Primary diffuse large B-cell lymphoma of bone

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Chapter 1

General introduction

History

Primary lymphoma of bone is a rare disease, first described by Oberling in 1928 as reticulum cell sarcoma of bone.[1] He accomplished a reliable and consistent separation of reticulum cell sarcoma of bone from Ewing's sarcoma, a histomorphological mimic. Reticulin fibers surrounding individual tumor cells are a characteristic finding in primary lymphoma of bone, hence the original terminology of reticulum cell sarcoma. It was not until 1939 that Parker and Jackson described 17 cases of so-called 'primary reticulum cell sarcoma of bone' and established this disease as a distinct and accepted clinical entity.[2] In 1963 Ivins and later in 1974 Boston et al. recognized the lymphoid origin of the tumor and named it malignant lymphoma of bone.[3, 4] In 1987 Vassallo introduced the use of immunohistochemistry to characterize the true cellular origin of these tumors.[5] Even today the histopathological diagnosis of primary lymphoma of bone can be challenging, it is often only with the help of lymphoma cell specific immunohistochemical markers that difficult cases can be diagnosed in a reliable manner. The subtype of lymphoma that is most often diagnosed in these cases is diffuse large B cell lymphoma. In this thesis we focus on primary diffuse large B cell lymphoma of bone. Other subtypes, such as primary follicular lymphoma of bone, primary Hodgkin lymphoma of bone, primary T cell lymphoma of bone or acute anaplastic large cell lymphoma in children do occur on rare occasions, but these subtypes were excluded in our studies.

Since 1974, when Boston gave primary lymphoma of bone its current name, multiple different systems of classification of non-Hodgkin lymphoma have been used. This has complicated research, as consensus on definitions and terminology is essential for both clinical practice and investigation. Poor reproducibility of morphologic criteria have helped to switch from the Kiel and Lukes-Collins classification via the Working Formulation system in 1982 to the REAL classification in 1994. From the REAL classification, in 2001 the World Health Organization (WHO) classification system was established. This classification system is used internationally nowadays. The diagnosis and classification of non-Hodgkin lymphoma has moved from a purely morphologically driven classification system then, into one in which a wide range of information, including immunophenotype and molecular genetic features, are part of the disease definitions today. In the WHO classification, primary diffuse large B cell lymphoma of bone is defined as a mono-ostotic disease with or without involvement of regional lymph nodes, or as a poly-ostotic disease affecting multiple skeletal sites without visceral- or lymph node involvement.[6] The percentage of primary diffuse large B cell lymphoma of bone is only 5% of all extranodal non-Hodgkin lymphomas and is less than 1% of all non-Hodgkin lymphomas.[6-8] As a consequence of this low incidence, scientific studies on this subject are uncommon.

In 1953, the Netherlands Committee on Bone Tumors was founded in Leiden. This Commission keeps a large national registry of bone tumors in the Netherlands containing more than 25.000 cases, including clinical data at presentation, histological slides of the tumor and complete radiographic documentation. This registry gave us the unique opportunity to assemble a large cohort of patients with primary diffuse large B cell lymphoma of bone, one of the largest

worldwide. Starting with this cohort, we initiated our research project on primary diffuse large B cell lymphoma of bone to increase the knowledge of this rare and poorly understood subtype of non-Hodgkin lymphoma.

Clinico-pathological characteristics

Primary diffuse large B cell lymphoma of bone often presents with pain or a palpable mass, most frequently in the long bones, especially the distal femur or the distal humerus. The median age at presentation is in the fifth decade, with male patients affected more often than female patients with a ratio of 1.8 : 1. Morphologically, almost all tumors are composed of large centroblast-like cells, often with multilobated nuclear features, but accurate morphological subclassification can be problematic due to mechanical crush, decalcification procedures or small sample size.[8, 9] In many cases the pathologic features of the tumor, often including intralesional fibrosis and prominent tumor cell spindling, make the histopathologic diagnosis even more complicated. In inexperienced hands misdiagnoses do occur, often classifying the tumor as a primary bone sarcoma.

Since 1971, the Ann Arbor system is used internationally for the staging of all Hodgkin and non-Hodgkin lymphomas **(see table 1).** [10] Although originally developed for staging patients with Hodgkin lymphoma, the Ann Arbor staging system provides the basis for anatomic staging in non-Hodgkin lymphomas as well. However, in non-Hodgkin lymphoma the Ann Arbor staging system has less prognostic value than in Hodgkin lymphoma.[11] The Ann Arbor staging system was not designed to take into account the different pattern of disease seen in the non-Hodgkin lymphomas, for example the often extranodal presentation.[12] It neither takes into account the grade of the tumor, which is prognostically relevant in non-Hodgkin lymphoma. Primary diffuse large B cell lymphoma of bone most often presents at one bone localization, which is classified as stage I. The stages II and III are rarely applicable in primary diffuse large B cell lymphoma of bone. In our studies, all stage IV cases had multiple bone localizations, none had bone marrow involvement. To avoid these staging problems for extranodal lymphoma, several authors choose to include only stage I patients in their bone lymphoma studies, which may lead to an imperfect representation of patients in literature.

Historically, a wide variety of therapies for primary diffuse large B cell lymphoma of bone has been used, including radiotherapy, surgery (amputation) and chemotherapy. For about twenty-five years most primary diffuse large B cell lymphoma of bone patients have been treated with a combination of radiotherapy and CHOP or CHOP-like chemotherapy. Probably the most important improvement for lymphoma patients in the last decade has been the addition of rituximab, a monoclonal antibody that specifically targets the CD20 positive B-cell, to the standard multiagent therapy regimen.[13] However, data on primary diffuse large B cell lymphoma of bone patients treated with this regimen are still scarce.[14] The five-year overall survival rate for primary diffuse large B cell lymphoma of bone is generally favorable compared to other intermediate grade, extranodal diffuse large B cell lymphoma, with a 5-years overall survival of 75% for the whole cohort in our studies

Table 1. Ann Arbor Staging Classification.

Stage I:	Involvement of a single lymph node region (I) or of a single extralymphatic organ or site. (IE)
Stage II:	Involvement of two or more lymph node regions on the same side of the diaphragm (II) or localized involvement of an extralymphatic organ or site and one or more lymph node regions on the same side of the diaphragm. (IIE)
Stage III:	Involvement of lymph node regions on both sides of the diaphragm (III), which may be accompanied by involvement of the spleen (IIIS) or by localized involvement of an extralymphatic organ or site (IIIE), or both. (IIISE)
Stage IV:	Diffuse or disseminated involvement of one or more extralymphatic organs or tissues with or without associated lymph node involvement.

The absence or presence of fever, night sweats, or unexplained loss of 10% or more of body weight in the 6 months preceding admission are to be denoted in all cases by the suffix letters A or B, respectively.

(see table 2).[9, 15, 16-18] Despite the improvement in diffuse large B cell lymphoma treatment with the addition of rituximab to CHOP, one quarter to one third of patients still die of their disease. The development of novel treatment regimens such as new anti-CD20 antibodies, proteasome inhibitors or lenalidomide are promising, but will require further study.[19-21]

Risk stratification in diffuse large B cell lymphoma

Diffuse large B cell lymphoma, including its extranodal subtype primary diffuse large B cell lymphoma of bone, represents a heterogeneous group of B-cell neoplasms, both clinically and morphologically. Paradoxically, the chemotherapy treatment regime for all of these patients is almost always the same: CHOP or CHOP-like chemotherapy, since 2001 in combination with rituximab. Strategies to intensify chemotherapy regimes, including autologous stem cell transplantation, have showed mixed results.[22, 23] Intensive chemotherapy plus autologous stem cell transplantation might be beneficial for a selected group of high risk young patients, but which subgroup will benefit the most is unclear. Clinicians have difficulties defining the optimal treatment regimen for their diffuse large B cell lymphoma patients, because it is impossible to accurately predict response to standard treatment regimens. Patients present with apparently similar diagnoses, but have markedly different clinical outcomes, with a five year overall survival ranging from about 20% to 80% **(see table 2).** To identify patients who will show progressive disease despite standard chemotherapy and who will benefit from more intensive or different treatment modalities, numerous studies have been undertaken to design risk stratifications and to define meaningful prognostic markers.

Clinical prognostic factors:

For risk stratification purposes lymphomas are traditionally divided into a group with primary tumor location in the lymph nodes and a group with primary extranodal presentation, which can occur at any site. It has long been held that overall survival for extranodal lymphomas as

a whole compares unfavorably with survival for nodal cases. Diffuse large B cell lymphoma of the testis and central nervous system for example - both lymphomas of immune-privileged sites - have been reported to have an unfavorable survival, with a 5-year overall survival below 50 %. In recent years, the survival of patients with lymphoma of immune-privileged sites has much improved, probably due to better diagnostic procedures and more tailored chemotherapy, including rituximab. [24-26] Primary diffuse large B cell lymphoma of bone has shown a much better survival, even in studies from more than ten years ago. It is therefore questionable whether this distinction between nodal lymphoma and extranodal lymphoma still holds its clinical relevance [27, 28 29, 30] **(see table 2).**

The recognition that the Ann Arbor anatomical staging system does not subdivide some types of non-Hodgkin lymphomas in a clinically useful way, and the recognition that other factors are important in predicting treatment outcome, has led to the development of the International Prognostic Index (IPI) in 1993. The IPI is based on a limited set of clinical and laboratory variables, which makes it a useful and affordable index in daily practice (**see table 3**). [43 44] Since one parameter of the index is the number of extranodal sites and another parameter is the Ann Arbor stage, this index is not directly intended for indexing primary extranodal lymphomas. However, some studies indicate that the IPI is nonetheless applicable for staging primary extranodal lymphomas. [45]

Molecular genetic prognostic factors:

In the last decade, several molecular techniques, e.g. using DNA and RNA derived from tumor cells, have been developed to study the pathogenesis of diffuse large B cell lymphoma in more detail. However, it is important to realize that most studies using these new techniques concern nodal diffuse large B cell lymphoma, as those tissue samples are more abundantly available than tissue samples from extranodal lymphomas. Moreover, in contrast to extranodal lymphomas, cell lines of nodal diffuse large B-cell lymphoma are generally available, allowing for functional testing. These new techniques are also increasingly being used in extranodal lymphomas and future studies will be necessary to investigate to what extend the results of nodal diffuse large B-cell lymphoma can be extrapolated to extranodal lymphoma.

Table 2. Overall 5 years survival (OS) in diffuse large B cell lymphoma

Primary location	Reference	5 year OS %
nodal	Sehn[32], Gutierrez[33]	69-70
nodal (elderly patients)	Feugier[34], Coiffier[35]	58-60
testis	Al-Abbadi[36], Vitolo[25], Mazloom[26]	60-86
central nervous system	Shenkier[37], Hattab[38], Yamanaka[39], Gavrilovic[27]	18-46
bone	Heyning[10], Beal[18], Ramadan[19]	62-88
stomach	Leopardo[40], Hung[41], Zhang[42]	50-100
skin (primary cutaneous large B-cell lymphoma, leg type)	Dijkman[28], Grange[43]	25-41

Table 3:. International Prognostic Index

One point is assigned for each of the following risk factors:
Age greater than 60 years
Stage III or IV disease
Elevated serum LDH
WHO performance status of 2 or more
More than 1 extranodal site
The sum of the points allotted correlates with a median three-year survival ranging from 91% to 59%.

Gene expression profiling can divide diffuse large B cell lymphoma into two histological indistinguishable molecular subtypes: a prognostically favorable group of germinal center B-cell (GCB) phenotype and a prognostically unfavorable group of activated B-cell (ABC or non-GC) phenotype.[46] The GCB subtype has a significantly better outcome, 80% five year overall survival versus 45% five year overall survival for the ABC subtype, independent from IPI classification.[47, 48] These two subtypes apparently arise from B-cells that are at separate stages of differentiation. Each subtype has a different process of malignant transformation and acquires distinct oncogenic abnormalities. In GCB phenotype lymphoma the germinal center B cells of the malignant clone continue to undergo somatic hypermutation. They avoid cell death by oncogenic translocations such as the t(14;18) translocation or TP53 mutations.[49] Anti-apoptotic pathways are activated by the overexpression of bcl-2 as a result of a t(14;18) resulting in a fusion of IgH promoter to BCL-2. However, the presence of this specific translocation does not have an inverse impact on prognosis. [50, 51] Secondary changes, such as TP53 mutations may occur in a subset of these patients. The overall survival is significantly worse for patients with TP53 mutations than for patients with wild type TP53 in GCB-type diffuse large B-cell lymphoma.[49, 52]

Few studies exist on BCL-2 gene rearrangement in primary diffuse large B cell lymphoma of bone. Gianelli et al. found BCL-2/IgH rearrangement in 5% of cases, Bhagavathi in 4 of 21 cases. [53, 54] No prognostic effect could be detected in these small cohorts **(see table 4)**.

As germinal-center B cells begin to differentiate into plasma cells, they upregulate interferon regulatory factor 4 (IRF4). This is the stage of differentiation where the malignant clone of ABC-type diffuse large B-cell lymphoma likely arises. The ABC subtype often carries a

Table 4. Gene rearrangements in primary diffuse large B cell lymphoma of bone patients

Gene(s)	Reference	Technique	Rearrangements
IgH	Bhagavathi[55]	PCR	10/17
IgH/BCL-2	Bhagavathi, Gianelli[54]	PCR	4/17, 2/41
	Bhagavathi	FISH	4/21
BCL-2	Lima[56]	FISH	9/32
BCL-6	Bhagavathi	FISH	3/21
C-MYC	Bhagavathi, Lima	FISH	2/21, 3/32

homozygous deletion of the CDKN2A (INK4A-ARF) locus, which encodes p16, an inhibitor of senescence and p14-ARF, an inhibitor of p53 activation.[49, 56] This deletion has a negative impact on prognosis. [57]

While in GCB type diffuse large B-cell lymphoma, BCL-2 is commonly deregulated by translocations, alternative mechanisms of BCL-2 up-regulation, such as gain or amplification of 18q21, are more frequently seen in the ABC subgroup. BCL-2 is a target gene for nuclear factor (NF)-κB, and in ABC type diffuse large cell B cell lymphoma, BCL-2 up-regulation may be mediated through the NF-κB pathway. In the ABC subgroup, bcl-2 expression is associated with poor survival.[58] BCL-2 may act as an anti-apoptotic factor in this subgroup, but it may also serve as a marker for events that are responsible for poor prognosis, such as NF-κB activation.[59, 60]

The NF-κB comprises a family of transcription factors that control genes implicated in B-cell activation, proliferation and resistance to apoptosis. The NF-κB family has five members: p65 (RelA), p50 (NF-κB1), p52 (NF-κB2), RelB and REL. Two signaling pathways account for the activation of NF-κB. The classical pathway activation is normally triggered in response to inflammatory stimuli. Signal transduction events lead to activation of the IκB kinase (IKK) complex resulting in phosphorylation and proteasomal degradation of IκB. Heterodimers and homodimers of p50 and p65 can then be translocated to the nucleus to regulate gene transcription. The alternative pathway is triggered, amongst others, by certain members of the tumor-necrosis factor (TNF) cytokine family, eventually leading to processing of the p52 precursor subunit into active p52 translocating to the nucleus and forming a heterodimeric complex with RelB.[61] Constitutive activation of NF-κB has been implicated to play a role in the pathogenesis of different types of haematological malignancies.[62, 63] It seems generally involved in ABC type diffuse large B cell lymphoma and is required for survival of ABC type diffuse large B cell lymphoma cells in vitro.[64] The role of constitutive NF-κB pathway activation in lymphomagenesis has raised interest in its potential as a target for therapeutic interventions, [65 66 61] specifically in ABC-type diffuse large B-cell lymphoma, as inhibition of the NF-κB pathway in vitro was shown to be selectively toxic to ABC type diffuse large B cell lymphoma cell lines and not to GCB type cell lines.[64]

Recent studies, including ours, have focused on the use of immunohistochemistry to identify risk groups. In 2004 Hans et al. developed an algorithm applying immunohistochemical parameters, the expression of CD10, BCL-6 and MUM1/IRF4, to define the two prognostic groups of germinal center B cell phenotype and activated B-cell phenotype of nodal diffuse large B-cell lymphoma, thus avoiding the limitations of fresh tissue and costly technology of gene expression profiling.[67] BCL-6 and CD10 proteins are considered germinal center (GC) markers, and MUM1/IRF4 denotes the final step of intra-GC B-cell differentiation and activation. More recently, a consortium of hematopathologists improved on the Hans method by employing a different combination of immunostains, i.e. GCET1, CD10, BCL-6, MUM1/IRF4 and FOXP1 and derived a new algorithm with 93% concordance with gene expression profiling.[68]

Concluding remarks

It is clear that diffuse large B cell lymphoma is a heterogeneous disease group. Even within the group of extranodal diffuse large B cell lymphoma, clinical outcome between the specific sites is quite variable. The scientific development in diffuse large B cell lymphoma research during the course of our project illustrates the ongoing evolution in risk stratification for lymphomas. The prognostic impact of grouping activated B cell type versus germinal center B-cell type diffuse large B-cell lymphoma has been described extensively in literature now, with usually a poorer prognosis for the ABC subtype. However, clinical evidence in the form of a large, prospective trial is still lacking. The knowledge on the role of BCL-2 up-regulation and its influence on prognosis depending on the lymphoma phenotype is increasing. Constitutive NF-κB pathway activation plays a major role in lymphomagenesis, but the exact mechanisms are still poorly understood. Moreover, many of the techniques necessary for subtyping diffuse large B cell lymphoma according to the various risk stratification models, such as cDNA microarray and array-comparative genomic hybridization (array-CGH), are not available in routine patient care yet. It is also still unclear whether pathogenetic models discovered in nodal diffuse large B-cell lymphoma are applicable to the different extranodal lymphoma subtypes, such as primary diffuse large B cell lymphoma of bone. The expanding knowledge on lymphoma-associated biologic processes can help identify targets for the development of new therapeutic agents. At this moment, however, the novel treatment regimes are still experimental. The progress in risk stratification has not yet changed the standard R-CHOP treatment regimen for the individual patient.

With this thesis, we hope to define the entity of primary diffuse large B cell lymphoma of bone more clearly by increasing the understanding of this very specific and rare subtype of diffuse large B cell lymphoma. Ultimately, ongoing lymphoma research will provide insights that lead to an improved, tailored treatment regimen for all diffuse large B cell lymphoma patients.

Scope and outline of the thesis

This thesis focuses on the clinico-pathological aspects of primary diffuse large B cell lymphoma of bone, a rare subtype of extranodal diffuse large B cell lymphoma. Improved insight of the pathophysiology of this bone tumor can be employed to acquire a better understanding of the wide range of different diseases grouped together under the name of diffuse large B cell lymphoma.

In **chapter two**, we investigated the clinical course of a large cohort of 60 primary diffuse large B cell lymphoma of bone patients, which we selected from the files of the Netherlands Committee on Bone Tumors. We completed follow-up data until time of death or date of last follow-up. At the time of our research, no uniform directives for classification, treatment and prognosis were available yet. The objective of the study was to document patient and tumor characteristics of this cohort, with emphasis on histological subtype, substantiated by

immunohistochemistry, and to define factors affecting prognosis, including the parameters of the IPI risk index.

In **chapter three**, we studied the MRI characteristics of 29 primary diffuse large B cell lymphoma of bone patients. Literature on the presentation of MR imaging features of primary diffuse large B cell lymphoma of bone is relatively scarce and contradictory. There is an ongoing debate as to whether there is a uniform imaging pattern in primary lymphoma of bone. We aimed to assess the imaging characteristics of primary lymphoma of bone and to asses the rate of a non-aggressive MR imaging appearance, by evaluating various features known from literature, with emphasis on cortical bone manifestation and marrow and soft-tissue signal characteristics.

In **chapter four,** we studied multiple immunohistochemical markers and their possible prognostic influence in primary diffuse large B cell lymphoma of bone. Immunohistochemical studies on primary diffuse large B cell lymphoma of bone are rare, probably because of the limited availability of tissue specimens of this disorder and technological difficulties in handling osseous tumor material. In nodal diffuse large B-cell lymphoma, individual markers have been shown to have a prognostic influence on survival. We determined the prognostic significance of BCL-6, CD10, MUM1, BCL-2, p53, CD30 and CD44. With the help of these markers we investigated the possible germinal center derivation in primary diffuse large B cell lymphoma of bone.

In **chapter five**, we described the first array-CGH study of primary diffuse large B cell lymphoma of bone. Recent studies report that this technique can be used to classify lymphomas into the clinically and biologically relevant phenotypes: GC-like and ABC/non-GC-like, and possibly this technique can reveal differences in oncogenic mechanisms. We investigated genomic alterations in 9 cases of primary diffuse large B cell lymphoma of bone using this technique and analysed the results in the context of data available from literature on studies of other distinct subtypes of extranodal diffuse large B cell lymphoma such as skin, central nervous system and testis.

In **chapter six**, we described the first study on the nuclear factor (NF)-κB signaling pathway in primary diffuse large B cell lymphoma of bone. The NF-κB comprises a family of transcription factors that control genes implicated in B-cell activation and proliferation. Constitutive activation of this pathway can contribute to tumorigenesis and chemotherapy resistance and is shown to be involved in tumor cell survival in several types of lymphomas, including nodal diffuse large B cell lymphoma. Two major pathways account for the activation of NF-κB, the classical pathway and the alternative pathway. We investigated both pathways of NF-κB activation by immunohistochemistry and evaluated its clinical parameters in a large cohort of 50 primary diffuse large B cell lymphoma of bone patients.

Finally the findings are summarized in **chapter seven.**

Reference List

1. Oberling C. Les réticulosarcomes et les réticulo-endothéliosarcomes de la moelle osseuse (sarcomes d'Ewing). Bull Assoc Fr Etud Cancer 1928;17(5):259-296.
2. Parker F, Jr., Jackson H, Jr. Primary reticulum cell sarcoma of bone. Surg Gynecol Obstet 1939;68:45-53.
3. Boston HC, Jr., Dahlin DC, Ivins JC, Cupps RE. Malignant lymphoma (so-called reticulum cell sarcoma) of bone. Cancer 1974;34(4):1131-1137.
4. Ivins JC, Dahlin DC. Malignant Lymphoma (Reticulum Cell Sarcoma) of Bone. Mayo Clinic Proceedings 1963;38(18):375-&.
5. Vassallo J, Roessner A, Vollmer E, Grundmann E. Malignant lymphomas with primary bone manifestation. Pathol Res Pract 1987;182(3):381-389.
6. k.k.Unni, P.C.W.Hogendoorn. World health organization classification of tumours.pathology and genetics of tumours of soft tissue and bone. lyon: IARC Press . 2002.
7. Desai S, Jambhekar NA, Soman CS, Advani SH. Primary lymphoma of bone: a clinicopathologic study of 25 cases reported over 10 years. J Surg Oncol 1991;46(4):265-269.
8. de Leval L, Braaten KM, Ancukiewicz M et al. Diffuse large B-cell lymphoma of bone: an analysis of differentiation-associated antigens with clinical correlation. Am J Surg Pathol 2003;27(9):1269-1277.
9. Heyning FH, Hogendoorn PC, Kramer MH et al. Primary non-Hodgkin's lymphoma of bone: a clinicopathological investigation of 60 cases. Leukemia 1999;13(12):2094-2098.
10. Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res 1971;31(11):1860-1861.
11. Rosenberg SA. Validity of the Ann Arbor staging classification for the non-Hodgkin's lymphomas. Cancer Treat Rep 1977;61(6):1023-1027.
12. Armitage JO. Staging non-Hodgkin lymphoma. CA Cancer J Clin 2005;55(6):368-376.
13. Vose JM, Link BK, Grossbard ML et al. Phase II study of rituximab in combination with chop chemotherapy in patients with previously untreated, aggressive non-Hodgkin's lymphoma. J Clin Oncol 2001;19(2):389-397.
14. Pellegrini C, Gandolfi L, Quirini F et al. Primary bone lymphoma: evaluation of chemoimmunotherapy as front-line treatment in 21 patients. Clin Lymphoma Myeloma Leuk 2011;11(4):321-325.
15. Alencar A, Pitcher D, Byrne G, Lossos IS. Primary bone lymphoma--the University of Miami experience. Leuk Lymphoma 2010;51(1):39-49.
16. Adams H, Tzankov A, d'Hondt S, Jundt G, Dirnhofer S, Went P. Primary diffuse large B-cell lymphomas of the bone: prognostic relevance of protein expression and clinical factors. Hum Pathol 2008;39(9):1323-1330.
17. Beal K, Allen L, Yahalom J. Primary bone lymphoma: treatment results and prognostic factors with long-term follow-up of 82 patients. Cancer 2006;106(12):2652-2656.
18. Ramadan KM, Shenkier T, Sehn LH, Gascoyne RD, Connors JM. A clinicopathological retrospective study of 131 patients with primary bone lymphoma: a population-based study of successively treated cohorts from the British Columbia Cancer Agency. Ann Oncol 2007;18(1):129-135.
19. Morschhauser F, Leonard JP, Fayad L et al. Humanized anti-CD20 antibody, veltuzumab, in refractory/recurrent non-Hodgkin's lymphoma: phase I/II results. J Clin Oncol 2009;27(20):3346-3353.
20. Dunleavy K, Pittaluga S, Czuczman MS et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. Blood 2009;113(24):6069-6076.
21. Wiernik PH, Lossos IS, Tuscano JM et al. Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma. J Clin Oncol 2008;26(30):4952-4957.
22. Martelli M, Gherlinzoni F, De RA et al. Early autologous stem-cell transplantation versus conventio-

nal chemotherapy as front-line therapy in high-risk, aggressive non-Hodgkin's lymphoma: an Italian multicenter randomized trial. J Clin Oncol 2003;21(7):1255-1262.
23. Intragumtornchai T, Bunworasate U, Nakorn TN, Rojnuckarin P. Rituximab-CHOP-ESHAP vs CHOP-ESHAP-high-dose therapy vs conventional CHOP chemotherapy in high-intermediate and high-risk aggressive non-Hodgkin's lymphoma. Leuk Lymphoma 2006;47(7):1306-1314.
24. Vitolo U, Chiappella A, Ferreri AJ et al. First-line treatment for primary testicular diffuse large B-cell lymphoma with rituximab-CHOP, CNS prophylaxis, and contralateral testis irradiation: final results of an international phase II trial. J Clin Oncol 2011;29(20):2766-2772.
25. Mazloom A, Fowler N, Medeiros LJ, Iyengar P, Horace P, Dabaja BS. Outcome of patients with diffuse large B-cell lymphoma of the testis by era of treatment: the M. D. Anderson Cancer Center experience. Leuk Lymphoma 2010;51(7):1217-1224.
26. Gavrilovic IT, Hormigo A, Yahalom J, DeAngelis LM, Abrey LE. Long-term follow-up of high-dose methotrexate-based therapy with and without whole brain irradiation for newly diagnosed primary CNS lymphoma. J Clin Oncol 2006;24(28):4570-4574.
27. Dijkman R, Tensen CP, Jordanova ES et al. Array-based comparative genomic hybridization analysis reveals recurrent chromosomal alterations and prognostic parameters in primary cutaneous large B-cell lymphoma. J Clin Oncol 2006;24(2):296-305.
28. Booman M, Szuhai K, Rosenwald A et al. Genomic alterations and gene expression in primary diffuse large B-cell lymphomas of immune-privileged sites: the importance of apoptosis and immunomodulatory pathways. J Pathol 2008;216(2):209-217.
29. Zucca E, Roggero E, Bertoni F, Conconi A, Cavalli F. Primary extranodal non-Hodgkin's lymphomas. Part 2: Head and neck, central nervous system and other less common sites. Ann Oncol 1999;10(9):1023-1033.
30. Zucca E, Roggero E, Bertoni F, Cavalli F. Primary extranodal non-Hodgkin's lymphomas. Part 1: Gastrointestinal, cutaneous and genitourinary lymphomas. Ann Oncol 1997;8(8):727-737.
31. Sehn LH, Berry B, Chhanabhai M et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood 2007;109(5):1857-1861.
32. Gutierrez-Garcia G, Colomo L, Villamor N et al. Clinico-biological characterization and outcome of primary nodal and extranodal diffuse large B-cell lymphoma in the rituximab era. Leuk Lymphoma 2010;51(7):1225-1232.
33. Feugier P, Van HA, Sebban C et al. Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol 2005;23(18):4117-4126.
34. Coiffier B, Thieblemont C, Van Den Neste E et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood 2010;116(12):2040-2045.
35. Al-Abbadi MA, Hattab EM, Tarawneh M, Orazi A, Ulbright TM. Primary testicular and paratesticular lymphoma: a retrospective clinicopathologic study of 34 cases with emphasis on differential diagnosis. Arch Pathol Lab Med 2007;131(7):1040-1046.
36. Shenkier TN, Voss N, Chhanabhai M et al. The treatment of primary central nervous system lymphoma in 122 immunocompetent patients: a population-based study of successively treated cohorts from the British Colombia Cancer Agency. Cancer 2005;103(5):1008-1017.
37. Hattab EM, Martin SE, Al-Khatib SM et al. Most primary central nervous system diffuse large B-cell lymphomas occurring in immunocompetent individuals belong to the nongerminal center subtype: a retrospective analysis of 31 cases. Mod Pathol 2010;23(2):235-243.

38. Yamanaka R, Morii K, Shinbo Y et al. Results of treatment of 112 cases of primary CNS lymphoma. Jpn J Clin Oncol 2008;38(5):373-380.
39. Leopardo D, Di LG, De RA et al. Efficacy of rituximab in gastric diffuse large B cell lymphoma patients. World J Gastroenterol 2010;16(20):2526-2530.
40. Hung YS, Lin TL, Kuo MC et al. Primary gastric diffuse large B-cell lymphoma. Chang Gung Med J 2008;31(2):159-166.
41. Zhang J, Wang MY, Xu LC et al. [Clinical analysis of primary gastric diffuse large B-cell lymphoma]. Zhonghua Zhong Liu Za Zhi 2010;32(8):614-618.
42. Grange F, Beylot-Barry M, Courville P et al. Primary cutaneous diffuse large B-cell lymphoma, leg type: clinicopathologic features and prognostic analysis in 60 cases. Arch Dermatol 2007;143(9):1144-1150.
43. Ziepert M, Hasenclever D, Kuhnt E et al. Standard International prognostic index remains a valid predictor of outcome for patients with aggressive CD20+ B-cell lymphoma in the rituximab era. J Clin Oncol 2010;28(14):2373-2380.
44. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 1993;329(14):987-994.
45. Mok TS, Steinberg J, Chan AT et al. Application of the international prognostic index in a study of Chinese patients with non-Hodgkin's lymphoma and a high incidence of primary extranodal lymphoma. Cancer 1998;82(12):2439-2448.
46. Alizadeh AA, Eisen MB, Davis RE et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403(6769):503-511.
47. Gutierrez-Garcia G, Cardesa-Salzmann T, Climent F et al. Gene-expression profiling and not immunophenotypic algorithms predicts prognosis in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. Blood 2011;117(18):4836-4843.
48. Fu K, Weisenburger DD, Choi WW et al. Addition of rituximab to standard chemotherapy improves the survival of both the germinal center B-cell-like and non-germinal center B-cell-like subtypes of diffuse large B-cell lymphoma. J Clin Oncol 2008;26(28):4587-4594.
49. Lenz G, Staudt LM. Aggressive lymphomas. N Engl J Med 2010;362(15):1417-1429.
50. Huang JZ, Sanger WG, Greiner TC et al. The t(14;18) defines a unique subset of diffuse large B-cell lymphoma with a germinal center B-cell gene expression profile. Blood 2002;99(7):2285-2290.
51. Abdel-Ghaffar H, El-Aziz SA, Shahin D et al. Prognostic value of the t(14;18)(q32;q21) in patients with diffuse large B-cell lymphoma. Cancer Invest 2010;28(4):376-380.
52. Young KH, Leroy K, Moller MB et al. Structural profiles of TP53 gene mutations predict clinical outcome in diffuse large B-cell lymphoma: an international collaborative study. Blood 2008;112(8):3088-3098.
53. Gianelli U, Patriarca C, Moro A et al. Lymphomas of the bone: a pathological and clinical study of 54 cases. Int J Surg Pathol 2002;10(4):257-266.
54. Bhagavathi S, Micale MA, Les K, Wilson JD, Wiggins ML, Fu K. Primary bone diffuse large B-cell lymphoma: clinicopathologic study of 21 cases and review of literature. Am J Surg Pathol 2009;33(10):1463-1469.
55. Lima FP, Bousquet M, Gomez-Brouchet A et al. Primary diffuse large B-cell lymphoma of bone displays preferential rearrangements of the c-MYC or BCL2 gene. Am J Clin Pathol 2008;129(5):723-726.
56. Tagawa H, Suguro M, Tsuzuki S et al. Comparison of genome profiles for identification of distinct subgroups of diffuse large B-cell lymphoma. Blood 2005;106(5):1770-1777.
57. Jardin F, Jais JP, Molina TJ et al. Diffuse large B-cell lymphomas with CDKN2A deletion have a distinct gene expression signature and a poor prognosis under R-CHOP treatment: a GELA study. Blood 2010;116(7):1092-1104.

58. Iqbal J, Neppalli VT, Wright G et al. BCL2 expression is a prognostic marker for the activated B-cell-like type of diffuse large B-cell lymphoma. J Clin Oncol 2006;24(6):961-968.
59. Wilson KS, Sehn LH, Berry B et al. CHOP-R therapy overcomes the adverse prognostic influence of BCL-2 expression in diffuse large B-cell lymphoma. Leuk Lymphoma 2007;48(6):1102-1109.
60. Dunleavy K, Staudt LM, Wilson WH. The BCL-2 biomarker in the era of molecular diagnosis of diffuse large B-cell lymphoma. Leuk Lymphoma 2007;48(6):1061-1063.
61. Pavan A, Spina M, Canzonieri V, Sansonno S, Toffoli G, De R, V. Recent prognostic factors in diffuse large B-cell lymphoma indicate NF-kappaB pathway as a target for new therapeutic strategies. Leuk Lymphoma 2008;49(11):2048-2058.
62. Rayet B, Gelinas C. Aberrant rel/nfkb genes and activity in human cancer. Oncogene 1999;18(49):6938-6947.
63. Courtois G, Gilmore TD. Mutations in the NF-kappaB signaling pathway: implications for human disease. Oncogene 2006;25(51):6831-6843.
64. Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. J Exp Med 2001;194(12):1861-1874.
65. Espinosa I, Briones J, Bordes R et al. Activation of the NF-kappaB signalling pathway in diffuse large B-cell lymphoma: clinical implications. Histopathology 2008;53(4):441-449.
66. Liu X, Wang B, Ma X, Guo Y. NF-kappaB activation through the alternative pathway correlates with chemoresistance and poor survival in extranodal NK/T-cell lymphoma, nasal type. Jpn J Clin Oncol 2009;39(7):418-424.
67. Hans CP, Weisenburger DD, Greiner TC et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004;103(1):275-282.
68. Choi WW, Weisenburger DD, Greiner TC et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clin Cancer Res 2009;15(17):5494-5502.