\textsuperscript{249}

Twenty to 60 percent of CHOP-treated patients achieve a complete response \textsuperscript{\textsuperscript{151,250,251}} with a median duration of remission of only one to three years.\textsuperscript{151,252-256} The addition of newer agents, such as fludarabine, taxanes, flavopirifol or bortezomib, used alone or in combination, either failed to show a substantial benefit, or the reports are too preliminary, as for the promising agent bortezomib,\textsuperscript{257,258} to allow firm conclusions. The only possible exception is rituximab, in particular when used in combination with chemotherapy. In a phase II study, approximately 30 percent of 25 informative chemo-therapy-naïve patients treated with rituximab and CHOP, achieved molecular remission in peripheral blood or bone marrow. However, the molecular complete responses were not predictive of improved progression-free survival.\textsuperscript{151} More R-CHOP was compared with CHOP in a randomized trial as first-line therapy for MCL.\textsuperscript{259}

A significantly higher rate of complete responses and longer of time-to-treatment-failure was observed, while the time-to-progression was not improved. An adequate follow-up is clearly needed for a final assessment of the long-term results with this approach. Rituximab has also been used in association with thalidomide with interesting results.\textsuperscript{260}

In contrast to the limited success with conventional-dose chemotherapy, very encouraging results were recently reported by two groups using more aggressive induction treatments as first-line therapy.\textsuperscript{261,262} Both studies made use of rituximab, in combination with either sequential administration of multiple high-dose chemotherapy cycles and stem cell autografting,\textsuperscript{261} or with an aggressive multiagent acute lymphoblastic leukemia-like induction therapy (Hyper-CVAD/M-A) without stem cell transplant.\textsuperscript{262} Of note, in the former study the use of rituximab had a critical role as an in vivo purging agent, and allowed reinfusion of tumor-free stem cells in the vast majority of treated patients.\textsuperscript{261} Both regimens achieved complete and durable responses in a very high proportion of patients, with a 54-month event-free survival rate of 87 percent following rituximab, high-dose sequential therapy and autografting and a 24-month freedom-from-survival rate of 80 percent, respectively. Toxicities were comparable and manageable, suggesting that high-dose or dose-intense chemo-immunotherapy has a role in the initial management of MCL, at least in clinically fit patients less than 65 years of age. A similarly promising approach is high-dose chemo-immunotherapy with 131I-tositumomab and autologous stem cell support.\textsuperscript{263}

For patients in chemosensitive relapse, allogeneic transplantation with reduced-intensity conditioning appears to be a potentially effective strategy, safer than full-dose allografting.\textsuperscript{263}

In summary, elderly patients with a MCL should be treated with a regimen containing rituximab, such as R-CHOP or equivalent. In treating young, otherwise healthy patients, it is important to consider that long-term remissions, and possibly cures, could be obtained with aggressive or high-dose rituximab-supplemented regimens. For relapsed chemosensitive patients, reduced-intensity allogeneic bone marrow transplantation should always be considered. In general, given the invariably dismal outlook for conventionally-treated patients with MCL, it is recommended to refer these patients to a center offering a clinical trial, or fully capable of safely delivering high-dose therapy with or without autologous or allogeneic transplantation in first remission.

**Lymphoblastic lymphoma**

Lymphoblastic lymphoma is a rare disease (2% of NHL) arising from immature T cells in 85-90% of cases and more rarely from immature B cells. It is more common in children and adolescents. Mediastinal involvement is present in 80% of patients while the bone marrow and CNS are infiltrated in about 20% of cases. With chemotherapeutic regimens designed for lymphoma patients, such as CHOP the clinical outcome is poor while the application of ALL-type protocols produces excellent responses. In children with lymphoblastic lymphoma, regimens similar to treatments for ALL produced 5-year disease-free survival rates ranging from 70-90%. In adults, leukemia-type regimens produce response rates usually higher than 70% but the 5-year disease-free survival rate in adults remains about 50%. In a recent paper summarizing the outcome of adult patients with T-lymphoblastic lym-

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**Nodal aggressive non Hodgkin's lymphoma**
Table 5. Main regimens for Burkitt's lymphoma.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Complete remission</th>
<th>Disease-free/event-free survival</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMB 81-84-86-89</td>
<td>83-89%</td>
<td>61-71% at 2-3 years</td>
<td>66-74% at 2-3 years</td>
</tr>
<tr>
<td>B-NHL 83-86272</td>
<td>63-74%</td>
<td>50-71% at 4-8 years</td>
<td>49-51% at 4-8 years</td>
</tr>
<tr>
<td>CODOX-M/IVAC 274-276</td>
<td>75-92%</td>
<td>65-85% at 21 months-2 years</td>
<td>73% at 3 years</td>
</tr>
<tr>
<td>Hyper-CVAD 281</td>
<td>81%</td>
<td>61% at 3 years</td>
<td>49% at 3 years</td>
</tr>
<tr>
<td>CALGB 272-280</td>
<td>80%</td>
<td>50% at 4 years</td>
<td>45-52% at 3-4 years</td>
</tr>
<tr>
<td>INT-MILAN 281</td>
<td>77%</td>
<td>68% median follow-up 28.7 months</td>
<td>78% median follow-up 28.7 months</td>
</tr>
</tbody>
</table>

Phoma treated according to ALL protocols of the German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia (GMALL) Holtzer and co-workers reported that 93% of patients achieved a complete remission. In this study, 36% of patients relapsed within 12 months. The majority of relapses (47%) occurred in the mediasinum despite irradiation. The estimates for overall survival, complete remission, and disease-free survival at 7 years were 51%, 65%, and 62%, respectively. More recently, Thomas et al. reported the results obtained at the MD Anderson Cancer Center in 33 patients treated with the hyper-CVAD regimens (fractionated cyclophosphamide, vincristine, adriamycin, and dexamethasone) followed by radiotherapy to the mediastinal disease. Using this approach, a high proportion of patients (91%) achieved complete remission, and 9% achieved partial response. Within a median of 13 months, 30% of patients had relapsed or progressed. Estimates for 3-year progression-free and overall survival for the 33 patients were 66% and 70%, respectively. No parameters (eg, age, stage, LDH, β2 microglobulin) appeared to influence outcome except for CNS disease at presentation.

Both autologous and allogeneic hematopoietic SCT have been used in an attempt to improve survival. Prolonged remissions and long-term survival of patients with lymphoblastic lymphoma have been obtained using either autologous or allogeneic transplantation for recurrent or refractory disease.

Local radiation therapy can significantly decreases the risk of mediastinal recurrence in adult patients with mediastinal T-cell lymphoblastic lymphoma and the benefit of adjuvant radiation therapy (26-39 Gy) seems particularly evident in patients treated with more intensive chemotherapy regimens.

Burkitt's lymphoma

Adult sporadic, non-immunodeficiency associated Burkitt's lymphoma (BL) and its variant, Burkitt's-like lymphoma (BLL), represent aggressive forms of B-NHL clinically characterized by rapid growth and frequent extra-nodal involvement. Despite their intrinsic aggressiveness, however, it is now well known that adult BL/BLL may have a favorable outcome if adequately treated with specific treatments. Indeed, during the last ten years, different generations of intensive chemotherapeutic regimens, generally derived from pediatric protocols initially found to be highly effective in the treatment of children with BL, and subsequently tailored to older patients, have been developed for adult BL (Table 5). These regimens differs from other conventional approaches to NHL as they are mainly based on the principles that: i) BL cells, due to their high proliferative activity, are very sensitive to S-phase specific drugs administered at high doses and at short intervals; ii) CNS prophylaxis is of paramount importance for successful treatment. Thus, current strategies for the treatment of BL generally represent a sort of dose-dense, multi-agent intensified approach, with tendentially shortened duration of therapy and minimal treatment delays. Such regimens usually include fractionated (or hyper-fractionated) cyclophosphamide or analogs (postulating that a fractionated schedule would ensure exposure of dividing tumor cells to the alkylating activity of these drugs) and high dose ara-C and methotrexate (to cross the blood-brain barrier), alternated with additional non-cross-resistant cytostatics. Adjunctive aggressive CNS prophylaxis with intrathecal triple administrations of cytostatics also represents an essential part of these regimens. The further administration of cranial irradiation is not recommended in patients without marrow involvement for CNS prophylaxis, in order to avoid severe neurological sequelae. Some of these regimens also include a cytoreductive phase to minimize the risk of tumor lysis syndrome. Some are based on a sequence of multiple induction, consolidation and maintenance cycles, while others have introduced the concept of alternated blocks of combined chemotherapy.

Overall, using these approaches, it has been estimated that 63–92% of patients achieve complete remission and 50–85% of them maintain these remissions for at least two years following therapy (Table 4). More than half of these patients may be considered cured. Older patients, however, show a worse outcome, due to lower percentages of subjects completing the treatment,
higher mortality and toxicity, and higher disease progression and relapse rates, which are probably not simply related to treatment toxicity. Advanced stage, bulky disease, poor performance status, and high LDH levels are also usually considered poor prognostic factors for this disease, whereas leukemic phase and CNS involvement do not seem to have additional prognostic relevance if patients are treated with modern schemes. Of interest, early stage BL (Ann Arbor I-II or St. Jude I-II) may be successfully treated with the same regimens applied to advanced stages, with shorter duration of the therapy and a reduced total number of cycles.

Up-front autologous SCT for BL has rarely been incorporated into clinical trials. In a retrospective study, the 3-year actuarial overall survival rate was 72% for patients transplanted in first complete remission. For these patients, disease bulk at the time of autologous SCT was the only factor predictive of progression-free survival and overall survival. However, since long-term overall survival following autologous SCT appears to be quite similar to that achieved by brief duration, intensive chemotherapy alone and transplant-related mortality may exceed that of chemotherapy alone, autologous SCT is not currently recommended as consolidation after first line CT in patients in complete remission.

Complete remission is usually achieved after 4-6 weeks of treatment. An early and careful assessment of response (after 6-8 weeks of therapy), including, if necessary, repeated biopsies or PET-imaging in doubtful cases, is mandatory, since failure to achieve complete remission is a very poor prognostic factor. In adults with BL who relapse, this usually occurs within the first year, although late relapses (up to 5 years after the completion of chemotherapy) have been described. These patients, as well as those who do not achieve complete remission, have a very poor prognosis even when treated with combined chemotherapy, including non-cross-reactive agents not employed in front-line therapy, such as ifosfamide and cisplatin. High-dose therapy followed by autologous SCT for patients with relapsed disease is superior to conventional-dose salvage regimens, with a 3-year overall survival of 37% for patients in chemo-sensitive relapse and 7% for chemoresistant patients. In a recent study, patients who failed to achieve complete remission after a very brief induction phase, did obtain complete remission after a high-dose, stem-cell-supported, sequential chemotherapy program.

Neoplastic cells of BL strongly express CD20 antigen on their surface in the great majority of cases. Therefore, an anti-CD20 monoclonal antibody (rituximab) has been integrated into the most recent versions of some of the previously described regimens. Preliminary data appear promising, suggesting the possibility of a further improvement of the percentages of complete remission and of long-survivors.

Peripheral T-cell lymphomas

PTCL have been difficult to classify and treat for several reasons. The first reason is their rarity, because in western countries PTCL account for 10-15% of aggressive lymphomas. The second reason is their morphologic heterogeneity, since it has been difficult to stratify them according to different histological subtypes. Indeed, the Working Formulation classification, devised in the 1980s, did not recognize T-cell lymphomas and clustered together many aggressive diseases of different biology under the general term of intermediate-grade lymphomas.

Therefore CHOP (or CHOP-like regimens), the standard chemotherapy for working formulation intermediate grade lymphoma, was considered for many years the optimal therapy for T-cell neoplasms. In 1994, the REAL classification gathered together the different T-cell subtypes under a single broad category termed PTCL. Gisselbrecht et al. reported the results of the largest prospective study published so far: 288 PTCL patients were treated with different anthracycline-containing regimens. The 5-year overall survival and event-free survivals were 41% and 33%, respectively; the outcome of PTCL was significantly worse than that of DLBCL. Other authors confirmed in smaller studies that second or third-generation chemotherapy regimens were not able to modify the natural history of PTCL, and the 5-year overall survival remained between 25-40%. When the main subgroups defined by the REAL classification were analyzed, only anaplastic large cell lymphoma (ALCL) associated with t(2;5) had a prognosis equivalent to or better than that of to DLBCL. For the other subtypes, most authors agreed on the adverse prognostic effect of the T-cell phenotype. Considering that standard chemotherapy regimens proved to be unsatisfactory for PTCL, many investigators began to explore the use of high dose chemotherapy (HDT) and autologous SCT. Rodriguez et al. reported 3-year overall survival and progression-free survivals of 39% and 32%, respectively, in a small cohort of patients who had relapsed after or who were refractory to conventional chemotherapy. Song et al. reviewed the experience of the Toronto University in a larger cohort of patients with relapsed or primary refractory PTCL, and compared their outcome with that of of DLBCL. These two studies agreed on the similar outcome of aggressive relapsed B and T lymphomas, but other authors have questioned the advantage of HDT in this subset of patients. Moreover, as was
observed with standard chemotherapy, even with HDT the prognosis of T-cell lymphomas differs among the various subtypes: in fact, while ALC with t(2;5) has a good prognosis, patients with unspecified PTCL have a poor outcome, with a 5-years event-free survival ranging between 16% and 23%.

To define the clinical outcome of unspecified PTCL better and to assess a prognostic model specifically devised for PTCL, the Intergruppo Italiano Linfomi (IIL) retrospectively analyzed 385 cases and identified four risk groups on the basis of four clinical parameters (age, performance status, LDH, bone marrow involvement): patients classified in groups 3 and 4 had a very dismal prognosis, with a 5-year survival probability of only 26%. The conclusion of the investigators was that for patients with unspecified PTCL and an adverse score novel therapeutic strategies (such as monoclonal antibodies and allogeneic transplantation) were required. A recent pilot study evaluating the efficacy of alemtuzumab (anti-CD52 humanized antibody) in heavily pre-treated PTCL patients showed a considerable antitumor activity, with a 36% overall response rate. However, infectious complications and hematologic toxicity were unacceptably high, leading to early discontinuation of the study. In conclusion, PTCL are a rare and heterogeneous group of malignancies with a dismal prognosis. Considering the disappointing results of standard and high-dose chemotherapy (except for ALCL) large prospective studies should be encouraged in order to investigate the efficacy of novel approaches, such as monoclonal antibodies or frontline allogeneicSCT.

**Post-transplant lymphoproliferative diseases**

PTLDs constitute a group of potentially life-threatening complications of solid organ transplantation. The incidence of PTLD differs in relation to the type of transplanted organ. At 10 years, the actuarial risk for developing this devastating complication is around 3% and 8% in the case of heart or kidney transplant, respectively. The type and the intensity of immune suppression significantly affect the risk of developing PTLD and this is particularly evident in the case of allogeneic hematopoietic stem cell transplantation if *in vitro* or *in vivo* T-cell depletion is used to decrease the severity of graft versus host disease. The PTLD classification has been recently updated by the World Health Organization and encompasses three main categories: a) early lesions (reactive plasmacytic hyperplasia and infectious mononucleosis-like lesions), b) polymorphic PTLD and c) monomorphic PTLD (to be classified according to the lymphoma classification). While the role of Epstein-Barr virus (EBV) is firmly established in the development of B-cell PTLD, this virus is not a common finding in the few cases of T cell-derived PTLD. Moreover, as time goes by from transplantation, the proportion of EBV-negative, B-cell derived PTLD increases remarkably and these cases generally have a worse prognosis than their EBV-positive counterparts. Most PTLD of B-cell phenotype originate from cells that have experienced the germinal center reaction, since the neoplastic clone carries clues of the somatic hypermutation process targeting immunoglobulin variable genes (IgV) in the germinal center microenvironment.

Therapeutic strategies for these lymphoproliferative disorders must be adapted first to the performance status of the patients and second to the type and stage of the PTLD. It is now well established that reduction or discontinuation of immunosuppressive drugs are the first options of PTLD treatment. This strategy alone, however, is fully effective only for polyclonal, EBV-driven, early lesions which may exhibit complete and durable responses. In this setting, evaluating the EBV viral load has been used as a tool for preventing PTLD and monitoring the clinical benefit of reducing or discontinuing of immunosuppressive drugs. Surveillance studies in patients with high EBV loads in peripheral blood have been found to be sensitive but not specific predictors of PTLD development. When reduction in immunosuppression fails, the use of the anti-CD20 chimeric monoclonal antibody, rituximab, represents an attractive second-line therapeutic option because of its low toxicity and its ability to reduce the number of B cells in which massive proliferation of EBV particles is occurring. Rituximab therapy can rapidly induce reduction of lymph node enlargement and this may be particularly evident in cases showing massive disease infiltration of the lungs.

For patients with monoclonal NHL, particularly if EBV-negative and late developing, reduction of immunosuppressive drugs is not sufficient. Surgical resection and local radiotherapy followed by rituximab administration, may represent a valid therapeutic option for patients with localized stage I or II NHL of B-cell origin and low performance status.

Cellular therapies have been tested based on the *in vitro* generation of EBV-specific T-cell lines. These innovative treatments were shown to be effective in reducing the viral load and were remarkably well tolerated. However, the recent availability of rituximab and the highly demanding requirements of Good Manufacturing Practices (GMP) in laboratories reduced the initial enthusiasm for these therapies, at least for the treatment of the relatively benign EBV-driven early lesions.

All patients with advanced stage disease and acceptable performance status should rapidly initiate aggressive chemotherapy soon after discontinuation of any immunosuppressive treatment. Conventional chemo-
therapy programs were initially described as less effective and more toxic in PTLD with often unacceptable mortality rates. More recently, the toxicity-related mortality rate has been decreasing (down to 25%) even when high dose chemotherapy programs and autologous SCT are used (personal data).

During the chemotherapeutic programs immune suppression with azathioprine, cyclosporine A or tacrolimus must remain withdrawn since the risk of organ rejection is negligible while the patient is under treatment with cytotoxic drugs. At the end of treatment, immunosuppressive treatments can be restarted, generally within one month, paying attention to T-cell counts in the peripheral blood.

**Allogeneic stem cell transplantation as salvage treatment for aggressive lymphomas**

The role of allogeneic SCT in the treatment of relapsed aggressive lymphomas is still a matter of debate. The existence of a graft–versus–tumor effect is largely accepted for myeloid leukemias. In lymphoid diseases, and in particular in aggressive lymphomas, the evidence for a graft–versus–lymphoma effect is less documented, being mainly supported by reports describing lymphoma regression after cyclosporine withdrawal or cell therapy with donor lymphocyte infusions. In addition, a case-controlled study of the European Blood and Marrow Transplantation (EBMT) group showed a significantly lower rate of relapse in patients with aggressive lymphoma developing chronic graft–versus-host disease after allogeneic SCT. However, a recent retrospective analysis, by Bierman et al., failed to show a lower relapse risk when allogeneic and syngeneic transplants were compared. That said, the study had several limitations because it was retrospective and some of the patients’ characteristics were different or missing among the allogeneic, syngeneic and autologous transplant groups.

The role of allogeneic SCT in aggressive NHL is difficult to assess due to the few prospective studies and a common bias in patient selection: in fact, the majority of single center studies have a very limited number of patients, often presenting with poor-risk factors, who are heavily pretreated (including a failed autologous SCT) and who are frequently affected by a refractory disease. Dhedin et al. evaluate the largest series of myeloablative allogeneic SCT for aggressive lymphomas, and reported 5-year overall and progression-free survival and PFS rates of 41% and 40%, respectively. It is noteworthy that the 5-year overall survival rate was 76% for patients allografted in complete remission. The treatment–related mortality, however, was relevant (44%). A retrospective analysis of the EBMT group on 242 patients with intermediate-grade disease reported an overall of 50% and a progression-free survival of 43%; in comparison with 9488 autologous SCT patients, relapse was reduced, but overall survival was significantly worse because of the higher transplant-related mortality in the allogeneic cohort. Taken together all these studies have suggested a potential role for allogeneic SCT in the salvage treatment of aggressive lymphomas, but have also highlighted that the major concern is transplant-related mortality, which is exceedingly high, and which limits the widespread application of the procedure.

It has recently been shown that reduced intensity conditioning regimens can be used to obtain the engraftment of allogeneic stem cells with limited organ toxicity, and a rather low transplant-related mortality. This strategy has been effective in producing clinical and molecular remissions in advanced hematologic malignancies, but the experience with reduced intensity conditioning regimens in lymphomas is still quite limited. Initially encouraging results were reported by Khouri et al.: transplant-related mortality at day 100 was 0% and the 2-year progression-free and overall survivals were 61% and 71%, respectively. An EBMT retrospective analysis on 188 lymphoma patients reported, in the subgroup of aggressive lymphomas, 1-year progression-free and overall survivals of 32% and 52%, respectively. Recently, Morris et al. reported the outcome of relapsed and refractory lymphoma patients receiving an alemtuzumab–containing reduced intensity conditioning regimen followed by allogeneic SCT: the 3-year transplant-related mortality and progression-free survival was better in low-grade histologies.

Finally, very few studies have addressed the role of allogeneic SCT in rare aggressive lymphoma subtypes such as Lbl, BL and PTCL. Prolonged remissions and long-term survival of patients with Lbl have been obtained after allogeneic SCT for relapsed or refractory disease.

A retrospective study was recently conducted on behalf of the Lymphoma Study Writing Committee of the International Bone Marrow Transplant Registry: the efficacy of allogeneic transplantation was offset by the high transplant-related mortality. Not surprisingly, a lower relapse rate was observed in allotransplant recipients at 1 and 5 years, but no difference was noted in overall survival between recipients of allotransplant recipients and allogeneic SCT. Allogeneic SCT was also an effective salvage treatment for BL patients in chemosensitive relapse. However, although the outcome after allogeneic transplants appeared better than that after autologous ones in terms of relapse rate, the transplant-related mortality was relevant and there was no improvement in the overall survival. A multicenter pilot study employing reduced intensity conditioning followed by allogeneic SCT has been recently conducted in relapsed PTCL: although the number of patients was
limited, the outcome was encouraging, with estimated 3-year progression-free and overall survival of 64% and 81%, respectively. The estimated probability of transplant-related mortality at 2 years was only 6%.  

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**Supportive therapy**

**Management of neutropenia**

Granulopoiesis-stimulating growth factors, namely G-CSF and GM-CSF, are usually employed to manage chemotherapy-induced neutropenia, in order to reduce its duration and severity. Meta-analyses of several clinical studies have documented that growth factors, when used as prophylaxis in patients with NHL undergoing conventional chemotherapy, reduce the risk of chemotherapy-induced neutropenia, febrile neutropenia, hospital admissions and hospital stay due to infection, treatment delays and documented infections. However, infection-related mortality, use of intravenous antibiotics, tumor response, and overall clinical outcome are not significantly modified by growth factors.

Predictive parameters for calculating the risk of developing febrile neutropenia in NHL are age, performance status, chemotherapy-intensity, bone marrow involvement, renal or cardiac co-morbidity, albumin, hemoglobin and LDH levels. The ASCO guidelines concerning the use of growth factors for managing neutropenia, currently under revision, suggest that they should not be used as primary prophylaxis for chemotherapy-induced neutropenia, febrile neutropenia unless the risk of febrile neutropenia is 40% or greater. More recent data, however, based on updated pharmaco-economic evaluations, support the possible clinical benefit and the cost neutrality of primary prophylaxis in settings in which the risk of febrile neutropenia is 30% or even 20%. In general, when indicated, primary prophylaxis with growth factors seems to be more effective than secondary prophylaxis in preventing febrile neutropenia. However, the cost-effectiveness of G-CSF has recently been disputed in the context of elderly patients receiving standard CHOP chemotherapy.

Peg-filgrastim is a compound created by addition of a poly-ethylene-glycol molecule to filgrastim with a novel, self-regulating phamacokinetic profile characterized by neutrophil-mediated clearance. As a result, serum levels of peg-filgrastim after a single administration remain elevated during chemotherapy-induced neutropenia, febrile neutropenia and decline rapidly as neutrophil count recovers. Data from randomized trials have evidenced that a single, fixed, once-per-cycle dose of peg-filgrastim (6 mg) administered 24 hours after chemotherapy is safe, well tolerated and has an efficacy similar to that provided by daily injections of filgrastim in patients receiving chemotherapy for NHL. Peg-filgrastim might be particularly indicated when the risk of severe neutropenia and associated infections is higher and when the nadir and duration of neutropenia are unpredictable, such as in patients who receive more intensive regimens. Data are being accumulated on the impact of chemotherapy-induced neutropenia, febrile neutropenia on clinical outcomes in terms of quality of life, delay in therapy and ultimately, on response and survival. In fact, treatment delays or decreasing dose-intensity due to myelotoxicity or infective episodes during standard chemotherapy can compromise outcomes in a potentially curable malignancy, such as aggressive NHL. In addition, G-CSF has recently been employed to make feasible dose-dense regimens which have been demonstrated to be superior to standard treatments in terms of responses and survival.

**Management of anemia**

Anemia profoundly affects the quality of life of NHL patients and may also have prognostic significance. It is present in more than one-third of patients at diagnosis. Although less frequently than in low grade NHL, severe anemia (grade 3-4) may be observed during treatment in about 20% of elderly patients with aggressive NHL receiving chemotherapy. As in other types of cancers, the pathogenesis of anemia in NHL is multifactorial; however, cytokine-mediated anemia of chronic disease, with associated inadequate production of endogenous erythropoietin (EPO), may play a relevant role.

Packed-red cell transfusions represent the standard treatment for severe and acute anemia. In all other cases, recombinant (r-) epoetins (α or β r-EPO, darbepoetin α) represent valuable options, with the major goals of improving quality of life and preventing or reducing transfusions. Although studies specifically focused on the use of epoetins for treating anemia in NHL are scarce, useful information may be extrapolated from recently published guidelines and meta-analyses, and some clinical trials including these malignancies alone or among other tumors. According to ASCO/ASH evidence-based guidelines, r-EPO is recommended for patients with chemo-therapy-associated anemia and a hemoglobin concentration below 10 g/dL (8-10 g/dL). Clinical circumstances should determine the use of r-EPO for patients with less severe anemia (Hb between 10 and 12 g/dL). The ASCO/ASH guidelines also suggest that r-EPO should be started only after tumor-reduction due to initial chemo-radiotherapy has not allowed improvement of baseline hemoglobin levels. Recent data, however, indi-
cate that early (frontline) r-EPO treatment in mildly anemic patients (hemoglobin 10-12 g/dL) undergoing chemotherapy could provide better improvement in terms of Hb and transfusion requirement, quality of life and care resource utilization and work with respect to delayed (when the hemoglobin declines to <9 g/dL) administration of r-EPO.234

The more recently published EORTC guidelines327 suggest that r-EPO may be initiated, in patients receiving chemo-radiotherapy, at hemoglobin values of 9-11 g/dL. Anemia-related symptoms should drive the choice of administering r-EPO to patients with the same levels of hemoglobin who are not receiving chemo-radiotherapy. In selected cases (i.e. for patients who are scheduled to receive intensive chemotherapy) r-EPO may be considered to prevent a further decline of hemoglobin. r-EPO is also recommended in cases of severe anemia in combination with transfusions. Although an optimal dose of 150 U/kg thrice weekly for at least 8-12 weeks was initially suggested for r-EPO,226 fixed, rather than body-weight adjusted doses, are currently recommended.227 Alternatively, weekly, equivalent single-dose schedules (30,000-40,000-60,000 qw) may represent further options. Dose-escalation can be useful in the absence of response in selected patients. The use of epoetins after autologous stem cell transplantation is not currently recommended, but positive experiences in this context have recently been published.335

Darbepoetin is a novel erythropoiesis stimulating protein (NESP) which, compared to human α or β r-EPO, has a greater sialylated carbohydrate content permitting a prolonged serum half-life.236 Darbepoetin once-weekly can be recommended at the initial dose of 2.25 µg/kg as an alternative to r-EPO α or β.237,332 There is currently less evidence on the value of administering darbepoetin α at higher dosages and at longer intervals (300-500 µg every 1-3 weeks) and these require strategies further studies.

Treatment with epoetins should be continued until target hemoglobin–levels or significant clinical improvement are achieved. Individualized titration of the lowest effective maintenance dose should be performed repeatedly. In patients with adequate iron stores, there is no indication to prolong r-EPO administration beyond 6-8 weeks in the absence of response. However, a supply of iron, particularly if administered intravenously, may optimize responses in patients developing functional or absolute iron deficiency while receiving r-EPO for chemotherapy-related anemia.337

According to published guidelines,326-327 the target hemoglobin concentration should be 12-13 g/dL.

Recently, the FDA recommended 12 g/dL as the upper limit to maintain, as higher levels have been found to be associated with a possible increased risk of thromboembolic events.238

A new, pegylated form of r-EPO with a prolonged half-life, also known as CERA (continuous erythropoietic receptor activator), allowing single administrations every one to three weeks, has demonstrated significant erythropoietic activity in chemotherapy-treated aggressive NHL in phase I-II studies.329 Confirmatory phase III studies are in progress.

The question of whether tumor response and clinical outcome of patients may be positively or negatively influenced by administrations of epoetins, is currently being debated.340,341 Some data suggest that hematology patients with documented anemia who received r-EPO along with chemotherapy might have better survival than those who did not.299 By contrast, in patients with solid cancer, some reports suggest the possibility that prophylaxis of anemia with r-EPO could negatively affect the patient’s outcome.342-343 Pending additional information in this setting, the prophylactic use of r-EPO in non-anemic patients with cancer should currently be discouraged.

Management of thrombocytopenia

As in other hematologic malignancies, prevention and treatment of hemorrhage secondary to thrombocytopenia in aggressive NHL is generally managed by the transfusion of platelets. The need for such transfusion is related to the degree and duration of critical thrombocytopenia. A platelet count greater than 10×10⁹/L is usually considered safe in the absence of factors associated with increased tendency to bleed.344

Interleukin-11 was successfully shown to reduce the incidence of severe thrombocytopenia in patients receiving intensive chemotherapy, and its use has now received approval from the FDA for this purpose.345,346 Initial clinical trials of recombinant thrombopoietin, the central regulator of megakaryocytopoiesis and thrombopoiesis, and its analogs, mainly Peg–MGDF (megakaryocytic growth and development factor) have been shown to enhance platelet recovery after chemotherapy.347 However, the clinical use of these agents has been largely limited by the observation that their administration, may induce the formation of antibodies that neutralize native thrombopoietin and cause thrombocytopenia.348

Among a number of second generation platelet growth factors recently developed, AMG 531 is a 60,000 Da Mpl receptor agonist made in E. Coli, currently under clinical investigation, containing four peptide sequences that have been molecularly engineered in a Fc carrier domain.349 These peptides have no homology to TPO but have been selected because they avidly bind to the Mpl receptor and stimulate megakaryocyte maturation and proliferation. Preliminary in vivo studies indicate that AMG 531 induces a

Nodal aggressive non Hodgkin’s lymphoma

dose-dependent rise in the platelet count that peaks 7-9 days after intravenous or subcutaneous injection in healthy subjects, without additional effects on other blood cell counts and no antibody formation to thrombopoietin.

**Other issues in supportive therapy**

Large tumor masses and high proliferation rate (as observed, for example, in Burkitt's lymphoma) enhance the risk of tumor lysis syndrome, which results in marked hyperuricemia and renal failure and occurs as large numbers of malignant cells are rapidly destroyed during cytotoxic chemotherapy. Standard treatment for tumor lysis syndrome includes adjustments in the chemotherapy regimen, vigorous hydration, administration of a uric acid synthesis inhibitor such as allopurinol, and alkalization. The use of rasburicase, a recombinant form of the enzyme urate-oxidase, has been shown to provide effective prophylaxis and/or treatment against tumor lysis syndrome in aggressive NHL.

Severe mucositis is common after chemotherapy and is generally managed by parental nutrition, topical therapies and narcotics. Recently, a recombinant keratinocyte growth factor (palifermin) has been successfully employed to reduce the duration and severity of oral mucositis after intensive chemotherapy including transplant regimens.

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Severe mucositis is common after chemotherapy and is generally managed by parental nutrition, topical therapies and narcotics. Recently, a recombinant keratinocyte growth factor (palifermin) has been successfully employed to reduce the duration and severity of oral mucositis after intensive chemotherapy and radiotherapy for hematologic cancers, including NHL.

NHL remains the second most common tumor in HIV-infected patients. Improvements in supportive care, in particular, highly active antiretroviral therapy (HAART), have allowed therapeutic approaches in these patients similar to those used in non-HIV-infected individuals, including transplant regimens. Likewise, lamivudine may prevent viral reactivation in NHL patients undergoing chemotherapy who are carriers of the hepatitis B virus.

Patients with aggressive NHL with concomitant HCV infection but without signs of liver dysfunction can receive standard therapies and even autologous transplantation. Recent reports suggest that antiviral therapy with (peg)-interferon and ribavirin may induce tumor remissions in some patients with HCV-positive low-grade NHL (mainly splenic lymphoma with villous lymphocytes and marginal zone). However, in patients with more aggressive NHL, this anti-viral treatment may provoke elevated myelotoxicity if combined with chemotherapy (personal observations). Clinical trials are in progress to test the role of antiviral therapy after completion of standard chemotherapy programs in these patients (Musto et al., submitted for publication).

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