haematologica
Journal of Hematology

ISSN 1592-8721
educational edition

Volume 88
Supplement no. 10
May 2003

Published by the
Ferrata-Storti
Foundation,
Pavia, Italy

s10

New Insights in Hematology

Venice, Italy
May 15-18, 2003

Guest Editor
Teodoro Chisesi

haematologica – Vol. 88 supplement n. 10 — May 2003 — pp. 1-118

haematologica

editorial board

official organ of

AEHH (Spanish Association of Hematology and Hemotherapy)
AIEOP (Italian Association of Pediatric Hematology/Oncology)
SETH (Spanish Society of Thrombosis and Hemostasis)
SIE (Italian Society of Hematology)
SIES (Italian Society of Experimental Hematology)
SISET (Italian Society for Studies on Hemostasis and Thrombosis)

Direttore responsabile: Prof. Edoardo Ascari; Autorizzazione del Tribunale di Pavia n. 63 del 5 marzo 1955.
Editing: Mikimos - Medical Editions via gen. C.A. Dalla Chiesa 22, Voghera, Italy
Printing: Tipografia PI-ME via Vigentina 136, Pavia, Italy

Printed in May 2003

Haematologica is sponsored by educational grants from the following institutions and companies

IRCCS Policlinico S. Matteo, Pavia, Italy

University of Pavia, Italy

José Carreras International Leukemia Foundation

haematologica

information for authors, readers and subscribers

Haematologica (print edition, ISSN 0390-6078) publishes peer-reviewed papers across all areas of experimental and clinical hematology. The journal is owned by a non-profit organization, the Ferrata Storti Foundation, and the way it serves the scientific community is detailed online: http://www.haematologica.org/main.htm (journal's policy).

Papers should be submitted online: http://www.haematologica.org/submission. For the time being the journal considers also papers submitted via surface mail (Editorial Office, Haematologica, Strada Nuova 134, 27100 Pavia, Italy) or as attachments to email messages (office@haematologica.org). However, these submission modalities are discouraged and will be abolished shortly.

Haematologica publishes **editorials, research papers, decision making & problem solving papers, review articles and scientific letters**. Manuscripts should be prepared according to the **Uniform Requirements for Manuscripts Submitted to Biomedical Journals**, prepared by the International Committee of Medical Journal Editors (ICMJE) and fully available online (http://www.icmje.org). Additional information is available online: http://www.haematologica.org/instructions.htm (instructions to authors).

Additional papers may be considered for the purely online journal (Haematologica on Internet, ISSN 1592-8721). Because there are no space constraints online, Haematologica on Internet will publish several items deemed by peer review to be scientifically sound and mainly useful as educational papers. These will include case reports, irreplaceable images, educational material from scientific meetings, meeting abstracts, and letters to the Editor.

Galley Proofs and Reprints. Galley proofs should be corrected and returned by email, fax or express delivery within 72 hours. Minor corrections or reasonable additions are permitted; however, excessive alterations will require editorial re-evaluation and will be possibly charged to the authors. Papers accepted for publication will be printed without cost. The cost of printing color figures will be communicated upon request. Preprints may be ordered at cost by returning the appropriate form sent by the Publisher.

Transfer of Copyright and Permission to Reproduce Parts of Published Papers. Authors will grant copyright of their articles to the Ferrata Storti Foundation. No formal permission will be required to reproduce parts (tables or illustrations) of published papers, provided the source is quoted appropriately and reproduction has no commercial intent. Reproductions with commercial intent will require written permission and payment of royalties.

Haematologica is published in two printed editions: International (worldwide except Spain, Portugal and Latin Americas) and Spanish (in Spain, Portugal and Latin Americas). Detailed information about subscriptions is available online: http://www.haematologica.org/subscribe.htm (subscriptions). While access to the online journal is free, online access to additional items of the website http://www.haematologica.org/ will require either institutional or personal subscription.
Rates of the International edition for the year 2003 are as following:

	Institutional	*Personal*
Print edition and full access to the online journal plus additional items of haematologica.org	Euro 350	Euro 150
Full access to the online journal plus additional items of haematologica.org	Euro 350	Euro 75

To subscribe to the International edition, please visit our web site http://www.haematologica.org/subscribe.htm or contact: Haematologica Journal Office, Strada Nuova 134, 27100 Pavia, Italy (phone +39.0382.531182, fax +39.0382.27721, E-mail office@haematologica.org). To subscribe to the Spanish print edition, please contact: Ediciones Doyma SA, Travesera de Gracia, 17-21, 08021 Barcelona, Spain (phone +34.3.4145706, fax +34.3.414-4911, E-mail: doyma@doyma.es).

Advertisments. Contact the Advertising Manager, Haematologica Journal Office, Strada Nuova 134, 27100 Pavia, Italy (phone +39.0382.531182, fax +39.0382.27721, E-mail: mikimos@haematologica.org).

Disclaimer. Whilst every effort is made by the publishers and the editorial board to see that no inaccurate or misleading data, opinion or statement appears in this journal, they wish to make it clear that the data and opinions appearing in the articles or advertisements herein are the responsibility of the contributor or advisor concerned. Accordingly, the publisher, the editorial board and their respective employees, officers and agents accept no liability whatsoever for the consequences of any inaccurate or misleading data, opinion or statement. Whilst all due care is taken to ensure that drug doses and other quantities are presented accurately, readers are advised that new methods and techniques involving drug usage, and described within this journal, should only be followed in conjunction with the drug manufacturer's own published literature.

Associated with USPI, Unione Stampa Periodica Italiana.
Premiato per l'alto valore culturale dal Ministero dei Beni Culturali ed Ambientali

Haematologica (ISSN 1592-8721) is an educational journal of hematology that publishes several items, including educational material from scientific meetings and meeting abstracts. The reader is advised that these items are peer reviewed by the meeting organizers and not by the journal's editorial staff. Accordingly, the guest editors and scientific committees concerned are entirely responsible for the quality of peer review. Although Haematologica (ISSN 1592-8721) is primarily an online journal, educational material from scientific meetings and meeting abstracts may also appear in print supplements.

haematologica

New Insights in Hematology

Venice, Italy
May 15-18, 2003

GUEST EDITOR

TEODORO CHISESI

2003; Volume 88; supplement No. 10 - May 2003
(indexed by Current Contents/Life Sciences and in Faxon Finder and Faxon XPRESS, also available on diskette with abstracts)
http://www.haematologica.org/

haematologica

CHRONIC LYMPHOCYTIC LEUKEMIA

Chairpersons: A. Polliack, M. Brugiatelli

HODGKIN'S DISEASE

Chairpersons: S.J. Horning, A. Santoro

MINIMAL RESIDUAL DISEASE

Chairpersons: G. Saglio, A. Lopez-Guillermo

ROUND TABLE: EXTRANODAL LYMPHOMAS

Chairman: F. Cavavvli, G. Canellos

2003; Volume 88; supplement No. 10 - May 2003
(indexed by Current Contents/Life Sciences and in Faxon Finder and Faxon XPRESS, also available on diskette with abstracts)
http://www.haematologica.org/

haematologica

2003; Volume 88; supplement No. 10 - May 2003
(indexed by Current Contents/Life Sciences and in Faxon Finder and Faxon XPRESS, also available on diskette with abstracts)
http://www.haematologica.org/

New Insights in Hematology

Foreword

Dear Colleagues,

I am pleased to invite you to the 5th Meeting "New Insights in Hematology". The Meeting, that has already become a traditional appointment, is conceived as an international workshop for Hematologists, Oncologists and Pathologists. The aim of the Meeting is to try and give answer to some of the most burning questions in the field of Hematology. You will also have the opportunity to spend some days in the unique city of Venice, that will be the set of this Meeting. I hope you will come and join us in this Meeting.

Teodoro Chisesi

Anti-idiotypic vaccination in B-cell lymphomas

GIUSEPPE SAGLIO, MONICA RINALDI, GISELLA VOLPE, SILVIA A. CIAFRÈ, PAOLA PARRELLA, EMANUELA SIGNORI, MARIA GIULIA FARACE, VITO M. FAZIO

Treatment of cancer with vaccines is an attractive prospect, but few tumors express suitable target antigens. Idiotypic determinants of the immunoglobulins expressed on the surface of B-cell lymphomas are tumor-specific antigens (TSAs), which can be targeted by immunotherapy.[1] Idiotypic antigenic determinants arise from the variable regions of the heavy and light chains of immunoglobulins. Private idiotypic determinants generally involve amino acids in the complementarity-determining regions (CDRs) which are generated during the recombination process of V, D and J genes. Complexity of D-gene usage and heterogeneity at the joint result in an essentially unique aminoacid sequence, which can be regarded as the clonal signature of the individual B cell. The induction of idiotype-specific immunity by active immunization with tumor derived-Ig has been demonstrated for a number of myeloma and B-cell lymphoma or leukemia models.[2,3] This approach usually required complete Freund adjuvant or conjugation to a strongly immunogenic carrier protein. In all cases both humoral and cellular specific responses were induced and protection against tumor challenge was clear. Clinical trials demonstrated that humoral and cellular immune responses against the tumor specific idiotype of one patient's tumor correlate with freedom from progression of disease.[2,4] Nevertheless, in most cases, although the tumors initially regressed, cells bearing Igs with mutations or with modulated surface Ig escaped and regrew.[5,6]

The traditional approach to B-cell lymphoma immunization is also limited by the need for large amounts of anti-idiotype antibodies or purified idiotypic protein that have to be prepared for each individual patient within an appropriate time-scale: idiotypic determinants are unique to each tumor, and the prospect of preparing individual protein vaccines for patients has made the application to the clinic difficult and expensive. Recently, because genes encoding immunoglobulin variable regions have been well characterized and custom DNA vaccines can be made rapidly, DNA immunization was proposed as an attractive alternative to protein immunization against B-cell lymphoma.[7]

It has been demonstrated that the in vivo intramuscular injection of recombinant genes under the form of non-replicating, *naked*, plasmid DNA, is generally applicable as an effective method for producing functional proteins *in vivo*.[8] This *in vivo* method of gene transfer found several efficient applications to modulate immune response against expressed proteins and it is now well established that *naked* DNA intramuscular injection reproducibly induces both humoral and cellular immune responses against the encoded antigens. Numerous antigens from micro-organisms (viruses, bacteria, protozoa, etc.) and cellular components were proved to induce significant titers of neutralizing antibodies, combined with an efficient cellular response. In addition to the establishment of vaccines against microorganisms, DNA vaccines were also proved successful against tumor-associated antigens. Several reports have documented that direct intramuscular injection of plasmids encoding tumor antigens causes both immunization and tumor rejection.[9]

To propose that patients with B-cell tumors should be treated by vaccination with personal vaccines containing idiotypic antigen from their tumor, a simple, rapid, and effective method for producing such vaccines must be identified. The experimental goal of our group is to set up a molecular biology method for approaching this issue, starting with the identification and isolation of the variable region genes from tumor biopsy material, aiming to produce potential personal vaccines originating from naked DNA plasmid injection by a rapid, simple, low-cost, highly efficient approach, using the short peptide encompassing the hypervariable region of CDR3 (third complementarity determining region) of Ig heavy chain as a target for tumor-specific immune response via DNA-based vaccination. In parallel, we developed an improved eukaryotic expression vector whose principal characteristic is the coexistence of two distinct, complete and differentially regulated transcription units: this vector is specifically designed for DNA vaccination, as it allows the coexpression of a cytokine, mouse interleukin-2, and of the patient's lymphoma-specific CDR-3 immunoglobulin region. In our

From the Dept. Of Clinical and Biological Sciences, University of Torino, Italy (GS, GV), Campus BioMedico, School of Medicine, Rome, Italy (MR, SAC, PP, ES, MGF, VMF); Istituto Medicina Sperimentale, CNR, Rome, Italy (MR, SAC, VMF); Biologia, Università "Tor Vergata", School of Medicine, Rome, Italy (MGF), Molecular Oncology and Gene Therapy, IRCCS H. "Casa Sollievo Sofferenza", San Giovanni Rotondo (FG), Italy (ES, VMF).

Correspondence: Giuseppe Saglio, Department Of Clinical and Biological SCiences, University of Turin, Italy.

experimental strategy, we direct idiotypic vaccine construction to the heavy chain CDR3 of tumor idiotype. Several reports have indicated the VH-CDR3 as the most relevant idiotypic determinant and T-dependent antigen of Ig variable region. This approach, involving the use of extremely rare (possibly individual) sequences, would also avoid the inclusion of large, relatively constant regions, which could generate cross-reactivity against these regions and could reasonably elicit autoimmune responses.[10]

References

1. Stevenson FK, Zhu D, King CA, Ashworth LJ, Kumar S, Hawkins RE. Idiotypic DNA vaccines against B-cell lymphoma. Immunol Rev 1995;145:211-28.
2. Kwak LW, Campbell MJ, Czerwinski D, Hart S, Miller RA, Levy R. Induction of immune responses in patients with B-cell lymphoma against the surface immunoglobulin idiotype expressed by their tumors. N Engl J Med 1992;327:1209-15.
3. George AJT, Tutt AL, Stevenson FK. Anti-idiotypic mechanism involved in suppression of a mouse B-cell lymphoma BCL1. J Immunol 1987;138:628-34.
4. Nelson EL, Li X, Hsu FJ, Kwak LW, Levy R, Clayberger C, et al. Tumor specific, cytotoxic T-lymphocyte response, after idiotype vaccination for B-cell, Non-Hodgkin's lymphoma. Blood 1996;88:580-9.
5. Dyke RJ, McBride H, George AJT, Hamblin TJ, Stevenson FK. Idiotypic vaccination against B-cell lymphoma leads to dormant tumour. Cell Immunol 1991;132:70-83
6. Meeker T, Lowder L, Cleary ML, Stewart S, Warnke R, Sklar J, et al. Emergence of idiotype variants during treatment of B-cell lymphoma with anti-idiotype antibodies. N Engl J Med 1985;312:1658-65.
7. Hawkins RE, Zhu D, Ovecka M, Winter G, Hamblin TJ, Long A, et al. Idiotypic vaccination against human B-cell lymphoma, rescue of variable region gene sequences from biopsy material for assembly as single-chain Fv personal vaccines. Blood 1994;83:3279-88.
8. Wolff JA, Malone RM, Williams P, Chong W, Acsadi G, Jani A, et al. Direct gene transfer into mouse muscle in vivo. Science 1990;247:1465-8.
9. Syrengelas AD, Chen TT, Levy R. DNA immunization induces protective immunity against B-cell lymphoma. Nat Med 1996;2:1038-41.
10. Rinaldi M, Ria F, Parrella P, Signori E, Serra A, Ciafre` SA, et al. Antibodies elicited by naked DNA vaccination against the complementary determining region 3 hypervariable region of immunoglobulin heavy chain idiotypic determinants of B-lymphoproliferative disorders specifically react with patients' tumor cells. Cancer Res 2001;61:1555-62

Biological aspects of cytotoxic cells in the lymphoproliferative disease of granular lymphocytes

G. Semenzato, A. Cabrelle, R. Zambello

Lymphoproliferative disease of granular lymphocytes (LDGL) is a well recognized disorder which encompasses a large spectrum of conditions, ranging from mild asymptomatic lymphocytosis to aggressive, usually fatal, disorders. The diagnosis of this disease is related to the demonstration that a discrete subset of granular lymphocytes (GL) is chronically expanded. The relevant findings characterizing LDGL patients can be summarized as follows: low to moderate lymphocytosis (usually below 20,000 cell/mm^3) sustained by GL; chronic, usually indolent clinical course; presence of neutropenia and/or anemia; association with chronic diseases, such as rheumatoid arthritis, chronic viral infections or neoplasms. A multiparameter analysis including clinical, hematologic, immunologic and molecular data should be used to assess the diagnosis of LDGL. Current concepts on the pathogenesis of this disease point out that proliferating cells represent in vivo primed cytotoxic lymphocytes triggered by a still unknown (viral?) antigen.

The immunologic classification of LDGL distinguishes a CD3$^+$ form of the disease which is more common, and a CD3$^-$ variant, this latter accounting for nearly 15% of LDGL cases. CD3$^+$ LDGL is symptomatic in approximately 50% of cases, neutropenia, infections and anemia being the most frequent clinical findings. Leukemic LGL constitutively express Fas and Fas-Ligand but they are resistant to Fas-induced apotosis. Clonality of the T-cell receptor is usually documented in these patients. Cytokines such as interleukin (IL)-2 and IL-15 have been claimed to play a role in the mechanisms accounting for cell proliferation in this disorder, as also suggested in animal models. CD3$^-$ LDGL are usually associated with viral infection of GL, in particular, Epstein Barr and human T lymphotropic virus I/II have been claimed to play a role. Analysis of NK receptors (both killer immunoglobulin-like receptors and natural cytotoxicity receptors) in these patients has contributed to characterizing the subsets of NK proliferating cells and given insights into the pathogenetic mechanisms accounting for cell proliferation. Clonality has rarely been demonstrated; however, when present, it correlates with an aggressive clinical course. Spontaneous regression of lymphocytosis has been reported in both CD3$^+$ and CD3$^-$ patients.

As far as the pathogenesis of neutropenia is concerned, the expression and functional activities of different chemokine receptors (CCR1 to CCR6; CXCR1 to CXCR5) were recently investigated in 12 patients with LDGL. Six patients were characterized by proliferation of CD3$^+$ GL and 6 patients by the expansion of CD3$^-$ GL. The interleukin 8 (IL-8/CXCL8) receptor CXCR1 was expressed in 12/12 patients, the CXCR4 in 6/12 patients (four CD3$^+$ and two CD3$^-$) and the CXCR3 in 3/12 patients (one CD3$^+$ and two CD3$^-$). CXCR1 was expressed only by proliferating GL.

Other CC and CXC receptors were not expressed on proliferating GL (< 2%). In functional assays, purified GL from the patients displayed significant migration in response to specific chemokines, indicating that CXCR1, CXCR3 and CXCR4 were functionally active in these patients. In addition, a significant reduction of IL-8/CXCL8-mediated cell migration was reported in the presence of anti-CXCR1 monoclonal antibody. Our results indicate that expanding cells from patients with LDGL express specific CXCR. These data may help to define functional properties of proliferating GL in patients with LDGL and contribute toward the understanding of the complex clinical features of this disease. In particular, since CXCR1 was expressed in all the patients studied, we speculate that abnormal expression of this receptor on proliferating GL might play a role in the pathogenesis of neutropenia, which represents a common feature in LDGL patients.

The ability of NK cells to recognize and kill susceptible targets and spare normal autologous cells is commonly determined by the concerted action of different surface receptors (NKR), that either trigger or inhibit the NK-mediated cytolytic activity. The inhibitory NK receptors include the killer Ig-like receptors (KIRs, i.e. KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL1 and KIR3DL2) each specific for a different group of HLA-class I alleles; the leukocyte Ig-like receptor

From the University of Padua, Academic Department of Clinical and Experimental Medicine, Clinical Immunology Branch, Venetian Institute of Molecular Medicine (VIMM), Padua, Italy.

Correspondence: G. Semenzato, MD, University of Padua, Academic Department of Clinical and Experimental Medicine, Clinical Immunology Branch, Venetian Institute of Molecular Medicine (VIMM), 35128 Padua, Italy

(LIR)-1/ILT2 that shows a broad HLA-class I specificity; and the lectin-like heterodimer CD94/NKG2-A that recognizes the HLA class Ib, HLA-E molecules. The triggering receptors include the activating form of the HLA class I-specific KIR (i.e., KIR2DS and KIR3DS), the CD94/NKG2-C heterodimer and a series of non-HLA specific receptors: the natural cytotoxicity receptors (NCR, i.e. NKp46, NKp30 and NKp44), the NKG2D receptor, and certain co-receptors (i.e., NKp80 and 2B4). While the HLA-specific receptors are clonally distributed, the non HLA-specific triggering receptors are expressed on all NK cells. Thus, while the HLA-specific inhibitory receptors allow NK cells to spare normal autologous HLA class I^+ cells, the non-HLA specific activating receptors are involved in NK-mediated killing of abnormal target cells that have undergone downregulation of HLA-class I expression. A precise role for the activating form of KIR has not yet been established although recent data, both in mice and in humans, suggest a possible involvement of MHC-specific activating NK receptors in the recognition of virally infected cells.

In a series of patients we recently showed that in most patients with NK-type LDGL the abnormal GL expansion can be identified as a population that homogeneously reacts with one or another anti-KIR monoclonal antibody. More importantly, functional studies indicate that KIRs that are homogeneously expressed in NK-type LDGL are characterized by an activating *in vitro* function. These features make pathologic NK-cell expansions different from normal NK-cell populations since, in normal individuals, NK cells usually show a diversified NKR repertoire and prevalently express inhibitory rather than activating KIR.

In terms of $CD3^+$ LDGL, only limited studies have been addressed to KIR expression and function in this subset of patients. We firstly demonstrated the expression of GL183 and EB6 antigens in a very small subset of $CD3^+$ LDGL (4 out of 44 analyzed). In a larger series of cases we were able to demonstrate the expression of KIR in 7 out of 64 patients studied, confirming that a low number of $CD3^+$ LDGL express KIR antigens.

The impressive progress in understanding the expression and functional role of NK receptors has contributed to our increased knowledge on the characteristics of proliferating cells in patients with LDGL. This conclusion is particularly consistent with $CD3^-$ NK type LDGL, for which a pathogenetic mechanism primarily involving NK receptors has been postulated. In addition, the pattern of expression of NKR has been reported to contribute to the diagnosis of disease, having recently been included among diagnostic criteria. Although less clear, a role for these receptors is also likely to be found in $CD3^+$ LDGL, and new data have been generated which contribute to a better definition of the properties of proliferating cells.

Future studies will help to obtain a more precise definition of the role of these receptors in this disease, and provide insights into the pathogenetic mechanisms leading to proliferation of GL. This goal can be persued taking advantage of a collaborative study on the Lymphoproliferative Disease of Granular Lymphocytes ongoing under the sponsorship of GIMEMA (Gruppo Italiano per le Malattie Ematologiche dell'Adulto).

References

1. Bigouret V, Hoffmann T, Arlettaz L, Villard J, Colonna M, Ticheli A, et al. Monoclonal T-cell expansions in asymptomatic individuals and in patients with large granular leukemia consist of cytotoxic effector T cells expressing the activating CD94:NKG2C/E and NKD2D killer cell receptors. Blood 2003;101:3198-204.
2. Costello RT, Sivori S, Mallet F, Sainty D, Arnoulet C, Reviron D, et al. A novel mechanism of antitumor response involving the expansion of $CD3^+/CD56^+$ large granular lymphocytes triggered by a tumor-expressed activating ligand. Leukemia 2002;16:855-60.
3. Lanier LL. NK cell receptors. Annu Rev Immunol 1998;16:359-93.
4. Loughran TP Jr. Clonal diseases of large granular lymphocytes. Blood 1993;82:1-14.
5. Loughran TP Jr, Hadlock KG, Yang Q, Perzova R, Zambello R, Semenzato G, et al. Seroreactivity to an envelope protein of human T-cell leukemia/lymphoma virus in patients with $CD3^-$ (natural killer) lymphoproliferative disease of granular lymphocytes. Blood 1997;90:1977-81.
6. Mingari MC, Schiavetti F, Ponte M, Vitale C, Maggi E, Romagnani S, et al. Human $CD8^+$ T lymphocyte subsets that express HLA class I-specific inhibitory receptors represent oligoclonally or monoclonally expanded cell populations. Proc Natl Acad Sci USA 1996;93:12433-8.
7. Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari MC, et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu Rev Immunol 2001;19:197-223.
8. Romagnani C, Pietra G, Falco M, Millo E, Mazzarino P, Biassoni R, et al. Identification of HLA-E-specific allloreactive T lymphocytes: a cell subset that undergoes preferential expansion in mixed lymphocyte culture and displays a broad cytolytic activity against allogeneic cells. Proc Natl Acad Sci USA 2002;99:11328-33.
9. Semenzato G, Pandolfi F, Chisesi T, De Rossi G, Pizzolo G, Zambello R, et al. The lymphoproliferative disease of granular lymphocytes. A heterogeneous disorder ranging from indolent to aggressive conditions. Cancer 1987;60:2971-8.
10. Semenzato G, Zambello R, Starkebaum G, Oshimi K, Loughran TP Jr. The lymphoproliferative disease of granular lymphocytes: updated criteria for diagnosis. Blood 1997;89:256-60.
11. Sivori S, Vitale M, Morelli L, Sanseverino L, Augugliaro R, Bottino C, et al. p46, a novel natural killer cell-specific surface molecule that mediates cell activation. J Exp Med 1997;186:1129-36.
12. Zambello R, Facco M, Trentin L, Sancetta R, Tassinari C, Perin A, et al. Interleukin-15 triggers the proliferation and cytotoxicity of granular lymphocytes in patients with lymphoproliferative disease of granular lympho-

cytes. Blood 1997;89:201-11.
13. Zambello R, Loughran TP Jr, Trentin L, Rassu M, Facco M, Bortolin M, et al. Spontaneous resolution of p58/EB6 antigen restricted NK-type lymphoproliferative disease of granular lymphocytes: role of Epstein Barr virus infection. Br J Haematol 1997;99:215-21.
14. Zambello R, Trentin L, Ciccone E, Bulian P, Agostini C, Moretta A, et al. Phenotypic diversity of natural killer (NK) populations in patients with NK-type lymphoproliferative disease of granular lymphocytes. Blood 1993;81:2381-5.
15. Zambello R, Trentin L, Facco M, Carollo D, Cabrelle A, Tosoni A, et al. Upregulation of CXCR1 by proliferating cells in patients with lymphoproliferative disease of granular lymphocytes. Br J Haematol 2003;120:765-73.

Immunodeficiency and lymphoma: from AIDS to post-transplant

Davide Rossi, Silvia Franceschetti, Chiara Vendramin, Federica Manfroi, Lorenzo De Paoli, Giulia Benevolo, Gianluca Gaidano

Human immunodeficiency virus-related non-Hodgkin's lymphomas (HIV-NHL) are markedly heterogeneous clinically, pathologically, histogenetically and pathogenetically. HIV-NHL may be classified into: i) systemic NHL, including Burkitt lymphoma (BL), Burkitt-like lymphoma (BLL) and diffuse large B-cell lymphoma (DLBCL); ii) primary central nervous system lymphoma (PCNSL); iv) primary effusion lymphoma (PEL); and v) plasmablastic lymphoma of the oral cavity (PBL).

The molecular histogenesis of HIV-NHL has been elucidated to a certain extent through the application of a model based on genotypic and phenotypic markers. Genotypic markers of histogenesis include heavy chain immunoglobulin variable region (IgV_H) and *BCL-6* gene mutations, both acquired during B-cell transit through the germinal center (GC). Phenotypic markers of histogenesis are represented by expression of BCL-6 protein, that is restricted to centroblasts; expression of MUM1/IRF4 (MUM1), which denotes late centrocytes, and subsequent steps of plasma cell maturation; and expression of CD138/syndecan-1 (CD138), which clusters with post-GC B-cells. The application of this histogenetic model has demonstrated that HIV-NHL derive from GC-related B-cells, since they carry both IgV_H and *BCL-6* hypermutation. However, three predominant phenotypic patterns have emerged among HIV-NHL: i) the $BCL6^+/MUM1^-/CD138^-$ pattern, that reflects normal centroblasts and clusters with DLBCL centroblastic and BL/BLL; ii) the $BCL6^-/MUM1^+/CD138^-$ pattern, which reflects normal centrocytes and is associated with a fraction of DLBCL -immunoblastic; and iii) the $BCL6^-/MUM1^+/CD138^+$ pattern, which reflects post-GC B-cells and is associated with PCNSL, PEL, PBL and a fraction of DLBCL-immunoblastic. The molecular histogenesis of PEL has been recently further clarified using gene expression profile technology. PEL display a plasmablastic gene expression profile, similar to that of immunoblasts and plasma cells, but clearly distinct from that of GC and memory B-cells. The gene expression profile of PEL has led to the identification of a set of genes specifically expressed in this lymphoma type and of potential pathogenetic and clinical significance.

The pathogenesis of HIV-NHL is a multistep process involving factors provided by the host, including HIV infection and antigen stimulation and selection, as well as alterations intrinsic to the tumor clone, namely infection by oncogenic viruses, activation of proto-oncogenes, inactivation of tumor suppressor genes, aberrant somatic hypermutation and DNA promoter hypermethylation.

Two oncogenic viruses have been related to HIV-NHL pathogenesis: EBV and HHV8. EBV infects all the clinico-pathologic entities of HIV-NHL with different frequencies, while HHV8 infection is restricted to PEL. More recently, infection by SV40 has been claimed to occur in 40% of HIV-NHL from the USA. However, our extensive analysis of SV40 infection in lymphoid malignancies was unable to confirm these data among HIV-NHL from southern Europe, since all 118 cases tested lacked SV40.

Genetic lesions identified until now among HIV-NHL are generally specific for a particular type of HIV-NHL and predominantly involve activation of proto-oncogenes by chromosomal translocation. For example, *c-MYC* rearrangement is restricted to a fraction of BL, whereas BCL-6 rearrangement associates with 20% of DLBCL. Recent evidence has documented that, because of malfunctioning of Ig somatic hypermutation process, DLBCL of immunocompetent hosts associates with an aberrant hypermutation activity that-elicits tumor associated lesions at multiple proto-oncogene sites, including PIM-1, PAX-5, RhoH/TTF and c-MYC. Analogous to DLBCL of the immunocompetent host, aberrant hypermutation is a predominant feature of HIV-NHL and represents a novel mechanism of lymphomagenesis in this context.

Aberrant DNA methylation is a mechanism alternative to mutations/deletions of the locus for tumor suppressor gene silencing. A fraction of HIV-NHL display a methylator phenotype resulting in the inactivation of multiple tumor suppressor genes, including the DNA repair gene O^6-methylguanosine DNA-methyltransferase (MGMT), the apoptotic signal transduction gene death-associated-protein-kinase (DAP-kinase) and the glutathione S-transferase p1 gene (GSTp1).

From the U.D.A. Ematologia, Dipartimento di Scienze Mediche & IRCAD, Università del Piemonte Orientale "Amedeo Avogadro", Novara, Italy.

Correspondence: Gianluca Gaidano, M.D., Ph.D., U.D.A. Ematologia, Dipartimento di Scienze Mediche & IRCAD, Università del Piemonte Orientale "Amedeo Avogadro", via Solaroli 17, 28100 Novara, Italy. E-mail: gaidano@med.unipmn.it

Post-transplant lymphopoliferative disorders (PTLD) are a severe complication of solid organ and bone marrow transplantation. Similar to HIV-NHL, PTLD too share the general features of lymphomas associated with immunodeficiency, namely B-cell phenotype, EBV infection, frequent involvement of extra-nodal sites, and aggressive clinical behavior. However, PTLD are heterogeneous, in terms of morphology, pattern of clonality, histogenesis and molecular pathogenesis.

The clinico-pathologic heterogeneity of PTLD has stimulated several classifications. Currently, the World Health Organization (WHO) classification distinguishes PTLD into: i) early lesions, which are polyclonal disorders; ii) polymorphic PTLD sharing either a polyclonal, oligoclonal or monoclonal pattern of clonality; and, finally, iii) monomorphic PTLD, which are monoclonal expansions and include DLBCL-centroblastic, DLBCL-immunoblastic, BL/BLL and multiple myeloma.

The molecular histogenesis of PTLD has been recently elucidated by applying the same histogenetic model developed for HIV-NHL. In general terms, similarly to HIV-NHL, PTLD derive from GC-related B-cells, since they carry both IgV_H and BCL-6 hypermutation. In this context, expression of BCL-6, MUM-1 and CD138 allows the identification of PTLD derived from: i) GC centroblasts, namely DLBCL-centroblastic and BL/BLL; ii) GC centrocytes, namely a fraction of polymorphic PTLD and of DLBCL-immunoblastic; and iii) post-GC B-cells, namely a fraction of polymorphic PTLD and of DLBCL-immunoblastic.

Analogous to HIV-NHL, the pathogenesis of PTLD is a multiphasic process involving both host predisposing factors and alterations intrinsic to the tumor clone. Host predisposing factors include reduced immunosurveillance and exalted antigenic stimulation, whereas tumor clone alterations are essentially represented by modifications of the cell genome, namely viral infection, activation of proto-oncogenes, inactivation of tumor suppressor genes, aberrant somatic hypermutation and DNA promoter hypermethylation. Almost 80% of PTLD harbor EBV infection. In this context, the pathogenetic role of EBV is documented by expression of the viral oncoprotein LMP-1, which is able to transform B cells through the activation of the tumor necrosis factor pathway of signal transduction.

To date, the primary genetic lesion involved in PTLD pathogenesis is still unknown. Early onset PTLD are typically devoid of detectable molecular lesions, in agreement with their polyclonal nature. Polymorphic PTLD display frequent mutations of *BCL-6*, in the absence of other known molecular lesions. In this context, mutations of BCL-6 are an unfavorable prognostic marker of survival. Monomorphic PTLD, which are mainly NHL, lack the primary genetic lesions commonly observed in NHL of the immunocompetent host and HIV-NHL, such as rearrangements of BCL-6, BCL-1 or BCL-2. In molecular terms, monomorphic PTLD associate frequently with mutations of the BCL-6 proto-oncogene, and, more rarely, with mutations of RAS and p53.

Analogous to DLBCL of the immunocompetent host and HIV-NHL, aberrant somatic hypermutation of the proto-oncogenes PIM-1, PAX-5, RhoH/TTF and c-MYC characterizes a fraction of PTLD and may represent a major contributor to disease pathogenesis.

Finally, PTLD frequently display a methylator phenotype resulting in inactivation through promoter hypermethylation of tumor-suppressor genes, including DAP-kinase, of DNA repair enzymes such as MGMT, and of drug metabolyzing molecules such as GSTp1.

Funding

Work by the authors was supported by Istituto Superiore di Sanità, Programma Nazionale di Ricerca sull'AIDS - Progetto Patologia, Clinica e Terapia dell'AIDS, Rome, Italy; Cofin 2000 and 2002 – MIUR, Rome, Italy; and Progetto Strategico Oncologia, CNR-MIUR, Rome, Italy.

Selected readings

Capello D, Rossi D, Gaudino G, Carbone A, Gaidano G. Simian virus 40 infection in lymphoproliferative disorders. Lancet 2003;361:88-9.

Carbone A, Gaidano G. Acquired immunodeficiency syndrome-related cancer. A study model for the mechanisms contributing to the genesis of cancer. Eur J Cancer 2001;37:1184-7.

Carbone A, Gloghini A, Larocca LM, Capello D, Pierconti F, Canzonieri V, et al. Expression profile of MUM1/IRF4, BCL-6, and CD138/syndecan-1 defines novel histogenetic subsets of human immunodeficiency virus-related lymphomas. Blood 2001;97:744-51.

Gaidano G, Carbone A. Primary effusion lymphoma. A liquid phase lymphoma of fluid filled body cavities. Adv Cancer Res 2001;80:115-46.

Klein U, Gloghini A, Gaidano G, Chadburn A, Cesarman E, Dalla-Favera R, et al. Gene expression profile analysis of AIDS-related primary effusion lymphoma (PEL) suggests a plasmablastic derivation and identifies PEL-specific transcripts. Blood 2003 (in press).

Rossi D, Gaidano G. Molecular heterogeneity of diffuse large B-cell lymphoma: implications for disease management and prognosis. Hematology 2002;7:239-52.

Rossi D, Gaidano G. Messengers of cell death: Apoptotic signaling in health and disease. Haematologica 2003;88:212-8.

Adult T-cell leukemia/lymphoma and human T lymphotropic virus type I: a still challenging problem

Daniela Saggioro, Laura Daprai, Lidia Acquasaliente, Luigi Chieco-Bianchi

Defects in the regulation of programmed cell death play a fundamental role in the pathogenesis of neoplasia, autoimmune disorders, and degenerative diseases of the nervous system; this is relevant to the pathogenesis of the human T lymphotropic virus type 1 (HTLV-1) given that the virus is associated to both leukemia/lymphoma and chronic degenerative diseases. Indeed, HTLV-1 is the etiological agent of the adult T-cell leukemia/lymphoma (ATLL) and the inflammatory neurodegenerative disorder named tropical spastic paraparesis/ HTLV-1 associated myelopathy (TSP/HAM). HTLV-1 encodes a 40 kDa phosphoprotein called Tax that has many properties typical for oncoproteins: a) Tax immortalizes primary rodent fibroblasts and cooperates with the ras oncogene in the transformation of these cells; b) it induces leukemia and neurofibromas in transgenic mice; c) it immortalizes primary human T-lymphocytes; d) it induces chromosomal instability. Tax stimulates viral transcription and acts as a transcriptional modulator by altering the expression of selected cellular genes, many of which are involved in cell cycle regulation. It has been suggested that it is through this ability to modulate cellular genes expression that Tax mediates HTLV-1 transformation. As cell cycle regulation and programmed cell death are closely related, Tax represents an attractive candidate for the deregulation of the normal apoptotic pathway. So far, contradictory data have been obtained concerning the apoptotic activity of Tax as the protein has been found to either induce or inhibit apoptotic cell death. These discordant reports concerning the influence of Tax on apoptosis might reflect differences in cell type or methods used to induce apoptosis or might suggest that Tax affects the apoptotic processes by multiple mechanisms. We analyzed the susceptibility of Tax^+ and Tax^- murine fibroblasts to apoptosis using stimuli such as growth factor withdrawal and TNF-α, which trigger cell death through two distinct pathways, i.e. the mitochondrial apoptotic pathway and the death receptor pathway, respectively. We found that Tax, although unable to protect from apoptosis induced by TNF-α, is able to interfere with apoptosis triggered by depletion of growth factors. This protective effect is due to a block in the apoptotic program regulated by mitochondria, given that Tax^+ cells grown in 0.1% serum do not undergo release of cytochrome c from the mitochondrial intermembrane space or redistribution of the pro-apoptotic Bax protein from the cytosol to mitochondria. These data indicate that Tax-expressing cells respond differently to apoptosis, depending on the apoptotic stimulus used and consequently on the apoptotic pathway activated.

Tax exerts its functions by activating major cellular signal transducing pathways including NF-kB, and CREB/ATF (cAMP-responsive element-binding protein/activating transcription factor). To elucidate the relevance of each transcriptional pathway activated by Tax in the observed resistance to apoptosis, we analyzed the apoptotic behavior of Tax variants which are mutated in specific sites that disrupt a single aspect of Tax function. For these studies we used the well characterized Tax mutants M22 (defective for NF-kB pathway activation), and M47 (defective for CREB/ATF pathway activation). We found that the CREB/ATF-competent mutant M22 is able to protect murine fibroblasts from serum depletion-induced apoptosis at levels comparable to that of wild-type Tax. In contrast, the CREB/ATF-defective M47 mutant was unable to prevents cell death.

Various evidences implicate the pro-apoptotic Bax protein as en essential regulator of apoptosis induced by growth factor depletion. Furthermore, in absence of any other apoptotic stimulus, over-expression of Bax in intact cells activates a mitochondrial apoptotic pathway by inducing the release of cytochrome c from the mitochondrial intermembrane space. To verify whether Tax is also able to counteract the apoptosis induced by Bax over-expression, we transfected the human HeLa and 293T cell lines with either M47 or M22 together with a plasmid expressing Bax protein. We found that activation of the CREB/ATF pathway is necessary to protect the cells from apoptosis induced by over-expression of Bax while NF-kB activation seems to be dispensable. Thus, our results indicate that CREB/ATF activity of Tax plays a relevant role in protecting cells from apoptosis triggered by both growth factor withdrawal and Bax over-expression. Furthermore, these data allow the hypothesis that the ability of Tax to inhibit certain apoptotic stimuli may be important in its role as a viral transforming protein.

From the Department of Oncology and Surgical Sciences-Oncology Section, University of Padova, Italy.

Correspondence: Luigi Chieco-Bianchi, Department of Oncology and Surgical Sciences-Oncology Section, University of Padova, Italy.

Molecular pathogenesis of B-cell lymphoma: the role of BCL6

Riccardo Dalla Favera

Non-Hodgkin's lymphoma (NHL) derive from mature B cells (85% of cases), and, in a minority of cases, from T cells. Most B-NHL types derive from the germinal center (GC), the structure where naïve B cells encounter the antigen, and undergo immunoglobulin (Ig) V region somatic hypermutation (SH) and isotype switching (S) and are selected to become memory B cells or plasma cells. SH and S mechanisms are involved in the generation of specific chromosomal translocations, which contribute to the pathogenesis of NHL by deregulating the expression of oncogenes such as *BCL2*, *c-MYC*, *BCL1*, and *BCL6*. Recent progress will be presented in three areas: i) analysis of the signaling pathways controlling normal and neoplastic GC formation by gene expression profiling;[1] ii) evidence that the somatic hypermutation mechanism misfires and targets multiple loci in diffuse large cell lymphoma;[2] iii) new results on the regulation and function of the *BCL6* oncogene and on strategies for its therapeutic inactivation in NHL.[3-5]

References

1. Klein, U, Tu Y, Stolovitzky GA, Keller JL, Haddad J Jr, Miljkovic V, et al. Transcriptional analysis of the B cell germinal center reaction. Proc Natl Acad Sci USA 2003;100:2639-44.
2. Pasqualucci, L, Migliazza A, Basso K, Houldsworth J, Chaganti RSK, Dalla-Favera R. Mutations of the BCL6 proto-oncogene disrupt its negative autoregulation in diffuse large B-cell lymphoma. Blood 2003;101:2914-23.
3. Pasqualucci L, Neumeister P, Goossens T, Gouri N, Chaganti RSK, Küppers R, et al. Hypermutation of multiple proto-oncogenes in B-Cell Diffuse Large Cell Lymphoma. Nature 2001;19:341-6.
4. Niu H, Cattoretti G, Dalla-Favera R. BCL6 controls the expression of the B7.1 co-stimulatory receptor in germinal-center B cells. J Exp Med (in press).
5. Bereshchenko O, Gu W, Dalla-Favera R. Acetylation inactivates the BCL6 transcriptional repressor. Nat Gen 2002;32:606-13.

Correspondence: Riccardo Dalla Favera, Director, Institute for Cancer Genetics, Columbia University, New York, NY, USA.
E-mail: rd10@columbia.edu

Novel perspectives on the interface between innate and adaptive immunity to pathogenic fungi

LUIGINA ROMANI

Host defense mechanisms against fungi are numerous and range from relatively primitive and constitutively expressed, non-specific defenses to sophisticated adaptive mechanisms that are specifically induced during infection. Although the role of innate immunity was originally considered to be a process defending of the host early in infection, it is now clear that there is an important reciprocal relationship between innate and adaptive immune responses. In this context, the present contribution attempts to relate the early innate resistance process to the development of adaptive T helper (Th) immunity to pathogenic fungi, with possible implications for prophylaxis and therapy of fungal infections. Innate immunity plays an essential role in orchestrating the subsequent adaptive antifungal immunity. Through the involvement of a set of germline-encoded receptors (referred to as pattern recognition receptors, PRRs), cells of the innate immune system not only discriminate between different fungi and different forms of them, but also contribute to discrimination between self and pathogens at the level of the adaptive Th immunity. Discrimination occurs though the involvement of PRRs, including the Toll-like receptors (TLRs), recognizing invariant molecular structures shared by fungi (also known as PAMPs, pathogen-associated molecular patterns), such as mannan and glucan. PRRs and TRLs appear to be involved in the recognition of PAMPs on different classes of pathogens. In fungal infections, the engagement of PRRs and TLRs with PAMPS creates signals that (i) induce opsonization for phagocytosis and/or the activation of the lectin pathways of complement, (ii) promote phagocytosis, and (iii) induce effector functions which initiate the expression of inflammatory cytokines and initiate Th adaptive immunity. As the different Th cell subsets are endowed with the ability to release a distinct panel of cytokines, capable of activating and deactivating signals to effector phagocytes, the activation of an appropriate Th subset may be instrumental in the generation of a successful immune response to the fungal pathogens. In its basic conception, the paradigm calls for (i) an association between Th1 responses and the onset/maintenance of phagocyte-dependent immunity, critical for opposing fungal infectivity or clearing pathogenic fungi from infected tissues; (ii) the occurrence of Th2 responses in infections and diseases; (iii) the reciprocal regulation of Th1 and Th2 cells, occurring either directly or through regulatory T cells, resulting in a dynamic balance between these two types of reactivity that may contribute to the induction and maintenance of protective memory antifungal responses with minimum cytokine-mediated regulation of antifungal Th cell development and effector function.

As clinical resistance represents a significant component of the overall drug resistance of the antifungal agents, one major strategy to overcome clinical resistance is to improve the immune functions of the immunocompromised host. The Th1/Th2 paradigm appears to be useful for identifying of cytokines suitable for clinical intervention. Redressing the Th balance by combination therapy with cytokines or cytokine antagonists proved to be beneficial in optimizing antifungal chemotherapy in experimental fungal infections. Finally, the identification of PRRs as a critical component of the innate recognition system is a fundamental breakthrough in immunology. The understanding of PRR functions and dysfunctions which are suitable for deliberate manipulation in fungal infections remains a future challenge.

Funding

Supported by Progetto Nazionale AIDS, ISS, 50D.27

From the Microbiology Section, Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Via del Giochetto, 06122 Perugia, Italy.

Correspondence: Luigina Romani, Department of Experimental Medicine and Biochemical Sciences, Microbiology Section, University of Perugia, Italy.

Antifungal drugs: from the pharmacokinetic/pharmacodynamic relationships to therapy

FEDERICO PEA

Antifungal agents are assuming an increasingly relevant role in daily clinical practice because of the considerable increase in the incidence of systemic fungal infections occurred in the last ten years in hospital settings. This upward trend appears to be related, on one hand, to an increase in the number of immunocompromised hosts, and, on the other hand, to the widespread use of broad-spectrum antibacterial agents in the empirical treatment of critically ill patients admitted in hematology wards and in intensive care units. The treatment of systemic mycosis is currently founded on antifungal agents characterized by well-documented efficacy, namely amphotericin B, fluconazole, itraconazole and flucytosine, which do, however, have some major flaws from a pharmacodynamic point of view: incomplete antifungal spectrum of activity and/or a severe toxicity. In the 1990s, thanks to advanced pharmaceutical techniques, several entrapping lipid formulations (liposomal, colloidal dispersion and lipid complex) became available, improving the tolerance to amphotericin B and leading to a substantial change in the pharmacokinetic behavior of this antifungal agent.

Small size and *in vivo* stability of the lipid formulation in blood were shown to be among the most important characteristics ensuring efficacy of encapsulated amphotericin B. More recently, research activity in the field of antifungal therapy has been considerably intensified in an attempt to identify new molecules with a broad spectrum of activity and better tolerability. These efforts have lead to the development of several new antifungal agents, some of which are chemically related to the previously available triazoles, e.g. voriconazole, which is structurally related to fluconazole, and posaconazole and ravuconazole, which are structurally related to itraconazole. Others, namely caspofungin, micafungin and anidulafungin, belong to a completely new class of antifungal agents, the echinocandins.

The echinocandins are characterized by an innovative mechanism of action, inhibition of the $\beta^{1,3}$ D-glucan synthesis in the fungal cell wall, thus making them attractive agents for potential combination therapy with amphotericin B or triazoles. Despite the intense research activity in this field, the best schedule regimen (in terms of dosage and frequency of administration) for the antifungal agents has not yet been defined. Recently, several *in vitro* (*time-kill curves*) and *in vivo* (animal models of experimental infections and clinical trials) studies have been carried out in order to better define the relationships between the kind of antifungal activity and the degree of systemic exposure to the antifungal agent.

These so-called pharmacokinetic-pharmacodynamic (PK/PD) relationships have been defined for each antifungal agent in order to understand the most adequate dosage and its division, and the appropriate pharmacodynamic breakpoints suitable for the achievable *in vivo* concentrations. Amphotericin B (either deoxycholate or in lipid formulations) shows a concentration-dependent antifungal activity; that is, its antifungal activity increases as the peak plasma concentration to the minimum inhibitory concentration for the etiological agent (C_{max}/MIC) ratio increases. The echinocandins also seem to have concentration-dependent antifungal activity.

In contrast, flucytosine and triazoles show time-dependent antifungal activity: that is, their efficacy is mainly related to the time during which the plasma concentration persists above the MIC (t>MIC) of the etiological agent. Moreover, several antifungal agents are characterized by a significant post-antifungal effect (PAFE), namely the ability to maintain fungal growth suppressed for some time even after the concentration of the antifungal agent has fallen below the MIC, a characteristic which may improve their *in vivo* efficacy. On the basis of these PK/PD relationships, it has been suggested that the best administration schedule for amphotericin B, and probably also for caspofungin, should be a once daily dose. Conversely, divided administration, in two or even more doses, should be preferred for flucytosine and for triazoles, especially those with a shorter half-life of elimination (e.g. voriconazole). Finally, it should not be overlooked that systemic exposure for those antifungal agents interfering with or metabolized by the cytochrome P450 system (especially by the CYP2C9, CYP2C19 and CYP3A4), namely triazoles, may be unpredictable. These pharmacokinetic interactions might have important consequences not only becau-

From the Institute of Clinical Pharmacology and Toxicology, DPMSC, Udine University Hospital, Italy.

Correspondence: Federico Pea, Institute of Clinical Pharmacology and Toxicology, DPMSC, Udine University Hospital, Italy

se they may sometimes cause undertreatment, but also because they may be responsible, due to their inhibitory activity against CYP isoenzymes, for an increased incidence of adverse events and toxicity. Therapeutic drug monitoring of plasma concentrations of triazoles, may be helpful in tailoring and optimizing antifungal therapy, as has just been shown for itraconazole in hematologic settings.

Caspofungin. A novel antifungal agent with great promise in patients with hematologic malignancies

RODRIGO MARTINO

For more than 30 years, amphotericin B (AmB) was the only drug available for the treatment of severe systemic yeast and filamentous fungal infections in patients with hematologic malignancies. However, this drug is in reality very toxic, and the only reason that it has stood the pass of time is that there were no other real therapeutic alternatives. The introduction of fluconazole in the early 1990s was an important step forward in the prophylaxis and therapy of yeast infections, although within one decade a shift toward fluconazole-resistant strains has gradually occurred. Itraconazole was viewed as a potential step forward in the therapy of filamentous fungi, but unfortunately this azole has not undergone thorough clinical research, and more than 10 years after its introduction, clinicians still do not know the exact role of itraconazole in any of its three formulations in the management of aspergillosis and other invasive fungal infections (IFI). Voriconazole and other new azoles (posaconazole, ravuconazole) have potent broad-spectrum antifungal activity and are being intensively studied in the management of IFI. In fact, voriconazole is the first drug to have shown a higher clinical efficacy than conventional AmB in the treatment of invasive candidiasis.[1] Lipid formulations of AmB are less nephrotoxic than conventional AmB, even when given at doses five times higher. However, they are also very expensive, and despite being in the market for more than six years now, they have not been proven to improve the outcome of any type of IFI when compared with conventional AmB. In fact, the only clear difference (with the exception of the much lower nephrotoxicity) between these drugs and AmB is the higher cost, as shown in Table 1.

Caspofungin belongs to a new class of antifungal agents, the echinocandins, which are in fact the first truly new class of antifungal drugs introduced in the past 15 years. The mechanism of action of caspofungin is inhibition of β-D-glucan synthesase, an enzyme involved in the generation of the fungal wall that is not present in mammalian cells. This explains why caspofungin has virtually no clinically relevant toxicity, an extremely important point in the area of hematology and stem cell transplantation. Another very important advantage of these drugs over the azoles is the lack of drug interactions, which can complicate the use of the new azoles in very debilitated patients with hematologic malignancies.

Caspofungin has a broad-spectrum fungistatic and fungicidal activity. It is active against all species of *Candida spp*., including fluconazole- and AmB-resistant strains.[2] Of note, *Cryptococcus neoformans* and other basidiomycetous yeasts (*Trichospooin spp*., *Rhodotorula spp*., etc) are resistant *in vitro* to the echinocandins. Caspofungin is also active against all species of *Aspergillus spp*., the most common cause of severe IFI in hematology in most centers. However, activity against other filamentous fungi is not assured, and some emerging species such as *Fusarium spp*., *Scedosporiuum prolificans* and *Mucorales* are resistant. Interestingly, caspofungin is highly active against Pneumocytis carinii.

Caspofungin acts synergistically with AmB and the azoles against both yeasts and filamentous fungi, and many clinicians have placed great hopes in the use of combination therapy to improve the outcome of patients with these infections. In fact, although there is no formal proof, many patients with aspergillosis are now treated with combinations of caspofungin with a triazole or an AmB preparation outside of clinical trials. Although this strategy may be considered ethically appropriate, these combinations are extremely expensive, and we should make strong efforts to use such combinations within clinical trials. Another important point that hematologists should remember is that a great deal of money and effort is being employed now in seeing which drug(s), if any, is better than others. This, of course, is of great interest to the drug industry. However, many of our patients who die with an IFI acquire the infection and succumb to it only because their immune status is so deteriorated that emergence of an opportunistic infection is the only logical end. Paradoxically, we are engaged in generating more and more such severely compromised patients, through highly complex stem cell transplant procedures or immune-ablating antitumor therapies. We need to realize that if we truly want to see a reduction of the mortality from IFI, besi-

From the Servicios de Hematología Clínica, Hospital de la Santa Creu i Sant Pau, Barcelona.

Correspondence: Dr. Rodrigo Martino, Servei d'Hematologia Clinica Hospital de la Santa Creu i Sant Pau, Av. Sant Antoni Maria Claret, 167, 08025 Barcelona. E-mail: rmartino@hsp.santpau.es

Table 1. Direct drug acquisition costs of the main systemic antifungal agents in Spain (in Euros and for a 65 kg adult).

Drug	*1 mg/kg-day*	*3 mg/kg-day*	*5 mg/kg-day*	*10 mg/kg-day*
c-AmB	15			
ABLC	66	198	330	
L-AmB	198	594	990	1980
Voriconazole		440*		
Caspofungin		450 (630)°		

c-AmB: conventional amphotericin B; ABLC: AmB lipid complex or Abelcet; L-AmB: liposomal AmB or Ambisome.
**Refers to the standard daily dose of 4 mg/kg/12 hrs.*
°Refers to the standard FDA-approved daily dose of 50 mg, although a daily 70 mg dose has been approved in Europe (in parentheses the cost of 70 mg/day).

des investing so much time and money in studying antifungal drugs, we must also try to develop therapeutic strategies that preserve a functional immune status in a larger number of patients.

Despite being a relatively new drug, caspofungin has been well studied in various IFI. Thus, caspofungin has been shown to be at least as effective as fluconazole and conventional AmB in invasive candidiasis and candida esophagitis and perhaps even more effective.[2-5] Thus, it is a reasonable agent for the treatment of these infections in patients with hematologic malignancies, although its exact role in the therapeutic algorithm of these infections in most hospitals will require a careful study of the particular needs in each setting. With respect to invasive aspergillosis, caspofungin has been shown to be more effective than other licensed antifungal drugs in the management of patients who are refractory or intolerant to other antifungals, mainly AmB.[6] In fact, caspofungin is now approved for this indication in most countries. Again, however, the exact use of this agent in the real world needs to be carefully analyzed in each institution, since other drugs are now available (mainly voriconazole), and the issue of the reasonable use of limited health resources is of utmost importance with these extremely expensive agents.

References

1. Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. Invasive Fungal Infections Group of the European Organisation for Research and Treatment of Cancer and the Global Aspergillus Study Group. N Engl J Med 2002;347:408-15.
2. Letscher-Bru V, Herbrecht R. Caspofungin: the first representative of a new antifungal class. J Antimicrob Chemother 2003;51:513-21.
3. Villanueva A, Arathoon EG, Gotuzzo E, Berman RS, DiNubile MJ, Sable CA. A randomized double-blind study of caspofungin versus amphotericin for the treatment of candidal esophagitis. Clin Infect Dis 2001;33: 1529-35.
4. Arathoon EG, Gotuzzo E, Noriega LM, Berman RS, DiNubile MJ, Sable CA. A randomized, double-blind, multicenter study of caspofungin versus amphotericin B for treatment of oropharyngeal and esophageal candidiasis. Antimicrob Agents Chemother 2002;46:451-7.
5. Villanueva A, Arathoon EG, Gotuzzo E, Berman RS, Di Nubile MJ, Sable CA. A randomized double-blind study of caspofungin versus amphotericin for the treatment of candidal esophagitis. Clin Infect Dis 2001;33:1529-35.
6. Mora-Duarte J, Betts R, Rotstein C, Colombo AL, Thompson-Moya L, Smietana J, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. N Engl J Med 2002;347:2020-9.
7. Maertens J. Raad I, Petrikos G. Update of a multicenter, noncomparative study of caspofungin in adults with invasive aspergillosis refractory or intolerant to other antifungal agents: an analysis of 90 patients. ASM, Washington, DC, USA. In: Programs and Abstracts of the 40th ICAAC, San Diego, CA, USA, 2002. Abstract M868, p. 388.

Viral, bacterial and mycotic infections in aplastic patients

ENZO RAISE, CHRISTIAN MANZARDO, SANDRO PASQUINUCCI, MARCO BECHI, PIETRO ROCCHETTO, ANDREA PETRUCCI, GIORGIO ROSINI, PIERANGELO CHINELLO, SILVIA PITTALIS, PIERLUIGI BRUGNARO, ROSARIA SANCETTA, PAOLA POLISTENA, MICHELE VESPIGNANI, MARGHERITA BERGAMASCO

Infections remain of major concern in the management of patients with hematologic malignancies.[1,2] These complications are frequent, potentially life-threatening, and particularly worrisome since the prognosis for patients with acute leukemia and lymphoma has improved over the last decade. The better outcome of these patients is due to several factors including more aggressive antineoplastic chemotherapy and procedures such as bone marrow transplantation, but is also due to better control of hemorrhage and infections.[3,4] The incidence of infections is directly related to the granulocyte count.[5] Patients with polymorphonuclear cell (PMN) counts below 500/mm^3 are considered at risk. For these patients rapidly fatal infectious complications (mainly septicemia) have been well documented. Therefore, close medical surveillance is essential. Rapid initiation of empirical antimicrobial therapy at the onset of fever is mandatory and should occur within 60 minutes of the first signs or symptoms of infection. This systematic approach has been shown to decrease the mortality due to septicemia caused by Gram-negative bacilli.[6,7]

Aim

Our aim was to evaluate infections occurring in patients who underwent bone marrow transplantation (BMT) and intensive chemotherapy with subsequent marrow aplasia. We studied the germs responsible for the infections, their pattern of antimicrobial resistance and the efficacy of empirical and etiologic therapy.

Methods

We evaluated all the autologous (auto-) BMTs performed in the Venice Hospital Hematology Center during the years 1998-2002 and the episodes of febrile neutropenia that required the opinion of an infectious diseases consultant in the year 2002. Standard prophylaxis was ciprofloxacin (500 mg *bid*), fluconazole (200 mg *qd*) and acyclovir (800 mg *tid*). At the appearance of fever 3 sequential blood cultures were performed as well as a chest X-ray and a physical examination of the patients. After the cultures, the empirical first-line therapy was ceftazidime + amikacin. If there was not a meaningful reduction of fever within 72 hours, those were substituted by piperacillin-tazobactam or imipenem-cilastatin or meropenem in association with teicoplanin or vancomicin. If there was not a reduction of temperature by the 5th -7th day of therapy, liposomal amphotericin B was associated.

Results

From 1998 to 2002, 140 auto-BMTs were performed in the Venice Hospital Hematology Center. The underlying malignancies were non Hodgkin's lymphoma (n=32), Hodgkin's lymphoma (n=37), myeloma (n=48), chronic lymphocytic leukemia (n=13), acute lymphocytic leukemia (n=4) and chronic myeloid leukemia (n=6). All the patients had a central venous catheter (CVC). The mean duration of aplasia (PMN < 500/mm^3) was 7±3 days. All the pts were treated with granulocyte colony-stimulating factor (G-CSF).

Fever appeared in 36% of auto-BMTs (51/140) within the first 30 days after the transplantation, with a mean body temperature of 38.5°C . Blood cultures resulted positive in 89% (45/51) of the febrile episodes. Gram-positive (G$^+$) bacteria represented 69% of isolates (31/45) and Gram-negative (G$^-$) 31% (14/45); there were also 2 *Aspergillus spp* infections and 1 hemorrhagic chickenpox. Single or multiple bacterial species were isolated from the same patient. The G$^+$ bacteria were 14 *S. epidermidis* (75% methicillin-resistant), 4 *S. aureus* (all methicillin-resistant), 6 *S. hominis*, 3 *S. capitis*, 1 *Micrococcus luteus*, 1 *Micrococcus lylae*, 1 *Oerskovia sp.*, 1 *E. faecalis*. The G- bacteria were: 4 *Ps. aeruginosa*, 3 *E. coli*, 2 *Kl. pneumoniae*, 1 *Kl. oxytoca*, 1 *Flavobacterium sp.*, 1 *Acinetobacter*, 1 *Alcaligenes sp.*, and 1 *B. cepacia*.

First-line antibiotic therapy (ceftazidime + amikacin) resolved the fever within 72 hrs in 54% (27/51) of the febrile episodes. The 2nd association with imipenem-cilastatin + vancomycin was administered, when the first-line therapy failed, in 14/24 cases, obtaining defervescence in all patients. In the remaining 10 cases the second-line treatment was piperacillin-tazobactam + teicoplanin (6 patients) or meropenem + teicoplanin (4 patients). Death occurred

From the Infectious Diseases Division (ER, CM, SP, MB, PR, AP, GR, PC, SP, PB), Haematology (RS, PP, MV) and Microbiology (MB), SS. Giovanni e Paolo and Umberto 1° Hospitals, Venice and Mestre, Italy.

Correspondence: Enzo Raise, Infectious Diseases Division, SS. Giovanni e Paolo and Umberto 1° Hospitals, Venice and Mestre, Italy.

within 30 days in 1 of the 45 documented infections (2.2%), be cause of necrotising fascitis. All the patients with fever had SIRS (sistemic inflammatory response syndrome); one patient developed mitralic endocarditis on a native valve (*S. aureus*) and there was 1 case (2%) of G- shock (*Ps. aeruginosa*). The two infections from *Aspergillus spp*. became clinically evident between the 18th and 25th day of aplasia with an escavative lesion in the lung; therapy with liposomal amphotericin B at a 3 mg/Kg dose followed by oral itraconazole resolved of the infection. The hemorrhagic chickenpox appeared 15 days after the auto-BMT. The treatment was acyclovir 15 mg/kg/*tid*. The patient died from relapse of lymphoma 3 months after the transplantation.

During the year 2002 there were 399 hospital admissions in Mestre and Venice Hematology Centers (179 females and 220 males). We analyzed 68 cases (17%) of fever complicating aplasia due to intensive chemotherapy, often requiring the opinion of an infectious diseases consultant. Fever occurred in 42 patients (29 males and 13 females) with a mean age of 53 years, whose underlying malignancies were: acute leukemia (n=13), chronic lymphocytic leukemia (n=3), non-Hodgkin's lymphoma (n=12), Hodgkin's lymphoma (n=2), multiple myeloma (n= 7), myelodysplasia (n=3), bone marrow aplasia (n=1), and myelofibrosis (n=1).

At the onset of fever the mean body temperature was 38.5°C and the mean number of WBC was 656/mm^3. In 58/68 cases (85%) the patients had a CVC (Groshong) and in 5/68 episodes (7%) the febrile episode started with septic shock. The mean duration of fever was 10,5 days (range 1-40). At the beginning of fever all patients had less than 1000 PMN/mm^3 and in 60/68 episodes the number of PMN fell below 500/mm^3. The mean duration of aplasia (PMN < 500) was 9 days. In 58/68 cases the patients were treated with G-CSF.

Blood cultures were positive in 33/68 episodes (48%). Forty-two germs were isolated, 27 G^+ (64%) and 15 G- bacteria (36%): 10 *S. epidermidis*, 4 *S. haemolyticus*, 4 *S. hominis*, 2 *S. warneri*, 1 *S. capitis*, 1 *S. saprophyticus*, 1 *S. aureus*, 1 *Str. mitis*, 1 *E. faecium*, 1 *Micrococcus luteus*, 1 *Corynebacterium aquaticum*, 9 *E. coli*, 4 *P. aeruginosa*, 1 *Acinetobacter lwoffi*, and 1 *Psychrobacter sp*. All the coagulase-negative staphylococci were methicillin-resistant and 77% (17/22) were ciprofloxacin-resistant. Eight out of 9 *E. coli* (89%) and 2/4 *P. aeruginosa* were ciprofloxacin-resistant. In 8/33 episodes (24%) blood cultures yielded multiple germs (polymicrobial sepsis).

Antibiotic prophylaxis was already being administered at the onset of fever in 38/68 cases (56%), usually with levofloxacin or ciprofloxacin, and antifungal prophylaxis was ongoing in 56/68 episodes (82%) with fluconazole or itraconazole, an anti-*Pneumocystis* pneumonia prophylaxis with cotrimoxazole in 24/68 cases (35%). Antiviral prophylaxis with aciclovir was being used in 16/68 episodes (23%).

The first-line therapy (piperacillin-tazobactam or ceftazidime with or without amikacin) was effective in 19/68 cases (28%), in which fever disappeared after a mean of 4 days; a second-line association, usually with the introduction of a carbapenem and a glycopeptide, was effective in 20/68 cases (29%) leading to defervescence after a mean of 5 days; in the remaining 29/68 cases (43%) further changes in therapy were needed.

The final diagnosis was a microbiologically determined infection (MDI) in 38/68 cases (56%): 33 bacteremia, 3 pneumonia (*P. aeruginosa* + *C. glabrata* in broncho-aspiration [BASP] in 1 case, 1 *C. albicans* in BASP in 1 case and *S. maltophilia* + *C. incospicua* in sputum in 1 case), 2 pulmonary aspergillosis, 1 *P. aeruginosa* pharyngitis, 1 gastrointestinal salmonellosis, 2 pseudomembranous colitis, with the determination of A and B toxin of *Clostridium difficile*. In 14/68 episodes (20%) there was a clinically determined infection (CDI): 3 cases of pulmonary aspergillosis (suspected on the basis of the radiographic findings), 3 interstitial pneumonia, 3 pneumonia, 4 cases of abscess (thoracic wall, kidney, axilla, perianal), 1 otitis with mastoiditis, 1 pharyngitis, 1 cholecystitis, 1 cellulitis of the leg, 1 chickenpox. The remaining 16/68 cases (24%) were defined as fever of unknown origin and were treated with empiric antimicrobial therapy.

Ten patients died within 30 days after the onset of fever: 5 of progression of malignancy, 2 of Gram-negative shock, 2 of pneumonia and 1 of cerebral hemorrhage. Infection-related mortality was therefore 5.8% (4/68).

Conclusions

The rate of infection in transplanted and aplastic patients appears to be in line with the literature data; G^+ bacteria represent the majority of the isolates and the use of ciprofloxacin in prophylaxis appears to be responsible for the common ciprofloxacin-resistance registered for both G^+ and G^- germs. Almost all the coagulase-negative staphylococci are methicillin-resistant, which suggests a great diffusion of nosocomial infections.

The first-line antibiotic therapy is often replaced by the carbapenem + glycopeptide association because both the *Staphylococcus spp* infections and the multi-bacterial ones are frequent. The fear of an abuse of glycopeptides, which could cause an increase in glycopeptide-resistan-

ce, is in conflict with the possibility of having a higher mortality rate. Undoubtedly, therapeutic associations should be suited to the bacterial ecosystem of each hospital.

A multidisciplinary approach to the febrile neutropenic patients seems to be very important for the cure of severe infections in immunocompromised hosts.

References

1. Kirk JL, Greenfield RA, Disloyal B, Epstein RB. Analysis of early infectious complications after autologous bone marrow transplantation. Cancer 1988;62:2445-50.
2. Mossad SB, Longworth DL, Goormastic M, Serkey JM, Keys TF, Bolwell BJ. Early infectious complications in autologous bone marrow transplantation: to review of 219 patients. Bone Marrow Transplant 1966;18:265-71.
3. Bow WJ, Leaven R, Change MS, Shore TB, Rubinger M, Shacter B. Cytotoxic therapy-induced D-xylose malabsorption and invasive infection during remission-induction therapy acute for myeloid leukaemia in adults. J Clin Onc 1997;15:2254-61.
4. Sayer HG, Longton G, Bowden R, Pepper M, Storb R. Increased risk of infection in marrow transplant patients receiving methylprednisolone for graft-versus-host disease prevention. Blood 1994;84:1328-32.
5. End PA. Management of fever in patients with cancer and treatment - induced neutropenia. N Engl J Med 1993;328:1323-32.
6. Lin SJ, Schranz J, Teush SM. Aspergillosis house-fatality installments: sistematic review of the literature. Clin Infect Dis 2001;32:358-65.
7. Wingard JR, White MH, Anaissie E, Raffalli J, Goodman J, Arrieta A. A randomised, comparative double-blind trial evaluating the safety of liposomial Amphotericin B versus Amphotericin B lipid complex in the empirical treatment of febrile neutropenia. Clin Infect Dis 2000; 31:1155-63.

Oral fludarabine: improving chemotherapy for lymphoproliferative disorders

PIER LUIGI ZINZANI

The advent of the purine analogs in the early 1990s, in particular of fludarabine phosphate has had a major impact on the management of chronic lymphocytic leukemia (CLL). The intravenous formulation is indicated for the treatment of patients with CLL who have not responded to, or whose disease has progressed during or after, treatment with at least one standard alkylating agent-containing regimen. Treatment with fludarabine has produced objective response rates of 40% to 60%. Response and survival after treatment with fludarabine in advanced CLL are strongly correlated with disease stage and the degree of previous chemotherapeutic treatment. Patients with relapsed CLL have higher response rates to treatment with fludarabine than do patients with previously resistant disease.

Intravenous chemotherapy with fludarabine entails regular visits to the hospital as an outpatient or admission for inpatient treatment, as a typical treatment cycle consists of 25 mg/m^2 given over 30 minutes every day for 5 days and repeated every 4 weeks. For this reason, an oral formulation would be more convenient for both patients and health care workers and may also be more cost-effective than IV therapy.

An oral formulation of fludarabine, comprising an immediate-release tablet containing 10 mg fludarabine has recently been developed. Pharmacokinetic studies have demonstrated that a once-daily oral dose of 40 mg/m^2 would provide a similar systemic exposure to 25 mg/m^2 given intravenously.[1] Bioavailability is unaffected by food.[2] The efficacy of oral fludarabine does not differ from that of its intravenous formulation and its safety profile is similar.[3] As observed with the intravenous formulation, myelosuppression and infections are the most common adverse events. Gastrointestinal toxicity is mild to moderate with a low incidence of nausea, vomiting or diarrhea.[3]

Synergistic effects have been demonstrated *in vitro* and confirmed *in vivo* with cyclophosphamide.[4] In addition, cyclophosphamide is currently given as an oral formulation. Recently, in a multicenter prospective study, previously untreated patients with Binet stage B and C CLL received fludarabine 30 mg/m^2 and cyclophosphamide 200 mg/m^2 on days 1 to 5 repeated every 4 weeks for 6 cycles.[5] A high response rate (77.5%), including a complete response rate of 46.5%, was achieved following this oral combination regimen. Gastrointestinal toxicity following the combination was higher than that seen following fludarabine alone but remained mild.

We are currently carrying out a study in our Institute we have an ongoing study on the use of an oral fludarabine-cyclophosphamide combination in heavily pretreated patients with indolent non-Hodgkin's lymphoma.

In summary, the advantages of the oral formulation include: i) avoiding hospitalization, which improves quality of life and lowers cost; ii) venous access may be difficult in heavily pretreated, older patients; iii) an oral formulation is more convenient for both patients and health care workers.

References

1. Foran JM, Oscier D, Orchard J, Johnson SA, Tighe M, Cullen MH, et al. Pharmacokinetic study of single doses of oral fludarabine phosphate in patients with "low-grade" non-Hodgkin's lymphoma and B-cell chronic lymphocytic leukemia. J Clin Oncol 1999;17:1574-9.
2. Oscier D, Orchard JA, Culligan D, Cunningham D, Johnson S, Parker A, et al. The bioavailability of oral fludarabine phosphate is unaffected by food. Hematol J 2001;2:316-21.
3. Boogaerts MA, Van Hoof A, Catovsky D, Kovacs M, Montillo M, Zinzani PL, et al. Activity of oral fludarabine phosphate in previously treated chronic lymphocytic leukemia. J Clin Oncol 2001;19:4252-8.
4. O'Brien SM, Kantarjian HM, Cortes J, Beran M, Koller CA, Giles FJ, et al. Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. J Clin Oncol 2001;19:1414-20.
5. Cazin JC. Oral fludarabine and cyclophosphamide in previously untreated CLL: preliminary data on 59 patients. Blood 2001;98:772a[absract].

From the Institute of Hematology and Medical Oncology "Seràgnoli" University of Bologna.

Correspondence: Pier Luigi Zinzani, Institute of Hematology and Medical Oncology "Seràgnoli", University of Bologna.

The new therapeutic indications for rituximab

FRANCESCO ZAJA, SALVATORE DE VITA, NICOLA VIANELLI, GIANFRANCO FERRACCIOLI, MICHELE BACCARANI, RENATO FANIN

B cells have a key role in the pathogenesis of some autoimmune disorders and a selective B-cell blockade could positively interfere with the production of autoantibodies. Thus, the availability of a targeted anti-B cell treatment may be an important alternative for the therapy of some autoimmune disorders. The efficacy of rituximab observed in patients with non-Hodgkin's lymphoma (NHL), together with its good safety profile, have encouraged in these last years investigations of the therapeutic activity of this drug in some autoimmune disorders with a prevalent *autoantibody-based* pathogenesis. Autoimmune cytopenias and, in particular, immune thrombocytopenias have been the most studied diseases. Nevertheless some other rheumatological, neurological and dermatological autoimmune diseases have also been investigated with interesting preliminary results.

Therapeutic schedule and pharmacokinetics

So far data from a single dose finding study are available.[1] Three dose levels of rituximab (1: 50 mg/m^2 in week 1 followed by 150 mg/m^2 in weeks 2-4 mg; 2: 150 mg/m^2 in week 1 followed by 375 mg/m^2 in weeks 2-4; 3: 375 mg/m^2 weekly $\times$ 4) were investigated and 19 patients enrolled. No responses were seen at the first dose levels. Three out 16 patients receiving dose levels 2 and 3 achieved a clinical response (2 complete and 1 partial response). The dose schedule otherwise reported was the traditional used in NHL (375 mg/m^2 every 7 days for 4 weeks). We have looked to the pharmacokinetic of rituximab in 7 patients with idiopathic trombocytopenic purpura. The rituximab serum concentrations appeared similar to that observed in previous experiences in lymphoma patients.[2,3] Despite previous pharmacokinetic studies having demonstrated an association between clinical response and serum rituximab accumulation both during and after treatment,[4] in our group of patients no differences were observed in serum levels between responders and non-responders.

From the Clinica Ematologica, Policlinico Universitario, Udine, Italy; Clinica Reumatologica, Policlinico Universitario, Udine, Italy; Istituto di Ematologia ed Oncologia Medica L. e A. Seragnoli, Bologna, Italy.

Correspondence: Francesco Zaja, Clinica Ematologica, Policlinico Universitario, Udine, Italy.

Results

Immune thrombocytopenias

The biggest surveys regard patients with immune thrombocytopenias (idiopathic or secondary to B-cell lymphoprolyferative disorders). A meta-analysis of data from the literature,[1,5-15] together with the results of our experience, indicate that rituximab is effective in about 50% of patients, with a 15-50% rate of complete remission (CR). In most cases the response is prompt and can be achieved after the first administration of rituximab, but sometimes the platelet increase is observed 4 to 6 weeks after the beginning of the treatment. The duration of response is quite long in a large percentage of patients. Recurrence occurs in about 20%. Older age appears to be correlated with a worse response rate. No other clinical variable (gender, time from diagnosis to treatment, previous splenectomy) or laboratory parameter (total and CD20^{+ve} lymphocytes count, level of CD20 expression) was found to be correlated.

Autoimmune hemolytic anemia

Although the case series are small,[16-23] interesting results have also been observed in immune hemolytic anemia, whether idiopathic or associated with B-cell chronic lymphoproliferative disorders. In particular, rituximab appeared to have efficacy in the treatment of cold agglutinin disease. In our experience, among 7 patients refractory to standard treatments (1 idiopathic autoimmune hemolytic anemia, 4 CLL-associated autoimmune hemolytic anemia, 2 cold agglutinin diseases), 2 achieved a sustained CR still present after 10 and 40 months.

Other autoimmune disorders

Preliminary results suggest a potential therapeutic activity of rituximab in a broad spectrum of other different autoimmune disorders such as pure red cell aplasia,[24-25] acquired thrombotic thrombocytopenic purpura,[26] acquired factor VIII deficiency,[27-28] rheumatoid arthritis,[29] Wegener's granulomatosis,[30] immune complex vasculitis,[31] Goodpasture's syndrome,[31] pemphigus,[32-34] myasthenia gravis,[35] and paraproteinemic neuropathies.[36]

Type II mixed cryoglobulinemia

Interesting results has been observed in type II mixed cryoglobulinemia (MC). This is a systemic vasculitis prevalently mediated by immune-complexes, usually associated with hepatitis C virus (HCV) infection, and characterized by a clonal non-neoplastic proliferation of rheumatoid factor-positive B-cell clones leading to cryoglobulin production. The treatment of HCV-associated MC remains difficult at present, and may target either the viral trigger (HCV), when present, or the downstream pathogenetic events by means of less specific approaches such as corticosteroids, immunosuppressants or plasmapheresis. However, these therapies are not always effective, they are often toxic and, in some cases, they may be contraindicated because of patients' age or the presence of comorbidities. Based on the hypothesis that rituximab could act selectively on cryoglobulinemic B-cell lymphoproliferation we have treated 15 consecutive patients with active disease, poorly controlled with standard treatments. Rituximab proved effective on vasculitic skin manifestations (ulcers, purpura or urticaria), subjective symptoms of peripheral neuropathy, low-grade B-cell lymphoma, arthralgias, and fever. Laboratory features, i.e. significantly decreased serum rheumatoid factor and cryoglobulins, and increased C4, were consistent with the observed clinical efficacy.[37]

Toxicity

The good safety profile of this agent was confirmed in the treatment of these conditions. In particular, infusion-related symptoms were quite uncommon. It is likely that the smaller number of B-cells, compared to the number in patients with NHL, is the main explanation of this fact. Furthermore, in our experience, there were no signs of medium or long-term toxicity and, in particular, no significant infectious events were recorded.

Conclusions

From these preliminary experiences, rituximab seems to be an effective and safe agent for the treatment of some *autoantobody-based* immune disorders. Further controlled studies will deepen our knowledge of the potential activity of this agent in these new indications and investigate the mechanisms of resistance. The efficacy of a selective B-cell blockade confirms the primary pathogenetic role of B cells in some autoimmune disorders and may constitute the ground for more targeted therapies of autoimmune disorders.

References

1. Saleh MN, Gutheil J, Moore M, Feinberg B, Bunch P, Butler J, et al. A pilot study of anti-CD20 MoAb Rituximab in patients with refractory immune thrombocytopenic purpura (ITP). Blood 2000;96:1086[abstract].
2. Iacona I, Lazzarino M, Avanzini MA, Rupolo M, Arcaini L, Astori C, et al. Rituximab (IDEC-C2B8): validation of a sensitive enzyme-linked immunoassay applied to a clinical pharmacokinetic study. Therapeutic Drug Monitoring 2000;22:295-301.
3. Lazzarino M, Arcaini L, Bernasconi P, Alessandrino EP, Gargantini L, Cairoli R, et al. A sequence of immunochemotherapy with Rituximab, mobilization of in vivo purged stem cells, high dose chemotherapy and autotransplant is an effective and non toxic treatment for advanced follicular and mantle cell lymphoma. Br J Haematol 2002;116:229-35.
4. Berinstein NL, Grillo-Lopez AJ, White CA, Bence-Bruckler I, Maloney D, Czuczman M, et al. Association of serum Rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. Ann Oncol 1998;9:995-1001.
5. Stasi R, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti-CD20 monolonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. Blood 2001;98:952-7.
6. Stasi R, Stipa E, Forte V, Meo P, Amadori S. Variable patterns of response to rituximab treatment in adults with chronic idiopathic thrombocytopenic purpura. Blood 2002;99:3872-3.
7. Cooper N, Bussel J. Rituxan treatment in patients with immune thrombocytopenic purpura (ITP). Blood 2001; 98:2180[abstract].
8. Giagounidis AA, Anhuf J, Schneider P, Germing U, Soehngen D, Quabeck K, et al. Treatment of relapsed idiopathic thrombocytopenic purpura with the anti-CD20 antibody Rituximab. Eur J Haematol 2002; 69:95-100.
9. Grossi A, Santini V, Longo G, Balestri F, Ferrini PR. Treatment with anti-CD20 antibodies of patients with autoimmune thrombocytopenia with or without haemolytic anemia: worsening of the hemoglobin level. Blood 2001;96:1089[abstract].
10. Mow M, Hook CC. Rituximab for the treatment of refractory immune thrombocytopenic purpura. Blood 2000;96:3526[abstract].
11. Mehta AC, Mtanos GJ, Gentile TC. Exacerbation Of lupus while receiving Rituximab for chronic refractory thrombocytopenia. Blood 2001;98:3682[abstract].
12. Perotta A, Sunnemberg JS, Ratanatharathorn V, Hook C, Attas L, Dawson D. et al. Rituxan in the treatment of chronic idiopathic thrombocytopenia purpura (ITP). Blood 1999;94:49[abstract].
13. Ratanatharathorn V, Carson E, Reynolds C, Ayash LJ, Levine J, Yanik G. et al. Anti-CD20 chimeric monoclonal antibody treatment of refractory immune-mediated thrombocytopenia in a patient with chronic graft-versus-host disease. Ann Intern Med 2000;133:275-9.
14. Rosenthal E, Karsenti JM, Pesce A, Cassutto JP. Anti-CD20 monoclonal antibody (Rituximab) administration in patients with refractory immunologic thrombocytopenic purpura. Blood 2001;98:2194[abstract].
15. Schuler US, Florek M, Mueller J, Haack A, Weise M, Lehmann HU, et al. Succesful Rituximab treatment of immune thrombocytopenia (ITP) in an elderly lady with systemic mastocytosis. Blood 2001;98:3875 [abstract].
16. Lee E, Zamkoff KW, Gentile TC, Zimrin A. Rituxan in the treatment of auto-immune haemolytic anemia (AIHA). Blood 2000;96:2560[abstract].
17. Berentsen S, Tjonnfjord GE, Gjertsen BT, Hammerstrom J, Langholm R, Sorbo JH, et al. Rituxan (Rituximab) therapy for chronic cold agglutinin disease. Blood 2000;96:3156[abstract].
18. Ahrens N, Kingreen D, Seltsam A, Salama A. Treatment of refractory autoimmune haemolytic anemia with anti-CD20 (Rituximab). Br J Haematol 2001;114:244-5.

19. Baduer F. Rituximab: a very efficient therapy in cold agglutinin and refractory autoimmune haemolytic anaemia associated with CD20-positive, low grade non Hodgkin's lymphoma. Br J Haematol 2001;112:1085-6.
20. Layios N, Van Den Neste E, Jost E, Deneys V, Scheiff JM, Ferrant A. Remission of severe cold agglutinin disease after Rituximab therapy. Leukemia 2001;15:187-8.
21. Reddy S, Schwartz JM, Hussain S, Fernandez G. Rituximab-based therapy in 3 patients with autoimmune hemolytic anemia. Blood 2001;98:3610[abstract].
22. Zaja F, Iacona I, Masolini P, Russo D, Sperotto A, Prosdocimo S, et al. B-cell depletion with rituximab as treatment for immune hemolytic anemia and chronic thrombocytopenia. Haematologica 2002;87:189-95.
23. Quartier P, Brethon B, Philippet P, Landman-Parker J, Le Deist F, Fischer A. Treatment of childhood autoimmune haemolytic anaemia with rituximab Lancet 2001;358:1511-3.
24. Zecca M, De Stefano P, Nobili B, Locatelli F. Anti-CD20 monoclonal antibody for the treatment of severe, immune-mediated, pure red cell aplasia and hemolytic anemia. Blood 2001;97:3995-7.
25. Ghazal H. Succesful treatment of pure red cell aplasia with rituximab in patients with chronic lymphocytic leukemia. Blood 2002;99:1092-4.
26. Chemnitz J, Draube A, Schulz A, Scheid C, Fuchs M, Diehl V et al. Rituximab in the treatment of severe thrombotic thrombocytopenic purpura. Blood 2001; 98:131[abstract].
27. Wiestner A, Cho HJ, Asch AS, Michelis MA, Zeller JA, Peerschke EIB. Rituximab in the treatment of acquired factor VIII inhibitors. Blood 2001;98:2233[abstract].
28. Karwal MW, Schlueter AJ, Zenk DW, Davis RT. Treatment of acquired factor VIII deficiency with rituximb. Blood 2001;98:2232[abstract].
29. De Vita S, Zaja F, Sacco S, De Candia A, Fanin R, Ferraccioli G. The efficacy of selective B-cell blockade in rheumatoid arthritis supports the pathogenetic role of B cells. Arthritis Rheum 2002;46:2029-33.
30. Specks U, Fervenza FC, McDonald TJ, Hogan MC. Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy. Arthritis Rheum 2001; 44:2836-40.
31. Arzoo K, Sadeghi S, Liebman HA. Remission induced by rituximab in autoimmune disorders. Blood 2001; 98: 3723[abstract].
32. Salopek TG, Logsetty S, Tredget EE. Anti-CD20 chimeric monoclonal antibody (rituximab) for the treatment of recalcitrant, life-threatening pemphigus vulgaris with implications in the pathogenesis of the disorder. J Am Acad Dermatol 2002; 47:785-8.
33. Heizmann M, Itin P, Wernli M, Borradori L, Bargetzi MJ. Successful treatment of paraneoplastic pemphigus in follicular NHL with rituximab: report of a case and review of treatment for paraneoplastic pemphigus in NHL and CLL. Am J Hematol 2001;66:142-4.
34. Borradori L, Lombardi T, Samson J, Girardet C, Saurat JH, Hugli A. Anti-CD20 monoclonal antibody (rituximab) for refractory erosive stomatitis secondary to CD20(+) follicular lymphoma-associated paraneoplastic pemphigus. Arch Dermatol 2001;137:269-72.
35. Zaja F, Russo D, Fuga G, Perella G, Baccarani M. Rituximab for myasthenia gravis developing after bone marrow transplant. Neurology 2000;55:1062-3.
36. Levine TD, Pestronk A. IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using rituximab. Neurolgy 1999; 52:1701-4.
37. Zaja F, De Vita S, Mazzaro C, Sacco S, Damiani D, De Marchi G, et al. Efficacy and safety of rituximab in type II mixed cryoglobulinemia. Blood 2003(in press).

Intramyocardial inoculation of autologous bone marrow transplantation in patients with chronic refractory ischemia

A. Porcellini, B. Reimers, G. Azzarello, P. Pascotto, O. Vinante

Recent studies in an animal model have demonstrated that bone marrow cells inoculated into ischemic hearths enhance angiogenesis and can generate *de novo* myocardium. The purpose of our study was to assess the feasibility and safety of direct intramyocardial inoculation of filtered whole autologous bone marrow (ABM) in patients with chronic refractory myocardial ischemia not suitable for the conventional revascularization strategies. Ten patients (mean age 68+/-10 years) with severe refractory angina were included. Catheter–based electromechanical mapping of the left ventricle (NOGA) was performed to guide intramyocardial ABM inoculations using the Myostar catheter (J&J Biosense). Eight to ten inoculations of 1 mL of ABM into the target ischemic area were performed. Myocardial perfusion was assessed at baseline and 1 month after the procedure with NH3 positron emission tomography (PET). Procedural or 30-day adverse events were not observed. At each injection site a mean of 24.8×10^6/mL of ABM nucleated cells (range 15.3 to 50.0) were injected. The mean percentage values of $CD34^+$ cells and the $CD\ 34^-\ CD117^+\ CD45^{+/-}\ CD4^{+/-}$ subset in the mononuclear fraction were, respectively, 2.9 (range 1.5- 4.5) and 0.21 (range 0.02-0.13). PET evaluation at 30 days was available in 5/10 patients: in 2/5 it showed qualitative improvement of perfusion in the target area and in 7/10 patients it showed subjective improvement of angina symptoms. These clinical studies are in a very early stage but they proved safe and feasible and in our view are necessary to build a platform for future more sophisticated cell therapy.

*From the Department of Oncology and Hematology Noale, VE (AP, GA, OV) *Department of Cardiology Mirano, VE (BR, PP).*

Correspondence: A. Porcellini, Department of Oncology and Hematology Noale, VE.

The impact of anaemia on the quality and quantity of life in anaemic patients with cancer

T.J. LITTLEWOOD

Anaemia is common in patients with malignant disease.[1] The most common causes include the cytokine driven anaemia of chronic disease compounded by the myelotoxic effects of chemo and radiotherapy. Fatigue is the most frequently reported symptom by patients with cancer.[2] The fatigue may be explained by both physiological and psychological factors and it is likely that anaemia is one of the possible causes.

Treatment of anaemia

Blood transfusion

Anemia may be ignored (on the basis that mild to moderate anaemia is not important) or treated with either blood transfusion[3] or recombinant erythropoietin (rHuEpo). Blood transfusions are generally considered to be a safe and effective treatment. However, there are some risks[4] and, to compensate for these risks, the transfusion *trigger*, ie, the level that Hb must fall to before the patient receives a transfusion, is often very low. The beneficial effects of transfusion last for 2-4 weeks when a further transfusion might be needed.

Impact of anemia correction on quality of life

About a decade ago, recombinant human erythropoietin became available for treating anemia associated with cancer and cancer treatment. The use of epoetin alfa to increase Hb levels in patients receiving chemotherapy has been shown to improve patients' sense of well-being in randomized, controlled trials[5,6] and large open-label, community-based studies.[7-9] A very recent study has emphasised that anaemic patients with cancer clearly have a worse quality of life than the normal population but, strikingly, the deficit is markedly reduced in the cancer patients whose haemoglobin concentration is increased by treatment with erythropoietin. The deficit in the placebo treated patients was unchanged.[10]

Epoetin α has also been shown to effectively increase Hb levels in patients receiving radiotherapy and radiotherapy/chemotherapy combined. Treatment with erythropoietin has been very safe although red cell aplasia has been recently reported in a small number of patients with renal failure. Anti erythropoietin antibodies were detected in these patients.11

Survival benefit

Anemia may not only have an adverse impact on quality of life but also have a negative influence on life expectancy. We know that anemia at diagnosis is an adverse prognostic factor for a number of haematological malignancies such as myeloma and chronic lymphatic leukemia. In addition, particularly in patients with solid tumours there is a relationship between anaemia and tumour hypoxia and hypoxic tumours have increased malignant potential and are more radio-resistant than normoxic ones.[12]

Finally, data from phase 2 and non-randomized trials strongly suggested that correcting anemia (by blood transfusion and treatment with erythropoietin) improved the patient's life expectancy.[13,14]

A large, placebo-controlled, randomized, double-blind clinical trial of epoetin α was conducted in 375 anemic (baseline Hb <10.5 g/dL, or >10.5 but <12.0 g/dL following an Hb decrease of >1.5 g/dL in the previous month) patients who received nonplatinum chemotherapy for nonmyeloid hematologic and solid tumours.[5] The original objectives of this trial were to assess the effects of epoetin alfa on transfusion requirements, Hb level, QOL, and safety. Before the study was unblinded, an additional objective was included to explore a possible relationship between increased Hb and survival. Patients were randomized to receive epoetin alfa 150-300 IU/kg body weight 3 times weekly for 12-24 weeks (n=251), or placebo (n=124).

Median survival times were 17 months for patients who received epoetin α, compared to 11 months for the placebo group. The Kaplan-Meier 12-month estimates showed a trend toward better overall survival favouring the epoetin α group (p=.13; log rank test). The investigators concluded that although the study was neither designed nor powered for survival as an endpoint, the results suggest a survival benefit with epoetin α.

Another trial designed to confirm a survival benefit is warranted, since other uncontrolled variables may have influenced survival, e.g., tumor stage, intensity of chemotherapy, extent of bone marrow involvement, and disease progression.

From the Department of Haematology , John Radcliffe Hospital, Oxford, OX3 9DU.

Correspondence: TJ Littlewood, Department of Haematology, John Radcliffe Hospital, UK.

Conclusions

The majority of physicians were trained to believe that mild to moderate anaemia in patients with cancer was to be expected, and that the anaemia did not cause the patient any serious harm until the Hb fell to <10.0 g/dL or even <8.0 g/dL.

We now know that this theory is incorrect, and study data strongly support the notion that anaemia has a negative impact on patients' QOL, and that correcting the anaemia will provide an objective improvement in the patients' well-being. Additional data also suggests that correcting anaemia may improve the patient's life expectancy but this latter observation requires further confirmation.

References

1. Groopman JE, Itri LM. Chemotherapy-induced anemia in adults: incidence and treatment. J Natl Cancer Inst 1999;91:1616-34.
2. Curt GA, Breitbart W, Cella D, Groopman JE, Horning SJ, Itri LM, et al. Impact of cancer-related fatigue on the lives of patients: new findings from the Fatigue Coalition. Oncologist 2000;5:353-60.
3. Barrett-Lee PJ, Bailey NP, O'Brien ME, Wager E. Large-scale UK audit of blood transfusion requirements and anaemia in patients receiving cytotoxic chemotherapy. Br J Cancer 2000;82:93-7.
4. Goodnough LT, Brecher ME, Kanter MH, AuBuchon JP. Transfusion medicine. First of two parts--blood transfusion. N Engl J Med 1999;340:438-47.
5. Littlewood TJ, Bajetta E, Nortier JW, Vercammen E, Rapoport B. Effects of epoetin alfa on hematologic parameters and quality of life in cancer patients receiving nonplatinum chemotherapy: results of a randomized,double-blind,placebo-controlled trial. J Clin Oncol 2001;19:2865-74.
6. Osterborg A, Brandberg Y, Molostova V, Iosava G, Abdulkadyrov K, Hedenus M, et al. Randomized, double-blind, placebo-controlled trial of recombinant erythropoietin, epoetin β, in hematologic malignancies. J Clin Oncol 2002;20:2486-94.
7. Demetri GD, Kris M, Wade J, Degos L, Cella D. Quality-of-life benefit in chemotherapy patients treated with epoetin α is independent of disease response or tumor type: results from a prospective community oncology study. Procrit Study Group. J Clin Oncol 1998;16:3412-25.
8. Gabrilove JL, Einhorn LH, Livingston RB, Winer E, Cleeland CS. Once-weekly dosing of epoetin α is similar to three-times-weekly dosing in increasing hemoglobin and quality of life. Proc Am Soc Clin Oncol 1999; 18:574a[abstract 2216].
9. Glaspy J, Bukowski R, Steinberg D, Taylor C, Tchekmedyian S, Vadhan-Raj S. Impact of therapy with epoetin α on clinical outcomes in patients with nonmyeloid malignancies during cancer chemotherapy in community oncology practice. Procrit Study Group. J Clin Oncol 1997;15:1218-34.
10. Cella D, Zagari MJ, Vandoros C, Gagnon DD, Hurtz H-J, Nortier JW. Epoetin α treatment results in clinically significant improvements in quality of life in anaemic cancer patients when referenced to the general population. J Clin Oncol 2003;21:366-73.
11. Casadevall N, Nataf J, Viron B, Kolta A, Kiladjian JJ, Martin-Dupont P, et al. Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. N Engl J Med 2002;346: 469-75.
12. Höckel M, Schlenger K, Hockel S, Vaupel P. Association between tumor hypoxia and Malignant progression: the clinical evidence in cancer of the uterine cervix. In: Vaupel P, Kelleher DK, editors. Tumor Hypoxia. Wissenschaftliche Verlagsgesellschaft MBH; Stuttgart. 1996 p. 65-74.
13. Grogan M, Thomas GM, Melamed I, Wong FL, Pearcey RG, Joseph PK, et al. The importance of hemoglobin levels during radiotherapy for carcinoma of the cervix. Cancer 1999;86:1528-36.
14. Glaser CM, Millesi W, Kornek GV, Lang S, Schull B, Watzinger F, et al. Impact of hemoglobin level and use of recombinant erythropoietin on efficacy of preoperative chemoradiation therapy for squamous cell carcinoma of the oral cavity and oropharynx. Int J Radiat Oncol Biol Phys 2001;50:705-15.

Recent views on B-cell chronic lymphocytic leukemia pathophysiology

MANLIO FERRARINI, MASSIMO MANGIOLA, GIOVANNA CUTRONA, SIMONA ZUPO

B-cell chronic lymphocytic leukemia (B-CLL) has, for a long time, been considered a homogeneous disease caused by the progressive accumulation of antigen-inexperienced lymphocytes, probably characterized by apoptotic defects. In recent years, accumulated data have challenged this widespread belief. The demonstration that B-CLL cells are not antigen-inexperienced lymphocytes came primarily from observations on IgV gene usage by the malignant cells. Indeed, VH and VL gene sequence studies have revealed that the malignant cells of as many as 50% of cases of B-CLL utilize genes that are extensively mutated.[1-3] Since point mutations in these genes occur only following antigenic stimulation with the assistance of T cells, the data provide a definite,indirect demonstration that antigenic stimulation may have occurred before, during or even after neoplastic transformation. Even in those case of B-CLL in which the cells do not utilize mutated genes, there is indirect evidence that they have been subjected to antigenic stimulation. Comparative analyses of the VH/VL gene repertoire of these malignant cells and of normal cells have demonstrated a skewed usage of certain VH/VL genes, a particular VH/VL combination, or special HCDR3 sequences.[4] Moreover, there are case reports of malignant cells from different patients sharing the same antibody combining site (and presumably specificity) formed by the combination of H and L chains.

The above observations raise the issue of whether antigenic stimulation (which probably played an important role in clonal expansion prior to transformation) continues to exert a promoting effect on the growth of the malignant cells following transformation. A key point regarding this issue is the question of whether the malignant cells retain a viable, BcR-mediated signal transduction pathway. Indeed, recent studies, including those from our laboratory, have demonstrated that approximately 50% of B-CLL are generated by cells with a viable surface IgM-initiated signal transduction pathway.[5,6] The cells from these patients generally express surface CD38 and have unmutated VH/VL region genes. Cross-linking of the surface IgM of these cells *in vitro* is rapidly followed by apoptosis. Since these CD38 positive, unmutated cases of B-CLL also are those with a more aggressive clinical course,[2,7-10] this observation raises the issue of how stimulation via surface IgM, which readily cause apoptosis, can have a promoting effect on tumor growth. The answer to this apparent contradiction is provided by considerations that stimulation via surface IgM provides a potential apoptotic stimulus, but also activates the cells and renders them sensitive to receive signals that are delivered by a number of accessory and stromal cells. These may be particularly efficient in the pseudofollicles of the bone marrow, or of certain lymphoid tissues where malignant cells and accessory cells are brought into close contact. Interestingly, stimulation of the same cells through surface IgD causes prolonged cell survival and some differentiation into plasma cells instead of apoptosis.[11] Therefore, it appears that there is also a balance between the signals initiated by surface Ig and that this balance can dictate the subsequent fate of the cells.

Most of the CD38 negative, mutated B-CLL cells do not respond to signals delivered to surface IgM. Indeed, it appears that these surface molecules are incapable of activating the appropriate signal transduction pathway, as assessed by Ca^{++} mobilization or tyrosine kinase phosophorylation. Surface IgD appears capable of delivering cellular signals although these are destined only to counterbalance the apoptotic signals. Therefore, it appears that the interaction between these cells and the environment via the BcR is much less marked than in the CD38 positive, unmutated cases.

Collectively, the above data are compatible with the following model: CD38 positive B-CLL cells are likely to be continuously stimulated via surface IgM. This is probably related to the fact that surface IgM (encoded for by unmutated VH/VL genes) retain *natural antibody* activity and hence react continuously with autoantigens *in vivo*. The subsequent fate of the cells depends upon its capacity to activate the anti-apoptotic signals and also on the signals received from surface IgD. In the case of surface CD38 negative, mutated B-CLL cells, it is unlikely that the BcR can exert a promoting role on cell expansion since the

From the Laboratory of Tumor Immunology and Dept of Internal Medicine, H. San Raffaele Scientific Institute.

Correspondence: Manlio Ferrarini, Laboratory of Tumor Immunology and Dept of Internal Medicine, H. San Raffaele Scientific Institute, Via Olgettina 60, I-20132, Milan, Italy.

IgM signal-transducing pathway is not viable. Moreover, Ig encoded for by mutated VH/VL genes rarely have *natural antibody* activity. Thus, these cases of B-CLL may have to rely on their their *intrinsic* genetic defect, which dictates a low proliferation rate, for their expansion.

References

1. Fais F, Ghiotto F, Hashimoto S, Sellars B, Valetto A, Allen SL, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest 1998;102:1515-25.
2. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94:1840-7.
3. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999;94:1848-54.
4. Chiorazzi N, Ferrarini M. In: Chronic Lymphoid Leukemias. Cheson BD, Editor. Marcel Dekker Inc., 2nd Edition. p. 81-109.
5. Zupo S, Isnardi L, Megna M, Massara R, Malavasi F, Dono M, et al. CD38 expression distinguishes two groups of B-cell chronic lymphocytic leukemias with different responses to anti-IgM antibodies and propensity to apoptosis. Blood 1996;88:1365-74.
6. Lanham S, Hamblin T, Oscier D, Ibbotson R, Stevenson F, Packham G. Differential signaling via surface IgM is associated with VH gene mutational status and CD38 expression in chronic lymphocytic leukemia. Blood 2003;101:1087-93.
7. Ibrahim S, Keating M, Do KA, O'Brien S, Huh YO, Jilani I, et al. CD38 expression as an important prognostic factor in B-cell chronic lymphocytic leukemia. Blood 2001;98:181-6.
8. Del Poeta G, Maurillo L, Venditti A, Buccisano F, Epiceno AM, Capelli G, et al. Clinical significance of CD38 expression in chronic lymphocytic leukemia. Blood 2001;98:2633-9.
9. Hamblin TJ, Orchard JA, Ibbotson RE, Davis Z, Thomas PW, Stevenson FK, et al. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. Blood 2002;99:1023-9.
10. Ghia P, Guida G, Stella S, Gottardi D, Geuna M, Strola G, et al. The pattern of CD38 expression defines a distinct subset of chronic lymphocytic leukemia (CLL) patients at risk of disease progression. Blood 2003;101: 1262-9.
11. Zupo S, Massara R, Dono M, Rossi E, Malavasi F, Cosulich ME, et al. Apoptosis or plasma cell differentiation of CD38-positive B-chronic lymphocytic leukemia cells induced by cross-linking of surface IgM or IgD. Blood 2000;95:1199-206.

Karyotype of chronic lymphocytic leukemia

G.L. CASTOLDI, A. CUNEO

B-cell chronic lymphocytic leukemia (CLL) has an incidence of 2.3-3.3 cases per 100,000 people and 10-15% of the cases are diagnosed in subjects younger than 50 years old. A growing body of evidence has been accumulated over the last decade demonstrating the variability of the clinical course, possibly reflecting differences in phenotypic and molecular cytogenetic features. The introduction of B-cell mitogens, along with the development of sensitive molecular cytogenetic techniques helped us to extend our knowledge on the cytogenetic profile of CLL significantly.

Cytogenetic findings

Twenty-fifty percent of CLL carry a clonal chromosome defect. The variability in the incidence of cytogenetic aberrations is accounted for by different culture conditions and by the timing of cytogenetic analysis, the highest probability of detecting abnormal metaphase cells being associated with disease progression. CLL is a cytogenetically stable disease with less that 20% of the patients acquiring additional defects during the course of the disease.

Approximately 50% of abnormal cases carry a single chromosomal defect, 25% show 1 or 2 defects and 25% carry a complex karyotype (3 or more defects in the same clone). In a multicenter study, 40-50% of the patients had a normal karyotype (NN karyotype), 40-50% cases had 1-99% abnormal metaphases (AN karyotype) and 10% showed only abnormal metaphases (AA karyotype). The number of clonal abnormalities (complex karyotype) and the AA karyotype status represent two important prognostic factors in univariate analysis, the latter maintaining significance in multivariate analysis. These findings support the contention that genetic stability is an important prognostic factor in CLL as is the case in other human neoplasias. Clearly, the *in vitro* mitotic index of the cytogenetically abnormal clone may reflect its *in vivo* growth potential, accounting for the association between AA karyotype and short survival.

Specific chromosome aberrations are associated with peculiar clinicobiological features: when correlating survival probability with specific chromosome anomalies, patients with a normal karyotype and with 13q- do better that patients with +12 and 11q.

Unequal distribution of cytogenetically abnormal cells at different sites involved by disease has recently been observed in CLL, possibly reflecting selective retention and/or destruction of leukemia cells due to as yet unclear mechanisms. The acquisition of +12 is an early cytogenetic event in the course of CLL, although it probably does not represent the primary anomaly. Indeed, the presence of +12 and of 13q deletion in two distinct populations of neoplastic lymphocytes belonging to the same patient would suggest that these cytogenetic aberrations may be superimposed on an, as yet, unidentified submicroscopic primary change. The finding that +12 cells preferentially home to the lymph node and bone marrow, that the cells are not reduced or eliminated by chemotherapy and that their population expands as disease progresses, clearly support the hypothesis that this anomaly plays an important role in the natural history of the disease.

There are other recurrent chromosome anomalies occurring in CLL at an approximate incidence of 1%, the significance of these is being clarified. 1p34-36; 4p16; 4q35; 9q and chromosome 7 represent novel sites of recurrent rearrangement in CLL. Transformation in these patients seemingly occurred through cytogenetic routes not involving the classical CLL-associated chromosome regions. These chromosome rearrangements may be associated with peculiar hematologic features. In a recent study aberrations involving 1p34-36 and 4p16 were preferentially associated with early stage disease; 4q35 anomalies were associated with a relatively aggressive disease, atypical morphology and with monoclonal gammopathy; and rearrangements of 9q were characterized by atypical morphology and aggressive disease with splenic involvement.

Molecular cytogenetic data

Correlation of cytogenetic findings and hematologic features Fluorescent *in situ* hybridization (FISH) is more sensitive than conventional karyotyping. A number of studies show that there is a correlation between specific chromosome lesions and hematologic features in CLL.

Convincing evidence was provided that i) 13q dele-

From the Dipartimento di Scienze Biomediche e Terapie Avanzate, Sezione di Ematologia, Università di Ferrara.

Correspondence: G. L. Castoldi, Dipartimento di Scienze Biomediche e Terapie Avanzate, Sezione di Ematologia, Università di Ferrara.

tion occurring as an isolated chromosome lesion is associated with typical morphology and a benign clinical course; ii) patients with trisomy 12 usually display an excess of large lymphocytes identifying the CLL mixed-cell type variant of the FAB classification; iii) 11q22-23 is associated with typical morphology and extensive adenopathy and iv) 17p13 deletion may be found more frequently in the CLL/PL variant and may characterize an aggressive disease refractory to purine analogs

The 6q-, occurring as a possible primary chromosome defect, may represent another recurrent anomaly identifying a distinct subset of CLL. The incidence of this anomaly was 3.2% in a recent study showing that CLL with 6q- may represent a cytogenetic and clinicobiological entity characterized by a distinct phenotypic and hematologic profile. Patients with 6q- usually have atypical morphology, high white cell count, classical immunophenotype, and a therapy-demanding disease. The occurrence of CD38 positivity and the presence of *IGVH* mutations in 50% of the cases are in keeping with the observed clinical outcome which places CLL with 6q- in an intermediate risk-group.

Chromosome lesions and prognosis

Using a panel of 4 probes detecting the 13q14 deletion distal to the Rb gene, the 11q22.3-23.1 deletion involving the ATM gene, the 17p13.3 deletion involving p53 and total/partial trisomy 12 centred around the 12q13 segment, up to 70% of CLL can be shown to carry a cytogenetic lesion. This figure may be as high as 82% provided that additional probes are employed that recognize 6q21 deletion, 14q32 translocations, 3q and 8q partial trisomy. Using a hierarchical classification giving primary importance to 17p, followed by 11q-, +12 and by 13q-, Dohner and colleagues found that these cytogenetic groups had an incidence of 7%, 17%, 14%, and 36%, respectively, the remaining cases having other aberrations (8%) or normal karyotype (18%). Clinical outcome in these cytogenetic groups is significantly different, the shortest survival being observed in those patients with 17p- (32 months) and 11q- (79 months). The remaining patients with normal karyotype, +12 and 13q- were found to have a median survival in the range of 111-133 months.

There are at least two possible explanations accounting for the discrepant prognostic significance of +12 detected by cytogenetic analysis (highly significant) and detected by FISH (not significant). First, cytogenetic analysis identifies those cases with +12 with a relatively high mitotic index, whereas FISH may also disclose those cases with +12 in interphase cells only. Second, FISH can detect minor clones which escape detection by cytogenetic analysis due to the limited number of metaphases analyzed.

A growing body of evidence has been provided over the last years showing that the variability of the clinical behavior in CLL is mirrored by biological heterogeneity. Two novel important markers have strong prognostic importance, namely the mutational status of the Ig gene variable regions and expression with the CD38 antigen. Approximately 50% of CLL harbor a hypermutated Ig gene configuration, reflecting origin in a post-germinal center B-cell, whereas the remaining cases do not show such mutations because they derive from a pre-germinal center $CD5^+$ B cell or from a cell that has encountered the antigen in a T-cell independent reaction. There is a preferential association between CD38 positivity and *unmutated* CLL. The group of CLL with CD38 negativity and hypermutation of the *IGVH* genes have a better outcome, whereas, as expected, the *unvavourable* cytogenetic categories (17p-; 11q-) tend to cluster in the unmutated $CD38^+$ category. It must be clearly stated that this association is far from being absolute.

Conclusions

Cytogenetic analysis and FISH studies provide important information in the work-up of CLL since they a) identify novel rearrangements; b) are associated with distinct disease subsets and, c) allow for a refinement of risk assessment.

The combination of classical clinical parameters (staging systems), and of immunologic, cytogenetic and genetic characteristics is likely to dissect CLL into different disease subsets requiring specific treatments.

Recent update of prognosis and staging of chronic lymphocytic leukemia

MAURA BRUGIATELLI, DONATO MANNINA, SANTO NERI, LAURA NOCILLI

It is well known that B-cell chronic lymphocytic leukemia (CLL) is the most common leukemia in western countries, accounting for 5-7% of all non-Hodgkin's lymphomas. It has an incidence of 2.3-3.3 cases per 100,000 people and, although it more frequently affects elderly people, a significant fraction of patients (15%) are younger than 50 years.[1] It is also a heterogeneous disease with median survival varying from a value comparable to that of normal matched population to a value comparable to that found in patients with acute leukemia. About one-third of patients never require treatment; in contrast, one-third need immediate therapy.[2]

Therefore is of crucial importance to define the specific prognostic risk of each single patient in order to decide whether to start therapy and, if so, to choose the appropriate treatment.

More than twenty years ago two staging systems for CLL were designed, on in the USA by Rai,[3] the other in Europe by Binet.[4] Both were based on the number of involved sites and on the presence of cytopenia. Their validity induced the International Workshop on CLL (IWCLL) to propose the use of both systems, although in common clinical practice Rai's staging was more employed in USA and Binet's one in Europe. Both systems are still currently used for prognostic classification and therapy planning for CLL patients, in spite of the large number of additional prognostic parameters validated for this disease in the subsequent years. Among other considerable prognostic features, already reported in the past, it is worth mentioning total tumor mass score (TTM), lymphocyte doubling time (LDT), peripheral and bone marrow lymphocytosis, bone marrow infiltration pattern, bulky disease, performance status, and response to therapy.

For many years, clinical staging systems were considered sufficient for prognostic stratification for therapeutic purpose since in the past therapy options for this disease were rather limited, consisting mainly in palliative approaches. More recently, many advances have been reported in CLL treatment, with remarkable impact on its prognosis. Just within the past decade, the possibility of achieving a complete clinical remission in previously untreated cases has increased from around 5% up to 60%.

Thus, both the the increase of therapeutic options, often aimed at disease eradication, and the advances in understanding the biology of CLL have suggested the choice of risk-adapted therapy in this disease, in which the risk should be evaluated on the basis of clinical and biological parameters. Indeed, the variability of clinical behavior may depend on the biological heterogeneity of the disease.

Two milestones have been reported in the biology and prognosis of CLL. First, the possibility of assessing CLL cytogenetics by fluorescent *in situ* hybridization methods which led to the extensive study of large series demonstrating the favorable or unfavorable significance of specific lesions. Normal karyotype or deletions at 13q14 are associated with longer survival, while cases with trisomy 12 and/or deletions at 11q23 or 17p15 show a poor outcome. Moreover, it has been demonstrated that cells with +12 profile accumulate especially in lymph nodes and bone marrow, are less sensitive to chemotherapy and increase with disease progression. A worse outcome is associated with 14q anomalies.

The second milestone is represented by the definition of the molecular profile of IgV_H genes in CLL with the demonstration of two patterns: one is characterized by IgV_H gene mutation and is associated with an unfavorable outcome; another with unmutated IgV_H genes shows a better prognosis. Mutational status of IgV_H genes appears as the best prognostic indicator in CLL in all Binet stages. A median survival of 26 years is reported in mutated cases as compared to 8 years for unmutated ones. IgV_H gene configuration was initially considered strictly related to CD38 expression on CLL cells. CD38 is an activation antigen much more frequently present in the unmutated than in mutated subset. Now it is known that this relationship is sometime less stringent, because CD38 expression can also change during the course of the disease. There is probably a strong correlation with sCD23 and sTK levels.

From a biological point of view, the two patterns of gene configuration may suggest the coexistence of two different diseases; however, recent gene expression profile analysis confirms the hypothesis that all

From the Divisione di Ematologia. Azienda Ospedaliera Papardo, Messina, Italy.

Correspondence: Maura Brugiatelli, Divisione di Ematologia. Azienda Ospedaliera Papardo, Messina, Italy.

cases of CLL have a common cellular precursor and origin through a common transforming mechanism.[6,7]

Taking into consideration clinical and biological findings, the time has come to design a new prognostic classification for CLL patients, in order both to plan new clinical therapeutic trials and to implement the appropriate treatment strategy in individual cases.

The demonstration of the real impact of these new aspects of CLL prognosis on clinical practice comes from the consideration that the current prospective trials of large French and German co-operative groups have been based not only on clinical stage but also on the presence of biological features such as IgV_H gene profile, thymidine kinase, β-2microglobulin, soluble CD23 and LDT values. In particular, the German CLL study group (GCLLSG) has defined a risk- and age- adapted strategy for the first-line therapy of CLL.[8]

The main obstacle to extensive use of cytogenetic and molecular parameters for the prognostic stratification in current clinical practice is the need for sophisticated and expensive technology. In this respect, for the time being, it would be helpful to validate *surrogat*e biological parameters, such as CD38 expression or ZAP. However, a more accurate prognostic classification would clarify whether patients in initial stages but with unfavorable features benefit from immediate therapy with new treatment options. Likewise, an updated risk evaluation should be mandatory for deciding CLL patients' eligibility for more aggressive treatments.[9]

References

1. Foon KA, Gale RP. Chronic lymphoid leukemias. In: Handin RI et al., eds. Blood. Principles and practice of hematology. Philadelphia: Lippincot Co.; 1995
2. Dighiero G et al. Chlorambucil in indolent chronic lymphocytic leukemia. N Engl J Med 1998; 338:1506-14.
3. Rai KR, et al. Clinical staging of chronic lymphocytic leukemia. Blood 1975; 46:219-34.
4. Binet JL, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 1981; 48:198-206.
5. Liso V et al. Simultaneous evaluation of trisomy 12 by fluorescence in situ hybridization in peripheral blood, bone marrow and lymph nodes of patients with B-cell chronic lymphocytic leukemia. Haematologica 1999; 84:212-7.
6. Klein U et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenothype related to memory B cells. J Exp Med 2001; 194:1625-38.
7. Rosenwald A et al. Relation of gene expression phenothype to immunoglobulin mutation genotype in chronic lymphocytic leukemia. J Exp Med 2001; 194: 1639-47.
8. Hallek M. Risk- and age-adapted management of chronic lymphocytic leukemia: current and future clinical trials of the German CLL study group (GCLLSG). Haematologica vol. 87 (supplement II to n. 10):october 2002.
9. Montserrat E. Classical and new prognostic factors in chronic lymphocytic leucemia:where to now? Hematol J 2002;3:7-9.

Current therapeutic options for subgroups of chronic lymphocytic leukemia. Planning risk-adapted treatment according to recognized prognostic factors

AARON POLLIACK

Chronic lymphocytic leukemia (CLL) is the most common of all adult leukemias and is not a homogeneous disorder.[1,2] Although some have argued that this may not be a single entity[3] it is probably one disease with different subgroups displaying different biological behavior patterns, manifesting as different clinical courses and varying responses to treatment.[4] Most recently physicians have acquired more confidence in their approach and have dared to ask the once feared question of whether *CLL is a curable disease?*[5] This change in approach is basically due to the fact that much has changed in our thinking about CLL in the last decade because of the knowledge and data which have accumulated regarding the biology, molecular genetics and prognostic factors, coupled with the development of novel drugs, new concepts of immunochemotherapy and the newer techniques for stem cell transplantation now available.[5] All the latter have allowed us to entertain *new ideas* for therapy and the concepts of complete (CR) and molecular remission (MR) have now readily been incorporated into our new mode of thinking of how best to treat CLL. Concepts of possible *clinical cure* have been entertained and questions are asked such as whether very early disease in *younger* patients should be treated without necessarily waiting for the classical indications of progressive disease before treatment is given. In the light of all the above it is indeed difficult to outline rigid guidelines for what is best for CLL patients and many of these basic questions are the subjects of ongoing clinical trials.[6,7] However it does seem that the correct questions are now being addressed and it is possible that in 5-10 years from now more answers will be available which may well alter the current concepts of therapy for many patients.

From the Lymphoma-Leukemia Unit, Department of Hematology, Hadassah University Hospital, Jerusalem, Israel.

Correspondence: Professor Aaron Polliack, MD, Lymphoma-Leukemia Unit, Department of Hematology, Hadassah University Hospital, Jerusalem, Israel. Phone: international +0972.2.5633795. Fax: international + 0972.2.5610201. Email- apol@md.huji.ac.il

Importante of prognostic indicators for treatment selection

Before therapy can be discussed it has to be understood that the clinical presentation and course in CLL is far from uniform and disease progression and individual response to treatment is unpredictable, differing from patient to patient. Nowadays it seems evident that a proportion of patients have a long survival without major progression while an equal number (about one third) have more aggressive disease with progressive clinical features, a shorter survival and require therapy earlier.[3,4] A similar proportion of patients have an indolent clinical course which will eventually progress and require treatment. Thus, it is obvious that is not possible or even wise to plan treatment for all categories of disease without taking into consideration the above variables. In this respect prognostic factors are important and can help to predict who should receive therapy and may also play a role in deciding what approach to use for different subgroups of CLL thereby helping to establish therapeutic guidelines for these patients.

In the past, prognostic factors and categories were always well defined starting from clinical staging systems (Rai and Binet), which were the classical guidelines used for so long. These also included classical clinical and laboratory findings indicative of more rapid progression and shortened survival, such as, lymphocytic doubling time, bone marrow pattern of involvement, lymphocyte morphology, serum β2 microglobulin and lactate dehydrogenase (LDH) levels, as well as thymidine kinase and sCD23 levels.[4,8-12] All the above were important in decision-making and in choosing appropriate treatment. However, the surface immunophenotype, particularly CD38 expression, cytogenetics (17p del, trisomy 12 and 11q del.), the detection of the unmutated status of the VH Ig genes and more recently the significant expression of the ZAP 70 protein have all been found to of the utmost importance as poor prognostic indicators which will eventually determine survival.[13-16] These can be used as surrogate markers for prognosis and possibly drug resistance and their presence provides an indication of who may have a more aggressive subtype of CLL requiring more effective and earlier therapy. In the light of the above data, decision-making may soon become easier for the treating physician than in the past as should also help to make the design of future clinical trials more logical and simpler.

Treatment choices in individual patients

Elderly patients (> 65 years)

Basically it seems that who to treat and which drug therapy to give will always remain the central issues. However it is clearly evident that elderly patients who have truly indolent disease will have a life expectancy well above 10 years and their chances of responding to therapy afterwards are not compromised by delaying/deferring therapy. This group are probably best treated with a *watch and wait* approach unless we can identify clear-cut evidence of progression by the classic criteria or if we utilize the novel *surrogate markers* - mutational status, $CD38^+$ expression or ZAP 70 positivity to predict who in this group is likely to have a stormier course and require treatment.[15-18] In the subgroup with poor risk factors treatment up front could be considered earlier in the course of the disease and because of their age one could, indeed, first consider therapy with a single oral agent (such a chlorambucil or fludarabine). The alternative issue of combination chemotherapy in this age group could be left open for further consideration depending on the initial response or whether they have progressive disease on therapy.

In this elderly age group another obvious and important issue remains the quality of life (QOL) of patients with CLL. Thus if these patients are not entered into clinical trials, in day to day practice, most physicians will take QOL into consideration as a guideline for when to start treatment. This will obviously affect the clinician's decision on what regimen to use in the elderly, particularly if QOL and performance status are poor. Thus in essence QOL, surrogate genetic markers and gene profiling with ZAP 70 expression will remain key indicators in respect to treatment.

Younger patients (<65 years). Should the concept of achieving CR or molecular remission (MR) influence decision-making in this subgroup of CLL?

In recent years CLL patients have been able to achieve CR and even MR after receiving combination chemo/immunotherapy. Earlier therapies were rarely able to achieve this status.[19-26] As a result of the concept of a meaningful clinical CR and MR, possible *clinical cure* with prolonged disease-free periods and freedom from progression for a relatively large number of patients has slowly penetrated into our thinking, for the first time in the history of CLL, enabling physicians to consider this as a goal for their patients. However it is still unclear whether achieving CR/MR translates into a meaningful increasing life expectancy and whether the concept of *clinical cure* is applicable as for other lymphoproliferative disorders such as lymphomas and lymphoblastic leukemias. Can we in fact consider this as our optimal goal in younger CLL patients when there is still inadequate long-term follow-up of the recent encouraging data?

Until the era of purine analogs, monoclonal antibody therapy and autologous and reduced intensity (RIC) allogeneic stem cell transplantation (SCT), there was indeed no real debate about whether we could perhaps *cure* a meaningful proportion of younger CLL patients. Because of this, achieving clinical remissions with oral alkylating agents or CHOP - like regimens was not considered that significant a goal, as we never really thought that CLL could be eradicated or controlled by these regimens for prolonged periods of time. Now, that we can achieve CR/MR and have other alternatives for therapy, choices of treatment become more important and correct decision-making at an earlier stage seems to be more crucial, particularly in younger patients. This makes it more difficult for the treating physician who now has to explain this to CLL patients who are aware of this information which is so readily available in the electronic media today. In fact if patients are not included in clinical trials today, the clinician now has to decide for himself whether more aggressive combined modality treatment including chemo-immunotherapy and followed by a curative SCT approach should be adopted, as primary therapy in individual cases.

It seems that careful selection of the appropriate subgroup of patients to receive primary therapy with a combination of a purine analog (fludarabine/cladribine), cyclophosphamide and possibly mitoxantrone together with or followed by rituximab or even another monoclonal antibody such as alemtuzumab (Campath 1H) may be the correct approach, in the light of recent reported data.[23-26] These combinations are indeed synergistic for patients with more advanced disease or for those who have predictable poor prognostic features. Side effects of the newer antibodies used are mostly seen at the time of infusion and are not really more than would be expected for single chemotherapeutic agents, particularly in previously untreated patients who are not immunosuppressed after multiple prior cycles of combination chemotherapy. The future will no doubt provide us with other additional strategies, perhaps even more effective antibodies, DNA vaccinations and antisense ant-Bcl2 therapy,[27,28] which can be added to the regimens concerned so that now for the first time we really have some effective therapeutic options to apply in the relatively near future which may translate into increased life expectancy and a

proportion of possible *clinical cures* with prolonged disease-free periods for CLL patients.

Concept of consolidation of remission and stem cell transplantrs after initial response

Whether achieving CR/ MR status has a real impact on the long-term outcome of CLL as in other lymphomas and leukemias remains to be proven but in principle this should become evident in the future. Basically this is so for almost all hemato-oncological disorders and we aspire to achieve CR/MR in most disorders because without this we are unable to offer patients meaningful prolonged survival and possible cure. This of course may not be true for all chronic lymphoid neoplasias but if a good PR /CR/MR is not obtained - meaningful prolonged survival and *cure* is probably not possible. Obviously patients who have responded will require a long future follow-up as median survival has not been reached as yet in most of the reported studies and trials. Despite some early impressions that this *cure* may indeed be possible, it is still too early to predict. This raises the possibility and feasibility of consolidation/maintenance of the CR/MR status using monoclonal antibodies such as rituximab, mabCampath, others or their combinations.[19-26] This could indeed be appropriate maintenance and could be considered in future trials.

Furthermore, stem cell transplant (SCT) is a possible curative option for CLL patients, particularly those who have achieved remission using fludarabine/2Cda - rituximab regimens and show early signs of relapse.[29-36] In this respect it seems logical to harvest stem cells from all patients in CR/MR so as to be able to use them for autologous SCT. Whether this approach should be used early on as part of the initial therapy as consolidation or only when younger patients relapse will obviously need to be tested in the framework of randomized controlled trials. Autotransplants could theoretically be used at an earlier stage as there is minimal morbidity/mortality associated with the procedure but they lack a plateau effect in terms of response which may improve in the future. In contrast the RIC - *mini* allogeneic transplants are the only currently curative modality in CLL but are associated with more initial morbidity and mortality. Nevertheless, because they are curative and in the light of improving techniques one could consider using such transplants as *consolidation* for younger patients, but probably only for those who show evidence of relapsing disease.

Thus, many of the crucial issues of the day in CLL will have to be tested and analyzed in controlled studies with adequate periods of follow-up as currently proposed.[6,7] Risk- and age-adjusted management of early disease as well as advanced disease and proposals for high - dose therapy with SCT as first and second line treatment will have to be tested for CLL patients in this setting.[6,7] The only logical way to achieve this and to advance towards the desired goals will be through large co-operative, randomized trials with maximal international participation and collaboration.

References

1. Caligaris-Cappio F, Hamblin TJ. B-cell chronic lymphocytic leukemia: a bird of a different feather. J Clin Oncol 1999;17:399-408.
2. Montserrat E, Rozman C. Chronic lymphocytic leukemia: present status. Ann Oncol 1996;6:219-35.
3. Dighiero G. Is chronic lymphocytic leukemia one disease? Haematologica 2002;87:1233-5.
4. Montserrat E. Natural history of chronic lymphocytic leukemia. Implications for clinical management. Haematologica 2002; 87 Suppl 11:9-11.
5. Proccedings of the meeting on chronic lymphocytic leukemia: Is it a curable disease? Haematologica 2002; Suppl 2:87:1-79.
6. Hallek M. Risk-and age-adapted management of CLL: current and future clinical trials of the German CLL study group. Hematologica 2002; 87 Suppl 11:37-8.
7. Brugiatelli M, Domenico M, Donato M, Neri S. B-cell chronic lymphocytic leukemia: different therapies for different didseases? Hematologica 2002; 87 Suppl 11: 34-6.
8. Keating M, Lerner S, Kantarjian HM. The serum β-2-microglobulin level is more powerful than stage in predicting response and survival in CLL (Abstract). Blood 1995; 86:606a[abstract].
9. Sarfati M, Chevret S, Chastang C, Biron G, Stryckmans P, Delespesse G, et al. Prognostic importance of serum soluble CD23 level in chronic lymphocytic leukemia. Blood. 1996;88:4259-64.
10. Hallek M, Langenmayer I, Nerl C, Knauf W, Dietzfelbinger H, Adorf D, et al. Elevated serum thymidine kinase levels identify a subgroup at high risk of disease progression in early non-smoldering CLL. Blood 1999; 93:1732-7.
11. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000 28;343:1910-6.
12. Oscier DG, Gardiner AC, Mould SJ, Glide S, Davis ZA, Ibbotson RE, et al. Multivariant analysis of prognostic factors in CLL: clinical stage, IV GH gene, mutational status are independent prognostic factors. Blood 2002; 100:1177-85.
13. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999 15;94:1848-54.
14. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94:1840-7.
15. Chen L, Widhopf II GF, Huynh L, Rassenti L, Rai KR, Weiss A, et al. Expression of ZAP-70 is associated with increased B cell receptor signaling in chronic lymphocytic leukemia. Blood 2001;100:a361[abstract].
16. Orchard JA, Ibbotson RE, Davis ZA, Gardiner AC, Hamblin TJ, Oscier DG. Zap-70 evaluation by flow cytometry is a significant prognostic marker in B-CLL. Blood 2001;100:a630[abstract].
17. Wiestner A, Rosenwald A, Barry T, Ibbotson RE, Stetler-Stevenson M, Orchard JA, et al. Zap70 Expression identifies B-CLL with unmutated immunoglobulin genes, worse clinical outcome and a distinct gene expression

profile. Blood 2001;100:a631[abstract].
18. Bosch F, Villamor N, Crespo M, Bellosillo B, Colomer D, Rozman M, et al. ZAP-70 expression is a reliable surrogate for immunoglobulin variable region mutations in chronic lymphocytic leukemia. Blood 2001;100:a633[abstract].
19. Byrd JC, Murphy T, Howard RS, Lucas MS, Goodrich A, Park K, et al. Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. J Clin Oncol 2001; 19: 2153-64.
20. Wierda W, O'Brien S, Albitar M, Lerner S, Plunkett W, Giles F, et al. Combined fludarabine, cyclophosphamide, and rituximab achieves a high complete remission rate as initial treatment for chronic lymphocytic leukemia. Blood 2001;98:a3210[abstract].
21. Byrd JC, Peterson BL, Morrison VA, Park K, Jacobson R, Hoke E, et al. Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukemia: results from Cancer and Leukemia Group B 9712 (CALGB 9712). Blood 2003; 101:6-14.
22. Schulz H, Klein SK, Rehwald U, Reiser M, Hinke A, Knauf WU, et al. Phase 2 study of a combined immunochemotherapy using rituximab and fludarabine in patients with chronic lymphocytic leukemia. Blood. 2002;100:3115-20.
23. Hillmen P. MabCampath in chronic lymphocytic leukemia. Hematologica 2002; 87 Suppl 11:47-9.
24. O'Brien SM. Mabthera in chronic lymphocytic leukemia. Haematologica 2002; 87 Suppl 11:50-3.
25. Keating M, Manshouri T, O'Brien S, Wierda W, Kantarjian H, Washington L, et al. A high proportion of molecular remission can be obtained with a fludarabine, cyclophosphamide, rituximab combination (FCR) in chronic lymphocytic leukemia (CLL). Blood 2002;100: a771[abstract].
26. Rai KR, Byrd JC, Peterson BL, Larson RA. A phase II trial of fludarabine followed by alemtuzumab (Campath-1H) in previously untreated chronic lymphocytic leukemia (CLL) patients with active disease: Cancer and Leukemia Group B (CALGB) Study 19901. Blood 2002; 100:a772[abstract].
27. Rai KR, O'Brien S, Cunningham C, Turkina AG, Ochoa L, Frankel SR, et al. Genasense (Bcl-2 Antisense) monotherapy in patients with relapsed or refractory chronic lymphocytic leukemia: phase 1 and 2 Results. Blood 2002;100:a1490[abstract].
28. Cheson BD. New drugs: can they cure CLL? Hematologica 2002;87 Suppl 11:56-8.
29. Esteve J, Villamor N, Colomer D, Cervantes F, Campo E, Carreras E, et al. Stem cell transplantation for chronic lymphocytic leukemia: different outcome after autologous and allogeneic transplantation and correlation with minimal residual disease status. Leukemia 2001; 15:445-51.
30. Esteve J, Montserrat E, Dreger P, Meloni G, Pavletic S, Catovsky, et al. Stem cell transplantation (SCT) for chronic lymphocytic leukemia (CLL): Outcome and prognostic factors after autologous and allogeneic transplants. Blood 2001;98 Suppl 1:859.
31. Khouri IF, Saliba RM, Giralt SA, Lee MS, Okoroji GJ, Hagemeister FB, et al. Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality. Blood 2001;98:3595-9.
32. Khouri IF. Reduced intensity regimens for CLL: The MD Anderson experience. Hematologica 2002; 87 Suppl 11:76-7.
33. Bandini G, Bonifazi F, Falcioni S. Allogeneic hematopoietic stem cell transplantation for CLL: Background and results from the EBMT Registry. Haematologica 2002; 87:68-75.
34. Michallet M, Chevret S, Brand R, van Biezen A, Lévy V, Dreger P, et al. Autologous and allogeneic hematopoietic stem cell transplantations (HSCT: AutoT and AlloT) versus conventional chemotherapy in chronic lymphocytic leukemia (CLL): An european blood and marrow transplant (EBMT) and French CLL Cooperative Group Study. Blood 2001;98:a137[abstract].
35. Michallet M. Hematopoietc stem cell transplants in chronic lymphocytic leukemia. Haematologica 2002; 87:63-7.
36. Montserrat M , Should we transplant CLL? Proceedings from LEUKEMIA 2002. Towards the Cure in Leukemia & Lymphoma. Suppl 44. 2003;S40.

CAMPATH-1H and autologous transplantation with *in vivo* purged peripheral blood stem cells in chronic lymphocytic leukemia: preliminary results of a pilot study

IGNAZIO MAJOLINO, MARCO LADETTO, GABRIEL ANGHEL, CLAUDIA PAPETTI, FABIO BENEDETTI, ANDREA GALLAMINI, TEODORO CHISESI, CORRADO TARELLA

Alemtuzumab (Campath-1H) is a humanized, genetically reshaped IgG1 monoclonal antibody directed against CD52 antigen.[1] *In vivo* it induces a rapid and effective clearance of normal and malignant lymphocytes. The effector mechanisms are still not fully understood. However, cell death might be induced by three different mechanisms: complement-mediated cell lysis, antibody-dependent cellular cytotoxicity, and induction of dendritic cell-mediated apoptosis.[2]

B-cell chronic lymphocytic leukemia (B-CLL) is the most common adult leukemia in North America and Europe.[3,4] It is still an incurable disease and is characterized by a clonal proliferation of $CD5^+$ mature B-lymphocytes that co-express CD19, CD20 and sIg. While patients belonging to favorable prognostic groups are projected to survive for up to 10 years, even with no specific treatment, those with adverse features may have a rapid clinical progression and survive less than 2 years.[2,5] High-dose therapy and autologous or allogeneic stem cell transplantation (SCT) may be employed in these poor-prognosis patients, inducing remission in a high proportion of cases. However, allograft is associated with a high transplant-related mortality (TRM), while autologous SCT carries a risk of neoplastic cell reinfusion. Campath-1H is effective against CLL, with an over 75% response rate in untreated patients and 30-40% in refractory/relapsed patients as assessed by rearrangement - amplification of specific DNA sequences or chromosomal translocations.[2,5] Furthermore, it has a remarkable effect in clearing neoplastic lymphocytes from the peripheral blood, which represents the basis for its *in vivo* purging application.[5,6]

Based on prior reports demonstrating that alemtuzumab is an effective salvage therapy for patients who have failed to benefit from fludarabine, we launched a pilot study of *in vivo* purging with Campath-1H and autologous transplantation.

From the Ospedale S.Camillo, Roma (IM, GA, CP), Ospedale S. Giovanni, Torino (ML, CT), Policlinico G.B. Rossi, Verona (FB), Ospedale S.Croce, Cuneo (AG), Ospedale Civile, Venezia (TC) for GITIL (Gruppo Italiano per le Terapie Innovative nei Linfomi).

Correspondence: Dr Ignazio Majolino, MD, Unità Operativa di Ematologia e Trapianti di Midollo Osseo, Azienda Ospedaliera S.Camillo-Forlanini, Circonvallazione Gianicolense 87, Roma. Phone: international + 06.58704418. Fax: international + 06.58704357. E-mail imajolino@libero.it

Design and Methods

A prospective, multi pilot study was conducted at five istitutions in Italy belonging to the center GITIL (Gruppo Italiano per le Terapie Innovative nei Linfomi). The aim was to evaluate the feasibility of a program including sequential chemotherapy, Campath-1H and autologous transplantation in patients with poor prognosis B-CLL.

Admission criteria were as follows: a) B-CLL; b) failure to achieve (or relapse from) a complete remission (CR), or partial remission (PR) with conventional therapy including fludarabine; c) age ≥ 18 years and <60 years; d) stage B(I)-C(IV) (IW-CLL).4

Since April 2002, 8 patients have been enrolled at 5 istitutions in Italy. Their median age was 53.5 years (range 37–60). Four of them had stage B-II, 2 had C-IV one B-I (LDT<6 months), and one C-III. The median time from onset of disease to protocol admission was 37.5 months (range 7-68). Lymphocyte counts at study entry were 2-46 (median 27)$\times 10^9$/L. All patients had been previously submitted to 1-3 chemotherapy lines (median 2). At present, all patients have been submitted to DHAP, attaining 1 CR, 5PR, and 2 NR.

All patients have received the Campath-1H with 4 CR, 3 PR and 1 NR. With Campath-1H, clonal CD5/19 lymphocytes rapidly dropped from a median of 4.7×10^9/L to 0.2×10^9/L. The median purification of the clonal double positive CD5/CD19 population was 99.95%.

At the time of reporting, 7 patients have been submitted to high-dose cyclophosphamide (4 gr/m^2 in 4, and 7 gr/m^2 in 3). Responses were as follows: 3 CR, 2 PD and 1 PR; one patient is not yet evaluable for clinical and hematologic response. $CD34^+$ mobilization failed in two cases and bone marrow harvesting was necessary. The median number of $CD34^+$ cells collected by apheresis was 1.7×10^6/L/kg (range 0.3- 4.7) with 2-4 apheretic procedures (median 3).

Autologous transplantation has been performed in 3 patients. One patient recovered 0.5×10^9/L polymorphonuclear cells and 20×10^9/L platelets at day +15, and is in clinical and hematologic remission 3 months after stem cell infusion. The second, achieved CR then relapsed 6 months after stem cell infusion. The third patient is not evaluable (too early).

In terms of toxicity, we have observed 10 infectious complications in 6 patients. There were 2 Aspergillus

infections, 1 case of severe dermatitis, 1 streptococcal infection, 1 case of autoimmune hemolitic anemia, 3 cytomegalovirus reactivations, and 2 herpes-zoster virus infections. There were also 4 episodes of unexplained fever.

Discussion

As already reported, in patients with B-CLL failing standard regimens, namely fludarabine, Campath-1H can still induce a response in a majority of cases.2, 5 This is also our experience with the present protocol. All patients responded to Campath, with 5 of them achieving CR, confirming that this drug offers excellent clearance of the malignant cells from blood and bone marrow. Our patients were not only poor responders to fludarabine, but also exhibited a non-mutated IgH phenotype, what is considered an adverse prognostic feature of CLL.

We are using Campath not only as a cytoreductive agent, but also as an *in vivo* purging drug, with the intent of reducing peripheral blood contamination at the time of stem cell collection. The preliminary data seem to confirm our hypothesis that apheretic procedures undertaken under Campath treatment are profoundly depleted of lymphoid cells. However, adverse events such as infectious complications, linked to the severe immuno-suppression induced by Campath-1H must be considered carefully.

Data on minimal residual disease, as detected by polymerase chain reaction assessment of IgH rearrangement on apheretic collections[6] and bone marrow samples obtained during patients follow-up will be available at the time of the meeting.

References

1. Gilleece MH, Dexter TM. Effect of Campath-1H antibody on human hematopoietic progenitors in vitro. Blood, 1993;82:807-12.
2. Keating MJ, Flinn I, Jain V, Binet JL, Hillmen P, Byrd J, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood 2002;99:3554-9.
3. Scimè R, Indovina A, Santoro A, Musso M, Olivieri A, Tringali S, et al. PBSC mobilization collection and positive selection in patients with chronic lymphocytic leukemia. Bone Marrow Transplant 1998;22:1159-65.
4. Cheson BD, Bennett JM, Grever M, Kay N, Keating MJ, O'Brien S, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia. Revised guidelines for diagnosis and treatment. Blood 1999;87:4990–7.
5. Majolino I. International conference on innovative therapies in lymphomas. Palermo 24-27 October, 2002.
6. Vuillier F, Claisse JF, Vandenvelde C, Travade P, Magnac C, Chevret S, t al. Evaluation of residual disease in B-cell chronic lymphocytic leukemia patients in clinical and bone marrow remission using CD5/CD19 markers and PCR study of gene rearrangements. Leuk Lymphoma 1992;7:195-204.

"Staging" Hodgkin's lymphoma: why and how?

PATRICE CARDE

The initial work-up of Hodgkin's lymphoma involves a highly variable set of procedures. It depends on the type of treatment planned, the endpoint of the treatment strategy, the typical failure pattern, and on which treatment complications are expected. As it also depends on the level of knowledge and curiosity of the individual physician in charge, it differs from one center to the other even more than treatment guidelines do.

The way staging procedures have evolved over the last 40 years shows how, although each technique has been replaced by a more sophisticated one, the same basic requirements remain. Indeed, even if one technique characteristic of a specific period has disappeared from routine use, each period has left intact a stratum of knowledge which is still valuable, or worth being re-discovered. Staging relates to the initial inventory of the presence of the disease, the reassessment of response at the end of treatment, or during treatment, the prognostic factors that can be used to select treatment, and the tools to measure (and to prevent?) short and long-term sequelae.

Initial disease inventory, laparotomy is still the best

The Sixties. How initial work-up is closely linked to a radiotherapy-based treatment is illustrated by the pioneering era of *exploratory* laparotomy, before this became a standard procedure, i.e. as the *staging* laparotomy. This story is worth telling in detail,[1] because the time for a meticulous and *comprehensive* inventory may have come back.

To analyze the characteristics of a man's life, Claude Bernard advised *enter the living organisms using vivisection procedures*: this advice was followed by S.A. Rosenberg, who required vivisection in the form of an exploratory laparotomy to resolve the case of a patient with an equivocal lymphangiogram preventing administration *appropriate* portals of radiation therapy (RT); this was a full success for this patient who was still in his first remission ~25 years later. Why did we do such a heavy procedure? In the Sixties, prophylactic irradiation of uninvolved areas (based just recently on megavoltage therapy and extended field techniques) was thought to be of value only in supradiaphragmatic areas. It was intended to avoid *recurrence developing in the immediate vicinity of a field too narrowly irradiated*. Infradiaphragmatic and visceral involvement, known to occur from autopsy series, was thought to characterize end stage disease. Although lymphangiography turned out to be positive more frequently than expected, only the first exploratory laparotomies revealed how frequently para-aortic nodes and the spleen were involved, and responsible for treatment failures.[1,2] The range of lymphangiograhy limited to para-aortic and iliac nodes, and the frequency (~20%) of false positive and negative findings had prevented understanding of the natural history of the disease. Laparotomy was, therefore, essential to catch how the disease was spreading. Still, this early work of Kaplan and Rosenberg uncovering the intimate mechanism of Hodgkin's disease propagation was not pursued long enough. The reason is that the oncologist became too confident, assuming that extensive RT and/or adjuvant chemotherapy (CT) would literally erase any remaining microscopic disease. So why should the attempts of Hutchison and Tubiana to investigate a pattern of spread by contiguity, or those of Smithers about a random distribution of the disease make any difference ?

The Seventies and the Ann Arbor staging procedure. Conventional laparotomy staging refers to radiation-based treatment. Laparotomy had some *a posteriori* impact on treatment by identifying and correcting erroneous evaluations of disease spread. But the reason why it was so popular was because physicians were persuaded that, in patients with localized disease, any relapse of Hodgkin's disease (considered at that time to be due to insufficient RT) would eventually be fatal. Laparotomy was considered the optimal safeguard to tailor abdominal irradiation in response to each individual patient's presentation. The Ann Arbor classification was based on the alleged benefit of laparotomy staging. Although symbols such as H $^{+/-}$, N $^{+/-}$, M $^{+/-}$ only pointed out the pathological stage (= biopsy of the organ), as compared to the clinical stage, the initial letter (S $^{+/-}$), denoting the

From the Institut Gustave Roussy, 39 rue Camille Desmoulins, Villejuif, France.

Correspondence: Patrice Carde, MD, Institut Gustave Roussy, 39 rue Camille Desmoulins, Villejuif, France.
Phone: international +33.1.42114321. Fax: international +33.1.42115270. E-mail:carde@igr.fr

spleen, indicated that the information had been obtained through a laparotomy. The abbreviation *PS* for *pathological stage*, as intended initially and written, prevailed until the Eighties, standing ambiguously for *post-surgical stage*, as many thought it meant.

The last twenty years and the disappearance of the staging laparotomy. In a first step, laparotomy was deleted from the staging of localized disease (*EORTC H5 Unfavorable patients trial*) when sufficient clinical evidence suggested a need for either extended irradiation or adjuvant chemotherapy, for instance in patients with poor prognosis.[3] Later on, even in patients with the most favorable outlook (EORTC H6 Favorable patients trial), laparotomy staging and treatment adaptation proved not to be very rewarding in terms of tumor control and indeed to be worrisome in terms of immediate and late effects, as compared to clinical staging and subtotal nodal with splenic irradiation (STNI).[4] The next step toward renouncing staging laparotomy came from the superior results of combined involved field (IF) RT to the supradiaphragmatic areas only + adjuvant *light* chemotherapy over STNI.[5] Indeed, adjuvant CT was of benefit to all cases of localized disease (~75% of HD patients) and rendered accurate infradiaphragmatic staging of no interest.

At the beginning of the new Millenium. The situation now is exactly opposite of what has been true these last twenty years, for two good reasons:[1] RT is still needed. Indeed, most relapses occur in involved non-irradiated nodal areas after treatment with chemotherapy alone; even in good prognosis early-stage HD, the relatively light chemotherapy combinations that are used in short courses are unable to eliminate the microscopic disease left between the areas treated with IF RT. This was highlighted in the EORTC H9F trial in which the chemotherapy-alone arm had to be stopped because of an unacceptably high rate of relapses.[2] The RT fields need to be made smaller because of the high price paid in terms of toxicity in previous trials that used full dose extended fields.[4-7]

In conclusion, irradiation to the involved nodal areas (or nodes?), at least in stages I-II, should be continued. Therefore it is essential to know which areas are involved.

Initial inventory in the absence of laparotomy

Several steps can be taken to reduce the toxic burden of RT. These are:

(a) to taper the doses in non-involved areas of the extended field (EF) irradiation, according to the results of the German Hodgkin Study Group HD-1 study 20 Gy ≅ 40 Gy (GHSG HD-1 trial) and ≅ 30 Gy (non-randomized arm GHSG HD-5 trial);

(b) to prefer IF RT (EORTC choice since the H7 trial in 1988; tested in a randomized trial against EF RT in the HD-8 trial by the GHSG);

(c) to reduce doses even for IF RT (EORTC H9F trial 20 Gy versus 36 Gy); HD-10 trial of the GHSG 20 Gy versus 30 Gy);

(d) to irradiate only the involved nodes, i.e. less than the involved area, as advocated by a few specialists. Intensity-modulated radiation therapy[8] may be the tool to achieve this task, provided that adequate safety is ensured by efficient quality-control programs.[9]

This is why better knowledge on the pattern of spread by contiguity, or on a random distribution, would now be so helpful. Nevertheless, only one study has been devoted to this subject.[10,11] Based on a series of laparotomy-staged patients, the study described the pathways of successive involvement of nodal areas according to the initial site involved by the disease, usually the right cervical area.

Unfortunately, although better knowledge of serial node involvement in HD would be very helpful, no additional series have been reported to confirm and expand the data produced by Roth.[17] And yet these data are needed because of the unacceptably high rate of cardiac complications and second tumors, which are responsible for extra early deaths in this population of young patients. To determine which nodes are involved, the only ones that ideally irradiation should encompass, and in the uncertainty about the likely path of this serial node invasion, the initial work-up should be reinforced.

Are newer diagnostic procedures validated? There have been attempts to correlate results from Gallium-^{67} scanning with those from a lymphangiogram. In 94 patients with localized Hodgkin's disease, including 51 patients who undergone a laparotomy, computed tomodensitometry (50 to 25%) and lymphangiogram (42%) were more sensitive than gallium-^{67} scanning at detecting nodal involvement (27%).[12] Unhappily, because of the disaffection with laparotomy, none of these new procedures, including immunoscintigraphy using radiolabeled anti-CD30 antibodies, could be studied extensively enough by comparison to lymphangiogram or to tomodensitometry.[13] The failure of newer techniques may be the reason why the place of laparotomy has been well preserved in the Cotswolds classification.[14] Conversely, fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) improved the diagnostic accuracy in the staging of HD, based on the metabolic signal of the lesions. For instance, FDG-PET detects supraclavicular, axillary and inguinal node involvement better. It is also sen-

sitive at detecting visceral and bone marrow involvement. especially medullary involvement.[15-17] Initial FDG-PET may not, however, yield more information about infradiaphragmatic nodal involvement than does CT-scan,[15] and certainly needs additional assessment. Nevertheless, it provides first order benefits when inserted in the initial staging. Indeed, its importance, in case of localized disease after conventional staging, comes from its ability (a) to detect additional nodal involvement worthy of irradiation (b) to rule out visceral involvement, since in case of stage IV disease both brief/light CT and irradiation would be detrimental to the patient.[18,19]

The ideal initial work-up should, therefore, include:

(a) a CT-scan of all nodal areas, particularly of cervical nodes, as is now mandatory in the EORTC staging for patients with localized disease;

(b) FDG-PET scan to design the irradiation fields before any treatment is started;

(c) image fusion integrating the PET scan.

These staging procedures would best allow the use of static and dynamic intensity-modulated radiation therapy and protect organs at risk.

Assessment of response to initial treatment

Cheson's criteria for the assessment of response at the end of treatment are based on CT-scanning. This crucial assessment usually relies on a comparison with the studies performed at initial work-up, although its results could stand by themselves. If the type of response directs the rest of the treatment (as in most current HD trials), then response criteria are of primary importance.[20] The EORTC advocates the use of *Cheson's criteria*. Although they were designed for non-Hodgkin's lymphoma, they also make a lot of sense for HD.[21] The main features are:

(a) after treatment a *normal* lymph node must not exceed 1.5 cm in maximum diameter on CT-scan;

(b) in previously involved nodes a complete response (CR) is defined as a decrease by more than 75% in the sum of the products of the greatest diameters (SPD);

(c) a complete response/unconfirmed (CRu) in patients is a residual mass but greater than 75% reduction in tumor size after therapy representing a non-active disease mass;

(d) use of CT scans as standard procedure for evaluation of nodal disease: *thoracic, abdominal, and pelvic CT scans are recommended even if those areas were not initially involved because of the unpredictable pattern of recurrence in NHL*;

(e) introduction of the concept of *modulated response assessment*, which means assessment at intervals depending on the type of treatment: *studies should be performed no later than 2 months after treatment has been completed to assess response. This interval may vary with the type of treatment: a longer period may be more appropriate for biologic agents where the anticipated time to response may be greater*;

(f) selection of event-free survival (time to treatment failure), which includes failure or death from any cause as the optimal end-point;

(g) concept of the utility of treatment reflected in response assessment: *for patients with an indolent NHL, response duration may be less clinically important than the point at which initiation of treatment is necessary*.

Cheson's criteria are based on two-dimensional measurements of one or several target lesions.[21] They are in line with the recommendations made by the WHO in 1979 for reporting treatment results .[22] These criteria have been challenged foir solid tumors by the RECIST (*Response Evaluation Criteria In Solid Tumor*), based on one-dimensional measurements: less bias, simpler, quicker for the radiologist. The RECIST have only been validated for solid tumors.[23] A recent study assessed the RECIST for HD.[24]

Gallium scanning (^{67}Ga) is part of Cheson's recommendations. It is best used in the presence of a residual mass on conventional imaging (CT-scan) to distinguish HD from non-specific changes and to correlate residual disease imaged with ^{67}Ga uptake and *eventual likelihood of recurrence*.[21] Single-photon emission computed tomography (SPECT) with gallium scanning demonstrated a higher sensitivity, specificity, and positive and negative predictive values. Nevertheless, predictability of cure (sensitivity) in the mediastinum, is not excellent.[25] A representative example in 62 lymphoma patients (n=52 HD) where ^{67}Ga scintigraphy was also performed after therapy (n=42) using 185-220 MBq ^{67}Ga citrate and planar and SPECT studies. In this study, a residual mass was observed in 31/42 CT scans and ^{67}Ga imaging was normal in 22 of these 31 cases (71%); only 4 of the 22 patients relapsed (8-45 months interval). Predictability of relapse (specificity) was excellent, since 8/9 patients with abnormal ^{67}Ga uptake in a large residual mass relapsed within 30 months.[26] Other studies confirmed that gallium scanning is helpful to avoid unnecessary complementary treatment or in directing a change of treatment modalities.[27,28]

Several studies compared CT-scan and FDG-PET for the diagnosis of residual masses.

(a) In 37 HD patients both CT-scan and FDG-PET were performed after treatment.[29] Sensitivity and specificity (detection of relapses) were much better for FDG-PET (91% and 69%, respectively) than for the CT-scan (72% and 21%, respecti-

vely). Furthermore, only the result of FDG-PET was positively correlated with event-free survival.

(b) Of 54 patients (HD and NHL) showed a residual masses on CT; ^{18}F-FDG PET was positive in 5 of those 24 patients with residual CT mass and in only 1 of 30 patients without. All 6 patients (100%) with positive FDG PET relapsed, whereas 5/19 patients (26%) with residual masses on CT but negative FDG PET, and 3/29 patients (10%) with negative CT scan and ^{18}F-FDG PET studies did so. The positive predictive value was much higher for the FDG-PET: 100% v 42%. Furthermore, a positive FDG PET was also associated with poorer 1-year survival than was a negative study: 50% ± 20% versus 92% ± 4% ($p < .0001$).[30]

The same property explains the probable superiority of FDG-PET over gallium scanning in the diagnosis of residual masses.[31]

Medicare in the USA recognized expansion of coverage (effective July 1, 2001) for usage of PET for the initial staging, and restaging of both Hodgkin's and non-Hodgkin's disease. More specifically the clinical situations covered are when *(i) the stage of the cancer remains in doubt after completion of a standard diagnostic work-up, including conventional imaging (computed tomography, magnetic resonance imaging, or ultrasound) or, (ii) the use of PET would also be considered reasonable and necessary if it could potentially replace one or more conventional imaging studies when it is expected that conventional study information is insufficient for the clinical management of the patient and, (iii) clinical management of the patient would differ depending on the stage of the cancer identified. PET will be covered for restaging after the completion of treatment for the purpose of detecting residual disease, for detecting suspected recurrence, or to determine the extent of a known recurrence.."*[32]

Additionally FDG-PET can be coupled with CT-scan (radiological image fusion) a promising technique to design irradiation strategies.

Assessment of prognostic factors

Consensus on prognostic factors still differs according to the apparent extent of the disease, localized or advanced presentation. One should note that in both circumstances the same characteristics tend to be retrieved, especially when analyses are adjusted for treatment.[32]

Localized disease presentation

(a) in the early seventies, very quickly after it was first reported, the EORTC challenged the therapeutic value of laparotomy. The H2 trial proved that, in the absence of treatment adaptation, event-free survival and survival were similar in patients randomized to clinical staging and STNI + spleen irradiation instead of laparotomy + splenectomy.[34] Conversely, staging laparotomy and splenectomy brought prognostic information which has been in use for 20 years: spleen involvement predicted further nodal relapse (13% relapses in non-irradiated areas, a 17-fold increase) and extranodal relapses (16%, a 2-fold increase),[34] but only apparent in the best prognostic group, and exclusively on freedom from progression.

(b) many teams attempted to stratify HD treatment according to a specific set of initial characteristics, beyond the Ann Arbor and Cotswolds staging classifications. For example, the EORTC proposed, on the basis of analysis of 1392 patients,[35] a simple stratification into 2 main groups (favorable and unfavorable), calling for registration of tumor-related factors (number of initial clinically involved areas, a combination of systemic symptoms and accelerated erythrocyte sedimentation rate, bulky mediastinum) and of patient-related factors (age < or ≥ 50 years, sex), that has been widely adopted in Europe (EORTC, GHSG, GELA) and in the USA.

Advanced disease presentation

The recent international prognostic score (IPS)36 lists 7 unfavorable factors: albumin < 4 g/L ; Hb < 10.5 g/L ; sex (male); stage IV ; age ≥ 45 years; WBC > = 15×10^9/L; lymphopenia < 0.6 $\times10^9$/L or < 8%; these factors are correlated with the event-free survival. Treatment stratification may be performed according to the number of factors present.

There can be pitfalls in all staging systems. These may concern:

(i) statistical methods on judgement criteria that rely on the time elapsed;

(ii) techniques for assessing the patient's and disease characteristics, initial work-up, response and treatment parameters;[33]

(iii) the prognostic models in which it is not known whether missing characteristics are due to lack of data or lack of significance; consensual characteristics are not being tested in multivariate analyses when new, odd and strange characteristics are put forward

(iv) standardization limitations: for instance there are many different ways to measure the bulk of a mediastinal mass, the nodal areas involved, the *B* symptoms, the marrow involvement, the biological markers (Cu^{++}, albumin, LDH). Standardization through fractions/multiples of normal or broad standard errors make differences; assessment of response (according to the type of work-up [CT-scan $^{+/-}$ ^{67}Gallium or FDG-PET]), the time elapsed from last treatment or last CT. Irradiation allows more time for mass resolution and increase, *ipso facto*, the CR rating. The Cotswolds classification induces more varia-

tion both through the concept of *CRu* and by allowing some flexibility in the time range in which the response needs to be recorded.[14]

In all stages, prognostic factor classifications are relatively easy to correlate to the progression/relapse, at difference to the survival endpoint, probably because of a stronger interaction with the patient's characteristics (age, immunosuppression, intercurrent diseases) and ability to deliver the more intense treatments properly. However, 3 sets of data may be of value to identify prognostic factors which influence response/relapse criteria on one hand and survival on the other.

Tumor mass. Bulky mediastinum is probably less reliable than the tumor burden, valid for both localized and advanced HD, and for supra- as well as infra-diaphragmatic presentations.[37] The most convincing results have been obtained when the volume of all disease sites have been taken into account in proportion to the body.[38]

Biological characteristics. It is tempting to investigate whether the particular environment Reed-Sternberg cells, these cells' extraordinary mechanisms of apoptosis resistance (NF-κB activation), and their system for immune escape (CD30L, CD40L, LMP1, TNF) can be correlated to the prognosis in the individual patient. A recent paper confirmed the value of sCD30 determination.[39] A prospective effort is being made to correlate some of these factors, in a reproducible and quantitative way, to standard prognostic end-points, and promising results have been observed with the combination of CD30s, IL1RA, IL6, as compared to the IPS.

Mid-treatment response. This is a powerful surrogate to predict outcome.[40] Two hundred and seven patients with stage IIIB-IV Hodgkin's disease underwent an EORTC study *to assess, prospectively, the interval to reach an apparent complete response, and its meaning, through repeated tumor measurements every 2 cycles. Patients who were assessed, on clinical, biological and imaging criteria, as complete responders before cycle n°5 (CR4 patients), as compared to the other responders had a higher 15-year freedom from progression (FFP) (61% versus 37%, p< 0.001) but also survival (61% versus 41%, p= 0.001). This observation is not due to patient-related confounding factors since the survival advantage in CR4 patients all comes from the avoidance of HD progression-related deaths (HD-specific survival = 85% in CR4 patients versus 60%, p< 0.001) and does not concern the other deaths (non HD-specific survival 74% versus 71%, p = 74)*. Assessment of early response can be used in CR4 patients to decrease the number of cycles to be given, or to avoid overtreatment; in poor responders it may help to switch early enough to another treatment. These surrogates have been applied successfully to the strategies developed in the subsequent 20884 advanced HD EORTC trial.[18] In NHL, early FDG-PET (after 2 - 3 cycles of chemotherapy), when positive, proved predictive of failure (4/5) or relapse (5/5), demonstrating a very high specificity.[18,41] Another study, in 30 patients with NHL or HD, confirmed this observation and suggested that very early FDG-PET assessment had greater sensitivity (less false negative) when performed during initial CT than after CT ended.[18,42] However, use of PET to monitor tumor response during the planned course of therapy (i.e. when no change in therapy is being contemplated) is not covered in the USA by Medicare: "*restaging only occurs after a course of treatment is completed, and this is covered..*".[18,32]

Assessment of treatment compliance and long-term sequelae

Assessment of treatment compliance, long-term sequelae,[6,7,18] and quality of life (QoL),[18,43] needs to be inserted in the initial work-up. For instance the EORTC has been prospectively monitoring pulmonary, cardiac and gonadal function since the H6 trial, started in 1982. Apart from standard assessment of the patient's history (biological work-up, HIV & hepatitis serology, etc.), the following tests have been performed repeatedly: thyroid function (T4, TSH); fertility tests (FSH, LH, estradiol, progesterone, testosterone, spermogram, andrological examination and sperm preservation); cardiac function (isotopic or ejection fraction at rest); pulmonary function (vital capacity, forced expiratory volume, functional residual capacity, CO diffusion capacity). If these studies may be of benefit to an individual patient, one must recognize that their yield concerning quantification of specific treatment toxicity,[44] and global treatment strategy remains dismal. The absence of standardized tests may account, in part, for the relatively poor compliance with test performance and unwillingness to retrieve the data. Monitoring quality of life, through longitudinal questionnaires, has been more successful.[45]

Second tumors[6] are increasingly being taken into account in the design of treatment strategies. However, co-factors are rarely recorded. Only the last Hodgkin Intergroup trial (#20012) records patints' smoking status and familial cancers. Although few prospective cohort studies are available, screening for cancer (breast) may be rewarding.[46]

Conclusion

The reason why the initial work-up for Hodgkin's lymphoma includes a set of procedures of primary importance is that these allow optimal control of the type of treatment planned, the endpoint in the treatment strategy, check on the fai-

lure pattern typical of the presentation, as well as evaluation and prevention of expected treatment complications.

Current techniques (CT-scans, biology, FDG-PET), and a little curiosity left about this peculiar disease and a global approach of the patient should allow even better results in the long-run than those observed so far.

References

1. Carde P, Glatstein E. Role of staging laparotomy in Hodgkin's disease. Mauch PM et al., Editors. Philadelphia: Lippincott, Williams & Wilkins; 1999. p. 273-95.
2. Glatstein E, Guernsey JM, Rosenberg SA, Kaplan HS. The value of laparotomy and splenectomy in the staging of Hodgkin's disease. Cancer 1969;24:709-18.
3. Carde P, Burgers JM, Henry-Amar M, Hayat M, Sizoo W, Van der Schueren E, et al. Clinical stages I and II Hodgkin's disease: a specifically tailored therapy according to prognostic factors. J Clin Oncol 1988;6:239-52.
4. Carde P, Hagenbeek A, Hayat M, Monconduit M, Thomas J, Burgers MJ, et al. Clinical staging versus laparotomy and combined modality with MOPP versus ABVD in early-stage Hodgkin's disease: the H6 twin randomized trials from the European Organization for Research and Treatment of Cancer Lymphoma Cooperative Group. J Clin Oncol 1993;11:2258-72.
5. Noordijk EM, Carde P, Mandard AM, Mellink WA, Monconduit M, Eghbali H, et al. Preliminary results of the EORTC-GPMC controlled clinical trial H7 in early-stage Hodgkin's disease. EORTC Lymphoma Cooperative Group. Groupe Pierre-et-Marie-Curie. Ann Oncol 1994;5 Suppl 2:107-12.
6. Henry-Amar M, Somers R. Survival outcome after Hodgkin's disease: a report from the international data base on Hodgkin's disease. Semin Oncol 1990;17:758-68.
7. Henry-Amar M, Joly F. Late complications after Hodgkin's disease. Ann.Oncol 1996;7 Suppl 4:115-26.
8. Vicini FA, Sharpe M, Kestin L, Martinez A, Mitchell CK, Wallace MF, et al. Optimizing breast cancer treatment efficacy with intensity-modulated radiotherapy. Int J Radiat Oncol Biol Phys 2002;54:1336-44.
9. Girinsky T, Pereira R, Bosq J, Ribrag V, Vantelon JM, Munck JN, et al. The quality assurance program for patients with Hodgkin's disease entered in the H9 randomized trial. Leuk Lymphoma 2001;Suppl 2:109.
10. Roth SL, Sack H, Havemann K, Willers R, Kocsis B, Schumacher V. Contiguous pattern spreading in patients with Hodgkin's disease. Radiother Oncol 1998; 47:7-16.
11. Carde P, Noordijk EM. Studying spreading pattern in Hodgkin's disease: is it relevant to modern cancer treatment? Radiother Oncol 1998;47:3-5.
12. Stomper PC, Cholewinski SP, Park J, Bakshi SP, Barcos MP. Abdominal staging of thoracic Hodgkin disease: CT-lymphangiography-Ga-67 scanning correlation. Radiology 1993;187:381-6.
13. Carde P, Da Costa L, Manil L, Pfreundschuh M, Lumbroso JD, Saccavini JC, et al. Immunoscintigraphy of Hodgkin's disease: in vivo use of radiolabelled monoclonal antibodies derived from Hodgkin cell lines. Eur J Cancer 1990;26:474-9.
14. Lister TA, Crowther D. Staging for Hodgkin's disease. Semin Oncol 1990;17:696-703.
15. Bangerter M, Griesshammer M, Binder T, Hafner M, Heimpel H, Reske SN, et al. New diagnostic imaging procedures in Hodgkin's disease. Ann Oncol 1996;7 Suppl 4:55-9.
16. Jerusalem G, Beguin Y, Fassotte MF, Najjar F, Paulus P, Rigo P, et al. Whole-body positron emission tomography using 18F-fluorodeoxyglucose compared to standard procedures for staging patients with Hodgkin's disease. Haematologica 2001;86:266-73.
17. Partridge S, Timothy A, O'Doherty MJ, Hain SF, Rankin S, Mikhaeel G. 2-Fluorine-18-fluoro-2-deoxy-D glucose positron emission tomography in the pretreatment staging of Hodgkin's disease: influence on patient management in a single institution. Ann Oncol 2000;11:1273-9.
18. Aleman BMP, Raemaekers JMM, van 't Veer MB, Meerwaldt JH, Carde P, Lybeert RT, et al. Involved field radiotherapy in patients with advanced Hodgkin's lymphoma: an EORTC Lymphoma Group randomized controlled trial. N Engl J Med 2003;(in press).
19. Ferme C, Sebban C, Hennequin C, Divine M, Lederlin P, Gabarre J, et al. Comparison of chemotherapy to radiotherapy as consolidation of complete or good partial response after six cycles of chemotherapy for patients with advanced Hodgkin's disease: results of the groupe d'etudes des lymphomes de l'Adulte H89 trial. Blood 2000;95:2246-52.
20. Grillo-Lopez AJ, Cheson BD, Horning SJ, Peterson BA, Carter WD, Varns CL, et al. Response criteria for NHL: importance of 'normal' lymph node size and correlations with response rates. Ann Oncol 2000;11:399-408.
21. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol 1999;17:1244.
22. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981;47: 207-14.
23. James K, Eisenhauer E, Christian M, Terenziani M, Vena D, Muldal A, et al. Measuring response in solid tumors: unidimensional versus bidimensional measurement. J Natl Cancer Inst 1999;91:523-8.
24. Guilbot S, et al. Comparison of Cheson's and RECIST criteria for response assessment in Hodgkin's lymphoma. Dis Inst Gustave Roussy;2003.
25. Ha CS, Choe JG, Kong JS, Allen PK, Oh YK, Cox JD, et al. Agreement rates among single photon emission computed tomography using gallium-67, computed axial tomography and lymphangiography for Hodgkin disease and correlation of image findings with clinical outcome. Cancer 2000;89:1371-9.
26. Delcambre C, Reman O, Henry-Amar M, Peny AM, Macro M, Cheze S, et al. Clinical relevance of gallium-67 scintigraphy in lymphoma before and after therapy. Eur J Nucl Med 2000;27:176-84.
27. Bogart JA, Chung CT, Mariados NF, Vermont AI, Lemke SM, Grethlein S, et al. The value of gallium imaging after therapy for Hodgkin's disease. Cancer 1998;82: 754-9.
28. Ionescu I, Brice P, Simon D, Guermazi A, Leblanc T, Rousselot P, et al. Restaging with gallium scan identifies chemosensitive patients and predicts survival of poor-prognosis mediastinal Hodgkin's disease patients. Med Oncol 2000;17:127-34.
29. de Wit M, Bohuslavizki KH, Buchert R, Bumann D, Clausen M, Hossfeld DK. 18FDG-PET following treatment as valid predictor for disease-free survival in Hodgkin's lymphoma. Ann Oncol 2001;12:29-37.
30. Jerusalem G, Beguin Y, Fassotte MF, Najjar F, Paulus P, Rigo P, et al. Whole-body positron emission tomography using 18F-fluorodeoxyglucose for posttreatment evaluation in Hodgkin's disease and non-Hodgkin's lymphoma has higher diagnostic and prognostic value than classical computed tomography scan imaging. Blood 1999;94:429-33.
31. Kostakoglu L, Leonard JP, Kuji I, Coleman M, Vallabhajosula S, Goldsmith SJ. Comparison of fluorine-18 fluorodeoxyglucose positron emission tomography and Ga-67 scintigraphy in evaluation of lymphoma. Cancer 2002;94:879-88.
32. Program Memorandum Human Services (DHHS) Intermediaries/Carriers Health Care Financing Administration (HCFA) Transmittal AB-01-54. 2001.
33. Carde P. Who are the high-risk patients with Hodgkin's disease? Leukemia 1996;10 Suppl 2:s62-s7.
34. Tubiana M, Hayat M, Henry-Amar M, Breur K, van der Werf Messing B, Burgers M. Five-year results of the E.O.R.T.C. randomized study of splenectomy and spleen irradiation in clinical stages I and II of Hodgkin's disease. Eur J Cancer 1981;17:355-63.
35. Tubiana M, Henry-Amar M, Carde P, Burgers JM, Hay-

at M, Van der Schueren E, et al. Toward comprehensive management tailored to prognostic factors of patients with clinical stages I and II in Hodgkin's disease. The EORTC Lymphoma Group controlled clinical trials: 1964-1987. Blood 1989;73:47-56.
36. Hasenclever, D, Diehl V. A prognostic score for advanced Hodgkin's disease. International Prognostic Factors Project on Advanced Hodgkin's Disease. N Engl J Med 1998;339:1506-14.
37. Specht L. Tumour burden as the main indicator of prognosis in Hodgkin's disease. Eur J Cancer 1992;28A:1982-5.
38. Gobbi PG, Ghirardelli ML, Solcia M, Di Giulio G, Merli F, Tavecchia L, et al. Image-aided estimate of tumor burden in Hodgkin's disease: evidence of its primary prognostic importance. J Clin Oncol 2001;19:1388-94.
39. Zanotti R, Trolese A, Ambrosetti A, Nadali G, Visco C, Ricetti MM, et al. Serum levels of soluble CD30 improve International Prognostic Score in predicting the outcome of advanced Hodgkin's lymphoma. Ann Oncol 2002;13:1908-14.
40. Carde P. Mid-treatment response is a powerful surrogate to predict outcome and MOPP/ABVD confirms its superiority over MOPP: 2 long term results of the 1981-86 EORTC trial in advanced Hodgkin's disease. 2000;[abstract589].
41. Jerusalem G, Beguin Y, Fassotte MF, Najjar F, Paulus P, Rigo P, et al. Persistent tumor 18F-FDG uptake after a few cycles of polychemotherapy is predictive of treatment failure in non-Hodgkin's lymphoma. Haematologica 2000;85:613-8.
42. Kostakoglu L, Coleman M, Leonard JP, Kuji I, Zoe H, Goldsmith SJ. PET predicts prognosis after 1 cycle of chemotherapy in aggressive lymphoma and Hodgkin's disease. J Nucl Med 2002;43:1018-27.
43. Flechtner H, Bottomley A. Fatigue and quality of life: lessons from the real world. Oncologist 2003;8 Suppl 1:5-9.
44. Kluin-Nelemans JC, Henry-Amar M, Carde P. Assessment of thyroid, pulmonary, cardiac and gonadal toxicity in stagesI-II Hodgkin's disease. Proc Third International Symposium Hodgkin's Lymphoma. Cologne, Germany, September 18-23. 2003;1995a[abstract].
45. Henry-Amar M. Assessing quality-of-life in patients with Hodgkin's disease: instruments and clinical trials. Hodgkin's disease. Philadelphia: Lippincott, Williams & Wilkins. 2003. p. 673-99.
46. Diller L, Medeiros Nancarrow C, Shaffer K, Matulonis U, Mauch P, Neuberg D, et al. Breast cancer screening in women previously treated for Hodgkin's disease: a prospective cohort study. J Clin Oncol 2002;20:2085-91.

Biological prognostic factors in Hodgkin's lymphoma

GIANPAOLO NADALI, GIOVANNI PIZZOLO

In spite of the dramatic improvement observed in recent years in the cure rate of patients with Hodgkin's lymphoma (HL), 15-30% of cases still either progress during front-line therapy or relapse. The recent introduction of dose-escaleted regimens (BEACOPP) resulted in an improved freedom from treatment failure of up to 89%. However, the risk of long term toxicity creates concerns about the possible overtreatment of a number of patients with advanced stage disease potentially cured with standard (ABVD) chemotherapy.

Several clinical and laboratory features have been used to predict failure-free survival (FFS) and overall survival (OS) and various prognostic models have been proposed over the years.[1-2] However, none of them can confidently identify sizeable populations of patients with FFS far below 50%. Additional prognostic factors related to the biology of HL are currently being evaluated to predict clinical outcome in order to provide a rational basis for investigational therapies.

Immunologic and molecular markers as prognostic factors

These include surface molecules and/or nuclear-cytoplasmic proteins expressed by Hodgkin and Reed-Sternberg (HRS) cells and bystander cells which are detected by immunohistologic or molecular techniques.

The clinical significance of the expression of surface markers by HRS cells in classical HL remains controversial. We recently reported that CD20 was expressed in 22% of 598 patients with classical HL cases but that this expression was not associated with different outcomes after treatment with equivalent regimens.[3] In a number of studies the expression of LMP-1, an EBV-encoded protein which interacts in the cellular apoptotic pathway, was shown to influence the prognosis of patients with HL but these observations were not confirmed by other authors.[4,5] Ki-67 and other proteins, such as PCNA, expressed by active proliferating cells and detected by immunohistologic methods have been reported to be of prognostic value in HL.[6] The detection of p53 mutated phenotype in HRS cells has also been reported to have a negative prognostic impact but this, too, remains controversial.[7]

The abnormal expression by HRS cells of proteins encoded by genes involved in the control of apoptotic events, such as Bcl-2 and BAX, have been reported to bear some prognostic significance. In a large series of patients with classical HL we observed Bcl-2-expressing HRS cells in 65% of cases with nodular sclerosis and in 47% of those of mixed cellularity. Failure-free survival of these patients was significantly inferior to that of Bcl-2 negative cases.[8] Contrarriwise, in 260 cases of HL, a high percentage of BAX-expressing HRS cells did not correlate with FFS of patients treated with ABVD or equivalent regimens.[9]

Multiple alterations in different pathways and checkpoints of the cell cycle have been identified by tissue microarray technology (TMA). Recently the overexpression of cyclin E, CDK2, CDK6, STAT3, Hdm2, Bcl-2, Bcl-X, survivin and NF-κB has been reported, confirming the complexity of the changes in the malignant transformation in HL.[10] Furthermore, shorter survival was related to the overexpression of Bcl-2, p53, Bcl-X and BAX.[10]

Aberrant expression of a number of genes, previously unknown to be expressed by HRS cells, has recently been detected by gene expression profiling (of about 9,000 genes) of a number of HL-derived cell lines.[11] In particular transcription factors GATA-3, ABF1, EAR3 and Nrf3 were showed to be aberrantly expressed. It is, therefore, conceivable that a number of new biological prognostic markers will be identified in the near future.

Soluble circulating molecules as prognostic factors

Soluble forms of different molecules were analyzed for their possible prognostic value in HL. The molecules investigated include β2-M, TNFα, IL-6, IL-10, sIL-2R, sCD8, sTNFRs, sICAM, sVCAM, sCD27, sCD30, VEGF and VEGF-Rs. Although the levels of these molecules often correlate with disease activity, stage and outcome (survival, FFS, freedom from disease progression) only occasionally do they maintain an independent prognostic significance (Table 1).

We previously reported the independent prognostic

From the Department of Clinical and Experimental Medicine, Section of Hematology, University of Verona, Italy.

Correspondence: Giovanni Pizzolo, Department of Clinical and Experimental Medicine, Section of Hematology, University of Verona, Italy.

Table 1. Soluble circulating molecules: evidences of prognostic role.

Molecule	*Published studies*	*Correlation with disease activity*	*Prognostic value*	*Independent value*
sIL-2Ra	12	++	±	–
SCD8	5	++	±	–
sTNFRs	7	+/±	±	–
sICAM-1	5	+/±	-/±	±
IL-6	9	+/-	±/-	–
sVCAM	1	++	+	+
b2-Micro	7	++	±	+/±
sCD30	7	++	+	+
IL-10	7	++	+	+
VEGF	Abstract	+	±	–

significance of sCD30, IL-10 and β2-microglobulin in large series of patients with HL.[12-16] We recently evaluated data from 595 patients with HL from the International Hodgkin's Study Group Database (participating centers: MDACC, Houston, TX, USA; Mayo Clinic, Rochester MN, USA; University of Athens, Greece; Istituto Tumori, Milan, Italy, University of Verona, Italy.) Soluble CD30 was increased in most patients. There was a direct correlation with LDH and β2-microglobulin and an inverse correlation with albumin. In 441 patients treated with ABVD or equivalent regimens multivariate analysis for event-free survival showed an independent prognostic significance for sCD30 serum level and presence of B-symptoms.

The role of the detection of certain soluble molecules in clinical/biological prognostic models still needs to be validated and multi-institutional joint studies aimed to collect large clinical and biological data sets are warrented.

The contribution of basic research and modern biotechnology to the understanding of the pathophysiology of HL is leading to the identification of new biological markers potentially useful, in combination with clinical and laboratory features, in selecting subgroups of patients with different prognoses.

References

1. Straus DJ, Gaynor JJ, Myers J, Merke DP, Caravelli J, Chapman D, et al. Prognostic factors among 185 adults with newly diagnosed advanced Hodgkin's disease treated with alternating potentially noncross-resistant chemotherapy and intermediate-dose radiation therapy. J Clin Oncol 1990;8:1173-86.
2. Hasenclever D, Diehl V. A prognostic score for advanced Hodgkin's disease. N Engl J Med 1998;339:1506-14.
3. Rassidakis GZ, Medeiros LJ, Viviani S, Bonfante V, Nadali GP, Vassilakopoulos TP, et al. CD20 expression in Hodgkin and Reed-Sternberg cells of classical Hodgkin's disease: associations with presenting features and clinical outcome. J Clin Oncol 2002;20:1278-87.
4. Montalban C, Abraira V, Morente M, Acevedo A, Aguilera B, Bellas C, et al. Epstein-Barr virus-latent membrane protein 1 expression has a favorable influence in the outcome of patients with Hodgkin's Disease treated with chemotherapy. Leuk Lymphoma 2000;39:563-72.
5. Von Wasielewski R, Wiebusch A, Mengel M, et al. Clinical relevance of EBV-detection in Hodgkin's disease. Leuk Lymphoma 2001;42 Suppl 2:82.
6. Morente MM, Piris MA, Abraira V, Acevedo A, Aguilera B, Bellas C, et al. Adverse clinical outcome in Hodgkin's disease is associated with loss of retinoblastoma protein expression, high Ki67 proliferation index, and absence of Epstein-Barr virus-latent membrane protein 1 expression. Blood 1997;90:2429-36.
7. Nieder C, Petersen S, Petersen C, Thames HD. The challenge of p53 as prognostic and predictive factor in Hodgkin's or non-Hodgkin's lymphoma. Ann Hematol 2001;80:2-8.
8. Rassidakis GZ, Medeiros LJ, Vassilakopoulos TP, Viviani S, Bonfante V, Nadali G, et al. BCL-2 expression in Hodgkin and Reed-Sternberg cells of classical Hodgkin disease predicts a poorer prognosis in patients treated with ABVD or equivalent regimens. Blood 2002;100:3935-41.
9. Rassidakis GZ, Medeiros LJ, McDonnell TJ, Viviani S, Bonfante V, Nadali G, et al. BAX expression in Hodgkin and Reed-Sternberg cells of Hodgkin's disease: correlation with clinical outcome. Clin Cancer Res 2002;8:488-93.
10. Garcia JF, Camacho FI, Morente M, Fraga M, Montalban C, Alvaro T, et al. Hodgkin and Reed-Sternberg cells harbor alterations in the major tumor suppressor pathways and cell-cycle checkpoints: analyses using tissue microarrays. Blood 2003;101:681-9.
11. Kuppers R, Klein U, Schwering I, Distler V, Brauninger A, Cattoretti G, et al. Identification of Hodgkin and Reed-Sternberg cell-specific genes by gene expression profiling. J Clin Invest 2003;111:529-37.
12. Zanotti R, Trolese A, Ambrosetti A, Nadali G, Visco C, Ricetti MM, et al. Serum levels of soluble CD30 improve International Prognostic Score in predicting the outcome of advanced Hodgkin's lymphoma. Ann Oncol 2002;13:1908-14.
13. Vassilakopoulos TP, Nadali G, Angelopoulou MK, Siakantaris MP, Dimopoulou MN, Kontopidou FN, et al. The prognostic significance of β(2)-microglobulin in patients with Hodgkin's lymphoma. Haematologica 2002;87:701-8.
14. Vassilakopoulos TP, Nadali G, Angelopoulou MK, Siakantaris MP, Dimopoulou MN, Kontopidou FN, et al. Serum interleukin-10 levels are an independent prognostic factor for patients with Hodgkin's lymphoma. Haematologica 2001;86:274-81.
15. Nadali G, Vinante F, Ambrosetti A, Todeschini G, Veneri D, Zanotti R, et al. Serum levels of soluble CD30 are elevated in the majority of untreated patients with Hodgkin's disease and correlate with clinical features and prognosis. J Clin Oncol 1994;12:793-7.
16. Nadali G, Tavecchia L, Zanolin E, Bonfante V, Viviani S, Camerini E, et al. Serum level of the soluble form of the CD30 molecule identifies patients with Hodgkin's disease at high risk of unfavorable outcome. Blood 1998;91:3011-6.

Is chemotherapy alone an option for managing early stage Hodgkin's disease?

DAVID J. STRAUS

Combined modality treatment (CMT) with chemotherapy and radiation therapy (RT) has largely replaced extended field radiation therapy alone as the standard treatment for most cases of early stage Hodgkin's disease. With the excellent results achieved, the focus of trials in recent years has been on reducing toxicity. The ABVD regimen appears to have superior results to MOPP with less toxicity in advanced stage Hodgkin's disease. Radiation therapy in the past has been associated with late complications including secondary solid tumors, coronary artery and other vascular damage and neuromuscular damage.

There have been few randomized trials of chemotherapy alone vs. CMT in early stage Hodgkin's disease. A variant of MOPP chemotherapy with low dose cyclophosphamide, vinblastine, procarbazine and prednisone (CVPP) alone for 6 cycles was compared to 6 cycles of CVPP and involved field (IF) RT for a subgroup of favorable clinical stage (CS) IA and IIA patients. The trial was underpowered and the results were suboptimal probably due to the suboptimal doses of CVPP, but no difference was seen in 7-year disease free (DFS) or overall survival (OS).[1] A second trial, which was also underpowered, compared 3 with 6 cycles of more optimal dose CVPP and demonstrated excellent and comparable results in both arms for patients with favorable stage I and II disease.[2] The Children's Cancer Group reported on trials on 829 pediatric Hodgkin's disease patients with all stages. The 501 patients who achieved a CR with chemotherapy were randomized to low-dose IF RT or no further treatment. An analysis by *intent-to-treat* demonstrated a borderline significant increase in 3-year event-free survival (p=0.057) in the group receiving CMT, and the increase was significant by an *as-treated* analysis (p=.0024).[3] There was no difference in overall survival. There are two multi-institutional randomized trials (EORTC-20982 and CANNCIC-HD6) that have recently been completed for favorable subgroups of stages I and II in which chemotherapy only arms are compared to combined modality treatment.

To test the hypothesis that CMT may be superior to chemotherapy alone (CT), we initiated a trial comparing ABVD followed by RT to ABVD alone (4). One hyndred and fifty-two patients with CS IA, IB, IIA, IIB, and IIIA without bulky nodal tumors (mediastinal mass ≤ 0.33 thoracic diameter, retroperitoneal/peripheral nodal mass ≤ 10 cm) were prospectively randomized prior to treatment to 6 cycles of ABVD alone or 6 cycles of ABVD followed by RT consisting of 3600cGy in 180 cGy fractions to either extended field regions (stages I,II: mantle or spleen/inverted Y; stage III: subtotal lymphoid or total lymphoid irradiation) from 1990-1999 or involved field regions from 1999-2000. The median follow-up time is 62 months (1-123 months). Distribution of age, gender, stage and histology was similar for patients in both arms. Results are reported according to intent-to-treat. For ABVD+RT complete remission (CR) was 94% and 6% failed. For ABVD alone, 94% achieved a CR, 1.5 % a partial response (PR) and 4.5% failed. At 60 months, 91% of randomized patients receiving ABVD + RT and 87% of patients receiving ABVD alone continue in CR (p=0.61, log-rank). Freedom from progression (FFP) at 60 mo. is 86% for ABVD + RT and 81% for ABVD alone (p=0.61, log-rank). At 60 mo. the overall survival (OS) is 97% for ABVD + RT and 90% for ABVD alone (p=0.08, log-rank). In general, short-term toxicity was mild. In conclusion, no statistically significant differences were seen in CR %, CR duration, FFP or OS at a median follow-up time of 5 years for patients randomized to either ABVD + RT or ABVD alone. Using 95% confidence intervals the maximum difference for CR duration, FFP and OS is estimated to be at most 20%. Larger studies would be necessary to demonstrate equivalence on the basis of smaller differences in outcome between CMT and CT in non-bulky early stage Hodgkin's disease.

A small study from Spain demonstrated a progression free survival of 86% and an OS of 92% at 56 months for 6 cycles of ABVD without RT for stages I and II Hodgkin's disease, results similar to the MSKCC experience.[5,6] Excellent results have been achieved in patients with early stage Hodgkin's disease with only four cycles of ABVD combined with involved field rather than extended field RT.[7,8] This has become the standard of care for many physicians

From the Sloan-Kettering Cancer Center, Weill Medical College of Cornell University, New York, N.Y., U.S.A.

Correspondence: David J. Straus, M.D., Attending Physician, Memorial Sloan-Kettering Cancer Center, Professor of Clinical Medicine, Weill Medical College of Cornell University, New York, N.Y., U.S.A.

treating patients with early stage Hodgkin's disease. A larger trial would be needed to determine if the results of chemotherapy alone will be equivalent to those with chemotherapy and RT. An alternative approach would be to identify which patients with non-bulky early-stage Hodgkin's disease will benefit from consolidative IF RT in addition to chemotherapy. This approach will be investigated in a protocol to be conducted in the U.S.A. by the Cancer and Acute Leukemia Group B (CALGB 50203).

References

1. Pavlovsky S, Maschio M, Santarelli MT, Muriel FS, Corrado C, Garcia I, et al. Randomized trial of chemotherapy versus chemotherapy plus radiotherapy for stage I-II Hodgkin's disease. J Natl Cancer Inst 1988;80:1466-73.
2. Pavlovsky S, Schvartzman E, Lastiri F, Magnasco H, Corrado C, Raslawski E, et al. Randomized trial of CVPP for three versus six cycles in favorable-prognosis and CVPP versus AOPE plus radiotherapy in intermediate-prognosis untreated Hodgkin's disease. J Clin Oncol 1997;15: 2652-8.
3. Nachman JB, Sposto R, Herzog P, Gilchrist GS, Wolden SL, Thomson J, et al. Randomized comparison of low-dose involved-field radiotherapy and no radiotherapy for children with Hodgkin's disease who achieve a complete response to chemotherapy. Children's Cancer Group. J Clin Oncol 2002;20:3765-71.
4. Straus DJ, et al. Blood 2001;98:160a[abstract].
5. Rueda A, Alba E, Ribelles N, Sevilla I, Ruiz I, Miramon J. Six cycles of ABVD in the treatment of stage I and II Hodgkin's lymphoma: a pilot study. J Clin Oncol 1997; 15:1118-22.
6. Rueda A, Ribelles N, Sevilla I, Ruiz I, Marquez A, Miramon J, et al. Six cycles of ABVD for stage I and II Hodgkin's lymphoma (HL). Proc Am Soc Clin Oncol 1998; 17:A139[abstract].
7. Bonfante V, Viviani S, Devizzi L, Santoro A, Di Russo A, Zanini M, et al. Ten-years experience with ABVD plus radiotherapy: subtotal nodal (STNI) vs involved field (IFRT) in early-stage Hodgkin's disease (Hd). Proc Am Soc Clin Oncol 20: 2001;A1120[abstract].
8. Brusamolino E, Lunghi F, Orlandi E, Astori C, Passamonti F, Barate C, et al. Treatment of early-stage Hodgkin's disease with four cycles of ABVD followed by adjuvant radio-therapy: analysis of efficacy and long-term toxicity. Haematologica 2000;85:1032-9.

The continuing role for radiation therapy in Hodgkin's disease

RICHARD T. HOPPE

Radiation therapy has a long-standing record of importance in treating Hodgkin's disease. The first reports of its efficacy were published more than one hundred years ago and radiation therapy provided the first reliably curative treatment for Hodgkin's disease.

In the past 30 years, there have been significant advances in the use of radiation therapy, including the routine use of megavoltage equipment, three-dimensional treatment planning, and conformal therapy. At the same time, there have been major advances in the use of chemotherapy, with the identification of new effective agents and combinations of drugs such as ABVD, BEACOPP, and Stanford V. Although the use of chemotherapy has become widespread and has supplanted radiation therapy in many situations, radiation therapy remains an essential component of therapy for many patients with this disease.

For patients with early stage (I-IIA), favorable prognosis disease (no large mediastinal mass), comprehensive radiation (subtotal lymphoid or mantle + paraortic irradiation) remains an effective form of management. Long term data from single institutions and cooperative clinical trials groups indicate a 10-year survival of 90-95% and 10 year freedom from relapse of 80-85%. However, due to the late risks, especially secondary solid tumors and cardiac disease that may occur beyond 10 years, the preferred management for these patients is now with combined modality therapy. The standard is to combine brief chemotherapy of 2-4 months duration with *involved field* irradiation, achieving 10-year survival and freedom-from relapse of 90-95%. The dose of radiation employed ranges from 20-35 Gy in different reports, and clinical trials are in progress to define the most effective dose of radiation in this setting. However, the radiation component of this therapy remains important. Thus far, clinical trials have not indicated that radiation can be safely eliminated.

From the Clinical Cancer Center, Stanford University, Stanford, CA, USA.

Correspondence: Richard T. Hoppe, MD, FACR, Clinical Cancer at Stanford, Dept. of Radiation Oncology, Stanford, USA.

In certain situations, limited radiation alone may be a very effective therapy in early stage disease. The most common setting where this is appropriate is in stage IA lymphocyte predominance Hodgkin's disease. Involved field irradiation to a dose of 30-35 Gy may be curative for patients who have peripheral presentations of disease. Disease-free survival of 75% or better may be expected after limited treatment of early stage disease and the peripheral sites treated are usually not associated with significant late toxicity.

Another common role for radiation is in combined modality treatment for patients with large mediastinal masses. In stage I-IIA/B disease, this approach is standard. Treatment with four to six months of chemotherapy is generally followed by irradiation of the mediastinal and supraclavicular areas. The dose employed is usually 30-36 Gy. With this treatment program, 10-year survival and freedom-from relapse is expected to be 85%.

The role of consolidative radiation for patients with stage III-IV disease is less clear. Although randomized clinical trials show no benefit for the routine use of chemotherapy after a full course of chemotherapy, its selective use for patients with bulky disease is still being studied. In addition, abbreviated chemotherapy programs such as Stanford V utilize radiation therapy as a component of therapy for initially bulky sites, thereby permitting the use of lower total doses of chemotherapy. In this program, the radiation is an integral component of the therapy.

Radiation therapy also enjoys a role in high dose therapy programs used for salvage after failure of primary chemotherapy. Analysis of failure sites after high dose therapy indicates that the majority of patients relapse in previous sites of disease, and often exclusively in those sites. Because of this, radiation therapy is often employed to sites of relapse pre- or post-high dose therapy. Doses range from 18-40 Gy and may include all sites of relapse, or may be restricted to bulky sites. However, the absolute value of this approach has not been proved.

A potential new role for radiation is in the management of patients treated initially with chemotherapy alone for favorable stage I-IIA disease who relapse in their initial sites of involvement. Argument may be made to approach these patients as having de novo Hodgkin's disease and treat them with comprehensive irradiation at that point. The prognosis of these patients may be similar to those treated initial-

ly with radiation for favorable prognosis disease. However, this remains an anecdotal experience at this time. Finally, radiation therapy may provide a very important palliative treatment for patients with multiply recurrent disease. They may be responsive to irradiation despite resistance to chemotherapy. Doses of 15-30 Gy are often sufficient to achieve palliative benefit.

Given the multiple roles of radiation therapy, it is not surprising that it has been referred to as *the most effective single agent in the treatment of Hodgkin's disease*.

Advanced Hodgkin's disease: least complicated cure

SANDRA J. HORNING

ABVD has emerged as the preferred treatment for advanced Hodgkin's disease, yielding a 5-year survival of x%. In studies conducted by the German Hodgkin Study Group, failure-free and overall survivals were better with the BEACOPP and RT regimen than with COPP/ABVD and radiotherapy, but at the cost of the late complication of sterility and an increased risk of second cancers. Similar outcomes were reported with the Stanford V protocol and radiotherapy, with notably fewer complications, in phase II studies but outcome with this chemotherapy alone was inferior in an Italian phase III study. These latter results may relate to study design, patient selection, and deviation from original protocol guidelines in the delivery of the radiotherapy. High dose chemotherapy and transplantation is curative in a proportion of patients who fail primary therapy such that overall survival reflects the success of primary and secondary therapy; this concept is important in defining the least complicated cure in advanced Hodgkin disease as it once was considered in early stage disease. New directions in therapy include the evaluation of dose-dense chemotherapy and incorporation of new drugs such as gemcitabine. However, pulmonary toxicity has complicated combinations of gemcitabine and bleomycin. Better definition of high risk disease beyond clinical features, based upon tissue characteristics or early assessment of functional response is needed to limit intensified treatment and its complications. In future, the combination of targeted and cytotoxic therapies and understanding of genetic predisposition to toxicity, should take us closer to the least complicated cure for advanced Hodgkin's disease.

From the Department of Medicine, Division of Medical Oncology, Stanford University Medical Center, Stanford, CA 94304, USA.

Correspondence:Sandra J. Horning, Department of Medicine, Division of Medical Oncology, Stanford University Medical Center, Stanford, CA 94304, USA. E-mail: sandra.horning@stanford.edu

IGEV as pretransplant and CD34 mobilizing regimen in relapsed/refractory Hodgkin's disease

MONICA BALZAROTTI, MASSIMO MAGAGNOLI, LUCA CASTAGNA, MICHELE SPINA, UMBERTO TIRELLI, ARMANDO SANTORO

The trend towards a better outcome for patients treated with Hodgkin's disease (HD) with high dose therapy (HDT), along with the low morbidity rate which is now reported to be less than 1%, was already known from retrospective or phase II trials, and has been confirmed by two randomized comparisons.[1-3] By examining the recent literature, a freedom from progression (FFP) of approximately 40-50% emerges despite the heterogeneity of case series in terms of the number of patients treated, induction and conditioning regimens, and eligibility criteria. In particular, data from two large co-operative groups, the French Society of Bone marrow Transplantation[4] and the IBMTR,[5] support the somewhat enthusiastic results reported by small single institution series. In conclusion, HDT with stem cell rescue is now applicable to a large proportion of patients with relapsed Hodgkin's disease, as the correct use of growth factor and other supportive care strategies have definitively modified fatal risks and the eligibility criteria. Even cases relapsing after long lasting remission can reasonably be candidates for HDT, except rare cases at very good risk (e.g. good performance status, relapse after more than 12 months and limited extension of disease: the prognosis in such patients is similar to that in patients treated with conventional regimens),[6] or those with unacceptable concomitant illnesses.

Among possible prognostic factor at relapse that have been analyzed,[7,8] disease status at transplantation emerges as of major importance.[6] Thus, further improvement of outcome for patients with relapsed Hodgkin's disease should focus first of all on increasing the percentage of complete remissions by induction therapy. This aim can be achieved by increasing the dose intensity of regimens , the number of courses delivered or by including new active drugs, such as vinorelbine[9] or gemcitabine[10] in well conducted, co-operative controlled trials. Furthermore, any new regimen should share clinical efficay and mobilizing potential in order to support high dose chemotherapy.

Methods: Since 1997 we have used the IGEV regimen, consisting in ifosfamide 2000 mg/m^2/day 1-4; gemcitabine 800 mg/m^2 day 1 & 4; vinorelbine 20 mg/m^2 day 1; and prednisolone 100 mg/day day 1 of each 3-week course. Granulocyte colony-stimulating factor (G-CSF) was administered from day 7 to day 12 of each course or up to apheresis during the mobilization course. Four courses of chemotherapy were planned, provided there was evidence of at least partial remission after the second cycle. Peripheral blood stem cell collection was performed after the first or second course in the first 13 patients , in order to test the mobilizing potential of the regimen, and after documentation of an objective response (third course) thereafter. A target yield of at least 3.0×10^6 CD34$^+$ cells/kg of body weight was planned to support HDT. Patients with very good partial response or complete response after 4 cycles of IGEV underwent single HDT with thiothepa (160 mg/m^2) and melphalan (160 mg/m^2). Starting from January 2001, patients were candidates for tandem transplant, conditioned by melphalan 200 mg/m^2 and by the BEAM regimen (BCNU, etoposide, ARA-C and melphalan), respectively.

Results: Up to October 2002, 61 patients from Istituto Clinico Humanitas- Milano and Centro di Riferimento Oncologico-Aviano, were treated with IGEV. Of them, 59 are evaluable after IGEV, 26 after single HDT and 10 after tandem transplant. Thirty-three were male and 26 females; their median age was 30 years (range 18-59). Twenty-six patients had refractory disease, as defined by non-complete remission with previous treatment, and 33 relapses after a median of one regimen (range 1-3). Thirty-eight patients had also received extensive radiation as part of prior therapy. After four IGEV courses, 59% of cases obtained CR, and 24% PR, for an overall response rate of 83%.

As far as concerns mobilization of hematopoietic progenitor cells, 40 out of 41 evaluable cases mobilized the minimum target yield of CD34$^+$ cells. The CD34$^+$ cell peak occurred a median of 12 days after the first day of chemotherapy and the median amount of CD34$^+$ cells collected was 6.6×10^6/Kilogram ranging from 0.9 to 23×10^6. Overall, 60% of patients yielded an adequate amount of CD34$^+$ cells with one procedure, and 95% with two procedures.

At the end of the single HDT procedure, 24 out of

from the Department of Clinical Oncology and Hematology, Istituto Clinico Humanitas, Rozzano, MI, Italy (MB, MM, LC, AS), Division of Medical Oncology A, Centro di Riferimento Oncologico, Aviano PN, Italy (MS, UT).

Correspondence: Armando Santoro, Sezione Trapianto Midollo OsseoUnitá Operativa Oncol.ed.Ematol. Via Manzoni 56,20089 Milano Rozzano, Italy. Phone: international + 39.02.82244540. Fax: international + 39.02.82244591. E-mail:armando.santoro@humanitas.it

26 evaluable patients were in CR, one in PR and one had progressed. In particular, response status was modified by HDT from PR to CR in four cases. After a median follow-up of 34 months, eight patients experienced further relapse, and six of them died. Thus, the two-year FFP and OS are 60% and 69%, respectively. In univariate analysis, response to IGEV (CR vs non CR) was statistically significant for both FFP and OS (p = value 0.005 and 0.01, respectively), whereas male sex was of borderline significance for only FFP. Response to IGEV was the only factor which maintained statistical power in multivariate analysis (p = 0.001).

After tandem HDT, eight out of ten patients were in CR and two had progressed. For this subset, the limited number of patients and the short follow-up did not allow any further consideration.

IGEV was globally well tolerated and always given on an outpatient basis. No hospitalization was necessary to manage toxicity and only five patients required blood product support. Grade IV neutropenia occurred in 38% of courses and grade III-IV thrombocytopenia in 28%. Fever of unknown cases of severe entity rarely complicated chemotherapy. There was no life-threatening episodes or toxic deaths during IGEV.

Conclusions: The IGEV protocol was proposed to increase CR rate at transplantion in patients with pretreated HD who are candidates for high-dose consolidation. As of today, despite HDT being considered a standard approach in this setting, induction chemotherapy usually consists in the DHAP regimen as indicated by non-Hodgkin's lymphoma protocols. To our knowledge, no attempt has been made for this purpose, with the risk of underestimating the value of CR achievement because of a non-specifically targeted protocol. This choice of IGEV, incorporating three drugs, one alkylator with known antilymphoma and mobilizing efficacy, and two so-called *new drugs* is being tested by our group in the Hodgkin's disease setting.

The good therapeutic results obtained with IGEV, along with its adequate mobilizing potential and acceptable toxicity, are of interest in candidates for HDT, as the rescue of good clinical conditions is of major concern at transplantation. In fact, the small number of patients undergoing double HDT seem to tolerate the treatment program well, and the complete recovery after IGEV has, of course, an important role in this setting. In conclusion, this is the first trial aiming to evaluate the role of an induction regimen in relapsed/refractory HD, and the encouraging results obtained suggest further investigation towards the role of double transplant.

References

1 Yuen AR, Rosenberg SA, Hoppe RT, Halpern JD, Horning SJ. Comparison between conventional salvage therapy and high-dose therapy with autografting for recurrent or refractory Hodgkin's disease.Blood 1997;89:814-22.

2 Linch DC, Winfield D, Goldstone AH, Moir D, Hancock B, McMillan A, et al. Dose intensification with autologous bone-marrow transplantation in relapsed and resistant Hodgkin's disease: results of a BNLI randomised trial. Lancet 1993;341:1051-4.

3 Schmitz N, Pfistner B, Sextro M, Sieber M, Carella AM, Haenel M, et al. Aggressive conventional chemotherapy compared with high-dose chemotherapy with autologous haemopoietic stem-cell transplantation for relapsed chemosensitive Hodgkin's disease: a randomised trial. German Hodgkin's Lymphoma Study Group. Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. Lancet 2002;359:2065-71.

4 Brice P, Bouabdallah R, Moreau P, Divine M, Andre M, Aoudjane M, et al. Prognostic factors for survival after high-dose therapy and autologous stem cell transplantation for patients with relapsing Hodgkin's disease: analysis of 280 patients from the French registry. Société Française de Greffe de Moelle. Bone Marrow Transplant 1997;20:21-6

5 Sweetenham JW, Taghipour G, Milligan D, Blystad AK, Caballero D, Fassas A, et al. High-dose therapy and autologous stem cell rescue for patients with Hodgkin's disease in first relapse after chemotherapy: results from the EBMT. Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant 1997;20:745-52.

6 Horning SJ, Chao NJ, Negrin RS, Hoppe RT, Long GD, Hu WW, et al. High-dose therapy and autologous hematopoietic progenitor cell transplantation for recurrent or refractory Hodgkin's disease: analysis of the Stanford University results and prognostic indices. Blood 1997;89:801-13.

7 Josting A, Franklin J, May M, Koch P, Beykirch MK, Heinz J, et al. New prognostic score based on treatment outcome of patients with relapsed Hodgkin's lymphoma registered in the database of the German Hodgkin's lymphoma study group. J Clin Oncol 2002;20:221-30.

8 Brice P, Bastion Y, Divine M, Nedellec G, Ferrant A, Gabarre J, et al. Analysis of prognostic factors after the first relapse of Hodgkin's disease in 187 patients. Cancer 1996;78:1293-9.

9 Devizzi L, Santoro A, Bonfante V, Viviani S, Balzarini L, Valagussa P, et al. Vinorelbine: an active drug for the management of patients with heavily pretreated Hodgkin's disease. Ann Oncol 1994;5:817-20.

10 Santoro A, Bredenfeld H, Devizzi L, Tesch H, Bonfante V, Viviani S, et al. Gemcitabine in the treatment of refractory Hodgkin's disease: results of a multicenter phase II study. J Clin Oncol 2000;18:2615-9.

Do we need high-dose therapy for initial treatment of high-risk Hodgkin's disease?

M. Federico, S. Luminari

Many authors have investigated the value of high-dose therapy (HDT) followed by autologous stem cell transplantation (ASCT) in the treatment of patients with Hodgkin's lymphoma (HL) since the first clinical data were reported in the early 1980s. This combination is now considered an effective strategy for relapsed or refractory HL, with better results when patients are transplanted at the first event and if they are still chemosensitive to salvage chemotherapy.[1] The encouraging results obtained with high-dose salvage therapy have led to an increase in the use of HDT in patients responding to initial therapy but considered at high risk of relapse. In 1991 Carella *et al*. published a pilot study of HDT and ASCT in patients with unfavorable HL who had achieved complete remission (CR) with conventional dose chemotherapy.[2] In this study 15 patients with very poor prognosis Hodgkin's disease in remission after a treatment with MOPP/ABVD regimen, were treated with HDT and ASCT immediately after achieving CR. Thirteen patients (86.6%) remained alive in unmaintained CR at a median time of 36 months (range 10-64 months) post-transplant.

In addition to Carella's experience, Sureda *et al*. reported a promising 78% continuous CR rate after a median follow-up of 2.5 years in a group of 27 patients with poor prognosis HL. Moreau *et al*. evaluated cure rate, toxicity and late effects of early intensive therapy followed by ASCT in a group of 130 patients with advanced HL registered in the French database and transplanted in first partial remission (PR) or first CR. The 5-year overall survival rate of patients in PR and CR was 82.8% and 76.3%, respectively. Similar results were reported by Nademanee et al. and, more recently, by Sperotto *et al*.[3-6] Based on these results, and in the absence of data from comparative phase III trials, an increasing number of patients with HL were treated with HDT after the achievement of a CR. For example, the EBMT registry contains data on more than 450 patients with HL who were treated with HDT and ASCT in first CR between 1990 and 2000.

However, standard dose chemotherapy (CT) has also led to encouraging results in the treatment of patients with advanced stage HL. In addition to the good results achieved with MOPP and ABVD in the past two decades, even more promising results in terms of CR and prolonged long-term survival rates have been obtained with newer regimens such as MOPPEBVCAD and BEACOPP, even in those patients considered at high risk of failure.[7-9]

Regardless of the encouraging results reported in all these studies the question whether HDT should be included in the initial treatment plan of patients with high risk HL is still a matter of debate. Two different, randomized trials that compared conventional chemotherapy with HDT as consolidation therapy for responding patients with poor risk HL have recently been concluded and have, in our opinion, clearly demonstrated that, in patients responding to initial therapy, HDT as consolidation is not superior to consolidation with conventional chemotherapy.

The study published by Proctor *et al*.,[10] carried out on behalf of the *Scotland and Newcastle Lymphoma Group* (SNLG) compared three courses of a continuous hybrid CHT regimen plus high dose melphalan and ASCT versus five courses of the same hybrid treatment in a prospective, randomized setting in patients with poor-risk HL. One hundred and twenty-six patients were registered between 1988 and 1999, and 65 of them (52%) accepted randomization. Based on an intention-to-treat analysis, after a median follow-up of 68 months the event-free survival rate of the whole group was 78% and there was no difference between the randomization arms.

The second trial was a large co-operative study, performed by the EBMT/GISL/ANZLG/SFGM/GELA HD01 Intergroup.[11] This cooperative study was designed to verify whether patients with initial features associated with a high-risk of failure after achieving CR or PR with 4 courses of standard-dose therapy would benefit from HDT-ASCT. A total of 208 patients were registered in the study and among those 163 achieving CR or PR with 4 initial courses of ABVD or other doxorubicin-containing regimens were randomized to receive either HDT followed by ASCT (arm A) or four courses of conventional chemotherapy (Arm B). At the end of the whole treat-

From the Dipartimento di oncologia ed ematologia Università di Modena e Reggio Emilia, Centro Oncologico Modenese, Policlinico, Modena, Italy.

Correspondence: M. Federico, Dipartimento di Oncologia ed Ematologia, Università di Modena e Reggio Emilia, Centro Oncologico Modenese, Policlinico, via del Pozzo 71, Modena, Italy.

ment program, 92% of patients in Arm A and 89% in Arm B achieved a CR (p=0.6). The 5-year failure free survival was 75% in Arm A and 82% in Arm B (p=0.4). The 5-year overall survival was similar in the two treatment groups (p=1.0), being 88% in Arm A and 88% in Arm B. Finally, the 5-year relapse free survival was 88% in Arm A and 94% in Arm B: this difference was not statistically significant (p=0.3). The HD01 trial has thus demonstrated that patients with advanced, unfavorable HL, responding to front-line therapy with conventional-dose chemotherapy and then receiving intensification with HDT-ASCT had an identical outcome (in terms of CR, overall survival, RFS, and FFS rates) to those patients who received four additional courses of conventional-dose CHT.

In conclusion, given the excellent outcome of patients treated with HDT-ASCT, in the absence of a control arm in a randomized study we would probably have concluded that HDT-ASCT should be considered the treatment of choice for patients with advanced, unfavorable HL. However, the right conclusion to be drawn is different; HD01 and HD3 data definitely support the view that for patients with HL, considered at high risk according to existing prognostic scores and who respond to initial conventional chemotherapy, *more is not better* (i.e. consolidation with high dose therapy is not better than consolidation with conventional dose therapy), and most importantly, these patients should no longer be offered HDT as consolidation therapy.

The identification of patients at high risk of failure remains a key question. If there is an indication for front line use of HDT in the treatment of patients with HL, it should come from a different evaluation of prognosis in HL and, probably, from the use of different drugs or conditioning regimens. Based on currently used prognostic scores patients with high risk disease are well treated with standard chemotherapy although there probably is group of patients with poor outcome in whom the HDT option should be tested. The application of new diagnostic modalities (e.g. PET scanning and studies of tumor volume) and new serum markers with prognostic value (e.g. sCD30) and in particular their modification in the early phase of treatment could contribute to better identification of patients with high risk disease eligible for investigational therapies.

References

1. Armitage JO. Early bone marrow transplantation in Hodgkin's disease. Ann Oncol 1994;5:161-3.
2. Carella AM, Carlier P, Congiu A, Occhini D, Nati S, Santini G, et al. Autologous bone marrow transplantation as adjuvant treatment for high-risk Hodgkin's disease in first complete remission after MOPP/ABVD protocol. Bone Marrow Transplant 1991;8:99-103.
3. Sureda A, Mataix R, Hernandez-Navarro F, Jarque I, Lahuerta JJ, Tomas JF, et al. Autologous stem cell transplantation for poor prognosis Hodgkin's disease in first complete remission: a retrospective study from the Spanish GEL-TAMO cooperative group. Bone Marrow Transplant 1997;20:283-8.
4. Moreau P, Fleury J, Brice P, Colombat P, Bouabdallah R, Lioure B, et al. Early intensive therapy with autologous stem cell transplantation in advanced Hodgkin's disease: retrospective analysis of 158 cases from the French registry. Bone Marrow Transplant 1998;21:787-93.
5. Nademanee A, Molina A, Fung H, Stein A, Parker P, Planas I, et al. High-dose chemo/radiotherapy and autologous bone marrow or stem cell transplantation for poor-risk advanced-stage Hodgkin's disease during first partial or complete remission. Biol Blood Marrow Transplant 1999;5:292-8.
6. Sperotto A, Zaja F, Damiani D, et al. High-dose therapy and autologous stem cell transplantation for high-risk hodgkin's lymphoma: a single center experience. Haematologica 2001;86:1317-8.
7. Canellos GP, Anderson JR, Propert KJ, et al. Chemotherapy of advanced Hodgkin's disease with MOPP, ABVD, or MOPP alternating with ABVD. N Engl J Med 1992; 327:1478-84.
8. Gobbi PG, Pieresca C, Federico M, Di Renzo N, Narni F, Iannitto E, et al. MOPP/EBV/CAD hybrid chemotherapy with or without limited radiotherapy in advanced or unfavorably presenting Hodgkin's disease: a report from the Italian Lymphoma Study Group. J Clin Oncol 1993; 11:712-9.
9. Diehl V, Sieber M, Ruffer U, et al. BEACOPP: an intensified chemotherapy regimen in advanced Hodgkin's disease. The German Hodgkin's Lymphoma Study Group. Ann Oncol 1997;8:143-8.
10. Proctor SJ, Mackie M, Dawson A, et al. A population-based study of intensive multi-agent chemotherapy with or without autotransplant for the highest risk Hodgkin's disease patients identified by the Scotland and Newcastle Lymphoma Group (SNLG) prognostic index: A Scotland and Newcastle Lymphoma Group study (SNLG HD III). Eur J Cancer 2002;38:795–806.
11. Federico M, Carella AM, Brice P. High dose therapy (HDT) and autologous stem cell transplantation (ASCT) versus conventional therapy for patients with advanced Hodgkin's disease (HD) responding to initial therapy. Proc Am Soc Clin Oncol 2002;21[abstract 1050].

Development of a human anti-CD30 antibody for the immunotherapy of Hodgkin's lymphoma

PETER BORCHMANN, ANDREAS ENGERT

Hodgkin's lymphoma (HL) has become a curable disease since the introduction of improved polychemotherapy regimens such as ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) and BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone).[1-3] Although most patients can be cured by standard approaches even in advanced stages of disease, fewer than 30% of those who relapse attain durable disease-free remissions after second-line treatment.[4] Data from HL and from non-Hodgkin's lymphoma (NHL) suggest that small numbers of residual tumor cells remaining after first-line treatment can give rise to late relapses.[5] Thus, eliminating residual malignant lymphoma cells after first-line treatment might further improve outcome in these diseases. Antibody-mediated cell lysis may be ideally suited to eliminating residual tumor cells since (i) Hodgkin-Reed/Sternberg (H-RS) cells consistently express high amounts of potential target antigens such as CD30, (ii) human Hodgkin's lymphomas are well vascularized, and (iii) the mechanism of cell killing and side effects of antibody mediated cell-lysis are completely different from conventional therapy. Of the various target antigens on Hodgkin-Reed/Sternberg cells, CD30 seems to be most promising since it is specifically expressed at very high levels.[6-9] Here, we summarize the characteristics of the first fully human anti-CD30 antibody, 5F11.

Design and results

The human CD30 hybridoma, 5F11, was derived from a HuMAb mouse (Medarex Inc., Bloomsbury, NJ, USA) which had been immunized CD30 positive L540 cells and then boosted with purified recombinant CD30. Hybridomas were generated according to standard procedures and clones were screened for CD30 reactivity by ELISA (enzyme linked immunosorbent assay). Hybridoma 5F11 secretes human IgG1 with κ light chains. Further binding studies for this fully human antibody 5F11 were performed using ELISA (with recombinant CD30-Fc coated microtiter plates) and FACS (fluorescence activated cell sorting) flow cytometry using the CD30-positive HL cell line L540. 5F11 demonstrated binding to L540 cells with saturating concentrations below 1 μg/mL. Competitive binding studies with murine antibodies showed partial cross reactivity with the anti-cluster A antibodies Ki-4 and BerH2. *In vitro* studies revealed dose-dependent lysis of L540 cells when incubated with IFN-α stimulated human monocytes using the ^{51}Cr-release assay. In addition, freshly isolated mononuclear cells were also able to kill L540 cells demonstrating the ability of this antibody to activate non-activated effector cells, most likely natural killer cells (NK cells), for Fc receptor mediated lysis. Apart from the Fc-mediated activation of human effector cells, after cross linking with a goat anti-human Fc-antibody, the 5F11 antibody showed a dose-dependent effect on cell-metabolism (as measured by the XTT-assay on the HL cell-line L540 and the CD30 positive anaplastic large cell lymphoma cell-line KARPAS-299). The IC_{50} was reached at a concentration of 10 μg/mL. Finally, these *in vitro* data translated into marked activity in xenografted *in vivo* models of HL. In established subcutaneous L540cy tumors, a substantial growth inhibition was observed in all treated mice with complete tumor regression in some animals. In a disseminated model, where the antibody is administered on day 1 after tumor cell inoculation, the cross linked 5F11 antibody was curative for all animals.

Discussion

CD30 is not only expressed specifically and at a very high density on H-RS cells, but - similar to other members of the tumor necrosi factor family - it is also involved in cell cycle regulation.[10,11] These characteristics make CD30 an ideal target for antibody-based immunotherapy and the potential clinical use led to the development of a vast number of murine anti-CD30 monoclonal antibodies (Moabs). However, Moabs containing murine components can generate a human anti-mouse antibody (HAMA) response when administered to patients: this can have a neutralizing activity or even result in anaphylactic reactions. In any case, the utility of murine antibodies is very limited.[9,12] In addition, these murine anti-CD30 antibodies showed no or only very little anti-

From the 1st Department of Internal Medicine, University of Cologne, Germany.

Correspondence: Andreas Engert, MD, Ph D, 1st Department of Internal Medicine, University of Cologne, Germany.

tumor effect in preclinical models and were therefore not promising candidates for clinical trials. Based on this experience, different antibody-based constructs have been developed for treatment of HL, including antibody-toxin constructs (IT), radioimmunoconjugates (RT), and bispecific molecules.[6,13-17] But so far, all of these approaches have shown some major disadvantages in clinical trials. Immunotoxins and radioimmunoconjugates both have major side effects, such as capillary leak syndrome (for ITs) or myelosuppression with long-lasting bone marrow aplasia and the need for platelet transfusions (for RT). In our experience, both treatment modalities have to be administered with very carefully monitoring of the patients. Production of bispecific molecules is very expensive and time consuming and therefore hardly feasible on a larger clinical scale. The focus of immunotherapy in HL was then directed to the development of human naïve antibodies. Apart from the different interactions between CD30 and anti-CD30 Moabs and direct growth inhibition of CD30 positive cells as could be shown for the 5F11 Moab, targeting this receptor with human antibodies might recruit effector mechanisms, including complement-dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC) resulting in tumor rejection. These immune mechanisms have be shown to have efficacy in the antibody-based treatment of indolent and aggressive B-cell NHL.[18,19] Here we have summarized the characteristics of the first fully human anti CD30 antibody, 5F11, which has potent *in vitro* and *in vivo* activity against HL cell lines.[20] Similar results have been reported for a humanized anti CD30 Moab.[12] Interestingly, in vitro activation of monocytes with the human 5F11 antibody is the same as that produced by the chemically linked anti-CD64 × anti-CD30 construct H22-Ki4, which has been tested in a clinical study.[16] But the complete antibody activates other human effector cells as well. In the phase I study of the bispecific molecule H22-Ki4, development of anti-bispecific molecule antibodies was observed in patients after longer treatment periods. The presumed absence of anti-antibodies using the 5F11 Moab allows schedules to be tested that have a longer treatment duration, e.g. over 4 weeks comparable to the duration of the anti-CD20 rituximab treatment schedule in NHL. Thus, the recruitment of different effector cells, suich as monocytes and NK-cells, and also the presumably abolished anti-antibody reaction in the patient should both help to improve the therapeutic activity of this human anti-CD30 antibody. An international clinical phase I/II study of the 5F11 antibody has been initiated.

References

1. Devita VT, Jr., Serpick AA, Carbone PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 1970;73:881-95.
2. Bonadonna G, Santoro A, Bonfante V, Valagussa P. Cyclic delivery of MOPP and ABVD combinations in Stage IV Hodgkin's disease: rationale, background studies, and recent results. Cancer Treat Rep 1982;66:881-7.
3. Diehl V, Franklin J, Hasenclever D, Tesch H, Pfreundschuh M, Lathan B, et al. BEACOPP, a new dose-escalated and accelerated regimen, is at least as effective as COPP/ABVD in patients with advanced-stage Hodgkin's lymphoma: interim report from a trial of the German Hodgkin's Lymphoma Study Group. J Clin Oncol 1998; 16:3810-21.
4. Carella AM. Indications for autologous bone marrow transplantation in Hodgkin's disease. Leuk Lymphoma 1992; 7 Suppl:21-2.
5. Kanzler H, Hansmann ML, Kapp U, Wolf J, Diehl V, Rajewsky K, et al. Molecular single cell analysis demonstrates the derivation of a peripheral blood-derived cell line (L1236) from the Hodgkin/Reed-Sternberg cells of a Hodgkin's lymphoma patient. Blood 1996; 87:3429-36.
6. Schnell R, Linnartz C, Katouzi AA, Schon G, Bohlen H, Horn-Lohrens O, et al. Development of new ricin A-chain immunotoxins with potent anti-tumor effects against human Hodgkin cells in vitro and disseminated Hodgkin tumors in SCID mice using high-affinity monoclonal antibodies directed against the CD30 antigen. Int J Cancer 1995;63:238-44.
7. Schwab U, Stein H, Gerdes J, Lemke H, Kirchner H, Schaadt M, et al. Production of a monoclonal antibody specific for Hodgkin and Sternberg-Reed cells of Hodgkin's disease and a subset of normal lymphoid cells. Nature 1982;299:65-7.
8. Horn-Lohrens O, Tiemann M, Lange H, Kobarg J, Hafner M, Hansen H, et al. Shedding of the soluble form of CD30 from the Hodgkin-analogous cell line L540 is strongly inhibited by a new CD30-specific antibody (Ki-4). Int J Cancer 1995;60:539-44.
9. Engert A, Burrows F, Jung W, Tazzari PL, Stein H, Pfreundschuh M, et al. Evaluation of ricin A chain-containing immunotoxins directed against the CD30 antigen as potential reagents for the treatment of Hodgkin's disease. Cancer Res 1990;50:84-8.
10. Wiley SR, Goodwin RG, Smith CA. Reverse signaling via CD30 ligand. J Immunol 1996;157:3635-9.
11. Horie R, Watanabe T. CD30: expression and function in health and disease. Semin Immunol 1998; 10:457-470.
12. Wahl AF, Klussman K, Thompson JD, Chen JH, Francisco LV, Risdon G, et al. The anti-CD30 monoclonal antibody SGN-30 promotes growth arrest and DNA fragmentation in vitro and affects antitumor activity in models of Hodgkin's disease. Cancer Res 2002; 62:3736-42.
13. Hartmann F, Renner C, Jung W, Deisting C, Juwana M, Eichentopf B, et al. Treatment of refractory Hodgkin's disease with an anti-CD16/CD30 bispecific antibody. Blood 1997;89:2042-7.
14. Schnell R, Staak O, Borchmann P, Schwartz C, Matthey B, Schindler J, et al. A Phase-I study with an anti-CD30 ricin A-chain immunotoxin (Ki-4.dgA) in patients with refractory CD30+ Hodgkin´s and Non-Hodgkin´s lymphoma. Clin Cancer Res 2001;8:1779-86
15. Schnell R, Vitetta E, Schindler J, Barth S, Winkler U, Borchmann P, et al. Clinical trials with an anti-CD25 ricin A-chain experimental and immunotoxin (RFT5-SMPT-dgA) in Hodgkin's lymphoma. Leuk Lymphoma 1998;30:525-37.
16. Borchmann P, Schnell R, Fuss I, Manzke O, Davis T, Lewis LD, et al. Phase 1 trial of the novel bispecific molecule H22xKi-4 in patients with refractory Hodgkin lymphoma. Blood 2002;100:3101-7.
17. Schnell R, Borchmann P, Schulz H, Engert A. Current strategies of antibody-based treatment in Hodgkin's disease. Ann Oncol 2002;13:57-66.

18. Rowan W, Tite J, Topley P, Brett SJ. Cross-linking of the CAMPATH-1 antigen (CD52) mediates growth inhibition in human B- and T-lymphoma cell lines, and subsequent emergence of CD52-deficient cells. Immunology 1998;95:427-36.
19. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002;346:235-42.
20. Borchmann P, Treml JF, Graziano RF, Schnell R, Engert A, Davis TA. The new fully human anti-CD30 antibody 5F11 shows in vitro and in vivo activity against Hodgkin lymphoma. Blood 2002;100:[abstract 2258].

Monitoring minimal residual disease after chemo-immunotherapy in low grade non-Hodgkin's lymphoma patient

ALESSANDRO RAMBALDI

Despite the great progress in understanding the molecular pathogenesis of low grade non-Hodgkin's lymphomas (NHL), conventional and most experimental therapeutic approaches have failed to modify the overall survival of patients with these diseases.[1] The use of polymerase chain reaction (PCR) analysis to detect the BCL-2/IgH chimeric gene in follicular NHL lymphoma patients bearing the t(14;18) chromosome aberration was proposed as a powerful tool to investigate minimal residual disease.[2] In general, protocols that allow a more thorough eradication of the neoplastic clone, such as those based on high-dose chemoradiotherapy and transplantation of i*n vitro* purged autologous hematopoietic stem cells, might provide some advantage at least in terms of freedom from disease progression.[3,4] However, in previously untreated follicular NHL, anthracycline-containing or fludarabine-based chemotherapeutic protocols can also induce a substantial proportion of complete clinical responses along with molecular negativization of the bone marrow (BM) and peripheral blood (PB).[5] The anti-CD20 chimeric monoclonal antibody, rituximab, has demonstrated remarkable efficacy in patients with various lymphoid malignancies and is the first antibody approved for the treatment of low and high grade NHL. Early studies showed that an extensive depletion of $CD20^+$ normal and lymphoma cells is rapidly induced *in vivo* by rituximab and several lines of evidence suggest that complement-dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC) are of crucial importance in the mechanism of action of this recombinant molecule.[6] Encouraging data are emerging on the use of rituximab alone as well as in combination with standard chemotherapeutic regimens.

From the Division of Hematology, Ospedali Riuniti Bergamo, Italy.

Correspondence: Alessandro Rambaldi, M.D., Division of Hematology, Ospedali Riuniti di Bergamo, Largo Barozzi 1, 24128 Bergamo, Italy. Phone: international +39.035.266808. Fax: international +39.035. 266147. E-mail: arambaldi@ospedaliriuniti.bergamo.it

Rituximab in combination with CHOP in low grade NHL

The first clinical trial of rituximab (375 mg/m^2 for 6 infusions) in combination with 6 cycles of CHOP chemotherapy in follicular low grade NHL, was conducted by Czuczman and colleagues in 40 patients (31 previously untreated). All patients responded (58% CR and 42% CR) and after a median observation time of 65^+ months a median progression-free survival has not been reached.[7] This and other similar studies provided evidence that this chemo-immunotherapy combination is highly effective in the treatment of indolent NHL and often capable of inducing a molecular Bcl-2 conversion in the bone marrow and peripheral blood. However, it was difficult to discriminate the relative merit of the antibody and chemotherapy in the molecular eradication of the neoplastic clone.

Sequential administration of Rituximab after CHOP in follicular lymphomas-NHL

To evaluate whether the sequential administration of rituximab can induce and maintain prolonged negativity of the Bcl-2/IgH chimeric gene in patients remaining persistently PCR-positive after CHOP chemotherapy, a phase II, open label, Italian multicenter study was conducted.[8] To be eligible for rituximab (4 weekly intravenous infusions of rituximab, 375 mg/m^2), at the end of CHOP chemotherapy patients had to be in partial or complete clinical response and still PCR-positive in the bone marrow and/or peripheral blood. A molecular follow-up was performed on bone marrow and peripheral blood samples, at 12, 28 and 44 weeks after the baseline. At the end of CHOP chemotherapy 57% had achieved CR, 37% PR and 6% were considered non-responsive (NR). The 41 patients, who proved PCR-negative in the BM and PB on 2 determinations (baseline), as well as the 8 clinically non-responsive patients, were excluded from rituximab treatment. The molecular analysis of minimal residual disease in the patients treated with rituximab showed that at the last molecular follow-up (+44 weeks), 63% of the patients remained PCR negative in the BM. Interestingly, provided that PCR-negative status in the BM was maintained until the last molecular follow-up (+44 weeks), an equally low frequency of recurrence or relapse was observed in patients who achieved the molecular response at the first molecular follow-up or later. Patients who never

converted to PCR negativity or who reverted to positivity after rituximab had a similarly poor clinical outcome.

The clinical significance of molecular remission

For many years it has been considered that molecular negativization of the tumor cell contamination in the bone marrow and peripheral blood was possible in follicular NHL only after high dose chemotherapy programs. Several studies performed over the last few years have produced convincing data that this result can be obtained even after conventional chemotherapy with or without the addition of the anti-CD20 monoclonal antibody, rituximab. However, the clinical significance of achieving a PCR-negative status in follicular NHL, still remains open. While most investigators found a positive and strong correlation between the molecular result and the clinical outcome, at least in term of failure-free survival, some others did not.[9] Most importantly, at variance from what was reported after high-dose chemotherapy and autologous transplantation with *in vitro* or *in vivo* purged peripheral blood stem cells, the FFR curve did not show a convincing plateau even among molecularly responding patients. Obviously, the technical methods of performing minimal residual disease analysis by PCR are far from homogeneous and harmonization and standardization of the laboratory tools are urgently needed. It is possible that the development of molecular monitoring by real time PCR will improve the value of these laboratory data, which probably remain useful surrogate markers of clinical response. Nonetheless, wise interpretation of information yielded by these tests remains a crucial duty of the skillful physician.

References

1. Federico M, Vitolo U, Zinzani PL, Chisesi T, Clò V, Bellesi G, et al. Prognosis of follicular lymphoma: a predictive model based on a retrospective analysis of 987 cases. Blood 2000;95:783-9.
2. Gribben JG, Neuberg D, Barber M, Moore J, Pesek KW, Freedman AS, et al. Detection of residual lymphoma cells by polymerase chain reaction in peripheral blood is significantly less predictive for relapse than detection in bone marrow. Blood 1994;83:3800-7.
3. Apostolidis J, Gupta RK, Grenzelias D, Johnson PW, Pappa VI, Summers KE, et al. High-dose therapy with autologous bone marrow support as consolidation of remission in follicular lymphoma: long-term clinical and molecular follow-up. J Clin Oncol 2000;18:527-36.
4. Freedman AS, Gribben JG, Nadler LM. High dose therapy and autologous stem cell transplantation in follicular non-Hodgkin's lymphoma. Leuk Lymphoma 1998; 28:219-30.
5. Lopez-Guillermo A, Cabanillas F, McLaughlin P, Smith T, Hagemeister F, Rodriguez M, Romaguera J, Younes A, Sarris A, Preti H, Pugh W, Lee M-S: The clinical significance of molecular response in indolent follicular lymphomas. Blood 1999;91:2955-60.
6. Golay J, Zaffaroni L, Vaccari T, Lazzari M, Borleri GM, Bernasconi S, et al. Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab in vitro: CD55 and CD59 regulate complement-mediated cell lysis. Blood 2000;95:3900-8.
7. Czuczman MA, Grillo-Lopez A, White CA, Saleh M, Gordon LI, LoBuglio AF, et al. Progression Free Survival (PFS) after Six Years (Median) follow-up of the first clinical trial of Rituximab/CHOP Chemoimmunotherapy. Blood 2001;98:601a[abstract].
8. Rambaldi A, Lazzari M, Manzoni C, Carlotti E, Arcaini L, Baccarani M, et al. Monitoring of minimal residual disease after CHOP and rituximab in previously untreated patients with follicular lymphoma. Blood 2002; 99:856-62.
9. Mandigers CM, Meijerink JP, Mensink EJ, Tonnissen EL, Hebeda KM, Bogman MJ, et al. Lack of correlation between numbers of circulating t(14;18)-positive cells and response to first-line treatment in follicular lymphoma. Blood 2001;98:940-4.

Molecular monitoring after allogeneic transplantation in B-cell malignancies: a surrogate marker for graft-versus-tumor effect?

PAOLO CORRADINI, ELENA RIZZO, LUCIA FARINA, MATTEO CARRABBA

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents not only a way to restore hematopoiesis after high-dose chemo/radiotherapy, but also a form of adoptive immunotherapy. The immune recognition and elimination of residual tumor cells by engrafted donor cells, known as the graft-versus-leukemia (GVL) effect, is the main curative modality of allo-HSCT.[1] Recently, several groups have developed regimens exploiting the graft-versus-tumor (GVT) effect while reducing the intensity of conditioning to minimize regimen-related toxicities.[2-4] This approach allows allo-HSCT as a treatment option also for those patients previously considered too old or medically infirm to qualify for conventional HSCT.[3]

Several reasons support the use of sensitive and specific techniques to monitor changes in tumor load after allo-HSCT: i) the achievement of molecular remission after chemotherapy and/or autografting in leukemia and lymphoma correlates with a better disease free survival, ii) GVT is more effective against a low tumor burden than massive disease, iii) post-transplant induction of the GVT effect by rapid discontinuation of immune suppression or donor lymphocyte infusion (DLI) can result in tumor reduction but it is a potentially harmful option because of the risk of acute graft-versus-host disease (GVHD).[4-5] Qualitative polymerase chain reaction (PCR) is a very sensitive method for detecting the presence of residual tumor cells. Its best sensitivity can detect one tumor cell in 10^6 normal cells. Different tumor-specific sequences, such as immunoglobulin heavy-chain gene (IgH) clonal rearrangements or fusion products (e.g. Bcr-Abl, Bcl2-IgH, Bcl1-IgH, PML-RARa), are used for minimal residual disease (MRD) monitoring. Clinical data in multiple myeloma (MM), chronic myeloid leukemia (CML), non-Hodgkin's lymphoma (NHL) and acute myeloid leukemia (AML) strongly support the role of GVT against residual tumor cells.

We have analyzed MRD in 17 patients with NHL and 4 with MM, who received reduced intensity conditioning and allo-HSCT as salvage treatment for relapsed/refractory disease. Our conditioning regimen included thiotepa, fludarabine and cyclophosphamide. At day 0 patients received lenograstim-mobilized peripheral blood cells from HLA matched siblings.[4] After a median follow-up of 360 days 12 out of 21 patients are in molecular remission. Five of them attained molecular remission by day +30 while in the other 7 the molecular remission was achieved between day 30 and 180 after transplantation, mostly in concomitance with the occurrence of GVHD. Notably, none of these patients has experienced a recurrence yet. Thus, our data suggest that the GVL effect after reduced intensity allo-HSCT is effective against molecular disease, leading to the elimination of residual tumor cells.

Although extremely sensitive, qualitative MRD monitoring by nested PCR, does not provide information about the residual tumor load and the kinetics of its possible elimination by the immune system. Recently real-time quantitative PCR methods (i.e. TaqMan PCR) have been proposed for MRD monitoring. Studies in CML show that the amount of BCR-ABL transcript detected by quantitative PCR early after allo-HSCT directly correlates with the probability of relapse.[6-7] We are currently investigating TaqMan PCR for MRD monitoring of lymphoma and MM patients, using Bcl-2 and IgH gene rearrangements. Since neither the reproducibility of quantitative data among different laboratories, nor the sensitivity of the technique, which can be influenced by the specific primer and probe combinations has been clarified yet, in a first phase we are simultaneously performing experiments in duplicate with standard nested PCR. In order to quantify tumor specific IgH rearrangements we are validating two different strategies:

- the first strategy, previously described by Ladetto *et al.*, relies on a panel of family-specific consensus probes annealing to the FR1 or the FR3 region of the IgH locus. In this case the specificity of the assay is given by the use CDR-specific primers;[8]
- the second strategy, which is more time-consuming and expensive, relies on the identification of patient-specific probes; the major advantage of this

from the Hematology/Bone Marrow Transplantation Unit, Istituto Nazionale dei Tumori, Dipartimento Scienze Mediche, University of Milano, Italy.

Correspondence: Professor Paolo Corradini, MD, Università degli Studi di Milano, Facoltà di Medicina e Chirurgia, Dipartimento di Scienze Mediche, Milan, Italy.

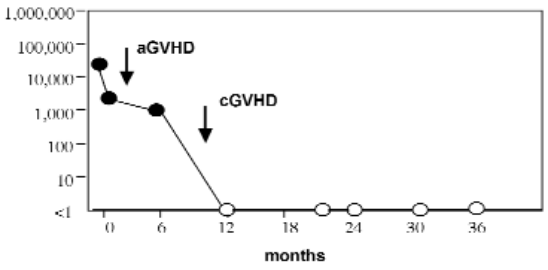


Figure 1.

approach is that it is independent of the pattern of somatic mutations of the IgH genes.[9]

For patients with follicular lymphoma we have based the assay on the detection of specific Bcl2-MBR rearrangements. TaqMan protocols that we are currently using detect 1 to 10 tumor cells diluted in 10^5 peripheral blood mononuclear cells. Our preliminary data indicate that a TaqMan PCR-based approach is feasible for monitoring patients with lymphoid malignancies after allo-HSCT. Figure 1 reports the quantitative MRD monitoring of a patient with MM who reached a complete molecular remission after myeloablative allo-HSCT. The progressive decrease of tumor-specific DNAs was concomitant to the occurrence of chronic GVHD.

In conclusion, molecular monitoring may be used to evaluate tumor burden in order to develop an *individualized* post-transplant immunotherapy, reducing the risk of GVHD and optimizing the chance of response.

References

1. Appelbaum FR. Haematopoietic cell transplantation as immunotherapy. Nature 2001;411:385-9.
2. Giralt S, Estey E, Albitar M, van Besien K, Rondon G, Anderlini P, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeolablative therapy. Blood 1997;89:4531-6.
3. McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft- versus- tumor effects. Blood 2001;97:3390-400.
4. Corradini P, Tarella C, Olivieri A, Gianni AM, Voena C, Zallio F, et al. Reduced-intensity conditioning followed by allografting of hematopoietic cells can produce clinical and molecular remissions in patients with poor-risk hematologic malignancies. Blood 2002;99:75-82.
5. Corradini P, Voena C, Tarella C, Astolfi M, Ladetto M, Palumbo A, et al. Molecular and clinical remissions in multiple myeloma: role of autologous and allogeneic transplantation of hematopoietic cells. J Clin Oncol 1999;17:208-15.
6. Olavarria E, Kanfer E, Szydlo R, Kaeda J, Rezvani K, Cwynarski K, et al. Early detection of BCR-ABL transcripts by quantitative reverse transcriptase-polymerase chain reaction predicts outcome after allogeneic stem cell transplantation for chronic myeloid leukemia. Blood 2001;97:1560-5.
7. Radich JP, Gooley T, Bryant E, Chauncey T, Clift R, Beppu L, et al. The significance of bcr-abl molecular detection in chronic myeloid leukemia patients "late," 18 months or more after transplantation. Blood 2001; 98:1701-7.
8. Ladetto M, Donovan JW, Harig S, Trojan A, Poor C, Schlossnan R, et al. Real-Time polymerase chain reaction of immunoglobulin rearrangements for quantitative evaluation of minimal residual disease in multiple myeloma. Biol Blood Marrow Transplant 2000;6:241-53.
9. Voena C, Locatelli G, Castellino C, Omede P, Ladetto M, Zappone E, et al. Qualitative and quantitative polymerase chain reaction detection of the residual myeloma cell contamination after positive selection of $CD34^+$ cells with small- and large-scale Miltenyi cell sorting system. Br J Haematol 2002;117:642-5.

Minimal residual disease in lymphoma

ARMANDO LÓPEZ-GUILLERMO

Non-Hodgkin's lymphomas are a very heterogeneous group of neoplasms derived from lymphoid cells at different steps of differentiation. Most patients with lymphoma may achieve a complete response [CR] with currently available treatments, including chemotherapy and other therapeutic approaches. However, a substantial proportion of these patients eventually have a relapse. The reason for this is most likely the persistence of lymphoma cells below the threshold of detection of standard methods (basically imaging techniques). The assessment of minimal residual disease [MRD] using highly sensitive methods may be of critical importance to assess the status of the disease after treatment and during the follow-up, to predict relapse and, perhaps in the future, to start new therapies before progression becomes clinically evident.

Two essential issues are necessary to detect and monitor MRD: 1) a specific tumor target and, 2) a highly sensitive technique to detect such a target. Cytogenetics (fluorescent *in situ* hubridization technique), flow cytometry and polymerase chain reaction (PCR) have been used to assess MRD. PCR has emerged in the last decade as one of the most sensitive of these techniques: using PCR one cell carrying a target can be detected in up to 10^5 to 10^6 normal cells. Moreover, PCR is easy and fast to perform, and can be carried out with small amounts of DNA or RNA. However, the lack of reproducibility of standard PCR among different institutions considerably limits the usefulness of this technique. More recently, quantitative PCR techniques (real-time PCR is the most common) opened up the possibility of quantifying MRD with a method that can be easily standardized. Translocations (e.g., t(14;18) in follicular lymphoma [FL], t(11;14) in mantle-cell lymphoma, t(8;14) in Burkitt's lymphoma, and t(2;5) in anaplastic $CD30^+$ large-cell lymphoma), as well as Ig rearrangements are the commonest targets for PCR. Flow cytometry is another excellent method to assess MRD when the lymphoma has specific combination markers previously assessed at diagnosis (e.g., lymphoid cells co-expressing CD38/bcl-2/CD19/ CD10 in FL). Flow cytometry is also very sensitive (detection of 1 tumor cell in 10^4 normal cells) and allows quantitative determinations.

Lymphoma is usually a disease of the lymph node. However, no lymph node is available for testing in patients in complete remission. Thus, peripheral blood [PB] and/or bone marrow [BM] must be used as the surrogate tissue source for MRD detection. In some subtypes, such as FL or mantle-cell lymphoma, detection of disease in PB and/or BM is almost invariable, whereas in other subtypes the disease may progress at nodal sites with no evidence of lymphoma in PB or BM. On the other hand, some new therapies, such as monoclonal antibodies, may clear preferentially lymphoma cells from PB and BM and yet have little effect on lymph nodes. This fact often makes difficult MRD assessment difficult.

In the lymphoma setting, the largest experience on MRD monitoring has been gained in FLs. This is mainly for two reasons: the high interest in MRD in FL patients, most of whom eventually relapse, and the existence of an excellent target, the t(14;18) translocation, which results in the bcl-2/JH rearrangement, assessable in up to 85% of FLs. In fact, it is possible to assess MRD along the follow-up in most patients with FL. The clinical significance of MRD remains controversial and ongoing trials are still studying this issue. In general, in both the setting of previously untreated patients and in that of patients submitted to transplantation, the patients in whom MRD is not detectable after treatment have a better disease-free survival than those in whom MRD is found. However, clinical relapses are not unusual in MRD-negative patients and no clear differences in terms of overall survival have been observed between MRD+ and MRD- patients. Quantitative techniques may provide more useful information on this issue. Finally, it is worth mentioning that the clinical significance of the bcl2/JH rearrangement has been called into question, since a small proportion of individuals without malignancies have detectable bcl-2/JH rearrangements.

From the Hospital Clínic, Barcelona, Spain.

Correspondence: Dr. Armando López-Guillermo, Hospital Clínic, Barcelona, Spain.

Tandem transplant in lymphomas

ANGELO M. CARELLA

Since immune-mediated graft-versus-malignancy effects can produce remissions in patients with hematologic neoplasia, we designed a strategy to induce these effects in patients with advanced lymphoma using nonmyeloablative allogeneic stem-cell transplantation (NST).

Twenty-seven consecutive patients with refractory or relapsed Hodgkin's disease (n=17) and non-Hodgkin's lymphoma (n=10), who had suitable donors, received nonmyeloablative conditioning with fludarabine and cyclophosphamide followed by an infusion of a fresh, non-T-depleted peripheral blood stem cell allograft from an HLA-identical sibling. Methotrexate and cyclosporine were employed to prevent graft rejection and graft-versus-host disease (GVHD). Cyclosporine was withdrawn early in patients with mixed chimerism or disease progression. If GVHD was absent, patients with no response received up to three infusions of donor lymphocytes.

At last follow-up, 17 of 27 patients (63%) were alive 330 to 1217 days after allografting (median follow-up, 690 days). Three patients died of transplantation-related causes, seven from progressive disease and one patient of combined extensive chronic GVHD and disease progression. Lymphoma regressed in 14 patients (52%): 12 patients achieved first or subsequent complete remission (CR) and 2 patients acheived partial remission (PR). Nine patients are alive in CCR at a median of 629 (range, 330-830) days after NST. Regression of lymphoma appeared late, occurring at a median of 165 days after transplantation (range, 65 - 313 days), and often followed donor lymphocyte infusion. Establishment of full donor chimerism was associated with GVHD.

These results are consistent with a graft-versus-lymphoma effect, and demonstrate that nonmyeloablative allografting can induce sustained engraftment with regression of lymphoma in patients who have had no response or multiple relapses to conventional chemotherapy and autografting.

From the Division of Hematology, BMT Unit, IRCCS "Casa Sollievo della Sofferenza.

Correspondence: Angelo M. Carella, , Division of Hematology, BMT Unit, IRCCS "Casa Sollievo della Sofferenza" San Giovanni Rotondo (FG), Italy.

Pathogenesis and treatment of extranodal lymphomas: the fascinating model of mucosa-associated lymphoid tissue lymphoma

E. Zucca, F. Bertoni, F. Cavalli

Extranodal lymphomas of the mucosa-associated lymphoid tissue (MALT) account for approximately 8% of all non-Hodgkin's lymphomas and comprise up to 50% of primary gastric lymphomas; they can, however, arise in virtually any extranodal site.[1-3] Their histologic features are similar regardless of the site of origin.[4]

MALT lymphoma usually arises in mucosal sites where lymphocytes are not normally present and where a MALT is acquired in response to either chronic infectious conditions or autoimmune processes (such as Helicobacter pylori gastritis, Hashimoto's thyroiditis, Sjögren's syndrome). Certain histologic features suggest that the cells of MALT lymphoma may be participating in an immune response. These include the presence of scattered transformed blasts, plasma cell differentiation, the presence of reactive T-cells, and follicular colonization.[1-3]

In the stomach the onset of MALT lymphoma is preceded by the acquisition of MALT as a result of *H pylori* infection and there is compelling evidence for a pathogenetic role of this infection in gastric lymphoma, supported by epidemiological, molecular and clinical findings.[5-8] The association of H pylori with gastric MALT lymphoma has led to the hypothesis that the micro-organism may provide the antigenic stimulus for sustaining the growth of the lymphoma in the stomach.[1-3] However, the tumor-derived immunoglobulin usually does not recognize *H. pylori* but recognises various autoantigens.[9]

Sequence analysis of the immunoglobulin genes expressed by gastric MALT lymphoma B cells shows a pattern of somatic hypermutation that suggests that the tumor cell has undergone antigen selection in germinal centers. In addition, ongoing mutations (intraclonal variation) of the immunoglobulin genes have been found in many cases.[10-12] This finding suggests that clonal expansion of tumor cells continues to be driven, at least partially, by a long-term antigen stimulation, which gives the B-cell clones with increased affinity a growth advantage over those that cannot respond or that respond less efficiently to the antigen.[10] Because of the persistent antigenic stimulation, the clone may become more susceptible to genetic alterations which can result in neoplastic transformation and tumor progression.

The most common non-random structural chromosomal aberration is the t(11;18)(q21;q21), which results in a fusion of the apoptosis inhibitor gene API2 on chromosome 11q21 with the *MALT1*, a *paracaspase* gene on chromosome 18q21.[13-16] The t(11;18) is present in at least one third of cases and has been found in several anatomic localizations of MALT lymphomas (lung, stomach, orbit), but not in nodal marginal zone lymphoma, splenic marginal zone lymphoma or mucosal diffuse large cell lymphoma. It is often the sole cytogenetic alteration. This latter finding suggests a major pathogenetic role for this translocation.[2] A second non-random translocation, much more rarely detected, the t(1;14)(p22;q32), has been shown to deregulate the expression of a survival-related gene, BCL10,[17-18] which is highly expressed in the nucleus of the neoplastic B cells of MALT lymphomas carrying this translocation. Nuclear expression of Bcl10 is also present in the MALT lymphomas carrying the t(11;18)(q21;q21). It appears that nuclear localization of Bcl10 can occur as the result of two apparently independent cytogenetic events, while Bcl10 is expressed only in the cytoplasm in MALT lymphomas without these translocations as well as in non-neoplastic germinal center and marginal zone B cells.[3,19-20]

Indeed these two seemingly disparate translocations that target *BCL10* and *MALT1* appear to affect the same signaling pathway, the result of which is the activation of NFκB. NfκB is a transcription factor with a central role in the activation of genes involved in immunity, inflammation and apoptosis. Under physiologic conditions, Bcl10 and MALT1 form a tight bond and synergize to increase activation of NFκB. Unlike wild type MALT1, which is dependent upon an interaction with Bcl10 as a mechanism for oligomerization and auto-activation, the API2-MALT1 fusion protein may possess a mechanism for self-oligomerization resulting in constitutive activation of the NFkB pathway independently of BCL10.[21-25] Thus, in MALT lymphoma, the t(1;14) or t(11;18) translocation leads to a dramatic increase in NFκB activity. This constitutive activation of the NFκB pathway is likely critical to lymphoma antigen-independent growth

From the Oncology Institute of Southern Switzerland, Bellinzona, Switzerland (EZ, FB, FC and Barts and The London, Queen Mary's School of Medicine, London, United Kingdom (FB).

Correspondence: E. Zucca, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland.

and lymphoma progression.[2-3] This may not only be important for the pathogenesis of MALT lymphomas but may also have a prognostic relevance. Indeed, the frequency of both t(11;18)(q21;q21) and nuclear BCL10 expression is significantly higher in tumors that have disseminated beyond the stomach.[3] Moreover, the t(11;18)(q21;q21) also seems strongly associated with failure to respond to eradication of *H. pylori*.[26]

Very recently a third non-random translocation has been described in MALT lymphomas, a t(14;18)(q32;q21), which is cytogenetically identical to the translocation involving BCL2 in follicular lymphoma, but involves MALT1 (which is localized about 5Mb centromeric of BCL2).[27-28] *MALT1* and the immunoglobulin heavy chain gene are rearranged in this t(14;18)(q32;q21), which was detected in approximately 20% of MALT lymphomas and appears to be more common at sites other than the gastrointestinal tract and lung. In contrast to t(11;18)(q21;21) - which is commonly found as a solitary genetic abnormality in MALT lymphomas of the stomach or the lung - tumors with t(14;18)(q32;q21) may also harbor additional genetic abnormalities.[27-28] These findings seem to suggest that site-specific pathogenetic pathways may sustain the growth of MALT lymphomas at different anatomic localizations. Deregulation of MALT1 due to genomic amplification was also found in some lymphoma cell lines, thus generating the hypothesis that MALT1 can be a dominant oncogene with a role in the pathogenesis of B-cell lymphomas.[27]

Understanding the role of *H. pylori* infection in the development of gastric MALT lymphoma has led to the successful use of antibiotic treatment in the cases localized to the stomach. A better comprehension of the additional pathogenetic events may result in further treatment improvement also at other anatomic sites.

Indeed, definite evidence indicates that eradication of *H. pylori* with antibiotics can be effectively employed as the sole initial treatment of localized gastric MALT lymphoma. Following the initial report of Wotherspoon *et al*.[28] several independent groups have confirmed the clinical efficacy of antibiotics, which can induce high rates of histologic lymphoma regression.[29-37] The lymphoma may take up to a year or more to regress. Histologic and endoscopic remission do not necessarily mean a cure. A polymerase chain reaction assay for the detection of monoclonal B cells can remain positive, without progression, in about half of histologic remissions, possibly related to small monoclonal aggregates of lymphocytes.[38-39] Histologic transformation into a diffuse large cell lymphoma has also been described in some cases. Therefore we recommend that strict follow-up is carried out (we perform a breath test 2 months after treatment to document *H. pylori* eradication and repeat post-treatment endoscopy with multiple biopsies every 6 months for 2 years, then yearly to monitor the histologic regression of the lymphoma).

In addition to the t(11;18) translocation,[26] other factors that predict a poor response to antibiotics are the presence of a bulky mass, deep infiltration of the gastric wall, involvement of perigastric lymph nodes and negative *H. pylori* immunostaining.[1-2] Several trials demonstrated the prognostic utility of endoscopic ultrasonography to identify locally advanced disease, which is unlikely to respond to *H. pylori* eradication therapy.[31-34]

No definite guidelines exist for the management of patients after antibiotic failure or for the subset of cases in which no evidence of *H. pylori* can be found. A choice can be made between conventional cancer treatments but there are no published randomized studies to help the decision.

Surgery has been widely and successfully used in the past.[40] The use of local treatment is evidently associated with excellent disease control, but the precise role for surgical resection must nowadays be redefined in view of the promising results of the conservative approach.[1-2]

Very encouraging results have been reported with low- to moderate-dose local radiotherapy in patients with stage I-II MALT lymphoma of the stomach, without evidence of *H. pylori* infection or with persistent lymphoma after antibiotics, as well as in those with localized disease at non-gastric sites.[41-45]

Chemotherapy has never been adequately evaluated in gastric MALT lymphomas because it was usually not administered, or given after surgery or radiotherapy.

Only few compounds have been tested specifically in MALT lymphomas. A non-randomized study reported that oral alkylatyng agents (either cyclophosphamide or chlorambucil, with a median treatment duration of one year) can result in a high rate of disease control with projected 5-year event-free and overall survival of 50% and 75%, respectively.[46] A more recent phase II study demonstrated some anti-tumor activity of the purine analog cladribine (2-CDA) with a complete remission rate of 84%. However, in this study, additional anti-Helicobacter treatment might have contributed to the very high remission rate in patients with gastric lymphoma since only 43% of patients with extragastric presentation achieved a remission.[47]

In the presence of disseminated or advanced disease, chemotherapy is an obvious choice. The

anti-CD20 monoclonal antibody rituximab has also been shown to be active in a phase II study (with a response rate of about 70%), and may represent an additional option for the treatment of systemic disease, but the efficacy of its combination with chemotherapy still needs to be explored in this histologic type.[48]

Most of the available information on the management of MALT lymphoma has been obtained from studies of gastric lymphoma. Non-gastric MALT lymphomas have been difficult to characterize because these tumors, numerous when considered together, are distributed so widely throughout the body that it is difficult to assemble adequate series of any given site. Yet, a few series have recently addressed the characteristics of non-gastric MALT lymphomas.[2] The *International Extranodal Lymphoma Study Group* (IELSG) published a retrospective survey of a large series of patients who were diagnosed as having non-gastric MALT lymphoma. The IELSG study confirmed the indolent course of non-gastric MALT lymphomas despite the fact that one quarter of cases presented with stage IV disease. Regardless of treatment type the 5-year survival was approximately 90%.[49]

The optimal management of non-gastric MALT lymphomas has not yet been clearly established. Retrospective series included patients treated with surgery, radiotherapy and chemotherapy, alone or in combination. Whether different sites have a different natural history remains an open question.[2,49] Location can be an important factor because of organ-specific problems, which result in particular management strategies. Since optimal management of MALT lymphomas has not yet been clearly defined the treatment choice should be *patient-tailored*, taking into account the site, the stage and the clinical characteristics of the individual patient.

References

1. Zucca E, Bertoni F, Roggero E, Cavalli F. The gastric marginal zone B-cell lymphoma of MALT type. Blood 2000; 96:410–9.
2. Cavalli F, Isaacson PG, Gascoyne RD, Zucca E. MALT Lymphomas. Hematology (Am Soc Hematol Educ Program. 2001;241-58.
3. Du MQ, Isaacson PG. Gastric MALT lymphoma from aetiology to treatment. Lancet Oncol 2002;3:97-104.
4. Isaacson PG, Müller-Hermelink HK, Piris MA. Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). In: Jaffe ES, Harris NL, Stein H, Vardiman JW, Editors. World Health Organization Classification of Tumors. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press, Lyon; 2001. p.157-60.
5. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. Lancet 1991;338:1175-6.
6. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, et al. Helicobacter pylori infection and gastric lymphoma. N Engl J Med 1994;330:1267-71.
7. Zucca E, Bertoni F, Roggero E, Bosshard G, Cazzaniga G, Pedrinis E, et al. Molecular analysis of the progression from Helicobacter pylori-associated chronic gastritis to mucosa-associated lymphoid-tissue lymphoma of the stomach. N Engl J Med 1998;338:804-10.
8. Roggero E, Zucca E, Pinotti G, Pascarella A, Capella C, Savio A, et al. Eradication of Helicobacter pylori infection in primary low-grade gastric lymphoma of mucosa-associated lymphoid tissue. Ann Intern Med 1995;122: 767-9.
9. Zucca E, Bertoni F, Roggero E, Cazzaniga G, Bosshard G, Biondi A, et al. Autoreactive B cell clones in marginal-zone B cell lymphoma (MALT lymphoma) of the stomach. Leukemia 1998;12:247-9.
10. Qin Y, Greiner A, Trunk MJ, Schmausser B, Ott MM, Muller-Hermelink HK. Somatic hypermutation in low-grade mucosa-associated lymphoid tissue-type B-cell lymphoma. Blood 1995;86:3528-34.
11. Du M, Diss TC, Xu C, Peng H, Isaacson PG, Pan L. Ongoing mutation in MALT lymphoma immunoglobulin gene suggests that antigen stimulation plays a role in the clonal expansion. Leukemia 1996;10:1190-7.
12. Bertoni F, Cazzaniga G, Bosshard G, Roggero E, Barbazza R, De Boni M, et al. Immunoglobulin heavy chain diversity genes rearrangement pattern indicates that MALT-type gastric lymphoma B cells have undergone an antigen selection process. Br J Haematol 1997;97: 830-6.
13. Rosenwald A, Ott G, Stilgenbauer S, Kalla J, Bredt M, Katzenberger T, et al. Exclusive detection of the t(11;18) (q21;q21) in extranodal marginal zone B cell lymphomas (MZBL) of MALT type in contrast to other MZBL and extranodal large B cell lymphomas. Am J Pathol 1999;155:1817-21.
14. Auer IA, Gascoyne RD, Connors JM, Cotter FE, Greiner TC, Sanger WG, et al. t(11;18)(q21;q21) is the most common translocation in MALT lymphomas. Ann Oncol 1997;8:979-85.
15. Dierlamm J, Baens M, Wlodarska I, Stefanova-Ouzounova M, Hernandez JM, Hossfeld DK, et al. The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18) (q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. Blood 1999;93:3601-9.
16. Remstein ED, James CD, Kurtin PJ. Incidence and subtype specificity of API2-MALT1 fusion translocations in extranodal, nodal, and splenic marginal zone lymphomas. Am J Pathol 2000;156:1183-8.
17. Willis TG, Jadayel DM, Du MQ, Peng H, Perry AR, Abdul-Rauf M, et al. Bcl10 is involved in t(1;14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. Cell 1999;96:35-45.
18. Zhang Q, Siebert R, Yan M, Hinzmann B, Cui X, Xue L, et al. Inactivating mutations and overexpression of BCL10, a caspase recruitment domain-containing gene, in MALT lymphoma with t(1;14)(p22;q32). Nat Genet 1999;22:63-8.
19. Ye H, Dogan A, Karran L, Willis TG, Chen L, Wlodarska, et al. BCL10 expression in normal and neoplastic lymphoid tissue. Nuclear localization in MALT lymphoma. Am J Pathol 2000;157:1147-54.
20. Liu H, Ye H, Dogan A, Ranaldi R, Hamoudi RA, Bearzi I, et al. T(11;18)(q21;q21) is associated with advanced mucosa-associated lymphoid tissue lymphoma that expresses nuclear BCL10. Blood 2001;98:1182-7.
21. Uren AG, O'Rourke K, Aravind LA, Pisabarro MT, Seshagiri S, Koonin EV, et al. Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. Mol Cell 2000;6:961-7.
22. Lucas PC, Yonezumi M, Inohara N, McAllister-Lucas LM, Abazeed ME, Chen FF, et al. Bcl10 and MALT1, independent targets of chromosomal translocation in MALT lymphoma, cooperate in a novel NF-κ B signaling pathway. J Biol Chem 2001;276:19012-9.
23. McAllister-Lucas LM, Inohara N, Lucas PC, Ruland J, Benito A, Li Q, et al. Bimp1, a MAGUK family member linking protein kinase C activation to Bcl10-mediated NF-κ B induction. J Biol Chem 2001;276:30589-97.
24. Muto A, Ruland J, McAllister-Lucas LM, Lucas PC, Yamaoka S, Chen FF, et al. Protein Kinase C-associated Kinase (PKK) Mediates Bcl10-independent NF-κ B Acti-

vation Induced by Phorbol Ester. J Biol Chem 2002; 277:31871-6.
25. Liu H, Ruskoné-Fourmestraux A, Lavergne-Slove A, Ye H, Molina T, Bouhnik Y, et al. Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to Helicobacter pylori eradication therapy. Lancet 2001;357:39-40.
26. Sanchez-Izquierdo D, Buchonnet G, Siebert R, et al. MALT1 is deregulated by both chromosomal translocation and amplification in B-cell non-Hodgkin lymphoma. Blood 2003(in press).
27. Streubel B, Lamprecht A, Dierlamm J, Cerroni L, Stolte M, Ott G, et al. T(14;18)(q32;q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma. Blood 2003;101:2335-9.
28. Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, et al. Regression of primary low-grade B-cell gastric lym-phoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. Lancet 1993;342:575-7.
29. Pinotti G, Zucca E, Roggero E, Pascarella A, Bertoni F, Savio A, et al. Clinical features, treatment and outcome in a series of 93 patients with low-grade gastric MALT lymphoma. Leuk Lymphoma 1997;26:527-37.
30. Neubauer A, Thiede C, Morgner A, Alpen B, Ritter M, Neubauer B, et al. Cure of Helicobacter pylori infection and duration of remission of low-grade gastric mucosa-associated lymphoid tissue lymphoma. J Natl Cancer Inst 1997;89:1350–5.
31. Nobre-Leitao C, Lage P, Cravo M, Cabecadas J, Chaves P, Alberto-Santos A, et al. Treatment of gastric MALT lymphoma by Helicobacter pylori eradication: a study controlled by endoscopic ultrasonography. Am J Gastroenterol 1998;93:732–6.
32. Ruskone-Fourmestraux A, Lavergne A, Aegerter PH, Megraud F, Palazzo L, de Mascarel A, et al. Predictive factors for regression of gastric MALT lymphoma after anti-Helicobacter pylori treatment. Gut 2001;48:297–303.
33. Nakamura S, Matsumoto T, Suekane H, Takeshita M, Hizawa K, Kawasaki M, et al. Predictive value of endoscopic ultrasonography for regression of gastric low-grade and high-grade MALT lymphomas after eradication of Helicobacter pylori. Gut 2001;48:454–60.
34. Steinbach G, Ford R, Glober G, Sample D, Hagemeister FB, Lynch PM, et al. Antibiotic treatment of gastric lymphoma of mucosa-associated lymphoid tissue: an uncontrolled trial. Ann Intern Med 1999;131:88–95.
35. Montalban C, Santon A, Boixeda D, Redondo C, Alvarez I, Calleja JL, et al. Treatment of low grade gastric mucosa-associated lymphoid tissue lymphoma in stage I with Helicobacter pylori eradication. Long-term results after sequential histologic and molecular follow-up. Haematologica 2001;86:609-17.
36. Zucca E, Roggero E, Traulle C. Early interim report of the LY03 randomised cooperative trial of observation vs chlorambucil after anti Helicobacter therapy in low-grade gastric lymphoma. Ann Oncol 1999;10 Suppl 3:25.
37. Levy M, Copie-Bergman C, Traulle C, Lavergne-Slove A, Brousse N, Flejou JF, et al. Conservative treatment of primary gastric low-grade B-cell lymphoma of mucosa-associated lymphoid tissue: predictive factors of response and outcome. Am J Gastroenterol 2002; 97: 292-7.
38. Bertoni F, Conconi A, Capella C, Motta T, Giardini R, Ponzoni M, et al. Molecular follow-up in gastric mucosa-associated lymphoid tissue lymphomas: early analysis of the LY03 cooperative trial. Blood 2002;99:2541-4.
39. Thiede C, Wundisch T, Alpen B, Neubauer B, Morgner A, Schmitz M, et al. Persistence of monoclonal B cells after cure of Helicobacter pylori infection and complete histologic remission in gastric mucosa- associated lymphoid tissue B-cell lymphoma. German MALT Lymphoma Study Group. J Clin Oncol 2001;19:1600-9.
40. Cogliatti SB, Schmid U, Schumacher U, Eckert F, Hansmann ML, Hedderich J, et al. Primary B-cell gastric lymphoma: a clinicopathological study of 145 patients. Gastroenterology 1991;101:1159–70.
41. Schechter NR, Portlock CS, Yahalom J. Treatment of mucosa-associated lymphoid tissue lymphoma of the stomach with radiation alone. J Clin Oncol 1998;16: 1916–21.
42. Tsang RW, Gospodarowicz MK, Pintilie M, Bezjak A, Wells W, Hodgson DC, et al. Stage I and II MALT lymphoma: results of treatment with radiotherapy. Int J Radiat Oncol Biol Phys 2001;50:1258-64.
43. Hitchcock S, Ng AK, Fisher DC, Silver B, Bernardo MP, Dorfman DM, et al. Treatment outcome of mucosa-associated lymphoid tissue/marginal zone non-Hodgkin's lymphoma. Int J Radiat Oncol Biol Phys 2002;52: 1058-66.
44. Fung CY, Grossbard ML, Linggood RM, Younger J, Flieder A, Harris NL, et al. Mucosa-associated lymphoid tissue lymphoma of the stomach: long term outcome after local treatment. Cancer 1999;85:9-17.
45. Gospodarowicz MK, Pintilie M, Tsang R, Patterson B, Bezjak A, Wells W. Primary gastric lymphoma: brief overview of the recent Princess Margaret Hospital experience. Recent Results Cancer Res 2000;156:108-15.
46. Hammel P, Haioun C, Chaumette MT, Gaulard P, Divine M, Reyes F, et al. Efficacy of single-agent chemotherapy in low-grade B-cell mucosa-associated lymphoid tissue lymphoma with prominent gastric expression. J Clin Oncol 1995;13:2524–9.
47. Conconi A, Thieblemont C, Martinelli G. IELSG phase II study of Rituximab in MALT lymphoma: final results. Proc Am Soc Clin Oncol 2002;21:267a[abstract 1067].
48. Jager G, Neumeister P, Brezinschek R, Hinterleitner T, Fiebiger W, Penz M, et al. Treatment of extranodal marginal zone B-cell lymphoma of mucosa- associated lymphoid tissue type with cladribine: a phase II study. J Clin Oncol 2002;20:3872-7.
49. Zucca E, Conconi A, Pedrinis E, Cortelazzo S, Motta T, Gospodarowicz MK, et al. Non-gastric marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. Blood 2003;101:2489-95.

How to manage gastric and intestinal primary presentation of diffuse large B-cell lymphoma

SERGIO CORTELAZZO

Primary non-Hodgkin's lymphoma (NHL) of the gastrointestinal (GI) tract is one of the most common lymphomas and accounts for about 5% of all NHL and 30% of extranodal presentations. The most frequent histologic subtypes in western countries are diffuse large B-cell lymphoma (DLBCL), which is the subject of this review and the mucosa-associated lymphoid tissue (MALT), generally associated with chronic Helicobacter pylori infection. Recently, it has been reported that incidence rates for NHL have been increasing for many years with this trend being more prevalent in extranodal disease, particularly in GI localisation, than in nodal NHL. However, in the spectrum of malignancies, GI lymphoma is relatively rare accounting for 1% to 10% of all GI tract malignancies and therefore, it remains the subject of many controversies with regard to the appropriate treatment.[1] The increasing possibility of endoscopic biopsies and the availability of endoscopic ultrasound which allow pre-operative diagnosis in most patients, particularly in those with gastric lymphoma, has raised much debate about the therapeutic value (separate from its diagnostic role) of surgical resection. Moreover, recent reports showing that treatment of primary high-grade B-cell gastric lymphoma by eradication of *Helicobacter pylori* infection resulted in complete lymphoma remission,[2,3] have futher differentiated and multiplied therapeutic options. In this regard, the promising results recently obtained with immunotherapy in patients with aggressive NHL[4] have suggested the possibility of extending this new therapeutic approach also to patients with primary GI lymphoma. In the setting of new therapeutic options evaluation of factors that might influence prognosis could be of great utility for a more coherent approach to the management of patients. The *International Prognostic Index* (IPI) is an effective prognostic model for advanced diffuse large cell lymphoma. Recently, a modified IPI (MIPI), including stage II within adverse features, was successfully used to predict the outcome of patients with early stage aggressive nodal and extranodal NHL[5] as well as primary gastric and intestinal DLBCL.[6,7] The aim of this brief review is to describe the value of different therapeutic strategies and the role of prognostic factors in predicting clinical outcome of these patients.

In the past, the treatment of localized GI lymphoma included surgery alone or surgery followed by radiation therapy and/or chemotherapy for patients with adverse prognostic factors. Surgery has been advocated to define stage better and to reduce the risk of perforation or hemorrhage related to other treatments. However, in the setting of gastric lymphoma ultrasound endoscopy is now sufficient to describe the depth of infiltration and the risk of perforation and hemorrhage appears not higher than the mortality rate (5-10%) after surgery with substantial immediate morbidity and long-term complications or discomfort related to gastrectomy. Moreover, when cohorts of patients with stage I or stage II disease were analyzed, no difference was observed between all types of treatment with or without surgery, the 5-year survival ranging around 75-79%.[6,8] The issue of a surgery-based approach to the management of primary gastric lymphoma versus chemotherapy-based approach has never been a subject of a randomized trial. However, with mature data showing the efficacy of a conservative approach such as chemotherapy alone or chemotherapy followed by radiation, gastrectomy has been virtually abandoned in the management of gastric lymphomas. Moreover, if surgery is considered, one must keep in mind that patients whose lymphoma is not radically resected, have a significantly worse prognosis than those managed with conservative treatment only, and that the extent of resection can only be judged afterwards.Thus, surgery in gastric lymphomas should be strictly limited to possible emergencies at presentation, such as perforation and macroscopic bleeding, but is no longer indicated for diagnostic purposes or for preventing complications, and can be avoided in the large majority of cases. Unlike this situation with gastric lymphomas, surgey still retains an important and largely unavoidable diagnostic role in primary intestinal lymphomas, thus the number of unresected patients tends to be low. Endoscopic biopsies are possible only in the duodenum and in the large intestine, while the majority of intestinal presentations involve the jejunum, ileum, and the cecum, a site which can be endoscopically biopsied only with some difficulty. Surgical resection should always be attempted for

From the Division of Hematology, Ospedali Riuniti di Bergamo, Italy.

Correspondence: Sergio Cortelazzo, Division of Hematology, Ospedali Riuniti di Bergamo, Italy.

localised disease, since recent data suggest that extensive resection may improve local control and eliminate early mortality from visceral perforation or hemorrhage in unresected lesions during adjuvant therapy. The 5-year survival rate for patients with primary intestinal DLBCL is lower than that of gastric lymphoma, ranging from 58 to 68%.[7,9,10] Patients not fit for surgery, but who are able to tolerate anthracycline chemotherapy, should be given chemotherapy and possibly adjuvant radiotherapy like those with gastric lymphoma. In fact, the two large prospective randomised trials conducted by SWOG and ECOG[5,11] established that chemotherapy followed by involved field radiation therapy offers better disease control and survival than treatment with chemotherapy alone in early stage aggressive nodal and extranodal NHL. However, in these analyses, no clear-cut information about the clinical outcome of patients with GI lymphoma was offered. Therefore, we are waiting for the results of a still ongoing prospective randomized trial conducted by *International Extranodal Lymphoma Study Group* (IELSG), which compares chemotherapy alone versus a short course of chemotherapy, followed by radiation therapy in patients with primary gastric DLBCL. Beside traditional therapeutic strategies, new treatment approaches have been recently proposed for GI lymphomas. Two studies demonstrated in a small series of patients with helicobacter pylori infection and high-grade MALT stage IE gastric lymphoma that the eradication of *H. pylori* resulted in durable long-term complete regression of lymphoma confirming several previous anecdotal reports.[2,3] These findings suggest that high-grade transformation is not necessarily associated with loss of H.pylori dependence and that the response of large cells to the cure of *H. pylori* infection may correspond to the antigen dependency of their low-grade counterpart in localized high-grade gastric MALT lymphomas. However, until further data are available, these tumors must be considered aggressive. Therefore, initial treatment that involves antibiotioc therapy in stage IE high-grade gastric MALT lymphoma should be given only when appropriate histologic grading, radiologic staging and an intensive endoscopic follow-up protocol can be strictly executed so that early initiation of systemic chemotherapy with or without radiotherapy can be offered to patients with tumors refractory to antibiotic treament. Moreover, similar to gastric low grade MALT lymphoma, an association has been established between intestinal low-grade MALT lymphoma and and *H. pylori* infection, with sporadic cases of remission achieved upon antibiotic treatment. However, the role of this treatment in patients with low and high-grade MALT primary intestinal lymphoma needs to be further clarified before it is recommended as a single-modality or combination treatment in clinical practice. An other therapeutic option for treatment of GI lymphomas could be offered by anti-CD 20 monoclonal antibody (Rituximab), which was successfully used in resistant and relapsed extranodal low-grade MALT lymphoma and in combination with CHOP chemotherapy as front-line treatment of diffuse aggressive NHL. Immunotherapy could potentiate conventional treatment modality increasing the rate of long-survivors, particularly in the setting of patients at high risk of resistance or relapse after font-line treatment. At this regard, conflicting results have been yelded from previous studies investigating the most important clinical factors that should guide treatment. Recently, the use of modified IPI (MIPI) as prognostic model for patients with primary GI lymphoma allowed us to identify cases with two or more adverse prognostic factors who fared worse than those with a more favorable presentation, independently on treatment.[6,7] Therefore, patients with a poor presentation according to MIPI could be the subgroup of GI lymphomas more suitable for innovative treatment such as immunotherapy.

In conclusion, primary GI DLBCLs are heterogeneous diseases. Gastric lymphoma has a better clinical outcome than intestinal lymphoma. Its management no longer routinely incorporates surgery, this being reserved for use in emergency situations, while antibiotic treatment to eradicate *H. pylori* should be given to *H. Pylori* positive cases. The recommendation for primary intestinal lymphoma it to adapt lymphoma resection in such a way as to make it as radical as is necessary. The choice of the most appropriate adjuvant treatment (e.g. chemotherapy, radiation therapy and/or immunotherapy), particularly in high-risk patients, should be further investigated in prospective, randomized trial.

References

1. Zucca E, Roggero E, Bertoni F, et al. Primary extranodal non-Hodgkin's lymphoma. Part 1: gastrointestinal, cutaneous and genitourinary lymphomas. Ann Oncol 1997;8:727-37.
2. Morgner A, Miehlke S, Fischbach W, et al. Complete remission of primary high-grade B-cell gastric lymphoma after cure of Helicobacter pylori infection. J Clin Oncol 2001;19:2041--8.
3. Chen LT, Lin JT, Shyu RY, et al. Prospective study of helicobacter pylori eradication. Therapy in stage IE high-grade mucosa-associated lymphoid tissue lymphma of the stomach. J Clin Invest 2001;19:4245-51.
4. Vose JM, Link BK, Grossbard ML, et al. Long-term follow-up of a phase II study of Rituximab in combination with CHOP chemotherapy in patients with previously untreated aggressive non-Hodgkin's lymphoma (NHL) Blood 2002;361a[abstract].
5. Miller TP, Dahlberg S, Cassady JR, et al. Chemotherapy alone compared with chemotherapy plus radiotherapy

for localized intermediate and high-grade non-Hodgkin's lymphoma. N Engl J Med 1998;339:21-6.
6. Cortelazzo S, Rossi A, Roggero E, et al. Stage-modified International Prognostic Index effectively predicts clinical outcome of localized primary gastric diffuse large B-cell lymphoma. Ann Oncol 1999;10:1433-40.
7. Cortelazzo S, Rossi A, Oldani E, et al. The modified International Prognostic index can predict the outcome of lovalized primary intestinal lymphoma of both extranodal marginal zone and diffuse large B-cell histologies. Br J Haematol 2002;118:218-28.
8. Koch P, del Valle F, Berdel WE, et al. Primary gastrointestinal non-Hodgkin's lymphoama: II. Combined surgical and conservative or conservative management only in localized gastric lymphoma-Results of the Prospective German Multicenter Study GIT NHL 01/92. J Clin Invest 2001;19:3874-83.
9. Koch P, del Valle F, Berdel WE, et al. Primary gastrointestinal non-Hodgkin's lymphoma: I. Anatomic and histologic distribution,clinical features, and survival data of 371 patients registered in the German Multicenter Study GIT NHL 01/92. J Clin Invest 2001,18:3861-73.
10. Ibrahim EM, Ezzat AA, El-Weshi AN. Primary intestinal diffuse large B-cell non-Hodgkin's lymphoma: clinical features, managemet, and prognosis of 66 patients. Ann Oncol 2001;12:53-8.
11. Glick J, Kim K, Earle J. An ECOG randomized phase III trial of CHOP vs CHOP + radiotherapy for intermediate grade early stage non-Hodgkin's lymphoma. Proc Am Soc Clin Oncol 1995;14:391[abstract 1221].

Questions and answers in the management of primary CNS and ocular lymphomas

ANDRÉS J. M. FERRERI, STEFANIA DELL'ORO, MICHELE RENI, MARCO FOPPOLI, NICOLETTA ANZALONE, SILVIA GOVI, PIERO PICOZZI, GIULIO TRUCI, MAURILIO PONZONI

Primary central nervous system lymphoma (PCNSL) is a rare form of extranodal non-Hodgkin's lymphoma (NHL) and represents 4% of all primary brain tumors.[1] PCNSL occurs in all age groups but mostly in individuals over 50 years of age, with a male:female ratio of 1.5:1.[2] The main histotype in immunocompetent patients is EBV-negative diffuse large B-cell lymphoma (Figure 1). In more than half of immunocompetent patients, PCNSL appears as a single lesion, usually localized in the periventricular regions, infiltrating the corpus callosum and the basal ganglia (Figure 2).[2] The clinical onset consists of non-specific motor and/or sensory focal deficits in about 50% of the cases; personality changes, headaches and other signs of intracranial hypertension, such as nausea, vomiting and papilledema, are also frequent. Systemic symptoms are present in 2% of cases.[2] PCNSL may arise in the cerebral, cerebellar and the brain stem parenchyma, in the eyes, the leptomeninges, and the spinal cord. Intraocular lymphoma (IOL) represents 5-20% of PCNSL, being more common among females with multifocal disease.[3] It is associated with other CNS lesions in 65% of cases,[4] and bilateral involvement of the eyes occurs in almost 80% of cases. Usually, IOL presents as a non-specific unilateral uveitis refractory to topical or systemic corticosteroids, associated with floaters or campimeter deficits, which precede cerebral symptoms by months or years.[5-7] To confirm the diagnosis of PCNSL, a staging workup completed by total-body computed tomography, bone marrow biopsy and cerebrospinal fluid cytology examination is mandatory;[8] ophthalmic ultrasonography, slit-lamp examination and indirect ophthalmoscopy are adjunctive diagnostic techniques useful for detecting an asymptomatic ocular localization in more than 5% of PCNSL patients.[9] The suspicion of infiltration of the vitreous humor should be confirmed through vitrectomy, which allows cytologic diagnosis in most cases.[10] In elderly males, staging work-up should include testicular ultrasonography.[11]

Prognosis of PCNSL is poor and the median survival of untreated patients is 1.5-3.3 months.[2] Patients submitted to surgical resection alone have a median survival of 3.5-5 months. Historically, radiotherapy alone has been the standard treatment for PCNSL; however, radiotherapy is rarely a curative treatment in PCNSL patients since response is usually short-lived, with a median survival of 12-14 months.[12,13] Moreover, the positive impact of chemotherapy progressively limited the indications for radiotherapy alone. Current therapeutic knowledge in PCNSL results from a limited number of non-randomized phase-II trials,[14] meta-analyses of published series[13,15] and large retrospective, multicenter series.[2] Despite the fact that literature on PCNSL has been progressively increasing, several therapeutic questions remain unanswered, and the use of divergent study designs and entry criteria lead to incomparable results and debatable conclusions.[14] The present article summarizes the most relevant open questions in PCNSL treatment, and analyzes the related literature to identify the most reliable answers.

What is the standard therapeutic approach to PCNSL?

Patients with newly diagnosed PCNSL must be enrolled in prospective trials. A small number of patients are not eligible for clinical trials, while other patients are being treated in institutes that do not participate in multicenter prospective studies. These patients should be treated with standard combined chemo-radiotherapy, while radiotherapy alone is the rational treatment when chemotherapy is contraindicated. Even though not confirmed by results from randomized trials, there is substantial consensus regarding the superiority of combined chemo-radiotherapy with respect to radiotherapy alone.[16] Data from a large, multicenter retrospective series[2] and a large single-arm phase II trial[16] suggest that high-dose methotrexate (HD-MTX)-based chemotherapy followed by whole-brain radiotherapy (WBRT) should be preferred to radiotherapy alone. This strategy is in accordance with the treatment recommendation for the majority of localized aggressive lymphomas, for which primary chemotherapy is followed by consolidation radiotherapy. Chemo-radiotherapy produces a 5-year survival of 22% - 40%[14,17,18] in comparison to the 3% - 26% reported

From the Departments of Radiochemotherapy, (AJMF, SDO, MR, SG) Internal Medicine (MF), Neuroradiology (NA), Neurosurgery (PP), Neurology (GT), and Pathology (MP), San Raffaele H Scientific Institute.

Correspondence: Andrés J. M. Ferreri, Department of Radiochemotherapy, San Raffaele H Scientific Institute, Via Olgettina 60, 20132, Milan, Italy. E-mail: ferreri.andres@hsr.it

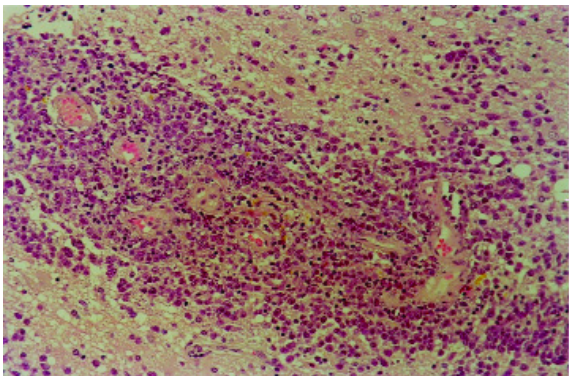


Figure 1. Diffuse large B-cell lymphoma of the brain. Diffuse perivascular proliferation with infiltration of cerebral parenchyma between the involved vessels. Neoplastic lymphocytic cuff in the perivascular space.

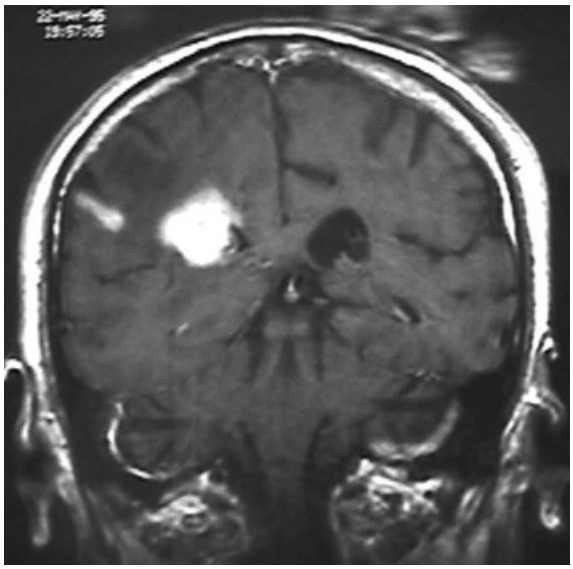


Figure 2. Magnetic resonance imaging of the brain after contrast infusion: a homogeneously enhanced lesion with polylobated limits in the deep left temporal area infiltrating the ventricular trigone, with evident perilesional edema can be seen.

with radiotherapy alone,[19,20] but it is not known whether more intensive combined treatment will improve outcome. Although a survival advantage for HD-MTX-based chemotherapy followed by radiotherapy has not been fully proven, a randomized trial comparing this strategy to radiotherapy alone would likely be unacceptable to the majority of clinicians,[21] and the combined approach should be retained as the first-choice strategy.

What is the best chemotherapy regimen for newly diagnosed PCNSL?

Blood-brain barrier (BBB) penetration and efficacy against systemic non-Hodgkin's lymphomas have been retained as the determining characteristics for choosing drugs to include in primary chemotherapy against PCNSL. HD-MTX (≥1 g/m^2) is the most effective drug against PCNSL.[13] HD-MTX, as monochemotherapy followed by radiotherapy, has shown a response rate of 80% - 90% and a 2-year survival of 60% - 65%.[22-25] Any regimen without HD-MTX is associated with outcomes no better than with radiotherapy alone.[26] The addition of a CHOP regimen to radiotherapy did not improve outcome, either when used as primary treatment or as post-radiation chemotherapy.[27-29]

Several studies attempted to improve survival by adding other drugs to MTX.[14] However, none of the used drugs had been previously evaluated as effective single-agents in patients with relapsed or refractory PCNSL. A recently reported survival improvement resulting from the addition of high-dose cytarabine to HD-MTX[2,15] deserves to be prospectively confirmed. Presently, primary chemotherapy against PCNSL should include HD-MTX, while there is no apparent reason to use additional drugs in ordinary clinical practice. The identification of new active drugs and combinations in phase I/II trials in relapsed or refractory PCNSL should receive high priority.

What is the best administration schedule for HD-MTX?

The efficacy of HD-MTX depends on the duration of exposure and drug concentration, which are determined by the administration schedule and pharmacokinetics. MTX enters the cells in part by an active transport mechanism and is bound as polyglutamate conjugates. During longer periods of drug exposure, a higher polyglutamate formation rate is observed and more cells enter into phase S, resulting in increased cytotoxicity. Since MTX clearance from plasma is triphasic,[30] an initial rapid administration to overcome the distribution phase of clearance, followed by a more prolonged infusion, appears the best administration schedule for this drug. The optimal duration of HD-MTX infusion is still unknown; in most trials using doses of 1–5 g/m^2, MTX has been administered in a 4-hour infusion,[31-33] while 24-hour infusions have been used for higher doses.[25,34] The use of a 3-hour

infusion has been associated with a significantly higher response rate and higher cerebrospinal fluid (CSF) levels compared to those achieved with a 6-hour infusion. CSF MTX concentration seems to be strictly related to the dose administered.[35] The optimal dose and timing of MTX have not been defined, but no significant difference in efficacy or toxicity was observed when MTX at 3.5 g/m^2 was administered every 3 weeks versus every 10 days.[22]

Is intrathecal chemotherapy necessary for all PCNSL patients?

PCNSL infiltrates the subarachnoid space in up to 40-50% of cases,[17,36,37] thus requiring adequate meningeal treatment, which may be achieved by cranio-spinal radiation, high-dose systemic chemotherapy or by intrathecal chemotherapy. The first strategy is associated with relevant myelotoxicity, while the indications for and efficacy of the other two strategies are debatable. Intrathecal administration of the most commonly used drugs, such as MTX, cytarabine and steroids, is associated with an increased risk of neurotoxicity and chemical meningitis,[2,22,31] while the efficacy of this strategy has not been prospectively assessed in PCNSL patients. As the majority of meningeal relapses occur in patients with positive CSF cytology at diagnosis,[2,22,32] some authors suggested that, to minimize toxicity, intrathecal chemotherapy should be reserved for this subgroup of patients.[22,29] On the other hand, preliminary data suggest that systemic HD-MTX is associated with eradication of neoplastic cells from CSF,[25,38] and some prospective[22,23,32] and retrospective[2,39] studies suggest that intrathecal chemotherapy does not improve outcome in patients treated with HD-MTX-based chemotherapy. Finally, the potential benefit of intrathecal chemotherapy is still a matter of debate because leptomeningeal relapse is almost always associated with brain recurrence, which constitutes the cardinal prognostic event in PCNSL, obscuring the effect of concurrent leptomeningeal relapse on survival, and, consequently, the potential benefit of intrathecal chemotherapy.

Is WBRT necessary for all patients with PCNSL?

Combined chemo-radiotherapy is associated with severe neurological impairment in 40% of cases and a neurotoxicity-related mortality of 30%,[16,17] especially in patients older than 60 years of age. Thus, some authors have proposed that consolidation radiotherapy should be avoided in elderly patients who achieve a complete remission following HD-MTX-based chemotherapy to minimize iatrogenic neurotoxicity.[40] Only a few prospective trials assessing the impact of chemotherapy alone on survival and toxicity have been reported, with response rates in excess of 90%, effective salvage therapy after relapse with additional chemotherapy or radiotherapy,[41] and 69% of patients alive at 4.5 years.[40] A non-randomized study suggested that, in elderly patients, WBRT suppression markedly reduces the risk of neurotoxicity, without having a detrimental effect on survival.[42] Similar results were observed in patients achieving complete remission after HD-MTX in a retrospective series of 378 patients.[2]

Exclusive chemotherapy is feasible in PCNSL patients, but its real efficacy has not yet been defined. An ongoing, randomized study comparing combined chemo-radiotherapy versus chemotherapy alone, with HD-MTX as the induction chemotherapy regimen, will provide valuable information regarding this important clinical question in a few years (*E. Thiel, Hamburg, Germany; personal communication*). In the meantime, chemotherapy alone should be considered an experimental approach, which could, however, be used in patients with a remarkably elevated risk of severe treatment-related neurotoxicity.

What is the best treatment for intraocular lymphoma?

In the past, almost all patients with IOL treated with radiotherapy alone developed early CNS progression and died.[43-45] Promising anecdotal results in small series of patients with concurrent brain and ocular lymphoma treated with chemotherapy have been reported.[46] The efficacy of chemotherapy is strongly conditioned by intraocular pharmacokinetics, which are not well understood. Clinical data show that it is very difficult to achieve therapeutic concentrations of MTX and cytarabine in the vitreous humor after intravenous injections,[47] so that persistence of disease and recurrence in the eyes are frequent events after chemotherapy alone.[40] On the other hand, the addition of adequate radiotherapy, i.e. irradiation of two thirds of both orbits with 30-36 Gy, to HD-MTX has been associated with a higher response rate without cases of ocular recurrence.[48] Considering the positive effect of ocular irradiation and the difficulties in achieving intraocular therapeutic concentrations of cytostatics, the use of chemotherapy alone should be the subject of experimental protocols and not considered a standard approach in patients with ocular disease.

Intriguing results with new therapeutic approaches, such as high-dose chemotherapy supported by autologous peripheral blood stem cell transplantation (APBSCT)[4] and intravitreal che-

motherapy,[49,50] have been reported. These experimental strategies may become valid alternatives in IOL, but their therapeutic role should be addressed in future studies.

What is the role of blood-brain barrier disruption?

Increasing drug delivery to the lymphoma-infiltrated brain could significantly enhance survival. Intra-arterial infusion of hypertonic mannitol results in reversible blood-brain barrier (BBB) disruption, which facilitates delivery of MTX- or carboplatin-based chemotherapy across the BBB, and produces high response and survival (5-year OS: 42%) rates, with excellent neurological tolerance.[51,52] BBB disruption may be an efficient strategy also in PCNSL patients who have a relapse after initial treatment with HD-MTX,[53] and may prove most useful in the delivery of agents unlikely to cross an intact BBB, such as unconjugated or radiolabeled monoclonal antibodies (*E. Neuwelt, Portland, USA; personal communication*). Despite its good efficacy and safety profiles, BBB disruption procedures are used in a limited number of cancer centers around the world, and randomized comparison with conventional chemotherapy should be considered in PCNSL patients.

What is the role of high-dose chemotherapy supported by autologous stem cell transplantation?

High-dose chemotherapy supported by autologous peripheral blood stem cell transplantation (APBSCT) has been used as one strategy to dose intensify the dose of chemotherapy given to patients with newly diagnosed or relapsed PCNSL. Theoretically, this strategy could be used to replace consolidation WBRT in an effort to avoid treatment-related neurotoxicity. There have been two small APBSCT phase II trials in patients with newly diagnosed PCNSL the two trials yielded discordant results. In one study,[54] 28 patients received intensive MTX and cytarabine, followed by BEAM consolidation chemotherapy; only 50% of patients had chemosensitive disease and a significant proportion relapsed after transplant, only 5 (18%) patients remained in remission at a median of 26 months after transplant. In another ongoing study,[55] a combination of MTX, thiotepa and cytarabine is being used as the induction regimen followed by high-dose chemotherapy with BCNU and thiotepa and hyperfractionated radiotherapy. Nineteen of 24 patients enrolled to date have achieved a complete remission and there have not been any unexpected acute toxicities. In a study on 22 patients with recurrent or refractory PCNSL or IOL,[56] induction cytarabine and etoposide followed by high-dose chemotherapy with thiotepa, busulfan and cyclophosphamide produced a complete remission rate of 72%, with a 3-year survival of 64%. However, there was a significant incidence of neurotoxicity as well as significant treatment-related morbidity/mortality in patients over the age of 60, particularly in those who had been previously irradiated. These preliminary results suggest that high-dose chemotherapy supported by APBSCT is feasible in PCNSL patients. Further studies will need to be done to identify the optimal induction and high-dose chemotherapy regimens and to define the best role of this strategy in PCNSL patients.

What is the optimal salvage treatment for relapsed PCNSL?

Salvage therapy significantly prolongs survival in relapsed and refractory PCNSL patients.[57-59] Conclusions regarding the optimum second-line treatment cannot be made because of the extremely heterogeneous modalities used in published series; however, relapses in the brain after combined treatment oblige the use of further chemotherapy. High-dose cytarabine is the most widely used cytostatic in patients who have relapsed after HD-MTX, but re-treatment with HD-MTX has also been proposed.[25] In patients who relapse after chemotherapy alone, radiotherapy appears to be the subsequent choice, but some authors have suggested using chemotherapy again as the salvage strategy.[40,60] In some cases, re-irradiation of relapsed lesions has also been indicated.[59] Ocular recurrence, which is associated with a slightly longer survival,[58] can be treated with radiotherapy, high-dose cytarabine or high-dose chemotherapy supported by APBSCT.[56] Meningeal relapse can be treated with systemic and/or intrathecal chemotherapy or with spinal-cord irradiation. As for other aggressive lymphomas, high-dose chemotherapy supported by autologous or allogeneic PBSCT can be retained as an interesting experimental alternative.[56,61,62]

Do reliable prognostic factors exist?

The identification of reliable prognostic factors may allow PCNSL patients to be divided into risk groups, which could result in the application of risk-adjusted therapeutic strategies. Among the parameters used for the *International Prognostic Index* (IPI), age, ECOG performance status and serum lactate dehydrogenase level are generally correlated to survival in retrospective series.[13,28,63] However, the use of the IPI does not discriminate between low and intermediate-low risk groups in PCNSL series,[64] which could be due to the influence of more specific prognostic variables. A significant association between survival and involvement of deep structures of the brain (peri-

ventricular areas, corpus callosum, basal ganglia, brainstem, cerebellum) and elevated CSF protein concentrations has been reported.[2,65] In the IELSG series of 378 case,[2] age, ECOG performance status, serum lactate dehydrogenase level, CSF protein level, and tumor location were established as independent predictors of response and survival.[65] These variables have been used to develop a prognostic scoring system that distinguishes three different risk groups based on the presence of 0-1, 2-3 or 4-5 unfavorable features.[65] The clinical relevance of this prognostic score should be validated in further studies. Histopathologic,[66] biological and molecular[67] markers with potential prognostic value are currently under investigation.

What are the best issues for future prospective clinical trials?

An international, multidisciplinary collaboration is really needed in PCNSL. This is the only setting in which to address clinical and biological research questions and to perform randomized trials that will yield definitive conclusions. Several fundamental challenges must, however, be addressed prior to initiation of randomized studies, which require substantial financial resources as well as several years for accrual and follow-up. Among others, the definition of fundamental questions, the more diffuse use of some specialized procedures, the chemotherapy regimen to be used as the control arm, and the role of emerging strategies against systemic NHL should be discussed and a consensus reached. An important dilemma in PCNSL treatment is the choice between strategies designed to increase dose intensity, to improve cure rate, versus strategies of treatment de-escalation, to avoid severe neurotoxicity. In fact, the evaluation of treatment impact on cognitive function and quality of life is a critical issue in these patients. Finally, international clinical trials will also be crucial to share archives of tumor tissue, which are relevant resources to explore new molecular and biological aspects of PCNSL.

References

1. CBTRUS. Statistical report: Primary brain tumors in the United States, 1992-1997. Published by the CBTRUS, 2000.
2. Ferreri AJ, Reni M, Pasini F, et al. A multicenter study of treatment of primary CNS lymphoma. Neurology 2002; 58:1513-20.
3. Deangelis LM, Yahalom J, Heinemann MH, Cirrincione C, Thaler HT, Krol G. Primary CNS lymphoma: combined treatment with chemotherapy and radiotherapy. Neurology 1990;40:80-6.
4. Soussain C, Merle-Beral H, Reux I, Sutton L, Fardeau C, Gerber S, et al. A single-center study of 11 patients with intraocular lymphoma treated with conventional chemotherapy followed by high-dose chemotherapy and autologous bone marrow transplantation in 5 cases. Leuk Lymphoma 1996;23:339-45.
5. Akpek EK, Ahmed I, Hochberg FH, et al. Intraocular-central nervous system lymphoma: clinical features, diagnosis, and outcomes. Ophthalmology 1999; 106:1805-10.
6. Rockwood EJ, Zakov ZN, Bay JW. Combined malignant lymphoma of the eye and CNS (reticulum-cell sarcoma). Report of three cases. J Neurosurg 1984;61:369-74.
7. Qualman SJ, Mendelsohn G, Mann RB, Green WR. Intraocular lymphomas. Natural history based on a clinicopathologic study of eight cases and review of the literature. Cancer 1983;52:878-86.
8. Ferreri AJ, Reni M, Zoldan MC, Terreni MR, Villa E. Importance of complete staging in non-Hodgkin's lymphoma presenting as a cerebral mass lesion. Cancer 1996;77:827-33.
9. O'Neill BP, Illig JJ. Primary central nervous system lymphoma. Mayo Clin.Proc 1989;64:1005-20.
10. Verbraeken HE, Hanssens M, Priem H, Lafaut BA, De Laey JJ. Ocular non-Hodgkin's lymphoma: a clinical study of nine cases. Br J Ophthalmol 1997;81:31-6.
11. O'Neill BP, Dinapoli RP, Kurtin PJ, Habermann TM. Occult systemic non-Hodgkin's lymphoma (NHL) in patients initially diagnosed as primary central nervous system lymphoma (PCNSL): how much staging is enough? J Neurooncol 1995;25:67-71.
12. Nelson DF. Radiotherapy in the treatment of primary central nervous system lymphoma (PCNSL). J Neurooncol 1999;43:241-7.
13. Reni M, Ferreri AJ, Garancini MP, Villa E. Therapeutic management of primary central nervous system lymphoma in immunocompetent patients: results of a critical review of the literature. Ann Oncol 1997; 8:227-34.
14. Ferreri AJ, Reni M, Villa E. Therapeutic management of primary central nervous system lymphoma: lessons from prospective trials. Ann Oncol 2000; 11:927-37.
15. Reni M, Ferreri AJ, Guha-Thakurta N, Blay JY, Dell'Oro S, Biron P, et al. Clinical relevance of consolidation radiotherapy and other main therapeutic issues in primary central nervous system lymphomas treated with upfront high-dose methotrexate. Int J Radiat Oncol Biol Phys 2001;51:419-25.
16. Deangelis LM, Seiferheld W, Schold SC, Fisher B, Schultz CJ. Combination chemotherapy and radiotherapy for primary central nervous system lymphoma: Radiation Therapy Oncology Group Study 93-10. J Clin Oncol 2002;20:4643-8.
17. Abrey LE, Deangelis LM, Yahalom J. Long-term survival in primary CNS lymphoma. J Clin Oncol 1998;16:859-63.
18. O'Brien P, Roos D, Pratt G, Liew K, Barton M, Poulsen M, et al. Phase II multicenter study of brief single-agent methotrexate followed by irradiation in primary CNS lymphoma. J Clin Oncol 2000;18:519-26.
19. Nelson DF, Martz KL, Bonner H, Nelson JS, Newall J, Kerman HD, et al. Non-Hodgkin's lymphoma of the brain: can high dose, large volume radiation therapy improve survival? Report on a prospective trial by the Radiation Therapy Oncology Group (RTOG): RTOG 8315. Int J Radiat Oncol Biol Phys 1992;23:9-17.
20. Laperriere NJ, Cerezo L, Milosevic MF, Wong CS, Patterson B, Panzarella T. Primary lymphoma of brain: results of management of a modern cohort with radiation therapy. Radiother Oncol 1997;43:247-52.
21. Hoang-Xuan K, Delattre JY. Ongoing protocols for non-AIDS primary central nervous system lymphoma. J Neurooncol 1999;43:287-91.
22. Glass J, Gruber ML, Cher L, Hochberg FH. Preirradiation methotrexate chemotherapy of primary central nervous system lymphoma: long-term outcome. J Neurosurg 1994;81:188-95.
23. Cher L, Glass J, Harsh GR, Hochberg FH. Therapy of primary CNS lymphoma with methotrexate-based chemotherapy and deferred radiotherapy: preliminary results. Neurology 1996;46:1757-9.
24. O'Brien PC, Roos DE, Liew KH, Trotter GE, Barton MB, Walker QJ, et al. Preliminary results of combined chemotherapy and radiotherapy for non-AIDS primary central nervous system lymphoma. Trans-Tasman Radiation Oncology Group (TROG). Med J Aus

1996;165:424-7.
25. Guha-Thakurta N, Damek D, Pollack C, Hochberg FH. Intravenous methotrexate as initial treatment for primary central nervous system lymphoma: response to therapy and quality of life of patients. J Neurooncol 1999;43:259-68.
26. Blay JY, Conroy T, Chevreau C, Thyss A, Quesnel N, Eghbali H, et al. High-dose methotrexate for the treatment of primary cerebral lymphomas: analysis of survival and late neurologic toxicity in a retrospective series. J Clin Oncol 1998;16:864-71.
27. Mead GM, Bleehen NM, Gregor A, Bullimore J, Shirley D, Rampling RP, et al. A medical research council randomized trial in patients with primary cerebral non-Hodgkin lymphoma: cerebral radiotherapy with and without cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy. Cancer 2000;89:1359-70.
28. O'Neill BP, O'Fallon JR, Earle JD, Colgan JP, Brown LD, Krigel RL. Primary central nervous system non-Hodgkin's lymphoma: survival advantages with combined initial therapy? Int J Radiat Oncol Biol Phys 1995;33:663-73.
29. Schultz C, Scott C, Sherman W, Donahue B, Fields J, Murray K, et al. Preirradiation chemotherapy with cyclophosphamide, doxorubicin, vincristine, and dexamethasone for primary CNS lymphomas: initial report of radiation therapy oncology group protocol 88-06. J Clin Oncol 1996;14:556-64.
30. Shapiro WR, Voorhies RM, Hiesiger EM, Sher PB, Basler GA, Lipschutz LE. Pharmacokinetics of tumor cell exposure to [14C]methotrexate after intracarotid administration without and with hyperosmotic opening of the blood-brain and blood-tumor barriers in rat brain tumors: a quantitative autoradiographic study. Cancer Res 1988; 48:694-701.
31. Bessell EM, Lopez-Guillermo A, Villa S, Verger E, Nomdedeu B, Petit J, et al. Importance of radiotherapy in the outcome of patients with primary CNS lymphoma: an analysis of the CHOD/BVAM regimen followed by two different radiotherapy treatments. J Clin Oncol 2002; 20:231-6.
32. Glass J, Shustik C, Hochberg FH, Cher L, Gruber ML. Therapy of primary central nervous system lymphoma with pre-irradiation methotrexate, cyclophosphamide, doxorubicin, vincristine, and dexamethasone (MCHOD). J Neurooncol 1996;30:257-65.
33. Dent S, Eapen L, Girard A, Hugenholtz H, DaSilva V, Stewart DJ. PROMACE-MOPP and intrathecal chemotherapy for CNS lymphomas. J Neurooncol 1996; 28: 25-30.
34. Ng S, Rosenthal MA, Ashley D, Cher L. High-dose methotrexate for primary CNS lymphoma in the elderly. Neuro-oncol 2000;2:40-4.
35. Hiraga S, Arita N, Ohnishi T, Kohmura E, Yamamoto K, Oku Y, et al. Rapid infusion of high-dose methotrexate resulting in enhanced penetration into cerebrospinal fluid and intensified tumor response in primary central nervous system lymphomas. J Neurosurg 1999;91:221-30.
36. Balmaceda C, Gaynor JJ, Sun M, Gluck JT, Deangelis LM. Leptomeningeal tumor in primary central nervous system lymphoma: recognition, significance, and implications. Ann Neurol 1995;38:202-9.
37. Bessell EM, Graus F, Punt JA, Firth JL, Hope DT, Moloney AJ, et al. Primary non-Hodgkin's lymphoma of the CNS treated with BVAM or CHOD/BVAM chemotherapy before radiotherapy. J Clin Oncol 1996; 14:945-54.
38. Ferreri AJ, Reni M, Dell'Oro S, Ciceri F, Bernardi M, Camba L, et al. Combined treatment with high-dose methotrexate, vincristine and procarbazine, without intrathecal chemotherapy, followed by consolidation radiotherapy for primary central nervous system lymphoma in immunocompetent patients. Oncology 2001;60:134-40.
39. Khan RB, Shi W, Thaler HT, Deangelis LM, Abrey LE. Is intrathecal methotrexate necessary in the treatment of primary CNS lymphoma? J Neurooncol 2002;58:175-8.
40. Sandor V, Stark-Vancs V, Pearson D, Nussenblat R, Whitcup SM, Brouwers P, et al. Phase II trial of chemotherapy alone for primary CNS and intraocular lymphoma. J Clin Oncol 1998;16:3000-6.
41. Freilich RJ, Delattre JY, Monjour A, Deangelis LM. Chemotherapy without radiation therapy as initial treatment for primary CNS lymphoma in older patients. Neurology 1996;46:435-9.
42. Abrey LE, Yahalom J, Deangelis LM. Treatment for primary CNS lymphoma: the next step. J Clin Oncol 2000; 18:3144-50.
43. Char DH, Ljung BM, Deschenes J, Miller TR. Intraocular lymphoma: immunological and cytological analysis. Br J Ophthalmol 1988;72:905-11.
44. Freeman LN, Schachat AP, Knox DL, Michels RG, Green WR. Clinical features, laboratory investigations, and survival in ocular reticulum cell sarcoma. Ophthalmology 1987;94:1631-9.
45. Fine HA, Mayer RJ. Primary central nervous system lymphoma. Ann Intern Med 1993;119:1093-104.
46. Whitcup SM, de Smet MD, Rubin BI, Palestine AG, Martin DF, Burnier M Jr, et al. Intraocular lymphoma. Clinical and histopathologic diagnosis. Ophthalmology 1993;100:1399-406.
47. Henson JW, Yang J, Batchelor T. Intraocular methotrexate level after high-dose intravenous infusion. J Clin Oncol 1999;17:1329.
48. Ferreri AJ, Blay JY, Reni M, Pasini F, Gubkin A, Tirelli U, et al. Relevance of intraocular involvement in the management of primary central nervous system lymphomas. International Extranodal Lymphoma Study Group (IELSG). Ann Oncol 2002;13:531-8.
49. Fishburne BC, Wilson DJ, Rosenbaum JT, Neuwelt EA. Intravitreal methotrexate as an adjunctive treatment of intraocular lymphoma. Arch Ophthalmol 1997; 115: 1152-6.
50. Smith JR, Rosenbaum JT, Wilson DJ, Doolittle ND, Siegal T, Neuwelt EA, et al. Role of intravitreal methotrexate in the management of primary central nervous system lymphoma with ocular involvement. Ophthalmology 2002;109:1709-16.
51. Doolittle ND, Miner ME, Hall WA, Siegal T, Jerome E, Osztie E, et al. Safety and efficacy of a multicenter study using intraarterial chemotherapy in conjunction with osmotic opening of the blood-brain barrier for the treatment of patients with malignant brain tumors. Cancer 2000;88:637-47.
52. Kraemer DF, Fortin D, Neuwelt EA. Chemotherapeutic dose intensification for treatment of malignant brain tumors: Recent developments and future directions. Curr Neurol Neurosci Rep 2002;2:216-24.
53. Tyson RM, Siegal T, Doolittle ND, Lacy C, Kraemer DF, Neuwelt EA. Current status and future of relapsed primary central nervous system lymphoma (PCNSL). Leuk Lymphoma (in press).
54. Abrey L, Moskowitz C, Mason W. A phase II study of intensive methotrexate and cytarabine followed by high dose beam chemotherapy with autologous stem cell transplantation (ASCT) in patients with newly diagnosed primary central nervous system lymphoma (PCNSL). Proc Am Soc Clin Oncol 2001; 20:53 [abstract].
55. Illerhaus G, Marks R, Derigs G. High-dose-chemotherapy with autologous PBSCT and hyperfractionated radiotherapy as first-line treatment for primary CNS lymphoma (PCNSL). Update of a multicenter phase II Study. Onkologie 2001;54:14[abstract].
56. Soussain C, Suzan F, Hoang-Xuan K, Cassoux N, Levy V, Azar N, et al. Results of intensive chemotherapy followed by hematopoietic stem-cell rescue in 22 patients with refractory or recurrent primary CNS lymphoma or intraocular lymphoma. J Clin Oncol 2001;19:742-9.
57. Pollack IF, Lunsford LD, Flickinger JC, Dameshek HL. Prognostic factors in the diagnosis and treatment of primary central nervous system lymphoma. Cancer 1989; 63:939-47.
58. Deangelis LM, Yahalom J, Thaler HT, Kher U. Combined modality therapy for primary CNS lymphoma. J Clin Oncol 1992;10:635-43.
59. Reni M, Ferreri AJ, Villa E. Second-line treatment for primary central nervous system lymphoma. Br J Cancer

1999;79:530-4.
60. Boiardi A, Silvani A, Pozzi A, Fariselli L, Broggi G, Salmaggi A. Chemotherapy is effective as early treatment for primary central nervous system lymphoma. J Neurol 1999;246:31-7.
61. Khalfallah S, Stamatoullas A, Fruchart C, Proust F, Delangre T, Tilly H. Durable remission of a relapsing primary central nervous system lymphoma after autologous bone marrow transplantation. Bone Marrow Transplant 1996; 18:1021-3.
62. Varadi G, Or R, Kapelushnik J, Naparstek E, Nagler A, Brautbar C, et al. Graft-versus-lymphoma effect after allogeneic peripheral blood stem cell transplantation for primary central nervous system lymphoma. Leuk Lymphoma 1999;34:185-90.
63. Lawler BE, Betensky R, Hochberg F, Batchelor TT. Primary central nervous system lymphoma: the MGH experience 1987-2001. Proc Am Soc Clin Oncol 2002; 21:75a[abstract].
64. Blay JY, Lasset C, Carrie C, Chauvin F, Coiffier B, Giselbrecht C, et al. Multivariate analysis of prognostic factors in patients with non HIV-related primary cerebral lymphoma. A proposal for a prognostic scoring. Br J Cancer 1993;67:1136-41.
65. Ferreri AJ, Blay JY, Reni M, Pasini F, Spina M, Ambrosetti A, et al. Prognostic scoring system for primary CNS lymphomas: the International Extranodal Lymphoma Study Group experience. J Clin Oncol 2003;21:266-72.
66. Ponzoni M, Berger F, Chassagne C, Tinguely M, Jouvet A. Reactive perivascular T-cell infiltrate is an independent favorable prognostic factor in primary central nervous system lymphomas (PCNSL) in immunocompetent patients. Ann Oncol 2002;13:264[abstract].
67. Braaten KM, Betensky RA, de Leval L, Okada Y, Hochberg FH, Louis DN, et al. BCL-6 expression predicts improved survival in patients with primary central nervous system lymphoma. Clin Cancer Res 2003;9:1063-9.

Primary testicular lymphoma

UMBERTO VITOLO

Primary malignant lymphoma of the testis (PTL) is a rare diseases that represents 1% to 2% of all non-Hodgkin's lymphomas, with an estimated incidence of 0.26/100,000 per year. The first case was reported by Malassez in 1877. PTLs account for no more than 5% of all testicular malignancies, altough they represent the most frequent testicular cancer in men older than 50 years of age.[1,2] PTL is typically a disease of the elderly, 85% of PTLs are diagnosed in men over 60 years old. The most common clinical presentation of PTL is a unilateral, painless scrotal swelling, rarely with sharp scrotal pain. Systemic conventional symptoms such as fever, night sweats, weight loss are usually present only in advanced stages in 25% to 41% of patients.[3,4] On physical examination there is usually a unilateral non-tender firm mass. Bilateral testicular involvement may be synchronous at diagnosis or, more frequently, asynchronous during the course of the disease. Bilateral involvement has been described in up to 35% of patients.[3,4] Monoclonal lymphoid cells have been shown in the contralateral testis in PTL patients, suggesting that bilateral testicular involvement is a pattern of a disease of the same origin.[5] Peculiar molecular features have been described in PTL such as somatic hypermutation of immunoglobulin heavy-chain gene, indicating a possible antigen-driven stimulation, analogous to what is seen in extranodal marginal zone lymphoma.[6]

Diagnosis

Histologically, 80% to 90% of PTLs are of diffuse large-cell type (DLCL) with B-cell phenotype, but isolated cases of other histologic subtypes have been described such as Burkitt and Burkitt's-like types in 10-20% of cases, mainly in HIV+ patients. Rarely T-cell or follicular lymphomas have been reported.[7] Ultrasound is the initial investigation of choice. For histopathologic diagnosis orchiectomy is the method of choice to obtain tissue and is better than fine needle ultrasound guided biopsy. Orchiectomy not only provides better histologic definition but it also removes the main tumor mass allowing good local tumor control.[8]

Staging

Testicular lymphoma may involve only the testicle or also structures within the scrotum and regional retroperitoneal lymph nodes. PTL has a propensity to disseminate systematically to several extranodal sites including the contralateral testis, central nervous system (CNS), skin, Waldeyer's ring, lung, pleura and soft tissue. Involvement of Waldeyer's ring is enigmatic. This may be because of a common embryonic origin, since the testis, oropharynx and nasopharynx are derived from the endoderm. Involvement of these sites may occur either concurrently or subsequently during the course of the disease. These data explain the high rate of relapses, the majority of them occurring in extranodal sites.

The disease is localized and in stages I and II in 70-80% of the patients (50-60% stage I and 20-30% stage II). Stage III is very rare (3-5%), whereas the precise incidence of stage IV is not easy to assess. A stage IV PTL is virtually undistinguishable from a nodal advanced stage lymphoma with testicular involvement. The rate of testicular involvement in advanced stage DLCL is 10-18% and 10-29% in Burkitt's lymphoma. In order to separate these two entities, a PTL is usually defined if the testicular mass is the primary site of the disease or the main site of involvement.

PTLs are staged according to Ann Arbor criteria with a few modifications:

Stage IE: unilateral or bilateral testicular involvement.

Stage IIE: unilateral or bilateral testicular involvement with locoregional lymph nodes (iliac and/or lumbo-aortic).

Advanced stage III/IV: unilateral or bilateral testicular involvement with involvement of distant lymph nodes and/or extranodal sites.

Staging procedures in PTL are similar to those applied in nodal lymphomas with some peculiar features. A thorough evaluation to determine the extent of lymphomatous involvement should be made, focusing specially to the central nervous system, contralateral testis, skin and Waldeyer's ring.

From the U.O.A. Ematologia, ASO S.Giovanni Battista, Torino.

Correspondence: Umberto Vitolo, U.O.A. Ematologia, ASO S.Giovanni Battista, Torino.

Treatment

The series reported so far include few patients with different clinical features and different therapeutic approaches. Orchiectomy alone has been advocated as the sole modality of therapy in stage IE patients. Although occasionally long-term survival may be achieved with orchiectomy alone, the majority of the patients have relapsed within two years suggesting that widespread microscopic disease is present at diagnosis.[9] PTL behaves aggressively with a poor outcome. Five-year survival ranged from 16% to 50% and median survival has been reported to be only 12-24 months in the different series of patients.[1-3,8] Radiation therapy on lomboaortic or iliac lymph nodes after orchiectomy has been used in stage IE and IIE patients. The results, however, have been disappointing. Despite a high response rate, the overall relapse rate exceeds 70%. In most cases relapses occurr in extranodal sites such as the CNS, skin, lung, pleura, soft tissue, and Waldeyer's ring.[10,11] One of the peculiar feature of PTL is contralateral testis relapse occurring in 5-35% of the patients.[4] Moreover CNS relapses are definitely more common than in other aggressive lymphomas and they have been reported up to 30% of the patients within 1-2 years from diagnosis. However occasionally late relapse have also been described, sometimes as CNS relapse alone.[12] CNS failures may occur both in brain parenchyma and in meninges.

Adjuvant chemotherapy in PTL was introduced in the 1980s. Doxorubicin-containing regimens have improvemed in the relapse-free survival compared to the relapse-free survival following orchiectomy ± radiotherapy, although the advantage on survival duration varied a lot among the different series published so far.[11,13,14] In a study of BCCA from Vancouver, patients treated with brief ACOPB or CHOP chemotherapy for three courses after orchiectomy and involved field radiotherapy to the scrotum ± pelvic and paraaortic lymph nodes, if involved, had a better outcome than a historical control group treated with only orchiectomy ± radiotherapy, with a relapse free-survival and overall survival of 93% versus 50%.[13] However the outcome was not so good in other series even with the use of CHOP-like regimens with median survival times ranging between 50 to only a few month.[12,14,15] The majority of patients with PTL relapse despite complete response to initial treatment. The pattern of relapse depends on previous primary treatment. When radiation is given to the retroperitoneal lymph nodes, failures are systemic. Very few cases of in-field relapses have been reported. After chemotherapy both systemic and regional relapses are see. Most relapses occurr in the first two years, but late relapses have also been described.[12,14]

Contralateral testicular relapses have been reported frequently.[4] In order to prevent this type of recurrence, prophylactic radiotherapy of the other testis has been administered at different doses (25-36 Gy). At the Princess Margaret Hospital in a group of 26 patients that were given scrotal radiotherapy, no contralateral testis relapse was observed.[16] The dose of prophylactic testicular radiotherapy has not yet been defined. This approach is feasible with low toxicity also in elderly patients and could reduce the risk of failure at this site.[12,17]

The high rate of CNS recurrence is troublesome and has led to a recommendation for routine CNS prophylaxis. Although the use of prophylactic intrathecal chemotherapy has been advocated, its value is controversial because CNS relapses occur more frequently in brain parenchyma than in meninges and also in patients who had received intrathecal chemotherapy.[10,12,18] Perhaps the incorporation of chemotherapy agents that have a better penetration into the CNS, as high-dose methotrexate, would allow a more effective control of the disease and may prevent CNS recurrence. However this type of chemotherapy is not easy to administer in elderly patients, as those with PTL usually are.

The rarity of PTL has prevented carefully designed prospective trials, thus treatment has not been standardized and the optimal management of the disease remains undefined. In order to clarify the natural history of PTL a retrospective international survey of patients with DLCL of the testis was co-ordinated by the International Extranodal Lymphoma Study Group (IELSG).[19]

IELSG retrospective study

The IELSG has recently completed a retrospective international survey of 373 adult patients with histologically confirmed aggressive non-lymphoblastic primary testicular lymphoma from 22 tertiary cancer canters and one co-operative group. The median age at presentation was 66 years (range 19-91 years). Most patients presented with limited stage disease (Ann Arbor stage I-II in 79%) and low to low-intermediate risk score according to the International Prognostic Index (IPI) in 81% of cases. Combination chemotherapy was administered to 279 (75%) patients and contained an anthracycline agent in 255 (68%). Prophylactic intrathecal chemotherapy was administered to 68 patients (18%). The outcome of patients was extremely poor with actuarial 5- and 10-year OS of 48% and 27% and actuarial 5- and 10-year progression-free survival (PFS) of 48% and 33%, respectively. The survival and PFS curves showed no clear evidence of a plateau, suggesting no cure

for patients affected by primary testicular lymphoma, even for those presenting with localized stage I/II (5- and 10-year OS in stage I 58% and 29%, stage II 46% and 29%). Fifty-two per cent of the patients relapsed, mainly in extranodal sites (72%). The commonest sites of relapse were: CNS (5 and 10-year risk of CNS relapse, 20% and 35%) and contralateral testis (15% at 3 years, 40% at 15 years) occurring in patients not receiving prophylactic scrotal radiotherapy. Clinical features significantly associated with a longer overall survival in multivariate analysis were: low/low-intermediate IPI score, no B symptoms, anthracycline-containing regimens, and prophylactic scrotal radiotherapy. Adequate CNS prophylaxis was associated with an improvement in progression-free survival, although it was not shown to prevent CNS relapse, perhaps because this prophylaxis was delivered to few patients. Retrospectively in this series, a limited number of 34 patients were selected who had received adequate treatment with systemic CHOP-like chemotherapy with contralateral testis and CNS prophylaxis with scrotal radiotherapy and intrathecal chemotherapy. These patients appeared to have a better outcome with a 3-years overall survival of 88%.

Testicular lymphoma is thus a unique extranodal presentation of DLCL. Unlike primary gastric lymphoma, patients with testicular lymphoma appear to have a poor prognosis and similar to that of patients who have primary central nervous system lymphoma. Given the rarity of the disease randomized trials to clarify the unsolved treatment issues are impossible. Hence international collaboration is essential to define the optimal management of PTL. Based on the results of its restrospective study, the IELSG has planned a prospective phase II trial in localized stage I-II primary testicular diffuse large cell lymphoma to evaluate the efficacy and toxicity of a combined treatment of CHOP with rituximab, intrathecal methotrexate and prophylactic scrotal radiotherapy or, for stage II patients, loco-regional radiotherapy. The study design is intended to define the standard therapy for this rare type of lymphoma in order to improve the outcome of these patients with the use of systemic chemotherapy along with CNS and contralateral testicular prophylaxis, thus helping to define a guideline treatment useful for future comparison.

References

1. Malassez M. Lymphadenome du testicle. Bull Soc Anta Paris 1877;52:176-8.
2. Zucca E, Roggero E, Bertoni F, Cavalli F. Primary extranodal non-Hodgkin's lymphomas. Part 1: gastrointestinal, cutaneous and genitourinary lymphomas. Ann Oncol. 1997;8:727-37.
3. Moller MB, D'Amore F, Christensen BE. Testicular lymphoma: A population-based study of incidence, clinicopathological correlations and prognosis. Eur J Cancer 1994;30A:1760-4.
4. Shahab N, Doll DC. Testicular lymphoma. Semin Oncol 1999; 6:259-69.
5. Leite KR, Garicochea B, Srougi M, Dzik C, Nesralhah L, de Moura RC, et al. Monoclonality of asynchronous bilateral lymphoma of the testis. Eur Urol 2000;38:774-7.
6. Hyland J, Lasota J, Jasinski M, Petersen RO, Nordling S, Miettinen M, et al. Molecular pathological analysis of testicular diffuse large cell lymphomas. Hum Pathol 1998;29:1231-9.
7. Ferry JA, Harris NL, Young RH, Coen J, Zietman A, Scully RE. Malignant lymphoma of the testis, epididymis, and spermatic cord. A clinicopathologic study of 69 cases with immunophenotypic analysis. Am J Surg Pathol 1994;18:376-90.
8. Salem YH, Miller HC. Lymphoma of genitourinary tract. J Urol 1994;151:1162-70.
9. Ciatto S, Cionini L. Malignant lymphoma of the testis. Acta Radiol Oncol Radiat Phys Biol 1979;18:572-6.
10. Fonseca R, Habermann TM, Colgan JP, O'Neill BP, White WL, Witzig TE, et al. Testicular lymphoma is associated with a high incidence of extranodal recurrence. Cancer 2000; 88:154-61.
11. Zietman AL, Coen JJ, Ferry JA, Scully RE, Kaufman DS, McGovern FG, et al. The management and outcome of stage IAE nonHodgkin's lymphoma of the testis. J Urol 1996;155:943-6.
12. Touroutoglou N, Dimopoulos MA, Younes A, Hess M, Pugh W, Cox J, et al. Testicular lymphoma: late relapses and poor outcome despite doxorubicin-based therapy. J Clin Oncol 1995; 13:1361-7.
13. Connors JM, Klimo P, Voss N, Fairey RN, Jackson S. Testicular lymphoma: improved outcome with early brief chemotherapy. J Clin Oncol 1988;6:776-81.
14. Tondini C, Ferreri AJ, Siracusano L, Valagussa P, Giardini R, Rampinelli I, et al. Diffuse large-cell lymphoma of the testis. J Clin Oncol 1999;17:2854-8.
15. Sasai K, Yamabe H, Tsutsui K, Dodo Y, Ishigaki T, Shibamoto Y, et al. Primary testicular non-Hodgkin's lymphoma: a clinical study and review of the literature. Am J Clin Oncol 1997;20:59-62.
16. Lippuner T, Gospodarowicz M, Pintilie M. Testicular lymphoma, pattern of failure after long-term follow-up. VII International Conference on Malignant Lymphoma, Lugano, Switzerland. Ann Oncol 1999;10-63 Suppl 3:[abstract 213].
17. Pectasides D, Economopoulos T, Kouvatseas G, Antoniou A, Zoumbos Z, Aravantinos G, et al. Anthracycline-based chemotherapy of primary non-Hodgkin's lymphoma of the testis: the hellenic cooperative oncology group experience. Oncology 2000;58:286-92.
18. Chua SL, Seymour JF, Streater J, Wolf MM, Janusewicz EH, Prince HM. Conventional prophylactic intrathecal chemotherapy is ineffective for the prevention of central nervous system (CNS) recurrence in patients with high-risk intermediate-grade non-Hodgkin's lymphoma (NHL). Proc Haematol Soc Aust N Z 2000;148.
19. Zucca E, Conconi A, Mughal TI, Sarris AH, Seymour JF, Vitolo U, et al. Patterns of outcome and prognostic factors in primary large-cell lymphoma of the testis in a survey by the International Extranodal Lymphoma Study Group. J Clin Oncol 2003;21:20-7.

Primary mediastinal (thymic) large B-cell lymphoma

FABIO MENESTRINA, MARCO CHILOSI, MAURIZIO LESTANI, ALBERTO ZAMÒ, ALDO SCARPA

Primary mediastinal large B-cell lymphoma (PMLBL) is a peculiar type of diffuse large B-cell lymphoma arising in the mediastinum and characterized by distinctive clinical, immunophenotypic and genotypic features.[2,12,19,20,22,35] Its acceptance as a separate entity was delayed until the second half of the 1980s when, in spite of the thymic localization of the neoplastic mass, immunhistochemical analysis documented the B-cell phenotype of the neoplastic cells.

PMLBL is an uncommon but not rare entity, accounting for about 2-3% of non-Hodgkin's lymphomas with a worldwide distribution; it occurs predominantly in young adults (third and fourth decade), with a slight female predominance.[1,5,8,17]

At presentation, the disease affects the antero-superior area of the mediastinum without superficial lymphadenopathy or hepato-splenomegaly but a supraclavicular extension, in contiguity with the mediastinal mass, is sometimes observed.[2,20,22] The mass is often *bulky* (>10cm in diameter) and can be locally invasive, infiltrating intra-thoracic structures such as lung, pleura, thoracic wall and pericardium. At progression, PMLBCL disseminates to extra-thoracic regions:[5] liver, kidney, adrenal are the most frequent sites of parenchymal involvement; retro-peritoneal lymph nodes, pancreas, gastro-intestinal tract, ovary, and central nervous system[4] are other reported sites. Bone marrow involvement is extremely rare.

Signs and symptoms are related to the mediastinal mass: superior vena cava syndrome (most frequently), airway obstruction, pleural and/or pericardial effusion. Systemic symptoms such as fever and weight loss or sweats or asthenia can also be present.[17] Imaging techniques are important in detecting the mass, in documenting the involvement of other intra-thoracic structures and in deciding on the best approach to obtain a diagnostic biopsy.

From the Azienda Ospedaliera Istituti Ospitalieri di Verona, Piazzale Aristide Stefani, 1, Verona.

Correspondence: Professor Fabio Menestrina, Dipartimento di Anatomia e Istologia Patologica, Ospedale Policlinico Gianbattista Rossi, Verona.

Radical surgery is not the procedure of choice. Diagnostic features might be lacking in small biopsies with crush artefacts or when only sclerosing tissue and/or necrosis is obtained.

Histologically, there is diffuse pattern of growth. PMLBCL has a broad range of cytomorphology: the cells may be medium-sized to (very) large, have abundant, frequently clear cytoplasm and irregularly round or ovoid (sometimes multilobated) nuclei usually with small nucleoli.[26] Mitotic activity is high. A variable number of reactive cells, such as lymphocytes, macrophages and granulocytes, may be present at the periphery of the mass. A frequent but not consistent feature is a distinctive fibrosis made up of irregular collagen bands compartmentalizing cellular areas of varying size.[20,22,26] The combination of different architectural patterns and cellular morphology of PMLBCL may be suggestive of thymoma, seminoma or Hodgkin's lymphoma.

Depending on the surgical approach and specimen size, thymic remnants can be observed, usually better highlighted by immunohistology. Lung, pleura and pericardial infiltration can also be present.

Immunophenotypically, PMLBCL expresses, in addition to the pan-leukocyte marker CD45, B-cell lineage-specific surface molecules such as CD19, CD20, CD22,[25] and the immunoglobulin-associated CD79a[27] molecule, but not lineage-restricted T-cell antigens, except for MAL,[6] not observed in other diffuse large B-cell lymphomas. CD10 is usually not expressed,[25,24] CD15 and CD21 are always negative. Molecules often found in/on PMLBCL cells, such as CD38, PC-1, MUM1 and PAX5[27] in the absence of CD138 favor a pre-plasma cell stage of maturation. With a few exceptions, PMLBCL does not express immunoglobulin (Ig).[20,22] In fact, the discrepancy between the lack of Ig and the constitutive CD79a[15] is characteristic of this disease. The lack of Ig expression is probably not related to a defect in Ig transcriptional machinery since Ig transcription factors such as Oct2 and BoB.1 are expressed.[27] Furthermore, there is frequently a defect of HLA class and/or II molecule expression.[23] CD30 expression, which is mainly restricted to subsets of tumor cells, has been observed in PMLBCL, especially when antigen retrieval techniques are used.[9] This characteristic draws attention to the differentiation between PMLBCL, Hodgkin's disease, and the so-called *gray zone* lymphomas of the mediastinum.[29]

Genetics

PMLBCL have been examined for alterations in genes commonly altered in other hematopoetic malignancies, including c-myc, $p16^{INK4}$, p53, bcl-1, bcl-2, bcl-6 and N-ras, as well as for the presence of EBV infection. The data on frequencies of BCL-6 mutations in MLBL are conflicting.[27,7,34] Bcl-2 is germ line, Bcl-1and N-ras are not altered while p16, c-myc, and p53 occasionally carry mutations.[27,31,32] As in other diffuse large B-cell lymphomas, Ig heavy-chain and light-chain genes have high loads of mutations.[11,16,18,30] Furthermore, the vast majority of heavy-chain V genes are potentially functional, showing evidence of selection for a functional antibody. Intraclonal variation has not been detected in the PMLBCL cases analyzed so far, indicating that continuing mutational activity is not a prominent feature.[18]

Different molecular genetic approaches have yielded a highly characteristic pattern of genomic alterations in PMLBCL: chromosomal gains (2p, 6p, 7q, 9p, 12, and X) are much more frequent than losses.[14,28,33] Most important is $9p^+$ which is detectable in up to 75 % of cases[3] and represents a chromosomal marker of PMLBCL, since 9^+, is very rare in other nodal and extranodal B-cell lymphomas but, interestingly, is detectable in about 25% of classic Hodgkin's disease.[13] Second essential genomic region in PMLBCL is chromosome X: aberrations, including high levels of DNA amplification, are present in up to 87% of cases of PMLBCL.[3]

Histologically, PMLBCL has been attributed to the asteroid variant of thymic medullary B-cells.[10] It is unrelated to EBV or other known tumor viruses.[21,34]

So far, there are no histologic,[26] immunophenotypic or genotypic features that are known to have prognostic potential.

References

1. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood 1997;89:3909-18.
2. Addis BJ, Isaacson PG. Large cell lymphoma of the mediastinum: a B-cell tumour of probable thymic origin. Histopathology 1986;10:379-90.
3. Bentz M, Barth TF, Bruderlein S, Bock D, Schwerer MJ, Baudis M, et al. Gain of chromosome arm 9p is characteristic of primary mediastinal b-cell lymphoma (MBL): Comprehensive molecular cytogenetic analysis and presentation of a novel MBL cell line. Genes Chromosomes Cancer 2001;30:393-401.
4. Bishop PC, Wilson WH, Pearson D, Janik J, Jaffe ES, Elwood PC. CNS involvement in primary mediastinal large B-cell lymphoma. J Clin Oncol 1999;17:2479-85.
5. Cazals-Hatem D, Lepage E, Brice P, Ferrant A, d'Agay MF, Baumelou E, et al. Primary mediastinal large B-cell lymphoma. A clinicopathologic study of 141 cases compared with 916 nonmediastinal large B-cell lymphomas, a GELA ("Groupe d'Etude des Lymphomes de l'Adulte") study. Am J Surg Pathol 1996;20:877-88.
6. Copie-Bergman C, Gaulard P, Maouche-Chretien L, Briere J, Haioun C, Alonso MA, et al. The MAL gene is expressed in primary mediastinal large B-cell lymphoma. Blood 1999;94:3567-75.
7. de Leval L, Ferry JA, Falini B, Shipp M, Harris NL. Expression of bcl-6 and CD10 in primary mediastinal large B-cell lymphoma: evidence for derivation from germinal center B cells? Am J Surg Pathol 2001;25:1277-82.
8. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994;84:1361-92.
9. Higgins JP, Warnke RA. CD30 expression is common in mediastinal large B-cell lymphoma. Am J Clin Pathol 1999;112:241-7.
10. Hofmann WJ, Momburg F, Moller P. Thymic medullary cells expressing B lymphocyte antigens. Hum Pathol 1988;19:1280-7.
11. Hsu FJ, Levy R. Preferential use of the VH4 Ig gene family by diffuse large-cell lymphoma. Blood 1995;86:3072-82.
12. Jaffe ES, Harris NL, Stein H, Vardiman J. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press: Lyon. 2001.
13. Joos S, Menz CK, Wrobel G, Siebert R, Gesk S, Ohl S, et al. Classical Hodgkin lymphoma is characterized by recurrent copy number gains of the short arm of chromosome 2. Blood 2002;99:1381-7.
14. Joos S, Otano-Joos MI, Ziegler S, Bruderlein S, du Manoir S, Bentz M, et al. Primary mediastinal (thymic) B-cell lymphoma is characterized by gains of chromosomal material including 9p and amplification of the REL gene. Blood 1996;87:1571-8.
15. Kanavaros P, Gaulard P, Charlotte F, Martin N, Ducos C, Lebezu M, et al. Discordant expression of immunoglobulin and its associated molecule mb-1/CD79a is frequently found in mediastinal large B cell lymphomas. Am J Pathol 1995;146:735-41.
16. Kuppers R, Rajewsky K, Hansmann ML. Diffuse large cell lymphomas are derived from mature B cells carrying V region genes with a high load of somatic mutation and evidence of selection for antibody expression. Eur J Immunol 1997;27:1398-405.
17. Lazzarino M, Orlandi E, Paulli M, Strater J, Klersy C, Gianelli U, et al. Treatment outcome and prognostic factors for primary mediastinal (thymic) B-cell lymphoma: a multicenter study of 106 patients. J Clin Oncol 1997;15:1646-53.
18. Leithauser F, Bäuerle M, Huynh MQ, Möller P. Isotype-switched immunoglobulin genes with a high load of somatic hypermutation and lack of ongoing mutational activity are prevalent in mediastinal B-cell lymphoma. Blood 2001;98:2762-70.
19. Levitt LJ, Aisenberg AC, Harris NL, Linggood RM, Poppema S. Primary non-Hodgkin's lymphoma of the mediastinum. Cancer 1982;50:2486-92.
20. Menestrina F, Chilosi M, Bonetti F, Lestani M, Scarpa A, Novelli P, et al. Mediastinal large-cell lymphoma of B-type, with sclerosis: histopathological and immunohistochemical study of eight cases. Histopathology 1986;10:589-600.
21. Moller P, Bruderlein S, Strater J, Leithauser F, Hasel C, Bataille F, et al. MedB-1, a human tumor cell line derived from a primary mediastinal large B-cell lymphoma. Int J Cancer 2001;92:348-53.
22. Moller P, Lammler B, Eberlein-Gonska M, Feichter GE, Hofmann WJ, Schmitteckert H, et al. Primary mediastinal clear cell lymphoma of B-cell type. Virchows Arch A Pathol Anat Histopathol 1986;409:79-92.
23. Möller P, Lammler B, Herrmann B, Otto HF, Moldenhauer G, Momburg F. The primary mediastinal clear cell lymphoma of B-cell type has variable defects in MHC antigen expression. Immunology 1986;59:411-7.
24. Möller P, Matthaei-Maurer DU, Hofmann WJ, Dorken B, Moldenhauer G. Immunophenotypic similarities of mediastinal clear-cell lymphoma and sinusoidal (monocytoid) B cells. Int J Cancer 1989;43:10-6.
25. Möller P, Moldenhauer G, Momburg F, Lammler B, Eberlein-Gonska M, Kiesel S, et al. Mediastinal lym-

phoma of clear cell type is a tumor corresponding to terminal steps of B cell differentiation. Blood 1987;69: 1087-95.
26. Paulli M, Strater J, Gianelli U, Rousset MT, Gambacorta M, Orlandi E, et al. Mediastinal B-cell lymphoma: a study of its histomorphologic spectrum based on 109 cases. Hum Pathol 1999;30:178-87.
27. Pileri SA, Gaidano G, Zinzani PL, Falini B, Gaulard P, Zucca E, et al. Primary mediastinal B-cell lymphoma: high frequency of BCL-6 mutations and consistent expression of the transcription factors OCT-2, BOB.1, and PU.1 in the absence of immunoglobulins. Am J Pathol 2003;162:243-53.
28. Rigaud G, Moore PS, Taruscio D, Scardoni M, Montresor M, Menestrina F, et al. Alteration of chromosome arm 6p is characteristic of primary mediastinal B-cell lymphoma, as identified by genome-wide allelotyping. Genes Chromosomes Cancer 2001;31:191-5.
29. Rudiger T, Jaffe ES, Delsol G, deWolf-Peeters C, Gascoyne RD, Georgii A, et al. Workshop report on Hodgkin's disease and related diseases ('grey zone' lymphoma). Ann Oncol 1998;9 Suppl 5:S31-S8.
30. Scarpa A, Bonetti F, Menestrina F, Menegazzi M, Chilosi M, Lestani M, et al. Mediastinal large-cell lymphoma with sclerosis. Genotypic analysis establishes its B nature. Virchows Arch A Pathol Anat Histopathol 1987; 412:17-21.
31. Scarpa A, Borgato L, Chilosi M, Capelli P, Menestrina F, Bonetti F, et al. Evidence of c-myc gene abnormalities in mediastinal large B-cell lymphoma of young adult age. Blood 1991;78:780-8.
32. Scarpa A, Moore PS, Rigaud G, Inghirami G, Montresor M, Menegazzi M, et al. Molecular features of primary mediastinal B-cell lymphoma: involvement of p16INK4A, p53 and c-myc. Br J Haematol 1999;107: 106-13.
33. Scarpa A, Taruscio D, Scardoni M, Iosi F, Paradisi S, Ennas MG, et al. Nonrandom chromosomal imbalances in primary mediastinal B-cell lymphoma detected by arbitrarily primed PCR fingerprinting. Genes Chromosomes Cancer 1999;26:203-9.
34. Tsang P, Cesarman E, Chadburn A, Liu YF, Knowles DM. Molecular characterization of primary mediastinal B cell lymphoma. Am J Pathol 1996;148:2017-25.
35. Yousem SA, Weiss LM, Warnke RA. Primary mediastinal non-Hodgkin's lymphomas: a morphologic and immunologic study of 19 cases. Am J Clin Pathol 1985; 83:676-80.

Is CHOP the best treatment for mediastinal large B-cell lymphoma?

GIUSEPPE TODESCHINI

Mediastinal Large B-Cell Lymphoma (MLBCL) was first reported as a possible distinctive NHL entity in the 80s.

MLBCL was finally defined as an entity in aggressive lymphoma by REAL and WHO classifications.[1,2]

Its peculiarity comes from the histologic pattern and clinical characters.

Histology is characterized by large cell with clear cytoplasm, divided by bands of fine compartimentalizing sclerosis. The mytotic rate is high.

Clinically, it is characterized by:

- young age of patients;
- female prevalence;
- bulky mediastinal involvement with frequent SVCS;
- frequent pleural / pericardial effusion;
- peculiar tropism for some organs when lymphoma overpasses the diaphragm;
- extremely rare bone marrow involvement;
- aggressive behaviour.

The initial therapeutic experiences were dismal as reported in the first series.[3]

The introduction in the late 80's of third generation regimens (notably MACOP-B)[4] seemed to improve its outcome.

In our first experience, we observed significantly better outcome in patients treated with MACOP-B as respect to those treated with CHOP.[5]

Although in a large randomized study[6] in DLC NHL no difference emerged among CHOP and other more intensive regimens, nevertheless this seems not to be the case in MLBCL. These findings came from various retrospective studies- the only available so far in the literature- where the results of third generation therapies were superior to CHOP.

From the Department of Hematology, Verona University School of Medicine, Ospedale Policlinico "G.B. Rossi", Piazzale A. Scuro,10, 37134, Verona. Fax: international +39.04.501807.

Correspondence: Giuseppe Todeschini, MD, Department of Hematology, Verona University School of Medicine, Ospedale Policlinico "G.B. Rossi", Verona. E-mail: giuseppe.todeschini1@tin.it

We recently collected data of 138 consecutive pts with newly diagnosed MLBCL treated with CHOP or MACOP-B/ VACOP-B from 14 Italian Centers.

The study was aimed:

- to assess the response and the long-term outcome of CHOP vs MACOP-B/VACOP-B
- to verify the possible role of IF-RT on the mediastinum after CR achievement.

Of note, the clinical characters and the IPI risk factors were balanced between the two chemotherapy arms which were uniformely distributed over time, thus avoiding biases due to different supportive care.

Significantly better results were obtained with MACOP-B/VACOP-B therapies as compared to CHOP. CR were 80 % with MACOP-B/VACOP-B and 51.7% with CHOP ($p < 0.001$).

After a median follow-up of 66.5 months (1^{-}–199^{+}), Event-Free patients were 75.7% with MACOP-B/VACOP-B, vs 39.5% with CHOP ($p<0.001$).

Patients who received IF-RT on the mediastinum after CR had a better outcome than those who received CT alone ($p=0.04$). The best results were observed in patients who received MACOP-B/VACOP-B+ IF RT.

At the multivariate analysis, achievement of CR and type of chemotherapy retained significant value for OS and EFS (*unpublished data*).

Similar results were reported by Zinzani in a large multicenter retrospective study[7](10-year projected PFS: 35% with CHOP; 67% with MACOP-B-V/VACOP-B, $p=<0.001$)

In MLBCL a poor outcome is common in advanced cases, when the disease overpasses the diaphragm. In these cases kidney, suprarenal gland, pancreas and liver are frequently involved.

Unlike other large cell lymphomas- where in refractory/relapsed patients margins of rescue exist, in MLBCL cure is exceptional in spite of intensive therapies including SCT, and patients almost invariably die in few months due to progressive disease.

The virtual absence of rescue possibilities underlines that every therapeutic effort should be made in the first-line chemotherapy.

Data in literature including ours, indicate that third generation therapies (notably MACOP-B/ VACOP-B) followed by IF RT on the mediastinum, provide better results in this lymphoma entity.

We obviously need randomized trials to definitely answer to two questions:

- the best CT approach;
- the role of post remission IF RT.

Waiting for these necessary studies,[8] which will require international cohoperation and years to be concluded, in the mean time, there is a sufficient body of data in the literature strongly recommending the use of MACOP-B or VACOP-B, followed by IF RT on the mediastinum.

References

1. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994;84:1361-92.
2. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. J Clin Oncol 1999;17:3835-49.
3. Haioun C, Gaulard P, Roudot-Thoraval F, Divine M, Jouault H, Lebourgeois JP, et al. Mediastinal diffuse large-cell lymphoma with sclerosis: a condition with a poor prognosis. Am J Clin Oncol 1989;12:425-9.
4. Klimo P, Connors JM. MACOP-B chemotherapy for the treatment of diffuse large-cell lymphoma. Ann Intern Med 1985;102:596-602.
5. Todeschini G, Ambrosetti A, Meneghini V, Pizzolo G, Menestrina F, Chilosi M, et al. Mediastinal large-B-cell lymphoma with sclerosis: a clinical study of 21 patients. J Clin Oncol 1990;8:804-8.
6. Fisher RI, Gaynor ER, Dahlberg S, Oken MM, Grogan TM, Mize EM, et al. Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N Engl J Med 1993;328:1002-6.
7. Zinzani PL, Martelli M, Bertini M, Gianni AM, Devizzi L, Federico M, et al. Development of reference ranges in Induction chemotherapy stategies for primary mediastinal large B-cell lymphoma with sclerosis: a retrospective multinational study on 426 previously untreated patients. Haematologica 2002;87:1258-64.
8. Arcaini L, Lazzarino M. Management of primary mediastinal B-cell lymphoma with sclerosis: advances and caveats. Haematologica 2002;87:1238-9.

Treatment of cutaneous non-Hodgkin's lymphomas

EMILIO FRANCESCO BERTI

Cutaneous lymphomas (CL) include a heterogeneous group of lymphoproliferative disorders originating from T- and B-lymphocytes or NK cells. In our experience and in most countries about 60-70% of primary cutaneous lymphomas are of T-cell origin (CTCL), while about 20-30% are B-cell derived; rare cases, 5-10%, derive from NK or NK/T cells. The recent WHO classification of lymphomas and particularly the EORTC classification of primary cutaneous lymphomas recognizes different clinical entities based on clinicopathologic, immunohistochemical and molecular data. CTCL include several entities; mycosis fungoides (MF), lymphomatoid papulosis (LYP) and $CD30^+$ large cell lymphoma (LCL), and rare cases, most originating from cytotoxic lymphocytes. B-cell lymphomas include most of the cases originating from germinal center cell (FCCL) or from marginal zone cells (MZL) and rare cases with large cells, plasmocytoid or lymphoblastic cytomorphology.

The indolent course of most primary cutaneous lymphomas is well known, although rare cases usually have an aggressive course. In this report we will consider the different therapies used in primary cutaneous lymphomas, in the various stages of these diseases.

CTCL (mycosis fungoides and variants)

Mycosis fungoides is a prototype of an indolent epidermotropic T-cell lymphoma showing an helper memory $CD3^+$, $CD4^+$, $CD45RO^+$ phenotype. Stages IA, IB, and IIA are manifested by cutaneous patches or plaques, without involvement of lymph nodes and can be treated by ultraviolet (UVA-UVB) radiation and Psoralen (PUVA, 8 joules, 2-3 times/week); topical corticosteroids are also useful, particularly in the case of inflammatory lesions. These therapies reduce the number of infiltrating lymphocytes and dendritic cells inducing apoptosis and may cause prolonged remission. In case of relapses or partial remission, retinoids (in a follicular variant) or interferon α (IFN-α), often in association with PUVA, can be used.

In some cases, topical nitrogen mustard (mechlorethamine, 10-20mg in 60mL of water or carmustine) can also be used.

Patients with stage IIB disease present with two or more cutaneous tumors in association with patches and plaques. Possible therapies include IFN-α, retinoids also in association with PUVA therapy or corticosteroids (topical or systemic), particularly in cases of eczematoid exudative lesions.

Furthermore local radiotherapy can be considered or, depending on the number of tumors, total skin electron beam therapy (TSEB).

IFN-α can be used for long periods (months or years) starting from 3,000,000-6,000,000 U/day and by using a maintenance dosage of 3,000,000 U two/three times a week. Retinoids can be used in association with UV or in association with IFN-α. The starting dose is 0.5 mg/ Kg of acitretin.

Another possibility for patients in this stage is to use *old* drugs, such as methotrexate (15-30 mg/week) or cyclophosphamide (50-100 mg/day) as single agents or in combination with other drugs (IFN-α, retinoids, corticosteroids) or UV therapy.

Stage IIIA disease (erythroderma) can be treated with the above cited therapies, but also with photopheresis (extracorporeal photochemotherapy). After apheresis, peripheral blood leukocytes are sensitized with psoralen and irradiated with UVA. The treatment can be repeated every 2 or 4 weeks. The efficiency of the treatment can be increased by also using IFN-α, retinoids or methotrexate.

Stage IIIA and particularly stage IIIb-IVB disease can be managed with TSEB (particularly in cases of lesions restricted to the skin and superficial lymph-nodes) and mono or polychemotherapy. The drugs used include chlorambucil, deoxycoformycin (also in combination with IFN), methotrexate, cyclophosphamide, fludarabine, cladribine, COP, CHOP, proMACE-CytaBOM, VICOP-B, or MACOP-B). Bone marrow transplantation may be considered for relapses. Unfortunately, in our experience and from the data reported in the literature, all cases treated with autologous bone marrow transplantation subsequently relapse a few months later.

However, there are a few cases reported in the literature of allogeneic bone marrow transplantation of obtaining long remission of CTCL.

Sézary syndrome must be considered as an aggressive CTCL and therapies include all those modalities cited for the management of stage IIIA-IVB of mycosis fungoides.

Lymphomatoid papulosis: no treatment, PUVA or a

From the Univ. degli Studi di Milano-Bicocca, Dip. Medicina Clinica Prevenzione e, Biotecnologie ad Indirizzo Sanitario.

Correspondence: Professor Emilio Francesco Berti, Prof. Straordinario di Dermatologia, Univ. degli Studi di Milano-Bicocca, Dip. Medicina Clinica Prevenzione e, Biotecnologie ad Indirizzo Sanitario, Via Cadore 38, 20052 Monza, MI. Phone: international +39.039.5200926. Fax: international +39.039 2004867. E-mail: emilio.berti@unimib.it

short course of systemic steroids should be used. In cases with borderline lesions or associated with nodules of $CD30^+$ LCL methotrexate could be used (starting from 15-30 mg/weekly, with a maintenance dose of 7.5-10 mg every 7-10 days).

$CD30^+$ LCL: local radiotherapy and surgical excision are the treatments of choice in case of single or localized lesions, whereas polychemotherapy must be administered for disseminated lesions.

Rare forms include cytotoxic panniculitis-like subcutaneous T-cell lymphomas and Epidermotropic cytotoxic T-cell lymphomas (showing $CD8^+$ or $CD4^+$ or γδ-T cell phenotype); NK/T nasal and nasal type $CD56^+$ lymphomas; $CD4^+$ $CD56^+$ blastic NK-like lymphomas or intravascular lymphoma (showing large intravascular cells expressing B-phenotype or less frequently a T- or NK-phenotype). In all these cases, which have an aggressive course and systemic involvement, polychemotherapy and bone marrow transplantation must be considered. The last rare form considered *provisional* in the EORTC classification is the pleomorphic small/medium T-cell lymphoma. We can distinguish two types of presentation: one is single or localized lesions that can be treated by local radiotherapy or surgery, the second type of presentation is more aggressive, showing superficial and deep nodules with systemic involvement that must be treated with radiotherapy and polychemotherapy.

Primary cutaneous B-cell lymphomas: FCCL and MZL show an indolent course and can be treated by local radiotherapy (also using local orthovolt radiotherapy) or surgery. Treatment with COP or CHOP –like regimens can be restricted to patients showing disseminated lesions, or those who are refractory to local radiotherapy or with signs of systemic involvement. IFN or a short course of monochemotherapy with chlorambucil or cyclophosphamide could also be considered in selected cases before using polychemotherapy.

New therapies: mainly because there are not *consensus* guidelines accepted for the therapy of CTCL the EORTC study group of cutaneous lymphomas stimulated the use of new drugs and new modalities of treatment for CTCL and at a recent meeting[1] several international trials were reported.

The Italian group (GILC) presented data about the use of *gemcitabine* in CTCL (mycosis fungoides), whereas other groups presented the results of international trials on the use of *bexarotene*, a new, very potent synthetic retinoid, recently marketed also in Europe. This retinoid produces partial or complete remission in about 33% of CTCLs, independently of the stage of the disease.

The use of different types of vaccine in CTCLs was also debated, as was the new modality of treatment with extracorporeal photochemotherapy (*transimmunization*),[2] with longer incubation time.

Pegylated IFN is currently being tested in other diseases and could be used in CTCLs.

Pegylated lyposomal doxorubicin (PEG-DOXO) has been found to be effective in relapsing or resistant CTCLs.

The use of low dose subcutaneous *recombinant interleukin-12* therapy, also in association with *interleukin-2* (in those patients refractory to *interleukin-12*) was recently evaluated in CTCL: it produced an improvement in the disease, probably due to the advantage of restoring normal immune function in patients and boosting cytotoxic lymphocytes whitin the lesions.

The use of *anti-CD52/alemtuzumab* in patients with advanced mycosis fungoides/Sézary syndrome was also reported. The data were very interesting, showing an overall response rate (OR) of 55%, with 32% achieving complete remission and 23% partial remission. The effect was better in patients who had received only 1-2 previous regimens (OR 80%).

The results of autologous or allogeneic stem cell transplantation and particularly of non-myeloablative stem cell transplantation (NST) were also evaluated. Our group showed the results of NST, using a classical conditioning regimen, in two patients with advanced mycosis fungoides/Sézary syndrome, in complete remission with mild graft-versus-host disease after 28 and 24 months. Recently a case of follicular MF was also transplanted with a good engraftment and complete remission after 4 months.

Anti-CD20/rituximab, administered systemically or locally, and in association with chlorambucil should, at present, be considered an experimental, very promising possible treatment of CBCL in patients with disseminated cutaneous lesions.

Finally other topical immunomodulatory or biological modifiers could be considered and are currently being tested in experimental trials: the retinoids bexarotene/tazarotene, high potency steroids (clobetasol), imiquimod and peldesine.

References

1. Cutaneous Lymphomas. Are they separated entities? Which is the best treatment? Editor: S. Tura, M. Baccarani. Haematologica 88;Suppl 5:2003.
2. Girardi M, Schechner J, Glusac E, Berger C, Edelson R. Transimmunization and the evolution of extracorporeal photochemotherapy. Transf Apher Sci 2002;26:181-90.

Update on multiple myeloma pathology

U. MAGRINI

In most cases, the diagnosis of myeloma in based on the finding of M-component in the blood or urine and on clinical data. The role of the pathologist is to confirm the diagnosis, to define the cytological grading and the histopathologic staging, to describe the microenvironmental lesions and to a monitor the course of the disease.

Cytology

The appearance of neoplastic plasma cells (PC) in myeloma varies from mature cells, quite similar to reactive PC, to very immature cells.

More commonly a range of atypical features can be observed, such as small size with lymphocyte-like nuclei, nucleo/cytoplasmic (N/C) asynchronysm with mature cytoplasm and immature nuclei with dispersed chromatin and prominent nucleoli. N/C asynchronysm is the most reliable cytologic parameter for myeloma diagnosis even when fewer than 5% PC are present.

Less frequently there may be abnormalities of nuclear outlines, e.g. cleaved cells, monocytoid cells and nuclear and cytoplasmic inclusions giving a polymorphous appearance. In some cases the predominant cells are plasmablasts characterized by large nuclei, finely dispersed chromatin, prominent nucleoli and scant blue cytoplasm. Nuclear and cytoplasmic inclusions such as Dutcher bodies and Mott cells can be found.

Flaming cells typical of IgA-secreting myeloma, various crystals and thesaurocytes can be observed. These features are not, however, sufficient for a diagnosis of myeloma, since they can be found in reactive conditions too. The spectrum of M cytology can be combined in three prognostic grades. The median survival times from the onset of symptoms are respectively 51, 23 and 9 months. These data derive from the studies by Bartl and his group, and remain a milestone in the histopathology of myeloma.

From the Dept. of Human and Hereditary Pathology, University of Pavia, Italy.

Correspondence: U. Magrini, Dept. of Human and Hereditary Pathology, University of Pavia, Italy.

Immunochemistry

The most reliable markers of PC are CD38 and CD138, but these are neither specific for PC nor differentiate benign from malignant PC. Most of the adhesion receptors that have been found in malignant PC are also present on normal PC. The notable exceptions are the apparent loss of CD31 and CD19 and the gain of CD28 and CD56 in malignant PC. The expression of these antigens is often variable within and among patient samples. For instance CD56 may be lost during leukemic transformation. Additionally myeloma cells may express antigens of the myelocytic, megakaryocytic and erythroid lineages.

Histochemical demonstration of clonality by light chain restriction is necessary only in a few cases. It has been proposed that, to be diagnostic, the ratio of the two light chains should be 16:1 or more. Histochemical demonstration of clonality is useful for the diagnosis of head and neck lesions where most extramedullary plasmacytomas occur and for the diagnosis of the very rare cases of non secretory myeloma.

Most myelomas show a low percentage of Ki67 positive cells at presentation. This finding suggests that enhanced survival of neoplastic cells through loss of apoptosis is more important than the loss of growth control. An elevated Ki-67 (> 20%) index predicts a short survival. According to this observation, it is well known that adhesion molecules not only have a pivotal role in anchoring and homing of at presentation cells in the bone marrow (BM), but also confer resistance to apoptosis. On the contrary Ki-67 has demonstrated high proliferative activity in myeloma precursor B cells. So, precursor B cells may be the stem cell accounting for myeloma self-renewal.

Quantity of PC

Three main patterns of BM infiltration, with possible combinations among them, can be identified: interstitial, nodular and diffuse. The patterns are strictly related to the amount of PC infiltration. Disease progression results in a packed marrow. Usually the more immature the PC are, the more extensive the BM involvement will appear and the more unfavorable the prognosis. Three histologic stages with progressively poorer prognosis have been defined evaluating the degree of BM replacement by neoplastic PC.

Histologic staging correlates very well with clinical

staging systems and appears complementary to them.

Microenvironment

Frequent features of the BM milieu are coarse fibrosis, which has been proven to be an unfavorable prognostic sign, and the evidence of osteoclasts. Osteoclastic activity is relevant particularly in patients with myeloma nodules, while patients with interstitial or packed marrow have preferentially generalized osteoporosis.

It has been demonstrated that angiogenesis is greater in patients with symptomatic myeloma than in patients with MGUS or other PC disorders and that the degree of angiogenesis correlates with overall survival. Vascular endothelial growth factor (VEGF) secretion is triggered by anchoring myeloma cells to BM stromal cells. VEGF induces angiogenesis, up-regulates interleukin (IL)-6 secretion, which, in turn, stimulates PC growth, inhibits dendritic cells, and stimulates osteoclasts. These data suggest the rationale for new treatments targeting the BM milieu and explain the successful use of thalidomide as an anti-angiogenetic factor in the treatment of refractory relapsed myeloma.

Lymphoid aggregates (T-TIL) are found particularly within dense myelomatous infiltration. Recent studies suggest that new treatments, such as thalidomide and its powerful immunomodulatory drug derivatives, stimulate autologous NK-cell-mediated anti-myeloma autoimmunity.

Finally the deposition of amyloid is an ominous feature that we encounter in 10% of myeloma patients.

Differential diagnosis

Striking reactive plasmacytosis may be observed in many conditions, including: liver disease, autoimmune disorders, hypersensitivity states, chronic granulomatous disorders, drug-related neutropenia, Hodgkin's lymphoma, viral infections, and metastasis. In reactive plasmacytosis a few immature PC may also be present, but the predominant cells have mature nuclei. Topography is an additional parameter distinguishing reactive from neoplastic PC. While PC in reactive plasmacytosis are mainly located around capillaries in an orderly fashion, in myeloma there is a random interstitial infiltration, with formation of rings around fat globules and of aggregates along endosteal surfaces, although pericapillary and perivascular aggregations with no orientation may also occur. Histochemically, a reactive plasmacytosis is characterized by a balanced population of κ and λ cells.

Monitoring

In the early treatment phase, BM alterations are characterized by a reduction of PC mass, marked edema and prominent vacuolization of residual PC.

In some patients a reduction of PC load cannot be obtained. Adriamycin and vincristine induce overexpression of drug resistance protein resulting in resistance to VAD regimen. This factor explains why some patients are non-responders.

In the treated patients the prognosis is related not only to the reduction of tumor load but also to the attainment of a plateau phase, in which the findings are similar to those of smoldering myeloma. In other words there is minimal interstitial infiltration of predominantly mature PC. The duration of the plateau phase is not predictable. Changes in PC grade and the development of nodularity are unfavorable signs of progression.

Finally, BM biopsies during the follow-up give information concerning the state of hematopoiesis, particularly the onset of myelodysplasia or the development of a second neoplasia, such as myelomonocytic acute leukemia.

Suggested readings

Bartl R, Frish B. Bone marrow histology in multiple myeloma: prognostic relevance of histologic characteristics. Hematol Rev 1989;3:87-108.

Dalton WS, Bergsagel PL, Kuehl WM, Anderson KC, Harousseau JL. Multiple myeloma. Hematology (Am Soc Hematol Educ Program) 2001;157-77.

Grogan TM, Van Camp B, Kyle RA. Plasma cell neoplasms. In: Tumours of Haematopoietic and Lymphoid Tissues. Jaffe ES et al, editors. IARC 2001. p. 142-56.

Anderson KC, Shaughnessy JD Jr, Barlogie B, Harousseau JL, Roodman GD. Multiple myeloma. Hematology (Am Soc Hematol Educ Program) 2002; 214-40.

Anemia in multiple myeloma: advancement in pathogenesis and treatment

FRANCO DAMMACCO, FRANCO SILVESTRIS

Chronic anemia is a recurrent feature in multiple myeloma (MM) since it occurs at diagnosis in over 20% of patients. The pathogenesis of this normochromic/normocytic anemia is multifactorial and includes an increased production of pro-inflammatory cytokines within the bone marrow microenvironment, enhanced eryhtroblast apoptosis induced by the malignant plasma cell clone and the chronic defect of erythropoietin (EPO), especially in patients with kidney involvement. However, failure of erythropoiesis can be reversed in a remarkable number of patients by long-term treatment with recombinant erythropoietin (EPO). This molecule has been shown in several clinical trials to induce a stable amelioration of the hemoglobin values as well as to improve the quality of life in MM patients receiving the conventional chemotherapy.

Multiple myeloma (MM) is a hematological disorder characterized by the expansion of a malignant plasma cell clone within the bone marrow, that leads to severe anemia and skeleton devastation. Anemia is of the "chronic disorders" (ACD) type and contributes to its morbidity and mortality in over 70% of patients.[1] A hemoglobin value of less than 8 g/dl is seen at presentation in about 20% of MM patients. Anemia is generally normochromic/normocytic and is associated with normal or increased iron stores despite low serum iron levels, decreased transferrin concentration and inappropriate erythropoietin (EPO) production.[2]

Pathogenesis of ineffective erythropoiesis in MM is multifactorial. Several pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and interferon (IFN)-γ as well as tumor necrosis factor (TNF)-α have been postulated to separately participate in the mechanism of progressive erythroid matrix exhaustion.[3] In addition, the increased susceptibility to apoptosis in erythroid progenitors as well as the cytotoxic properties of malignant plasma cell clones within the bone marrow may also exert a critical role in the inefficient maturation of red cell progenitors. Other events promoting the erythroblast default include shortening of red blood cell survival, renal failure, occasional bleedings or a variable combination of these features.

Correspondence: Prof. FRANCO DAMMACCO, M. D., DIMO, University of Bari, Section of Internal Medicine and Clinical Oncology, Piazza G. Cesare, 11 – 70124 Bari, Italy. Phone: +39-080-547-8862; Fax: +39-080-5478-820. E-mail: f.dammacco@dimo.uniba.it

Since anemia is usually worsened by conventional chemotherapy and tumor progression, blood transfusions were the inevitable therapeutic remedy for its treatment, although they were recurrently associated with important side effects such as allergic reactions, infections and iron overload. The availability of recombinant human erythropoietin-α (epoetin α or rHu-EPO-α), provided a new therapeutic option in the treatment of anemia associated to malignancies.[4] EPO is a glycoprotein growth factor synthesized by cells adjacent to the proximal renal tubule. EPO increases the production of red blood cells by activating specific receptors on the erythroid progenitor cells and promoting their differentiation while inhibiting apoptosis by molecules belonging to the bcl-2 family.[5] Clinical evidence from a wide number of trials demonstrated that EPO-a can prevent or ameliorate anemia, reduce transfusion requirements and improve the quality of life in a large number of patients with malignant diseases, including MM.[6]

Mechanisms of anemia in MM and role of EPO

The short red cell survival in MM is associated with the failure of the bone marrow to compensate for anemia by raising erythroid maturation. Several cytokines participate in this inhibitory effect exerted on the erythroid progenitors. In particular, the high levels of both TNFα and IFNγ produced by myeloma cells within the bone marrow are suspected to play a major role in both priming the erythroblast apoptosis and suppressing the differentiative effect operated by circulating EPO.[3,7]

Recent studies have demonstrated that erythroblasts at the pre-basophilic or basophilic stage of maturation show a high susceptibility to apoptosis, induced by the concurrent overexpression by mature erythroblasts of apoptogenic molecules, which include Fas-L and TRAIL.[8] These receptors trigger apoptosis by the proteolytic cleavage of cytoplasmic proteases in immature erythroblasts to counterbalance the excess of erythropoiesis associated to physiological conditions such as muscular hyperactivity or the altitude exposition. However, both Fas-L and TRAIL are also upregulated by malignant myeloma cells, which operate a suppressive effect on the erythroid progenitors similar to that exerted by mature erythroblasts. As a result of the chronic EPO defect of in MM, this inhibitory mechanism may lead to the progressive failure of the erythroblast matrix.[9,10]

EPO is the principal growth factor involved in the differentiation of erythroid progenitors into erythrocytes. This effect appears to be limited in time, since it has been shown that neither EPO nor its receptor are necessary for the erythroid lineage commitment as well as for the proliferation of burst forming erythroid units (BFU-E) progenitors. EPO receptor, indeed, is down-regulated in the late erythroblast stage and the natural hormone exerts its primary role on mature colony-forming erythroid units (CFU-E) as well as on early erythroblasts, whereas its deprivation rapidly induces their apoptosis.

Another interesting mechanism concerning the survival of erythroid cells is related to the defective expression of transcription factors involved in erythropoiesis in immature erythroblasts during active MM. These factors regulate several members of the GATA family. GATA-1 is a zinc-finger protein which binds a "GATA motif" expressed in many red cell genes. The defective expression of GATA-1 or its suppression during erythroblast differentiation promptly induces maturative arrest at the basophilic stage, cleavage of caspases and activation of apoptosis. It has been postulated that GATA-1 prevents erythroblast apoptosis by interacting with EPO in the overexpression of negative cell death regulators such as bcl-xL molecule.[11]

Thus, EPO may exert a pivotal role as negative regulator of apoptosis in erythroid progenitors. Its chronic defect may account for the major deregulation in the erythroid differentiation and maturation in MM and is dependent on the renal failure associated to the tubular deposition of monoclonal light chains. These pathogenetic aspects of erythropoiesis exhaustion in MM are supported by extensive clinical evidence correlating the most severe pictures of anemia with minimal endogenous EPO levels.

Other mechanisms have been implicated in the pathogenesis of chronic anemia in MM. Increased bleeding associated with trombocytopenia, uremia, heparin-like anticoagulants, formation of complexes involving the monoclonal component and coagulation factors as well as platelet dysfunction shorten the mean half-life of erythrocytes. In addition, the diluting effect of both intravenous therapies and serum accumulation of the monoclonal component may induce hypervolemia with reduction of the hemoglobin values.

rHu-EPO-α in MM-associated anemia

In the majority of MM patients, anemia improves when the disease is responsive to chemotherapy. When this does not occur or chemotherapy is not required, treatment options for anemia are blood transfusions or the administration of EPO-α. This recombinant form of the hormone ameliorates the erythropoiesis by stimulating the expansion of erythroid progenitor cells and decreasing apoptosis in CFU-E. Extensive clinical studies have demonstrated that rHu-EPO-α can prevent anemia and reduce transfusion requirements in anemic patients with a variety of cancers, including MM.[12,13] Furthermore, studies over the past few years carried out on a total number of nearly 5,000 evaluable patients have shown that correction of anemia with the recombinant hormone can improve the quality of life in patients with cancer. A special mention deserve indeed three recent placebo-controlled and double-blind trials, enrolled MM patients regardless of whether they were receiving concurrent chemotherapy.[12,14,15] Each study compared the outcomes of EPO-α treatment supplemented with transfusions if required, or with transfusions alone. Results definitely support the hematological benefits of long-term EPO-α treatment, though the percentage of successful response varied. Clinical use and efficacy of EPO-α have been extensively documented in MM as well as in non-myeloid malignancies. Figure 1 depicts the results obtained by comparing patients not receiving chemotherapy with respect to those on chemotherapy. A significant improvement of hemoglobin values was observed in untreated patients with smoldering myeloma as compared to those with either refractory or relapsed MM. Erythropoiesis ameliorated in more than 75% of patients who completed their eight to twelve-months' chemotherapic regimens without receiving concurrent blood transfusions. However, a moderate improvement was also observed in the transfused group since a minor number of blood red cell transfusions was required whereas in the placebo-treated patients no improvement of erythropoiesis was demonstrated in the presence of unchanged transfusional needs.

In a recent double-blind, placebo-controlled study EPO-α significantly reduced transfusion requirements and maintained higher hemoglobin values long after the trial. This study also included an open-label period of 12 weeks. An increase of hemoglobin of at least 2 g/dL after eight weeks of treatment (150 to 300 IU/Kg administered three times weekly), was considered as positive response.[16] Figure 2 shows the mean hemoglobin levels during the study in the EPO-a treated patients as well as in the placebo group. During the double-blind phase, hemoglobin values were significantly ($p<0.02$) increased in the group receiving EPO-α, whereas the placebo group showed a minor increase presumably due to a response to chemotherapy as well as to a higher transfusion rate or both. By contrast, when this group received EPO-α in the open-

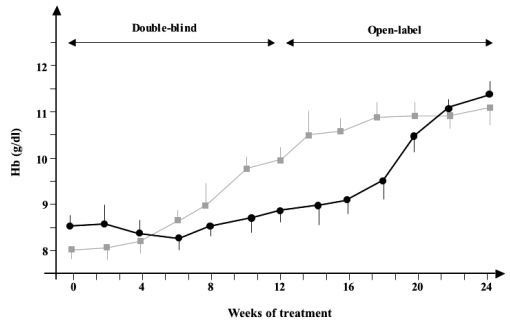


Figure 1. Percent of MM patients showing a positive response to rHu-EPO-a during (left) and off (right) chemotherapy regimens with respect to relative control groups. Positive responses were higher in patients with smoldering myeloma as compared to those with refractory disease (p<0.05), whereas both groups receiving chemotherapy showed a variable response in function of their transfusion needs. MM patients without transfusion requirements during chemotherapy showed the highest percentage of positive responses defined as an increase of more than 2 g/dl of Hb values and a variable improvement of the quality of life.

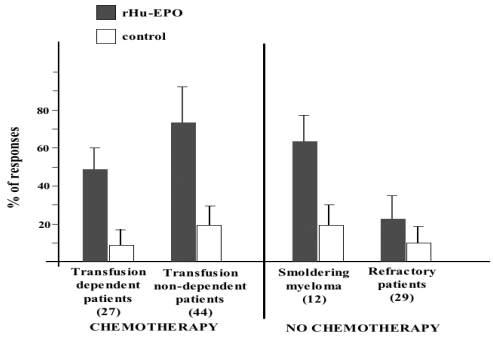


Figure 2. Treatment of MM patients with rHu-EPO-a in a 24-week trial including both a double-blind and an open-label phase. Hemoglobin increments were evident by the eighth week in the treated group (boxes), whereas the placebo-treated patients (circles) maintained ineffective levels of erythropoiesis. However, these patients showed a prompt response to the recombinant hormone in the open-label phase since their hemoglobin values rose to the levels observed in the other group towards the end of the trial.

label period, the hemoglobin became rose after 7-8 weeks and values comparable to those detected in the initially EPO-α-treated patients towards the end of the 24-months period of the study. Interestingly, more than 80% of the patients who received blood transfusions prior to EPO-α were no longer transfused after 10 weeks and, as expected, maintained their improved erythropoiesis in the open-label phase.

Open studies have evaluated the effect of chronic anemia on the quality of life. Measurement of functional status during EPO-α treatment has revealed substantial changes including general amelioration or social interactions, energy and the ability to cope with day-to-day life.[17] Changes in the performance scores have suggested a substantial rehabilitation in MM patients receiving EPO-α treatment. By contrast, placebo-controlled patients showed only minimal changes in performance score without improvement in the quality-of-life. When these patients received the recombinant hormone in the open-label phase, an improvement was recorded in the majority of quality of life variables, although they were not statistically significant. In the same study, more than 50% of patients receiving EPO-α showed stable performance scores and improvement of several measures including activity, feeling of well-being and amelioration in exercise tolerance.

The effect of long-term treatment with EPO-α has been investigated in terms of adverse effects. The majority of studies have shown the substantial absence of side effects, whereas in patients with renal failure undergoing chronic dialytic treatment it has been reported that treatment with EPO-α may induce in rare instances the production of anti-EPO neutralizing antibodies leading to progressive erythroid default and red cell aplasia.[18,19] These antibodies were mostly directed to both conformation and linear epitopes in the protein moiety of EPO.

Scanty information is so far available on the novel erythropoietin-stimulating protein (NESP), a hyperglycosylated rHu-EPO analog, with threefold longer half-life due to 5 aminoacid changes. It remains to be established whether NESP, in spite of its advantage of less frequent administration (once a week), may have similar or higher immunogenic properties as compared with EPO-a.

Conclusions

Overwhelming data from the literature and our own experience consistently support the effectiveness of EPO-α in the treatment of anemia in MM. The recombinant hormone enables the prosecution of chemotherapy and ameliorates the quality of life in the majority of these patients. Alternative therapies include the reconstitution of the erythroid matrix by autologous stem cell transplantation. However, although this procedure is apparently helpful in improving the prognosis, it is not widely adopted since methods to expand *in vitro* the hematopoietic progenitors have not been standardized.

Acknowledgments

This work was supported in part by the Ministry for Education, the Universities and Research (MIUR), Rome, and by AIRC (Associazione Italiana per la Ricerca sul Cancro), Milan.

References

1. Means RT Jr, Krantz SB. Progress in understanding the pathogenesis of the anemia of chronic disease. Blood 1992; 80: 1639-47.
2. MacLennan IC, Drayson M, Dunn J. Multiple myeloma. Br. Med. J. 1994; 308: 1033-6.
3. Merico FL, Bergui MG, Gregoretti P, et al. Cytokines involved in the progression of multiple myeloma. Clin Exp Immunol 1993; 92: 27-31.
4. Ludwig H, Fritz E. Anemia in cancer patients. Semin Oncol 1998; 25 (Suppl 7): 2-6.
5. Morioka K, Tone S, Mukaida M, Takano-Ohmuro H. The apoptotic and nonapoptotic nature of the terminal differentiation of erythroid cells. Exp Cell Res 1998; 240: 206-17.
6. Ludwig H, Fritz E, Leitgeb C, et al. Erythropoietin treatment for chronic anemia of selected hematological malignancies and solid tumors. Ann Oncol 1993; 4: 161-7.
7. Denizot Y, Fixe P, Liozon E, Praloran V. Serum interleukin-8 (IL-8) and IL-6 concentrations in patients with hematologic malignancies. Blood 1996; 87: 4016-7.
8. De Maria R, Testa U, Luchetti L, et al. Apoptotic role of Fas/Fas ligand system in the regulation of erythropoiesis. Blood 1999; 93: 796-803.
9. Silvestris F, Tucci M, Cafforio P, DammaccoF. Fas-L upregulation by highly malignant myeloma plasma cells: role in the pathogenesis of anemia and disease progression. Blood 2001; 97:1155-64.
10. Silvestris F, Cafforio P, Tucci M, Dammacco F. Negative regulation of erythroblast maturation by Fas-L(+)/TRAIL(+) highly malignant plasma cells: a major pathogenetic mechanism of anemia in multiple myeloma. Blood 2002; 99: 1305-13.
11. De Maria R, Zeuner A, Eramo A, et al. Negative regulation of erythropoiesis by caspase-mediated cleavage of GATA-1. Nature 1999; 401: 489-93.
12. Dammacco F, Silvestris F, Castoldi GL, et al. The effectiveness and tolerability of epoetin alfa in patients with multiple myeloma refractory to chemotherapy. Int J Clin Lab Res 1998; 28:127-34.
13. Abels R. Erythropoietin for anaemia in cancer patients. Eur J Cancer 1993; 29A Suppl 2: S2-8.
14. Cazzola M, Messinger D, Battistel V, et al. Recombinant human erythropoietin in the anemia associated with multiple myeloma or non-Hodgkin's lymphoma: dose finding and identification of predictors of response. Blood 1995; 86:4446-53.
15. Garton JP, Gertz MA, Witzig TE, et al. Epoetin alfa for the treatment of the anemia of multiple myeloma. A prospective, randomized, placebo-controlled, double-blind trial. Arch Intern Med 1995; 155: 2069-74.
16. Dammacco F, Castoldi G, Rodjer S. Efficacy of epoetin alfa in the treatment of anaemia of multiple myeloma. Br J Haematol 2001; 113: 172-9.
17. Demetri GD, Kris M, Wade J, Degos L, Cella D. Quality-of-life benefit in chemotherapy patients treated with epoetin alfa is independent of disease response or tumor type: results from a prospective community oncology study. Procrit Study Group. J Clin Oncol 1998; 16: 3412-25.
18. Casadevall N, Nataf J, Viron B, et al. Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. N Engl J Med 2002; 346:469-75.
19. Casadevall N. Antibodies against rHuEPO: native and recombinant. Nephrol Dial Transplant 2002; 17 Suppl 5:42-7.

Impact of negative selection (B-cell depletion) in multiple myeloma autologous transplantation. Final analysis of a prospective comparative trial by the Bolzano-Münich Study Group

P. Coser, M. Mitterer, M. Svaldi, N. Pescosta, B. Emmerich, C. Straka

High-dose chemotherapy, followed by autologous or allogeneic transplantation of hematopoietic stem-cells, is now the therapy of choice for patients affected by multiple myeloma. Autologous transplantation with peripheral blood stem cells (PBSC) improves the survival and the quality of life of myeloma patients, but relapse remains the major problem. This can be explained by the persistence of myeloma cells after high-dose chemotherapy and/or by reinfusion of clonal cells with the leukoapheretic products. To reduce the risk of relapse after autograft, positive selection of $CD34^+$ cells and negative selection with B cell depletion, are the two most useful techniques for stem cell purging. Until recently positive selection of $CD34^+$ cells did not show any correlation with improved overall or event-free survival. In our study we compared the outcome of patients transplanted with or without *ex vivo* purged stem cells using an immunologic approach of B cell depletion (negative selection) in an attempt to obtain tumor-free products.

Between 1995 and 2000, 110 consecutive patients, median age 53 years, median serum β2 microglubulin 2,7 mg/L (range 1.4 - 14.3) in advanced stage of disease, were treated with sequential chemotherapy and tandem transplantation, according to the *total therapy* concept of Barlogie, which consisted of 3 courses of VAD (vincristine, doxorubicin, dexamethasone), 1 course of IEV + G-CSF (ifosfamide 2500 mg/m^2 iv days 1-3, epirubicin 100 mg/m^2 iv day 1, etoposide 150 mg/m^2 iv days 1-3) administered on an outpatient basis with MESNA and hydration support therapy for mobilizing the PBSC, 1 course of EDAP (etoposide; dexamethasone, Ara-C, cisplatin) and then tandem high-dose therapy with melphalan 200 mg/m^2 iv within 3-6 months followed by autotransplantation of peripheral blood stem cells. Immunomagnetic *ex vivo* B-cell purging of PBSC, using a cocktail of CD10, CD19, CD20, CD22, CD37 antibodies and immunomagnetic beads (MaxSep System Baxter, Unterschleißheim, Germany) was applied in 53 patients out of the 110 in order to remove clonogenic B cells. Whether the PBSC autograft of a patient was purged or not depended on the availability of the purging kit (monoclonal antibodies and immunomagnetic beads) at the treatment Center, but not on the patient's individual characteristics. Contamination of the apheresis products and the minimal residual disease (MRD) were controlled by Gene Scan Analysis after CDR III and CDR I polymerase chain reaction (PCR).

Out of 110 patients 32 were in stage II and 78 in stage III. The purging efficacy using a panel of 3, 4 or 5 anti-B monoclonal antibodies did not differ (3 log) and the engraftment after the first transplantation (unmanipulated) and the second transplantation (purged) was identical (10 days to reach 0.5×10^9/L neutrophils, 11 days to reach 20×10^9/L platelets and 15 vs 17 days to reach 50×10^9/L platelets). One patient had a transitory graft failure due to reactivation of CMV infection after the second transplant. The treatment-related mortality for all patients was 5%. With PCR analysis of the CDR III and CDR I region, we documented that the immunomagnetic bead B-cell fractions isolated from the apheresis products were contaminated by myeloma precursor cells in 86% of the collections. The outcome of the transplanted patients was strictly correlated with the clonal pattern of the B-cell fraction revealed by PCR analysis on the magnetic beads. Patients showing a predominantly monoclonal B-cell population in their apheresis products had an event-free survial (EFS) of 20% at 40 months, in comparison to 50% for patients showing a polyclonal or oligoclonal pattern. Complete remission (CR) (bone marrow, Bence Jones, immunofixation: negative) and partial remission (PR) were obtained in 48% and in 37%, respectively. Examining the whole population, the impact of purging was favorable: in fact the median EFS for 53 patients transplanted with purged PBSC was 40 months versus 22 months for the patients transplanted with unmanipulated PBSC (p = 0.04). The median overall survival (OS) was not reached for purged patients, being 72% at 4 years after the first autograft, versus 48 months for not purged patients (p = 0.04). Our results confirm that multi-regimen induction and tandem transplantation is now a favorable therapy option for newly diagnosed myeloma patients and that purging with negative selection might improve the outcome of the patients. The final analysis of this study will be presented at the meeting.

From the Haematology and Bone Marrow Transplantation Unit, Regional Hospital Bolzano, Italy (PC, MM, MS, NP, Dept. of Haematology and Oncology, Ludwig-Maximillians-University, Münich-Germany (BE, CS).

Correspondence: Paolo Coser, Haematology and Bone Marrow Transplantation Unit, Regional Hospital Bolzano, Italy.

Reduced intensity conditioning with thiotepa, fludarabine and melphalan for allogeneic transplantation in multiple myeloma

I. Majolino, K. P. Urago, M. Riccardi, A. Locasciulli, A. Bacigalupo, P. Di Bartolomeo, R. Scimè, A. Olivieri, F. Narni, P.Corradini

In multiple myeloma (MM), high-dose therapy followed by single or autologous stem cell transplantation (auto-SCT) represents the treatment of choice for patients <60 years of age.[1,2] However, auto-SCT is not able to eradicate the disease. In a retrospective study of GITMO the event-free survival after auto-SCT failed to show a plateau. In practice, all patients relapsed within 3-5 years and later became resistant to any available treatment. In the meantime, molecular biology studies confirm that the myeloma cell clone seldom disappears after autologous transplantation.[3,4] The reason is the persistence of a sizeable number of neoplastic cells both in the patient and in the graft.

Allogeneic stem cell transplantation (alloSCT) is employed much less frequently than autologous transplantation, due in part to the limited availability of HLA-identical donors and in part to the higher transplant-related mortality (TRM).[5] Nonetheless, a retrospective study within the EBMT[6] has documented a TRM reduction over the last few years, as result of better selection of patients and improvements in technologies, as may be the preferential use of chemotherapy-based conditioning instead of total-body irradiation and the use of stem cells from the peripheral blood rather from bone marrow.[7]

The emerging concept is that suppression of the neoplastic clone may be obtained (in selected diseases) even without a mega-dose of chemo-radiotherapy as administered in the traditional transplantation regimens. An immunosuppressive protocol, in combination with the infusion of a large number of stem cells, will ensure stable engraftment and promote a graft-versus-tumor effect with little toxicity.[8] Such methodology is currently under evaluation in a variety of hematologic disorders, including MM.[9] MM is typically an immune-sensitive disease, as witnessed by the efficacy of donor-lymphocyte infusions (DLI) following allo-SCT and by the results of vaccination.[10] A rapid reduction of the M component, with disappearance of the marrow plasma-cell infiltration has been documented late after allo-SCT, at the time of chronic graft-versus-host disease (GVHD) onset.[7]

From the Hematology and BMT, S.Camillo-Forlanini Hospital, Rome (IM, KPU, AL), Hematology Department, S.Martino Hospital, Genoa (AB), BMT Center, Pescara (PDB), BMT Unit, "V.Cervello" Hospital, Palermo (RS), Hematology Clinic, Torrette Hospital, Ancona (AO), Hematology Service, University of Modena (FN), Transplant Unit, National Cancer Institute, Milan, Italy (PC).

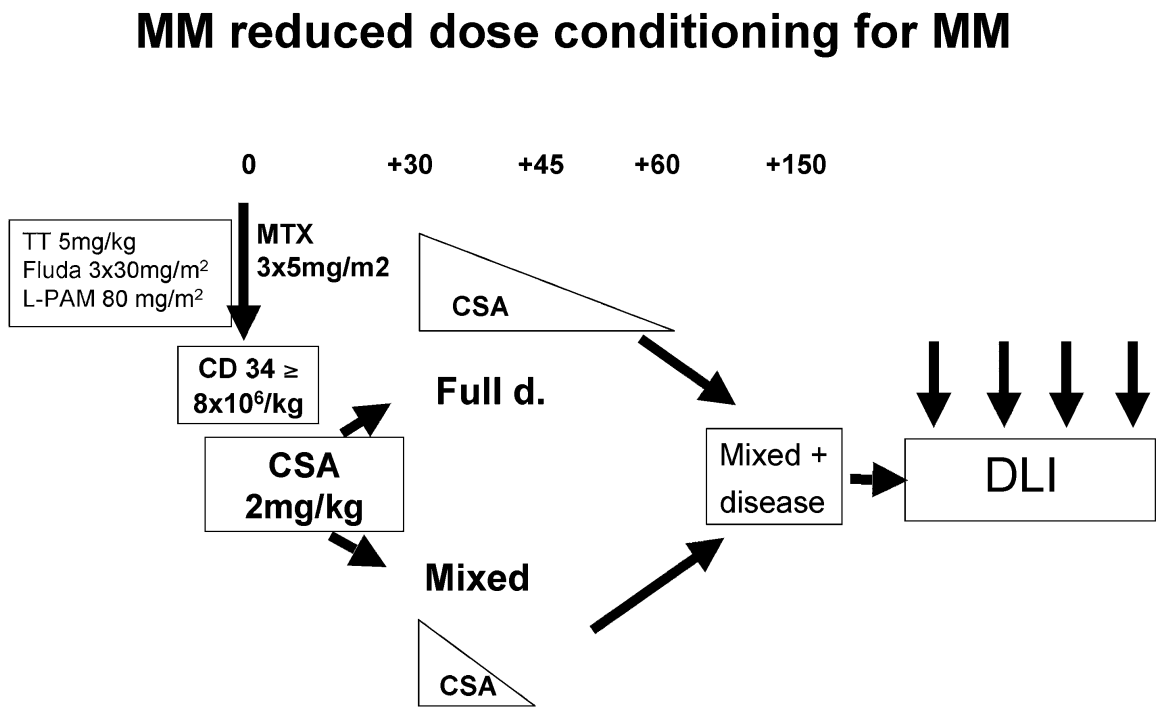
Correspondence: Dr Ignazio Majolino, Unità Operativa di Ematologia e Trapianti di Midollo Osseo, Azienda Ospedaliera S.Camillo-Forlanini, Circonvallazione Gianicolense 87, Rome, Italy. Phone: international +39.06.58704418. Fax: international +39.06.58704357. E-mail: imajolino@libero.it

Design and methods

For patients with MM we have recently designed a program of reduced-intensity conditioning transplant from HLA-identical sibling donors. The conditioning combines fludarabine, thiotepa and melphalan (Figure 1). GVHD prophylaxis is based on methotrexate-cyclosporine, but the latter is rapidly tapered following transplantation to favor the emergence of an immune-mediated suppression of tumor. DLI are employed in those patients who remain GVHD-free but still harbor detectable tumor following cyclosporine tapering. The study is supported by a molecular analysis of bone marrow cells to detect IgH gene mutation as a marker of miminal residual disease.[4]

Results

Twenty patients have been enrolled so far. We have data on 18 of them. Their characteristics are reported in Table 1. Seven were transplanted early in the course of their disease, while 11 had the allograft as treatment for disease progression or relapse. As transplant, they received a median of 5.1×10^6/Kg $CD34^+$ cells (range 0.2-10.2), and 3.0×10^8/Kg $CD3^+$ cells (range 0.4- 4.2) from bone marrow or granulocyte colony-stimulating factor (G-CSF)-primed peripheral blood. Full engraftment occurred in all, with 14 days to recover $>0.5\times10^9$/L granulocytes (range 10-18) and 12 days to recover $>20\times10^9$/L platelets (range 4-22). Acute GVHD is evaluable in 16. Grade 1 GVHD developed in 4, grade 2 in 3 (18%). None developed grade >2 acute GVHD. Of the 11 patients evaluable, 6 (54%) developed chronic GVHD. Clinical results are summarized in Table 2. Thirteen patients are evaluable for transplant response. Three of them were already in complete remission (CR) at the time of transplantation. Another 2 achieved CR after the allograft, while 6 reached only a partial remission and 2 were refractory. Twelve patients are currently in follow-up, since 1 died of disease progression soon after transplantation. Until now there has been a single relapse. Four patients remain in CR at a median of 12 months follow-up (range 5-15 months).



Design of the protocol.

Table 1. Patients' characteristics.

Patients (No.)	18
Age (years)	median 53 (21-64)
Disease phase	
early	7
advanced	11
Previous autotransplant (No.)	10
Time from diagnosis to allo (months)	median 8 (3-66)

Table 2. Clinical results.

	No. patients
Evaluable	13
In CR at transplantation	3
Evaluable for response to transplant	10
CR	2
PR	6
NR	2
In CR following transplantation	5 (38.4%)
In follow-up	12
Continuous CR	4
Relapse	1
Death	1

Conclusions and comments

The preliminary results of the present protocol show that reduced-intensity conditioning with fludarabine, thiotepa and melphalan is well tolerated even in patients who have a long disease history or who have had previous autograft(s). In fact, no patient died of transplant-related complications. For this reason this scheme seems to be applicable also in elderly patients, or when comorbidities would discourage the use of transplantation.

In terms of GVHD, our experience is encouraging. Acute GVHD ≥ grade 2 occurred in less than 20% of patients and we did not observe grade 3 or 4. Chronic GVHD is expected to occur in

over 50% of cases, but this is not to be envisaged as a negative factor in a disease that is known to be sensitive to immune aggression.

Our protocol is able to induce a response in a sizeable proportion of patients. Eighty percent showed a response, with over 30% CR. Of the 5 patients in CR after transplantation only one has relapsed. The follow-up is, however, too short to draw any conclusions on remission duration. Data on IgH-gene rearrangement will be available in the next months, and will probably shed more light on the significance of CR after this treatment program.

In conclusion, the reduced-intensity conditioning transplant presented here is considerably less toxic than conventional conditioning. The incidence of acute GVHD is limited, and clinical results appear to be encouraging, as nearly 30% of patients achieve a complete remission and the majority maintain this status, at least in the short-term. We currently offer this program to patients with an HLA-identical sibling donor at the time of induction, after 3-4 courses of VAD.

Funding

The present work is in part supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC).

References

1. Attal M, Harousseau JL, Stoppa AM, Sotto JJ, Fuzibet JG, Rossi JF, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Français du Myelome. N Engl J Med 1996;335:91-7.
2. Attal M, Harousseau JL, Facon T. Double autologous transplantation improves survival of multiple myeloma patients: final analysis of a prospective randomized study of the "Inter Groupe Français du Myelome". Blood 2002; 100 Suppl 1:5a[abstract].
3. Majolino I, Vignetti M, Meloni G, Vegna ML, Scime R, Tringali S, et al. Autologous transplantation in multiple myeloma: a GITMO retrospective analysis on 290 patients. Haematologica 1999;84:844-52.
4. Corradini P, Voena C, Tarella C, Astolfi M, Ladetto M, Palumb, et al. Molecular and clinical remissions in multiple myeloma: the role of autologous and allogeneic transplantation of hematopoietic cells. J Clin Oncol 1999;17:208-15.
5. Bensinger WI, Clift R, Martin P, Appelbaum FR, Demirer T, Gooley, et al. Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: a retrospective comparison with marrow transplantation. Blood 1996;88:2794-800.
6. Gahrton G, Svensson H, Cavo M, Apperely J, Bacigalupo A, Bjorkstrand B, et al. Progress in allogenic bone marrow and peripheral blood stem cell transplantation for multiple myeloma: a comparison between transplants performed 1983-93 and 1994-8 at European Group for Blood and Marrow Transplantation centres. Br J Haematol 2001;113:209-16.
7. Majolino I, Corradini P, Scimè R. High rate of remission and low rate of disease recurrence in patients with multiple myeloma allografted with PBSC from their HLA-identical sibling donors. Bone Marrow Transplant 2003; in press.
8. Slavin S, Nagler A, Naparstek E. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and non-malignant hematologic diseases. Blood 1998;91:756-63.
9. Badros A, Barlogie B, Siegel E, Cottler-Fox M, Zangari M, Fassas A, et al. Improved outcome of allogeneic transplantation in high-risk multiple myeloma patients after nonmyeloablative conditioning. J Clin Oncol 2002;20: 1295-303.
10. Bellucci R, Alyea EP, Weller E, Chillemi A, Hochberg E, Wu CJ, et al. Immunologic effects of prophylactic donor lymphocyte infusion after allogeneic marrow transplantation for multiple myeloma. Blood 2002;99:4610-7.

Diffuse large cell lymphomas

G. Palestro, R.Chiarle

The morphologic, immunophenotypic, molecular and genetic modifications of lymphoid cells during differentiation and activation processes, which take place in the primary as well as secondary lymphoid organs, are maintained over the entire life span of both animals and humans. As a consequence, distinct lymphoid cells have been identified as counterparts of corresponding non-Hodgkin's lymphomas (NHL). Over the last ten years a great many biological features of normal lymphocytes and genetic abnormalities characterizing malignant transformation have been identified. This has resulted in recognition of new entities and refinement of previously recognized tumor categories, thus allowing more precise diagnostic approaches and identification of factors that can predict prognosis and influence therapeutic strategies.

However, these extensive studies have not yet provided a certain and complete definition of lymphoid cell compartments and the relationships among them. As a consequence, some well-defined lymphoma varieties do not correspond to obvious normal counterparts.

Therefore, there is an international agreement in simply defining diseases on the grounds of the currently available morphologic, immunologic, molecular and genetic information.[1] This is the reason why the revised European-American lymphoma (REAL) classification[1] proposed including under the generic term of *diffuse large B-cell lymphomas* (DLBCL) all B-cell neoplasias independently of the histogenesis, provided that the neoplastic component was made up of large sized-cells and the disease showed an aggressive behavior.

In this paper we will focus on diffuse non-Hodgkin's large B-cell lymphomas (DLBCL) because of the striking predominance of this complex entity over diffuse large cell lymphoma of T-cell origin which is a separate entity displaying anaplastic morphology. DLBCL represents a most convincing example of the difficulty of establishing a correlation between the various entities included in this group and a precise normal cell compartment. Indeed, the category of DLBCL is characterized by marked heterogeneity in general phenotype and clinical course suggesting that it probably includes multiple disease entities not clearly defined by morphologic, immunological, molecular and cytogenetic parameters. The new World Health Organization (WHO) classification[2] substantially reflects the REAL scheme with additional subentities such as: mediastinal large B-cell lymphoma, intravascular large-cell lymphoma and primary effusion lymphomas (PEL).

Diffuse large B-cell lymphoma

Epidemiological features

DLBCLs represent 30-40% of adult non-Hodgkin's lymphomas (NHL) in western countries. The incidence of the disease has been increasing over the past few decades independently of the risk of HIV infection. Although these lymphomas are predominantly prevalent in the 7th decade, the age-range affected is broad, and not even children are spared.

Clinico-pathologic features

The nodal presentation is predominant, however up to 40% of the cases may primarily develop in extranodal sites. Although any extranodal organ may be involved, the stomach and ileocecum are the sites most commonly involved. The bone marrow, as a primary location, is rarely involved and if so, more often shows multilobated nuclei.[3]

Typically, the patient has a rapidly enlarging, symptomless mass involving a nodal or extranodal site. However, at staging evaluation the disease is often discovered to be disseminated.

Etiology

No factors have so far been defined as responsible for the development of DLBCL. The disease more often originates *de novo*, but can also represent a progression or transformation of a less aggressive lymphoma such as chronic lymphocytic leukemia/lymphoma, follicular lymphoma, marginal-zone B-cell lymphoma and the nodular variant of Hodgkin's disease with lymphocyte predominance (NLPHD). DLBCL which develop in the setting of immunodeficiency often have Epstein-Barr virus as a marker.

Morphology

The nodal structure is usually completely effaced. When extranodal, the tumor mass is often firm due

to fibrosis.

Histologically, the normal nodal or extranodal structure is diffusely replaced by the lymphoma. Some times the lymph node structure is not completely replaced by the tumoral infiltration which develops in between the follicles or, rarely involves the sinusoids.

From the cytological point of view, DLBCL are made up of large transformed lymphoid cells whose various features allow distinction of the disease into different morphologic variants. It must be stressed that purely morphologic identification of these variants does not have a good inter-intraobserver reproducibility[1] and that genetics and immunophenotype are not helpful.

Common morphologic variants

Centroblastic

This is the most frequent variant and is made up of centroblast-like cells with a proportion of large immunoblast-like cells varying from 0 to 90% of the entire neoplastic cell population. This heterogeneous cell composition makes the distinction between the centroblastic and immunoblastic variants problematic and arbitrary in routine diagnosis. Moreover, the centroblastic variant may be further divided into monomorphic, multilobated and polymorphic subvariants.

Immunoblastic

According to the Kiel classification[4] this variant accounts for only 4% of DLBCL. Immunoblasts and immunoblast-like cells represent more than 90% of the entire neoplastic population with a varying number of admixed plasmablasts or plasma cells. Patients with this variant are reported to have a significantly shorter than those with centroblastic variant.[5-6]

Anaplastic

This lymphoma, originally designated as Ki-1 lymphoma, has since been classified as CD30 lymphoma because of the reactivity of the neoplastic cells to this molecule. It is generally accepted that the B-cell anaplastic variant of large cell lymphoma should be removed from the group of non-B anaplastic large cell lymphomas (ALCL) which represents a distinct entity including only T/null-cell phenotypes with specific clinicopathological and molecular features. As to B-cell anaplastic lymphoma, this has been grouped within the DLBCL category by the REAL and WHO classifications because of similar clinical features. The neoplastic infiltration of cells with large, bizarre, anaplastic morphology is the main hallmark of this variant.

T-cell /histiocyte rich

The main feature that makes this variant unique is a high predominance of non-neoplastic T cells with a varying component of histiocytes while the large neoplastic B-cell component does not exceed 10% of the entire population. However, the cases reported in the literature are very heterogeneous with regard to the growth pattern, number of T cells and the relative number and morphology of the large cells. Furthermore, the neoplastic large cells may show features ranging from L&H cells, to centroblasts, immunoblasts or Reed-Sternberg cells. Although the growth pattern is predominantly diffuse, a vaguely nodular pattern may be found in many cases, hinting a possible association with NLPHD. However, T-cell/histiocyte rich B-cell leymphoma (T/HRBCL) is usually associated with a more advanced clinical stage at presentation and a more aggressive behavior than that of LPHD. The disease, when it does not develop de novo may originate from or transform into other lymphomas. In particular, the disease may appear as a secondary lymphoma following follicular lymphoma or DLBCL or form LPHD, but the reverse is also possible. Coexistence with follicular lymphoma has also been seen.[7] The possible occurrence of these pathological conditions prompted the REAL classification to adopt the concept that T/HRBCL is not an entity but includes a heterogeneous group of B-cell lymphomas or Hodgkin's disease. As to the relationship of T/HRBCL and LPHD, this has recently been investigated with comparative genomic hybridization analysis. The differences in the genomic imbalances between T/HRBCL and LPHD found in this study favor the hypothesis that the two diseases are distinct lymphomatous entities, possibly originating from the same precursor cells.[8]

As far as concerns the clinicopathological presentation, the disease is usually in advanced stage at first diagnosis, predominantly stage III or IV, with frequent involvement of spleen and bone marrow where infiltrates exibit a paratrabecular or diffuse pattern.[9]

Prognosis

Despite their clinical aggressiveness DLBCL are potentially curable with combined chemotherapy. Biological indicators of adverse prognosis are considered to be: a high proliferative rate, BCL2 expression, and p53 overxpression.[2] By contrast, it has been reported that BCL6 translocation is associated with better prognosis.[2] Genetic studies with microarrays for gene expression profiling have demonstrated that germinal center B-like DLBCL have better survival than activated B-like DLBCL.[6]

Immunophenotype

DLBCL usually express the conventional markers which characterize B cells, such as CD19, CD20, CD22, and CD79a. However, due to various biological anomalies, one or more of these markers may be lacking in the neoplastic cells. Surface and/or cytoplasmic Ig can be found in 50-75% of cases in the common variants of DLBCL with the following pattern: IgM> IgG> IgA. The CD30 mocelule can be occasionally demonstrated in non-anaplastic variants. By contrast, CD30 is expressed by the vast majority of neoplastic cells in the anaplastic variant.

Some cases (10%) express CD5 or CD10 (20-25%).[2] BCL 2 is overxpressed in 30-50% of cases; BCL 6 is found in a very high percentage of cases. p53 is expressed and often mutated in a minority of cases. The proliferative rate, as determined immunohistochemically by Ki 67 is usually over 40%, being greater than 90% in some cases.[10]

Genetics

Ig heavy and light chain genes are rearranged in the majority of cases and show somatic mutations in the V regions. *BCL-2* gene translocation, corresponding to t(14;18), occurs in 20-30% of cases. Chromosomal translocations involving band 3q27 are detectable in 35% of DLBCL and involve the *BCL 6* gene. In addition, up to 75% of DLBCL display multiple somatic mutations clustering in the *BCL 6* 5' regulatory sequences, independently of chromosomal translocations11. Recently, it has been reported that apparently aberrant activity of the somatic hypermutation mechanism, which usually involves the Ig, *BCL 6* and *FAS* genes, can produce tumor-associated lesions at multiple genetic loci of DLBCL.[11]

New morphologic variants included in the WHO scheme

Mediastinal (thymic) large B-cell lymphoma

This is a subtype of DBLCL whose putative cell counterpart is a thymic B cell with distinct clinical, morphologic, immunphenotypic and genotypic features.[12-16] It affects relatively young adult patients (third to fifth decade) with a female predominance.[13-17] The disease presents with symptoms relating to a large anterior mediastinal mass that may then disseminate involving nodal and other extranodal sites.[15,17,18]

A diffuse proliferation of medium-large B cells with pale abundant and cytoplasm infiltrating a dense stromal fibrosis represents the main morphological hallmark of the tumor. The immunohistochemical identification of thymic remnants is of help in defining the thymic origin of the disease. A rare association with nodular sclerosis-HD has been reported.[19]

The disease is usually succesfully cured when treated with intensive chemotherapy with or without associated radiotherapy. However, the prognosis is closely related to the initial disease stage. Diffusion into adjacent thoracic or infradiaphragmatic organs is predictive of an unfavorable course.[13,15,18]

Immunophenotype: the lymphoma expresses B-cell markers such as CD19 and CD20. Both Ig and HLA class I and II are often incompletely expressed or absent and CD30 molecule is often weakly expressed.[2]

Genetics: the hyperdiploid karyotypes, often with gains in chromosome 9p and amplification of the *REL* gene, suggest that this subvariant of DLBCL is distinct from those arising in other sites.[2]

Intravascular large B-cell lymphoma

This is a rare and highly aggressive variant of DLBCL which affects adult patients. It responds poorly to chemotherapy evolving in most cases rapidly to death. The hallmark of the disease consists of the presence of neoplastic cells only in the lumina of small blood vessels and capillaries: these cells then disseminate to extranodal sites often including the bone marrow. The lesion presents with skin plaques and nodules. Macroscopical hemorrhage, thrombosis and necrosis can also be observed. The wide dissemination of the disease may be responsible for various symptoms, which are related both to the presence of the tumor and to the vessel occlusion by neoplastic cells, e.g. dementia and focal neurological symptoms, nephrotic syndrome, hypertension, autoimmune hemolytic anemia, leukopenia, pancytopenia, and disseminated intravascular coagulation.

The scarcity of cases reported in the literature makes it difficult to collect conclusive epidemiological and etiopathological data. However, the disease has been hypothesized to develop as a consequence of a defect in homing receptors of transformed peripheral B-neoplastic cells,[20] in particular CE29 and CD54 adhesion molecules.[21]

Primary effusion lymphoma (PEL)

This variant of DLBCL is also named body cavity-based lymphoma.[2] The disease may be found in two distinct epidemiological conditions: i) in most young or middle-aged homosexual males the disease develops in the context of HIV infection;[22] ii) the disease may develop in Mediterranean areas with a high prevalence of HHV-8 infection[23] and in this case tends to affect elderly males. The lymphoma characteristically involves primarily a serous cavity, such as pleura, pericardium or peritoneum. Much more rarely the disease may involve the gastrointestinal tract, soft tissue or other extranodal sites.

The most important clinical feature consists of effusions without lymphadenopathy or organomegaly. In some cases a pre-existing Kaposi's sarcoma can be found as well as multicentric Castleman's disease.[24] Cytologically, the lymphoma is made up of cells that, more evidently in blood smears, show immunoblastic, plasmablastic, anaplastic and, sometimes, Reed-Sternberg features. The neoplastic cells adhere to serous surfaces and occasionally invade the tissue. This distribution distinguishes the disease from other DLBCLs which secondarily invade serous tissue as a mass.

Genetics: HHV-8 viral genomes are present in all cases; gain in sequences of chromosomes 12 and X, which are also displayed by other HIV-associated lymphomas, have been shown by comparative genomic analysis.[2]

Concluding remarks and perspectives

It is well known that carcinogenesis is closely related to genetic alterations produced by mistakes during normal cell function or by unrepaired physical or chemical damage to the genome. The combination of various genetic lesions, when reaching a certain level, may generate cells with full potential to generate a malignant tumor. Additional genetic lesions may impart characteristics that provide the tumor with different aggressivity and/or resistance to treatment. It has recently been postulated that the characteristics of a tumor and its clinical behavior are determined by specific sets of genetic alterations retained by the neoplastic cells. These alterations are expressed by gene profiles and are regarded as the *molecular signature* or *fingerprints* of the tumor. Thus, it can be foreseen that the future classification of lymphomas, alongside the traditional morphologic and immunophenotypic parameters, will also take into account gene expression profile analysis. This analysis may be of great help in establishing a clinicopathologically relevant and biologically meaningful cancer classification; it will be of help in determining prognosis and help therapeutic decision making in individual patients. It will also make it possible to identify genes that are important determinants of the behavior of lymphomas.[25] This new approach will allow the identification of clinically significant subtypes, so far not detected, among this very heterogeneous lymphoma category.

References

1. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, et al. Blood 1994;84:1361-92.
2. World Health Organization Classification of tumours (WHO). Pathology and genetics of tumours of haemopoietic and lymphoid tissues. ES Jaffe, NL Harris, H Stein, JW Vardiman, Editors. IARC press; Lyon. 2000.
3. Pettit CK, Zukerberg LR, Gray MH, Ferry JA, Rosenberg AE, Hermon DC, et al. A B-cell neoplasm with a frequency of multilobated cells. Am J Surg Pathol 1990;14:329-34.
4. Lennert K, Feller A. Histopatholoogy of non-Hodgkin lymphomas (based on the updated Kiel classification), 2nd ed. New York: Springer-Verlag. 1990.
5. Engelhard M, Brittinger G, Huhn D, et al. Subclassification of diffuse large B-cell lymphoma according to the Kiel classification: distinction of centroblastic andf immunoblastic lymphomas is a significant prognostic risk factor. Blood 1997;89;2291-7.
6. Alizadeh AA, Elson MB, David RE, et al. distinct tuypes of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403;503-11.
7. Schmidt U, and Leder LD. T-cell-rich-B-cell lymphoma-a distinct clinicopathological entity? Leuk Lymphoma 1996;23;17-24.
8. Franke S, Wlodarska I, Maes B, et al. Comparative genomic hybridization pattern distinguishes T-cell/Histiocyte-rich B-cell lymphoma from nodular lympèhocyte predominance Hodgkin's lymphoma. Am J Pathol 2002;161:1861-7.
9. Skinnider BF, Connors JM, Gascoyne RD. Bome marrow involvemeent in T-cell-rich B-cell lymphoma (see comments). Am J Clin Pathol 1997;108;570-8.
10. Miller TP, Grogan TM, Dahlberg S, et al. Prognostic significance of the Ki 67-associated proliferative antigen in aggressive non-Hodgkin's lymphomas: a prospective Southwest Oncology Group trial. Blood 1994;83:1460-6.
11. Pasqualucci L, Migliazza A, Fracchiolla N, et al. BCL-6 mutations in normal germinal center B cells: evidenced of somatic hypermutation acting outside Ig loci. Proc Natl Acad Sci USA 1998;95:11816-21.
12. Moller P, Lammier B, Eberlein-Gonska, et al. Primary mediaqstinal clear cell lymphoma of B-cell type. Virchows Arch A Pathol Anat Histopathol 1986;9:79-92.
13. Perrone T, Frizzera G, Rosai J. Mediastinal diffuse large-cell lymphoma with sclerosis. A clicnicopathologic study of 60 cases. Am J Surg Pathol 10;176-191,1986.
14. Isaacson PG, Norton AJ, Addis BJ: The human contains a novel population of B lymphocytes. Lancet 2;688-691,1987.
15. Kirn D, Mauch P, Shaffer K et al: Large-cell and immunoblastic lymphoma of the mediastinum: prognostic features and treatment outcome in 57 patients. J Clin Oncol 11;1336-1343,1993.
16. Davis RE, Dorfman RF, Warnke RA, et al:Primary large-cell lymphoma of the thymus: a diffuse B-cell neoplasm presenting as primary mediastinal lymphoma. Hum Pathol 21;1262-1268,1990.
17. Lazzarino M, Orlandi E, Paulli M et al: Treatmen outcome and prognostic factors for primary mediastinal (thymic) B-cell lymphoma: a multicenter study of 106 patients. J Clin Oncol 15;1646-1653,1997.
18. Cazals-Hatem D, Lepage E, Brice P, et al: Primary mediastinal large B-cell lymphoma. A clinicopathological study of 141 cases compared qith 916 nonmediastinal large B-cell lymphomas, a GELA ("Group d'Etude des Lymphomas de l'Adulte") study. Am J Surg Pathol 20;877-888,1996.
19. Rudinger T, Jaffe ES, Delsol G et al.: Workshop report on Hodgkin's disease and related diseases ('grey zone' lymphoma). Ann Oncol 9 Suppl 5;S31-S38,1998.
20. Ferry JA, Harris NL, Picker LJ et al: Intravascular lymphomatosis (malignant angioendotheliomatosis). A B-cell neoplasm expressing surface homing receptors. Mod Pathol 1;444-452,1988.
21. Ponzoni M, Arrigoni G, Gould VE et al: Lack of CD 29 (beta1 integrin) and CD54 (ICAM-1) adhesion molecules in intravascular lymphomatosis. Hum Pathol 31;220-226,2000.
22. Nodor RG, Cesarman E, Chadburn A et al: Primary effusion lymphoma: a distinct clinicopathologic entity asso-

ciated with the Kaposi's sarcoma-associated herpes virus. Blood 66;645-656,1996.
23. Said W, Chien K, Tacìkeuchi S et al: Kaposi's sarcoma-associated herpesvirus (KSHV or HHV8) in primary effusion lymphoma: ultrastructural demonstration of herpesvirus in lymphoma cells. Blood 87;4937-4943,1996.
24. Teruya-Feldstein J, Zauber P, Setsuda JE et al: Expression of human erpesvirus-8 oncogene and cytokine homologoues in an HIV-seronegative patient with multicentric Castleman's disease and primary effusion lymphoma. Lab Invest 76;1637-1642,1998.
25. Harris NL, Stein H, Coupland SE et al: New approaches to lymphoma diagnosis. Hematology 2001; 1:194-220.

Autologous stem cell transplantation: is there still a role for high-dose therapy in the treatment of aggressive non-Hodgkin's lymphoma?

GINO SANTINI, MARINA CONGIU, SANDRO NATI, GENNARO MARINO, VALENTINA NARDI, MAURO SPRIANO, RENATO VIMERCATI, ALESSANDRA RUBAGOTTI

Over the last ten years, claims have been made that second and third generation chemotherapy (CT) regimens have improved survival in advanced stage, intermediate or high-grade malignancy non-Hodgkin's lymphomas (NHL) (Groups F-G-H-K/Working Formulation, excluding lymphoblastic and Burkitt's lymphoma).[1] In spite of this, the percentage of achievable complete remissions (CR) is between 50% to 70%, and about 50% of the patients later relapse.[2-4] Consequently, the probability of long-term real cure is about 35%.

Negative prognostic factors, together with histologic subtypes and advanced stage, affect both the possibility of obtaining CR and survival, and on the other hand, CR maintenance and disease-free survival (DFS).[5]

Failure to obtain CR, or subsequent relapse, has serious consequences for the course of these aggressive lymphomas, because second-line therapies offer poor possibility of salvage.[6-8]

Autologous stem cell transplantation (ASCT) has been seen to overcome resistance, allowing an increase in the dose of available drugs and radiotherapy. Stem cell rescue can shorten the hypoplastic period decreasing life-threatening risks. Initially used after first-line therapy for relapsed or refractory NHL, ASCT has since been used in more favorable clinical conditions such as partial remission (PR), first CR, and as front-line therapy following CT.

ASCT as salvage treatment in relapsed or refractory NHL

The conventional management of refractory or relapsing aggressive NHL is usually associated with poor results. About 30% of patients achieve CR, but the median survival time is less than 1 year, 3-year probability of survival is from less than 20% to 30%, and the 3-year probability of not having treatment failure is less than 10%.[6-8]

From the Department of Haematology, Division of Haematology I, San Martino Hospital, Genova, Italy.

Correspondence: Professor Gino Santini, Department of Haematology Division of Haematology I, Azienda Ospedaliera San Martino e delle Cliniche Universitarie Convenzionate, Largo Rosanna Benzi 10, 1613 Genova, Italy. E-mail: gino.santini@Hsanmartino.liguria.it

The first data referred to relapsed or refractory patients treated with ASCT by various authors and showed that these patients had a CR rate of about 50% - 60% but, following relapse, only 25% of patients maintained CR status. An initial stratification of these patients into two groups, those with true chemoresistance on the one hand, and chemosensitive patients on the other (sensitive relapse and PR), showed that the probability of achieving and maintaining CR was very poor (less than 10%) in chemoresistant patients. This initial observation was well defined by Philip *et al.* (1987),[9] who identified three groups of patients with different outcomes after ASCT. Sensitive relapse patients, after CR, had a 3-year probability of disease-free survival (DFS) of 36%, while patients with resistant relapse and refractory patients had a DFS of 14% and of 0%, respectively. The problem of patients with true resistance (resistant relapse and refractory patients) remains unresolved. Our personal experience[10] in this subset of patients, using high-dose cyclophosphamide (7 gr/m^2) followed by the BEAM regimen and ASCT, shows a 5-year probability of progression-free survival of 11%.

The Parma randomized trial, published by Philip *et al.* in 1995,[11] clarifies the role of ASCT in relapsed patients. Two-hundred and fifteen patients in relapse, with aggressive NHL, were treated with two courses of DHAP chemotherapy. One-hundred and nine chemosensitive patients were randomly assigned to receive 4 courses of DHAP plus radiotherapy *versus* the BEAC regimen and ASCT. Five-year probability of survival was 53% vs 32% in the two arms (p=0.03), in favour of the intensified therapy arm. Similar results were observed in terms of event-free survival: 46% in the ASCT arm, and 12% in the conventional arm (p=0.001).

Following these results, ASCT has been considered the standard treatment for aggressive NHL in sensitive relapse.

ASCT as the primary treatment for aggressive non-Hodgkin's lymphoma

In partial remission. Survival of patients responding to initial chemotherapy but not in remission after induction is very poor, in spite of salvage treatment. The median survival duration ranges between 5 and 14 months, with a 2-year survival probability of less than 30%.[6-8,12]

Following the concept that ASCT can cure about 50% of chemosensitive relapsed patients, the ASCT procedure was applied in a subset of patients who partially responded to front-line therapy. In 1988 Philip *et al.*[13] reported interesting results in 17 patients treated with high-dose therapy and ASCT while in PR after conventional CT. Thirteen patients (76%) are disease-free, with a 6-year survival probability of 75% after ASCT. In 1993 the *Non-Hodgkin's Lymphoma Cooperative Study* showed an overall probability of DFS of 36% at 5 years in 21 patients, that about one in three patients autotransplanted in PR after front-line therapy had potentially been cured.[14]

Only two randomized studies have been published in this field. In 1995, Verdonck *et al.*[15] evaluated the impact of ASCT in patients who failed to achieved CR after 3 courses of CHOP therapy. Patients were randomized to receive high-dose therapy plus ASCT or 5 additional courses of CHOP chemotherapy. The majority of patients enrolled in this trial had low and low to intermediate risk disease.[5] The study failed to demonstrate any benefit from ASCT in patients in first PR. On the contrary, a trend in favor of conventional therapy was reported.

In 1996, Martelli *et al.*[16] reported results of a randomized study designed to evaluate, by a second randomization, the effect of DHAP versus ASCT in aggressive NHL in early PR after first-line therapy. A group of 286 patients entered first randomization and 49 second randomization. Twenty-seven patients entered the DHAP arm and 22 the ASCT arm. CR was achieved in 59% of DHAP patients and in 96% of ASCT patients. The probability of progression-free survival (PFS) was 73% for ASCT and 52% for DHAP, with a probability of survival of 73% and 59%, respectively. However, because of the small number of patients involved, the study was unable to determine whether ASCT or a standard salvage therapy is better for patients in PR. We must conclude that the problem of ASCT in PR patients remains unresolved.

In 1st complete remission. Following the failure of 2nd and 3rd generation regimens over 1st generation ones in improving outcome in aggressive advanced stage NHL, a series of studies were carried out in favorable situations on patients in CR after front-line therapy. In these CR patients we expect a relapse rate ranging from 40 to 50%, in spite of received treatment.[2-4]

In 1994, following single phase II studies suggesting a potential benefit of ASCT for 1st CR aggressive NHL,[17,18] the French Group published a randomized study of 790 patients with aggressive NHL who had at least one adverse prognostic factor.[19] Following an initial randomization on anthracycline, 464 patients in 1st CR after induction therapy were randomized to receive high-dose therapy (CVB) plus ASCT or a consolidative sequential therapy including ifosfamide, etoposide, aspariginase, and cytarabine. With a median follow-up of 28 months, the 3-year DFS was similar in both arms: 52% in the sequential arm and 59% in the ASCT arm (p=0.46). Overall survival was once again similar, at 71% and 69%, respectively, (p=0.60). A retrospective analysis showed a positive trend in favor of higher-risk patients (2-3 negative factors at diagnosis), as defined by the I.P.I. adjusted for age < 60 years,[5] who had received ASCT. Successive, intermediate and final analyses published by the same Group in 1997 and in 2000 confirm a statistical benefit of ASCT over sequential therapy in higher-risk NHL in terms of DFS (59% and 39% at 5 years, respectively, p=0.01),[20] and in terms of overall survival (64% and 49% at 8 years, respectively, p=0.04).[21]

Evidence that ASCT could improve outcome in higher-risk patients has also been reported by Pettengell *et al.*[22] in 1996 and by Santini for the NHLCSG[23] in 1998.

In 2001 a randomized study proposed by Vitolo *et al.*[24] did not confirm any difference in response rate and outcome of higher-risk patients treated with high-dose sequential therapy (HDS: APO, high-dose/cyclophosphamide, high-dose methotrexate, high-dose VP16) plus high-dose therapy and ASCT compared with response rate and oucome in patients treated with an intensified outpatient CT. A second retrospective analysis of a randomized study presented in 2002 by Santini for the NHLCSG[25] does not show any difference in terms of survival and PFS for patients treated with VACOP-B + high-dose sequential therapy (high-dose/cytoxan, high-dose VP16), BEAM regimen and ASCT *versus* patients treated with VACOP-B (plus HDS and ASCT in case of persistent disease after front-line therapy).

A more recent study published in 2002 by Gisselbrecht *et al.*[26] compared an experimental, shortened treatment followed by high-dose therapy and ASCT versus ACVBP regimen plus sequential consolidation therapy in 370 higher-risk patients younger than 60 years. Results showed a statistically better survival and event-free survival in favor of patients treated with the ACVBP conventional treatment. The concluding comment was that the dose-intensity received before high-dose therapy was too low and that ASCT was given too early.

In conclusion, the real benefit of ASCT over conventional therapy for higher-risk patients is still open to debate.

After full-course standard induction therapy. In 1997, Vitolo *et al.*[27] reported a phase II study in

which 50 high-risk patients, presenting advanced stage disease at diagnosis with high tumor burden and elevated lactate dehydrogenase levels or bone marrow involvement, were treated with escalating sequential therapy. Patients received MACOP-B for 8 weeks followed by intensified therapy (mitoxantrone, high-dose ARA-C, dexamethasone), the BEAM regimen and ASCT. This study showed a progressively increased response rate according to the number of chemotherapy steps, with a final CR rate of 72%. With a median follow-up of 32 months from the start of treatment, overall survival and failure-free survival rates were 56% and 50%, respectively. In conclusion, the sequential scheme with intensified and high-dose CT with ASCT was seen to be feasible, with an improved outcome in a category of patients with a usually poor outcome.

In 2000, the NHLCSG28 reported similar results in 40 patients with bone marrow involvement at diagnosis. Patients received VACOP-B for 8 weeks, high-dose cyclophosphamide (7 gr/m^2), the BEAM regimen and ASCT. CR rate improved according to the various steps and, at the completion of treatment was 72.5%. The actuarial 3-year overall survival, DFS and failure-free survival probabilities were 48%, 55% and 40%, respectively.

Randomized studies are currently in progress, and in 1997 Gianni *et al.*[29] reported on 98 patients with diffuse high-risk, large B-cell NHL (Groups G and H/WF), who were randomized to either standard therapy with MACOP-B, or HDS (six chemotherapeutic agents administered sequentially at a high dose) followed by high-dose therapy and ASCT. Patients with T-cell lymphoma or bone marrow involvement were excluded from this study. After a median follow-up of 55 months, patients treated with HDS had a significantly better outcome than did those receiving MACOP-B. The CR rate was 96% vs 49% (p=0.001), freedom from progression 84% vs 49% (p<0.001), freedom from relapse 88% vs 70% (p=0.055), and event-free survival 76% vs 49% (p=0.004) in the two arms, respectively. Overall survival, in spite of a large trend in favor of HDS, was not statistically different (81% vs 55%, p=0.09). The conclusion was that HDS is superior to standard therapy for patients with diffuse large B-cell NHL.

In 1998, Santini for the NHLCSG[23] published results on 124 patients with diffuse, mixed and large-cell type NHL, randomized at study entry to receive standard induction VACOP-B therapy alone or the same regimen followed by ASCT. Patients were less than 60 years of age and had stage II bulky (tumor > 10 cm) or stage III-IV disease. Patients with initial bone marrow involvement were excluded. Patients who were randomized to receive standard induction therapy and achieved a CR simply went to follow-up. Patients with persistent disease after induction or who relapsed underwent DHAP salvage regimen. Patients randomized to receive VACOP-B and ASCT, proceeded to ASCT after induction therapy. Complete remission was similar in the two arms (75% and 73%) respectively. With a median follow-up observation of 42 months, 6-year survival probability was 65% in both arms. There was no difference in DFS or PFS between the two groups of patients. However, as reported before, when outcome was analyzed on the basis of age-adjusted IPI at diagnosis, patients with high-intermediate or high-risk disease were more likely to remain disease-free if they received additional ASCT (3-year DFS rate: 87% for ASCT vs 48% for standard therapy, p=0.008). In conclusion, this study showed an apparent improvement in survival (65% in both arms) compared with the survival of about 50% to be expected with a conventional front-line therapy.

Following all these considerations, in 2002 the NHLCSG25 reported interim results of a new study in which patients with aggressive, advanced stage NHL were randomized to receive VACOP-B (+ HDS in case of persistent disease) vs VACOP-B + HDS (CY, 7 gr/m^2; VP-16, 2 gr/m^2 and BEAM + PBPC rescue) in all cases. The aims of the study were: a) to confirm the Milan group's data; and b) to evaluate the possible use of HDS only when necessary. Two-hundred and twenty-three patients with mixed and large-cell NHL (Groups F/G/H/K-WF) aged from 15 to 59 years, with stage II bulky (> 10 cm), III and IV were included. All categories of patients, with B- and T-cell phenotype, and with initial bone marrow involvement were entered. When results were analyzed, 223 patients were evaluable for response. A third interim analysis shows CR of 65% and 67%, respectively. With a median observation period of 37 months, actuarial curves show a 6-year probability of survival and of PFS of 51% and 47% respectively, with no difference between the two arms. When only B-cell type, G and H/WF NHL without BM involvement were analyzed, probability of survival improved to 70% (conventional arm) and 80% (intensified arm), and PFS to 50% and 64%, respectively. Patients with T-cell type NHL and with BM involvement showed the poorest results. When patients with BM involvement were excluded, the probability of survival and PFS were 57% and 66%, and 48% and 54% in the two arms respectively. When outcome was analyzed according to age-adjusted IPI at diagnosis, lower-risk patients (0-1 negative factors at diagnosis) showed a statistically better outcome than those at higher-risk (2-3 negative factors at diagnosis).

Survival was 74% vs 46% (p=0.0001) and PFS 57% vs 40% (p=0.0001), respectively. When these two groups were analyzed no difference was seen between them. This analysis seems to confirm that strategies including high-dose sequential therapy plus ASCT can give very good results in selected group of patients, and suggests that results achieved with conventional treatment plus HDS and ASCT are similar to those reported with the simple use of conventional therapy and ASCT. On final observation is that there is no apparent difference in using intensified therapy after CT in all cases or only in cases of persistent disease, even in higher-risk patients.

In conclusion, this study suggests that differences between methods to select patients in various reported studies probably have an impact on the interpretation of the different results.

Conclusions

High-dose chemotherapy and autologous stem-cell transplantation has now became the standard care for eligible patients with recurrent, chemosensitive aggressive NHL. Primary refractory disease and resistant relapse are not good indications and patients with these should be considered a group eligible for phase II studies.

There may also be a role for high-dose chemotherapy and ASCT in patients with partially responsive disease. However new and larger randomized studies are needed to clarify this question.

A challenge for lymphoma management is the evaluation of the role of high-dose therapy and ASCT as an initial treatment in aggressive NHL, identifying patients who will be not cured with standard therapy. A series of concurrent or retrospective analyses would indicate so- called *higher-risk patients*, as defined by the IPI, as potential targets for intensified therapy. However, according to recently published data, the problem remains open to debate. Larger, randomized studies are necessary and welcome and these should be considered a high priority.

We hope that in the future, increased knowledge of the different biological properties of aggressive NHL and the addition of biological modifiers in the pre- and post- auto transplantation phase will enable us to improve the management of these categories of patients.

References

1. The non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classification of non-Hodgkin's lymphoma : Summary and description of a working formulation for clinical usage. Cancer 1982;49:2112-35.
2. Gordon LI, Harrington D, Andersen J, Colgan J, Glick J, Neiman R, et al. Comparison of a second-generation combination chemotherapeutic regimen (m-BACOD) with a standard regimen (CHOP) for advanced diffuse non-Hodgkin's lymphoma. N Engl J Med 1992;327: 1342-9
3. Fisher RI, Gaynor ER, Dahlberg S. Comparison of standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N Engl J Med 1993;328:1002-06
4. Sertoli MR, Santini G, Chisesi T, Congiu AM, Rubagotti A, Contu A, et al. MACOP-B versus ProMACE-MOPP in the treatment of advanced diffuse non-Hodgkin's Lymphomas: results of a prospective randomized trial by the Non-Hodgkin's Lymphoma Cooperative Study Group. J Clin Oncol 1994;12:1366-74.
5. The International Non-Hodgkin's Lymphoma Prognostic Factors Project: a predictive model for aggressive non-Hodgkin's lymphomas. N Engl J Med 1993;329:987-94.
6. Cabanillas F, Hagemeister FB, Mc Laughlin P, Velasquez WS, Riggs S, Fuller L, et al. Results of MIME salvage regimen for recurrent or refractory lymphoma. J Clin Oncol 1987;5:407-10.
7. Velasquez WS, Cabanillas F, Salvador P, McLaughlin P, Fridrik M, Tucker S, et al. Effective salvage therapy for lymphoma with cisplatin in combination with high-dose Ara-C and dexamethasone (DHAP). Blood 1988; 71:117-22.
8. Velasquez WS, Mc Laughlin P, Tucker S, Hagemeister FB, Swan F, Rodriguez MA, et al. ESHAP - an effective chemotherapy regimen in refractory and relapsing lymphoma: a 4-year follow-up study. J Clin Oncol 1994; 12:1169-76.
9. Philip T, Armitage JO, Spitzer G. High-dose therapy and autologous bone marrow transplantation after failure of conventional chemotherapy in adults with intermediate-grade or high-grade non-Hodgkin's lymphoma. N Engl J Med 1987;316:1493-8.
10. Santini G, De Souza C, Congiu A, Nati S, Marino G, Soracco M, et al. High-dose cyclophosphamide followed by autografting can improve the outcome of relapsed or resistant non-Hodgkin's lymphomas with involved or hypoplastic bone marrow. Leuk Lymphoma 1999;33: 321-30.
11. Philip T, Guglielmi C, Hagenbeek A, Somers R, Van der Lelie H, Bron A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med 1995;333:1540-5.
12. Haw R, Sawka CA, Franssen E, Berinstein NL. Significance of a partial or slow response to front-line chemotherapy in the management of intermediate-grade or high-grade non-Hodgkin's lymphoma: A literature review. J Clin Oncol 1994;12:1074-84.
13. Philip T, Hartmann O, Biron P. High-dose therapy and autologous bone marrow transplantation in partial remission after first-line induction therapy for diffuse non-Hodgkin's lymphoma. J Clin Oncol 1988;6:1118-24.
14. Santini G, Congiu AM, Coser P. Autologous bone marrow transplantation in 100 cases of poor-prognosis non-Hodgkin's lymphoma. A report of the Non-Hodgkin's Lymphoma Co-operative Study Group. In: Autologous Bone Marrow Transplantation, Proc of the Sixth International Symposium, Dicke KA and Keating A, editors. Arlington, Texas, USA. 1993. p. 75-82.
15. Verdonck LF, Van Putten WLJ, Hagenbeek A. Comparison of CHOP chemotherapy with autologous bone marrow transplantation for slowly responding patients with aggressive non-Hodgkin's lymphoma. N Engl J Med 1995;332:1045-51.
16. Martelli M, Vignetti M, Zinzani PL, Gherlinzoni F, Meloni G, Fiacchini M, et al. High-dose chemotherapy followed by autologous bone marrow transplantation versus dexamethasone, cisplatin, and cytarabine in aggressive non-Hodgkin's lymphoma with partial response to front-line chemotherapy: a prospective randomized Italian Multicenter Study. J Clin Oncol 1996;14:534-42.
17. Gulati SC, Shank B, Black P. Autologous bone marrow transplantation for patients with poor prognosis lymphoma. J Clin Oncol 1988;6:1303-13.
18. Nademanee A, Schmidt G, O' Donnell MR, Snyder DS, Parker PA, Stein A, et al. High dose chemoradiotherapy

followed by autologous bone marrow transplantation as consolidation therapy during first complete remission in adult patients with poor-risk aggressive lymphoma: a pilot study. Blood 1992;80:1130-4.
19. Haioun C, Lepage E, Gisselbrecht C. Comparison of autologous bone marrow transplantation with sequential chemotherapy for intermediate-grade and high-grade non-Hodgkin's lymphoma in first complete remission: a study of 464 patients. J Clin Oncol 1994; 12:2543-51.
20. Haioun C, Lepage E, Gisselbrecht C, Bastion Y, Coiffier B, Brice P, et al. Benefit of autologous bone marrow transplantation over sequential chemotherapy in poor-risk aggressive non-Hodgkin's lymphoma: Updated results of the prospective study LNH87-2. J Clin Oncol 1997;15:1131-7.
21. Haioun C, Lepage E, Gisselbrecht C, Salles G, Coiffier B, Brice P, et al. Survival benefit of high-dose therapy in poor-risk aggressive non-Hodgkin's lymphoma: Final analysis of the prospective study LNH87-2 protocol. A Groupe d'Etude des Lymphomes de l'Adult Study. J Clin Oncol 2000;18:3025-30.
22. Pettengell R, Radford GR, Morgenstern JH, Scarffe JH, Harris M, Woll PJ, et al. Survival benefit from high-dose therapy with autologous blood progenitor-cell transplantation in poor prognosis non-Hodgkin's lymphoma. J Clin Oncol 1996;14:586-92.
23. Santini G, Salvano L, Leoni P. VACOP-B versus VACOP-B plus autologous bone marrow transplantation for advanced diffuse non-Hodgkin's lymphoma: Results of a randomized trial by the Non-Hodgkin's Lymphoma Cooperative Study Group. J Clin Oncol 1998;16:2796-802.
24. Vitolo U, Liberati AM, Lambertenghi Deliliers G. A multicenter randomized trial by the Italian Lymphoma Intergroup (ILI) comparing high dose chemotherapy (HDS) with autologous stem cell transplantation (ASCT) vs intensified chemotherapy MegaCEOP in high risk diffuse large cell lymphoma (DLCL): no difference in outcome and toxicity. Blood 2001;98:3028.
25. Santini G, Congiu A, Olivieri A. Vacop-B vs Vacop-B + high-dose sequential therapy (HDS) for aggressive non-Hodgkin's lymphoma. Bone Marrow Transplant 2002; 29 Suppl 2110:[abstract].
26. Gisselbrecht C, Lepage E, Molina T, Quesnel B, Fillet G, Lederlin P, et al. Shortened first-line high-dose chemotherapy for patients with poor-prognosis aggressive lymphomas. J Clin Oncol 2002;20:2472-9.
27. Vitolo U, Cortellazzo S, Liberati AM, Freilone R, Falda M, Bertini M, et al. Intensified and high-dose chemotherapy with granulocyte colony-stimulating factors and autologous stem-cell transplantation support as first-line therapy in high-risk diffuse large-cell lymphoma. J Clin Oncol 1997;15:491-8.
28. Santini G, Coser P, Congiu AM. VACOP-B, high-dose cyclophosphamide and high-dose therapy with peripheral blood progenitor cell rescue for aggressive non-Hodgkin's lymphoma with bone marrow involvement: a study by the non-Hodgkin's Lymphoma Co-operative Study Group. Haematologica 2000;85:160-6.
29. Gianni AM, Bregni M, Siena S, Brambilla C, Di Nicola M, Lombardi F, et al. High-dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. N Engl J Med 1997;336:1290-7.

Issues in analysis of prognostic factors and lymphoma. Clinical vs. molecular: does it improve therapy?

GEORGE P. CANELLOS

The current standards for determining prognosis for most non-Hodgkin's lymphomas and Hodgkin's disease are based on clinical features present at diagnosis. The most widely applied scheme for large cell lymphoma is the International Prognostic Index (IPI). This was calculated from data supplied by European and North American centers and groups that identified five features which were statistically independent predictive factors. These factors are: age, stage, performance status, tumor bulk and lactate dehydrogenase level (LDH). The IPI successfully separates subgroups with very poor prognosis (16% of the group) or a good outcome (35% of the group). Approximately half of the patients are in the intermediate group with a median five-year survival of 43.5%, which approximates the group as a whole. Therefore, the IPI cannot separate patients within these groups and so more measurable biological information is necessary in order to subdivide groups of patients further.

In the last ten years, advances in molecular biology have resulted in the measurement of a number of biological markers, many of which correlate with outcome. Many of the *significant* biologic measures of prognosis have been determined in retrospect from patients' specimens with only cursory information available on the type, intensity and duration of chemotherapy. Many series have relatively small numbers of patients to provide adequate statistical power. Recently identified biological prognostic factors include: 1) proliferation indices, such as Ki^{67} reactivity with a specific antibody for nuclear proliferation antigen or tritiated thymidine uptake; 2) circulating biological factors in plasma, such as ß-2 microglobulin, LDH, tumor necrosis factor and its circulating receptors, VEGF, FGF, the protein product of the gene, nm-23 HI; 3) immunophenotypic markers of unique biological significance, such as CD5, adhesion molecular ICAM-1, T-cell markers, CD10; 4) mutated tumor suppressor genes such as p53; 5) excessive protein expression of regulatory molecules such as bcl-2, bcl-6, p53, c-myc, bax, and survivin; 6) cloned novel risk-related genes such as HGAL and BAL; 7) molecular profiling using cDNA or oligonucleatide arrays.

The technically more sohpisticated approach of gene microarrays has added a new dimension, separating patients with diffuse large cell lymphoma into two or possibly three prognostic groups with significant differences in five-year survivals. In a pilot study and large confirmatory analysis, patients with the germinal center B-cell-like gene pattern had a better survival than those with a peripheral blood activated B-cell-like pattern. In another supervised learning analysis, a molecular model separated DLBCL patients into favorable and unfavorable categories.

Ultimately, large numbers of patients will have to be studied prospectively to determine the clinical applicability of the interesting, early findings. Furthermore, this technology would have to be more generally available and of reasonable cost to benefit the largest number of patients. In addition, it will be important to link insights regarding molecular heterogenity of disease with more rational therapeutic strategies.

Prognosis may also be predicted by studying post-treatment state using PET scans with ^{18}F-FDG. The PET accumulation status of residual masses detected by computed tomography appears to have a secondary (post-treatment) predictive value, especially in large cell lymphoma.

From the Dana Farber Cancer Institute,44 Binney Street Dana 1B14, Boston, MA 02115 USA.

Correspondence: George P. Canellos, Medical Director for Network Development, Dana-Farber/Partners CancerCare William Rosenberg Professor of Medicine, Harvard Medical School, USA.

Rescue therapy in aggressive lymphomas

ATTILIO OLIVIERI

Lymphoma patients relapsing after autologous transplantation (ABMT) have a very poor prognosis, especially those affected by aggressive non-Hodgkin's lymphoma (NHL); in this setting, salvage chemotherapy or a second ABMT cannot substantially modify the very poor outcome which is characterized by a median overall survival (OS) of 3 months for patients with large cell lymphoma.[1]

A second transplant attempt in these patients is rarely feasible; in fact in the allogeneic setting the lack of a suitable donor and the very high transplant-related mortality (TRM) reported with myeloablative conditioning consistently reduce the number of patients who are candidates for this approach; on the other hand, it is very hard to obtain a sufficient peripheral blood stem cell (PBSC) harvest in patients who are candidates for a second ABMT and even though this procedure is feasible, the efficacy of a second attempt is often limited, due to lack of a grfat-versus-transplant effect.[2-5]

As a matter of fact, in these patients both poor performance status and low hematologic tolerance to chemotherapy often jeopardize any salvage options.

Rituximab has demonstrated high activity both in follicular lymphoma, as a single agent[6] and in aggressive NHL[7] including mantle cell lymphoma.[8] The combination of rituximab with CHOP chemotherapy has allowed a significant increase in the response rate both in follicular[9] and in high-grade NHL,[10] even though this association has never been tested in the post-transplant setting.

The mechanisms by which rituximab induces B-cell death include antibody-dependent complement cytotoxicity, complement-mediated toxicity and apoptosis also against chemoresistant B-lymphoma cells.[11]

On the other hand some observations suggest that the outcome of patients receiving rituximab can be improved if the number and the activity of their immune effector cells (in particular, natural killer cells) are preserved or enhanced.[12]

From the Clinica di Ematologia-Università di Ancona.

Correspondence: Attilio Olivieri, Clinica di Ematologia-Università di Ancona, Italy.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine which strongly increases the number and the activity of polymorphonuclear cells and macrophages against opsonized targets and recently some preliminary reports suggest that this cytokine can up regulate CD20 expression on lymphoid B-cells *in vitro* and *in vivo* .[13,14]

Our study was aimed to evaluate the safety and activity of an immunochemotherapeutic approach including CHOP, rituximab and GM-CSF, in a group of lymphoma patients after ASCT failure. Thirty-two lymphoma patients failing AMBT were rescued with CHOP-rituximab and GM-CSF.

Twenty-three patients received rituximab + CHOP chemotherapy, with a median number of 4 rituximab infusions (range 1-14); the GM-CSF administration was planned in order to up-regulate CD20 expression and to increase the phagocytic activity against the opsonized lymphoma cells, but also to reduce the leukopenia. Nine patients received rituximab GM-CSF alone because of a low blood count or poor performance status.

There were 2 early deaths (ED). Relevant non-fatal toxicity (WHO grade 3-4) consisted mainly in myelosuppression (rituximab alone 14.3% and rituximab + CHOP 28.3%).

The overall response rate (ORR), according to the intention-to-treat, was 72% (47% CR and 25% PR). After the end of treatment, twenty-two patients are still alive with a median follow-up of 13 months (range 1-44 months); 13 are alive and still in continuous CR, two died while in continuous CR, 1 patient has stable disease, 10 patients showed progressive and 4 patients relapsed. Ten patients died, two of treatment-related causes (ED), one patient relapsed and died in CR of an infectious complication post-allogeneic transplant, two patients died while in remission and 5 died of progressive disease. The projected PFS was 37% and the OS 60% at 44 months, with a nearly significant difference (p=0.07) between those treated with rituximab + CHOP (69%) and those treated with rituximab alone (42%).

In patients with a suitable donor a second allogeneic transplantation can offer a better chance of cure than a second ABMT, thanks to the potential graft-versus-leukemia effect, but this procedure is affected by unacceptable TRM.[15]

Recently, the use of the so-called reduced intensity conditioning (RIC) regimens has allowed a signifi-

cant reduction of TRM with very promising OS and PFS even in heavily pre-treated patients with hematologic malignancies[16] and the role of RIC after failure of ASCT has been investigated in 38 patients with lymphoproliferative malignancies;[17] these patients received a RIC including melphalan, fludarabine and Campath-1H. The TRM at 14 months was 20%, with an OS of 53% and PFS of 50%. Nevertheless only a minority of patients can benefit from this chance after ASCT failure, and a longer follow-up is needed.

In contrast, the association rituximab + CHOP + GM-CSF was feasible in about 70% of our patients after ABMT failure; the overall toxicity of this association was not negligible, but manageable even in this group of heavily pre-treated patients.

Our experience shows for the first time that there is an effective salvage treatment for patients with CD-20$^+$ B-lymphoma, who have failed to benefit from ABMT and who are often not eligible for allogeneic transplantation.

These data are particularly relevant for patients with high grade NHL; in fact the number of such patients reported here and their long follow-up strongly suggest that about one third of them have been cured while no definitive conclusions can be made for the few patients affected by mantle cell lymphoma and follicular cell lymphoma.

The good response rate and the promising outcome observed in this subset of patients could be due to a possible synergy between chemotherapy, rituximab and GM-CSF; the possibility of increasing the CD20 expression and *in vivo* ADCC by low dose GM-CSF administration after CHOP + rituximab, while at the same time increasing hematologic tolerance, seem very promising features in these poor prognosis patients, but the results should be confirmed in a larger population of patients and assessed in different subgroups, stratified according to histologic subtype.

References

1. Vose JM, Bierman PJ, Anderson JR, Kessinger A, Pierson J, Nelson J, et al. Progressive disease after high-dose therapy and autologous transplantation for lymphoid malignancy: clinical course and patient follow-up. Blood 1992;15:2142-8.
2. Tsai T, Goodman S, Saez R, Schiller G, Adkins D, Callander N, et al. Allogenic bone marrow transplantation in patients who relapse after autologous transplantation. Bone Marrow Transplant 1997;20:859-63.
3. Radich JP, Gooley T, Sanders JE, Anasetti C, Chauncey T, Appelbaum FR. Second allogenic transplantation after failure of first autologous transplantation. Biol Blood Marrow Transplant 2000;6:272-9.
4. De Lima M, van Besien KW, Giralt SA, Khouri IF, Mehra R, Andersson BS, et al. Bone marrow transplantation after failure of autologous transplant for non Hodgkin's lymphoma. Bone Marrow transplant 1997;19:121-7.
5. Haas R, Mohle R, Fruhauf S, Goldschmidt H, Witt B, Flentje M, et al. Patient characteristics associated with succesfull mobilizing and autografting of peripheral blood progenitor cells in malignant lymphoma. Blood 1994; 83:3787-94.
6. McLaughlin P, Hagemeister FB, Grillo-Lopez AJ. Rituximab in indolent lymphoma: the single-agent pivotal trial. Semin Oncol 1999;26 Suppl 14:79-87.
7. Coiffier B, Haioun C, Ketterer N, Engert A, Tilly H, Ma D, et al. Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. Blood 1998;92:1927-32.
8. Howard OM, Gribben JG, Neuberg DS, Grossbard M, Poor C, Janicek MJ, et al. Rituximab and CHOP induction therapy for newly diagnosed mantle-cell lymphoma: molecular responses are not predictive of progression free survival. J Clin Oncol 2002;20:1288-94.
9. Czuczman MS, Grillo-Lopez AJ, White CA, Saleh M, Gordon L, LoBuglio AF, et al. Treatment of patients with low grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. J Clin Oncol 1999;17:268-76.
10. Coiffier B, Lapage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus Rituximab compared with CHOP alone in elderly patients with diffuse large cell lymphoma. N Engl J Med 2002; 346:235-42.
11. Wilson W. Chemotherapy sensitization by Rituximab:experimental and clinical evidence. Semin Oncol 2000;27 Suppl 12:30-6.
12. Wilson S, Hurst D, Yuen A, Gluck L, Dayton M, Gockerman JP, et al. IL-2-mediated NK cell expansion correlates with clinical response to rituximab: results of 2 phase I trials of the combination of IL-2 and Rituximab. Blood 2001;98:2525[abstract].
13. Venugopal P, Sivaraman S, Huang X, Chopra H, O'Brien T, Jajeh A, et al. Upregulation of CD20 antigen expression in chronic lymphocitic leukemia (CLL) cells by in vitro exposure to cytokines. Blood 1998;92 Suppl 1: 1009[abstract].
14. Olivieri A, Lucesole M, Capelli D, Montanari M, Brunori M, Gini G, et al. GM-CSF addition to rituximab improves the response rate and survival in patients with non-Hodgkin's lymphoma refractory or relapsed after bone marrow transplantation. Haematologica 2001;86 Suppl 10:31.
15. Van Besien K, Sobocinski KA, Rowlings PA, Murphy SC, Armitage JO, Bishop MR,et al. Allogenic bone marrow transplantation for low-grade lymphoma. Blood 1998; 92:1832.
16. Corradini P, Tarella C, Olivieri A, Gianni AM, Voena C, Zallio F, et al. Reduced intensity conditioning followed by allografting of hematopoietic cells can produce clinical and molecular remissions in patients with poor-risk hematologic malignancies. Blood 2002;99:75-82.
17. Branson K, Chopra R, Kottaridis PD, McQuaker G, Parker A, Chakraverty RK, et al. Role of nonmyeloablative allogeneic stem-cell transplantation after failure of autologous transplantation in patients with lymphoproliferative malignancies. J Clin Oncol 2002;20:4022-31.

Indolent lymphoma: the pathologist view point

STEFANO A. PILERI, ELENA SABATTINI, STEFANO ASCANI, FRANCESCO BACCI, PHILIP WENT

In 1994, the members of the International Lymphoma Study Group (ILSG) proposed the *revised European American lymphoma (REAL) classification*,[1] aiming at overcoming the different approaches (e.g. the Kiel Classification[2] and Working Formulation)[3] employed in Europe and the USA, which hampered comparison among clinico-pathologic trials. The proposal of the REAL classification was followed by a validation study, carried out by pathologists external to the ILSG and based on a series of cases collected world-wide, which showed that the ILSG scheme was superior to both the Kiel Classification and Working Formulation in terms of interpersonal and intrapersonal reproducibility.[4] More recently, the World Health Organisation (WHO) adopted the REAL classification as a model for the categorization of all the tumors of lymphoid and hematopoietic tissues.[5]

The REAL/WHO classification consists in a list of distinct entities, which are defined by an amalgamation of morphologic, phenotypic, genotypic, and clinical findings and can be updated on the basis of new evidence emerging from the literature. Unlike previous schemes, the REAL/WHO classification does not provide grades of malignancy, as the clinical behavior and response to therapy are not influenced by cell size and number of mitotic figures, as postulated in the Kiel Classification and Working Formulation, but depend on the category the tumor belongs to and within each category on a series of biological mechanisms, which work differently in each individual patient. On one hand, this explains the artificiality of certain distinctions of the past, such as the ones between low and high-grade peripheral T-cell lymphomas, all types of T-cell tumor showing a poor prognosis with the exception of mycosis fungoides and ALK-positive anaplastic large cell lymphoma. On the other hand, such an approach has favored research to define the risk factors within each lymphoma category, which has found its cutting edge in tissue micro-array and gene expression profiling techniques.

In 1995 Dan Longo proposed the usage of some terms such as indolent, aggressive and very aggressive lymphoma, which do actually refer to the natural history of the disease irrespectively of its response to therapy.[6] Although they have no room in the REAL/WHO classification, these terms are commonly used in the daily practice. In particular, the label *indolent* lymphoma applies to lymphoid tumors with a survival measurable in years, independently of whether or not any therapy is given. These lymphoproliferative disorders have very variable clinical presentations. Some are constantly systemic diseases, often with leukemic manifestations. Others have an extranodal primary presentation and can remain localized for long periods, even in the absence of any therapy. Yet others correspond to tumors with nodal presentation, which can have widespread immune system involvement at the time of diagnosis. This has led to the basic distinction of three fundamental subtypes of indolent lymphoma: disseminated leukemias/lymphomas, extranodal forms and nodal ones (Table 1). Interestingly, all these neoplasms are derived from the B-cell system, but one: T large granular lymphocyte leukemia. In spite of the fact that they

Table 1. List of indolent lymphomas.

Disseminated lymphomas/leukaemias
B-cell chronic lymphocytic leukaemia
Lymphoplasmacytic lymphoma
Hairy cell leukaemia
Splenic marginal zone B-cell lymphoma (+/- villous lymphocytes)
Plasma cell myeloma/plasmacytoma
T large granular lymphocyte leukaemia
Extranodal lymphomas
Extranodal marginal zone B-cell lymphoma of MALT type
Nodal lymphomas
Small lymphocytic lymphoma
Follicular lymphoma
Nodal marginal zone B-cell lymphoma (+/- monocytoid B cells)

From the Chair of Pathologic Anatomy & Haematopathology Unit, Institute of Haematology and Clinical Oncology "L. & A. Seràgnoli", Bologna University, Bologna, Italy. 1Institute of Pathology, University Hospital Basel, Basel, Switzerland.

Correspondence: Stefano A. Pileri, Chair of Pathologic Anatomy & Haematopathology Unit, Institute of Haematology and Clinical Oncology "L. & A. Seràgnoli", Bologna University, Bologna, Italy.

correspond to entities widely acknowledged in the literature, most of them have recently been the object of challenging molecular studies, which have focused on new aspects relevant to both better understanding of their histogenesis and the identification of new therapeutic strategies.[7,8]

References

1. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994;84:1361-92.
2. Stansfeld AG, Diebold J, Noel H, Kapanci Y, Rilke F, Kelenyi G, et al. Updated Kiel classification for lymphomas. Lancet 1988; 1:292-3.
3. Non-Hodgkin's lymphoma pathologic classification project. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. Cancer 1982; 49:2112-35.
4. The Non-Hodgkin's Lymphoma Classification Project: a clinical evaluation of the International Lymphoma Study Group Classification of Non-Hodgkin's Lymphoma. Blood 1997; 89:3909-18.
5. Jaffe ES, Harris NL, Stein H, Vardiman JW. Tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press, International Agency for Research on Cancer. 2001; 175-6.
6. Longo DL. The REAL Classification of lymphoid neoplasms: one clinician's view. In: PPO Updates. Rosenberg S, Editor. Philadelphia: Lippincott; 1995. p. 9:1-12.
7. Harris NL, Stein H, Coupland SE, Hummel M, Favera RD, Pasqualucci L, et al. New approaches to lymphoma diagnosis. Haematology Am Soc Hematol Educ Program 2001;194-220.
8. Liu H, Ye H, Ruskone-Fourmestraux A, De Jong D, Pileri S, Thiede C, et al. T(11;18) is a marker for all stage gastric MALT lymphomas that will not respond to H. pylori eradication. Gastroenterology 2002;122:1286-94.

True complete remission is a reasonable goal of therapy in chronic lymphocytic leukemia

MICHAEL J. KEATING

Chronic lymphocytic leukemia (CLL) is a disease in which palliation has been a goal for many years. Simple therapy with chlorambucil or other alkylating-agent programs were associated with short, multiple remissions with eventual relapse. The development of the purine analogs, particularly fludarabine and 2-chlorodeoxyadenosine (2-CDA), have improved the complete remission rate and remission duration but not had any demonstrated effect on long-term survival. Combinations of purine analogs and alkylating agents have been developed based on the inhibition of DNA repair, which occurs as a consequence of fludarabine inhibiting DNA repair enzymes. The development of monoclonal antibodies has led to new opportunities in the management of CLL. The response rate to rituximab as a single agent at conventional doses is low. However, the addition of rituximab to cell lines improves cell killing by chemotherapeutic agents including fludarabine and cyclophosphamide.

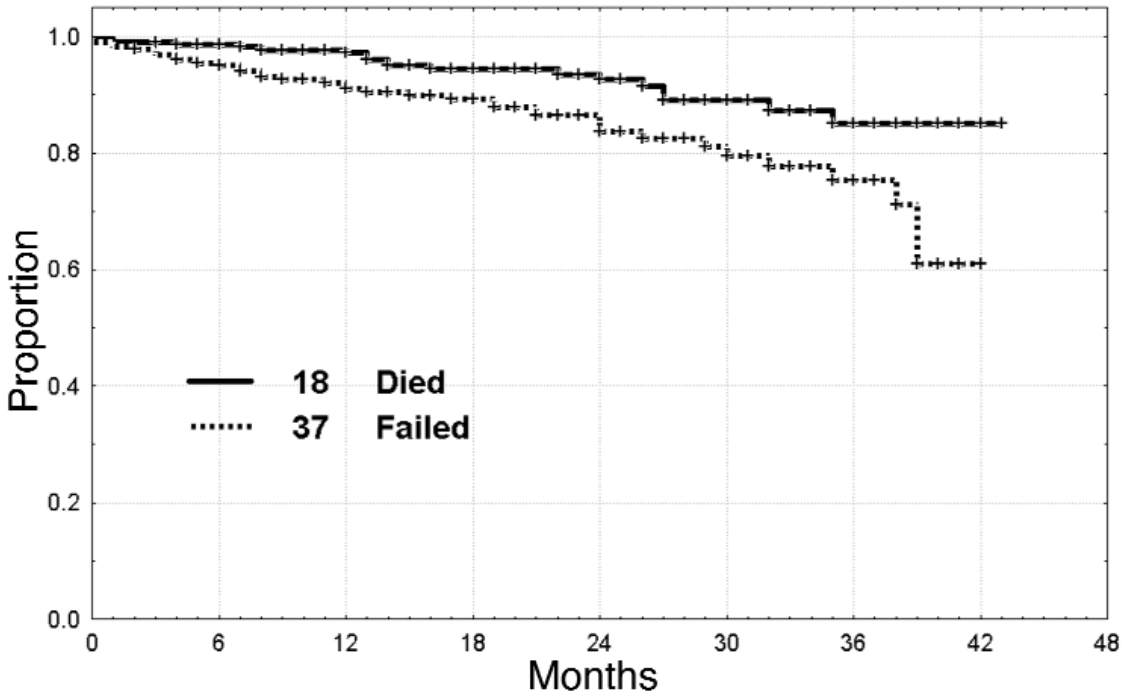
Thus a chemo-immunotherapy protocol of fludarabine, cyclophosphamide, and rituximab (FCR) was developed. The doses are rituximab 375 mg/m^2 on day 1 of the course and 500 mg/m^2 on day 1 of courses 2 – 6. Courses are repeated every four weeks. The fludarabine dose is 25 mg/m^2 per day for three days and that of cyclophosphamide 250 mg/m^2 for three days. Two hundred and two patients have been treated using this protocol as initial therapy and the overall complete response rate is 68%. The early death rate is 1%. The majority of patients are able to achieve flow cytometry-defined remission with < 1% of cells co-expressing CD5 and CD19. Fifty percent of the complete responders have achieved a polymerase chain reaction (PCR) negative state. The gene, which is amplified, is the variable immunoglobulin heavy chain sequence, which is a signature gene of the CLL cells. A ratio is developed where the IgVH is compared to the amplified *ras* gene. There is a clear relationship between the probability of staying in remission and whether the patients achieve a National Cancer Institute Working Group (NCIWG)-defined complete remission or not. A stronger relationship is present with the flow cytometry-defined remissions. Only one patient who has become PCR-negative has had a subsequent relapse. The regimen is well tolerated with only 2% of courses being associated with major infections (pneumonia or septicemia). The first infusion of rituximab is associated with a 10% incidence of grade 3 or 4 reactions. Subsequent doses of rituximab have minimal toxicity. The major hematologic toxicity is neutropenia. Anemia and thrombocytopenia are much less common. The incidence of Coombs' positive hemolytic anemia or pure red cell aplasia is approximately 3 – 4%. At a maximum follow-up of 42 months, 85% of the patients are still alive and 75% of the patients are still in remission. Myelosuppression is dose-limiting in older patients and those with high white blood cell counts and serum β-2-microglobulin.

The same regimen has been administered to 177 patients who have had previous therapy. The complete remission rate is 25% and the overall response rate 73%. The patients who achieve complete remission have significantly longer remission duration and survival than patients treeated with previous protocols. The response rate, remission duration and survival produced by FCR are significantly better than those produced by FC. There is evidence that treating of patients with minimal residual disease with Campath-1H can achieve PCR negativity in approximately

Response to FC + Rituximab (NCI-WG).

	Untreated (202 Pts.)		Prior Rx (177 Pts.)	
Response	#Pts.	(%)	#Pts.	(%)
CR	137	(68)	45	(25)
Nodular PR	26	(18)	28	(16)
PR	29	(14)	56	(32)
No Response	8	(4)	42	(24)
Early Death	2	(1)	6	(3)

Correspondence: Michael J. Keating, M.B., B.S, Associate head for Clinical research, Chair in clinical leukemia research, Professor of Medicine, Department of Leukemia, Division of Medicine, University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA.



FCR–survival and time to treatment failure (202 pts.)

half of the cases. Thus by strategic use of chemo-immunotherapy programs in the management of CLL a very significant number of patients can now achieve PCR negativity. All of these treatments can be conducted on an outpatient basis. These strategies hold promise for improving the overall survival of patients with CLL.

References

1. Keating MJ, Manshouri T, O'Brien S, Wierda W, Kantarjian H, Lerner S, et al. A high proportion of true complete remission can be obtained with a fludarabine, cyclophosphamide, Rituximab combination (FCR) in chronic lymphocytic leukemia. Proc Am Soc Clin Oncol 2003;
2. Wierda W, Garcia-Manero G, O'Brien S, Faderl S, Lerner S, Albitar M, et al. Molecular remissions achieved in patients with relapsed or refractory chronic lymphocytic leukemia treated with combined fludarabine, cyclophosphamide, and Rituximab. Proc Am Soc Clin Oncol 2003;

The use of purine analogs in the therapy of indolent lymphomas

PIER LUIGI ZINZANI

From a therapeutic viewpoint, indolent lymphomas are particularly controversial diseases. Treatment options range from monochemotherapeutic palliative treatments to front-line high-dose therapy followed by autologous hematopoietic rescue. The purine analogs, particularly fludarabine, are a novel group of antimetabolite compounds that are highly active in lymphoid malignancies. They broaden the therapeutic armory and may provide the basis for more effective and potentially curative lymphoma treatment. Non-comparative studies evaluating fludarabine monotherapy have reported objective overall and complete response (CR) rates ranging from 30% to 70% and from 10% to 38%, respectively. In general, previously treated patients showed lower response rates than do untreated ones. Recently, experimental evidence that fludarabine is a potent inhibitor of repair of DNA damage has encouraged the use of this purine analog in combination with other agents such as mitoxantrone or idarubicin; higher overall responses (70%-90%) and especially CR rates (20%-45%) have been observed in pretreated patients. Moreover, phase II trials using fludarabine in combination with cyclophosphamide in patients with previously untreated indolent lymphoma resulted in overall response rates approaching 90%-95%. In particular, over 60% of previously untreated patients with follicular lymphoma obtained CR after combination therapy with fludarabine and mitoxantrone. In a multicenter trial comparing fludarabine alone vs. fludarabine plus idarubicin (FLU-ID) the final data suggest two main considerations: i) in patients with follicular lymphomas, FLU-ID initially produces an inferior CR rate (39%) but eventually confers a higher quality of response, as indicated by the superior RFS rate; ii) in patients with the other histologic subtypes, FLU-ID seems to produce either better (in the cases of small lymphocytic and immunocytomas) or equivalent (in the case of mantle cell) CR rates to those achieved with fludarabine alone. As regards the use of fludarabine in combination with cyclophosphamide, recent data have shown CR rates ranging from 60% to 80% in untreated follicular lymphoma patients, with 5-year overall survival and RFS rates as high as those reported for other therapeutic approaches in untreated patients. The *Eastern Co-operative Oncology Group* and *Italian Co-operative Study Group* are currently performing two separate randomized trials (fludarabine plus cyclophosphamide vs. CVP conventional chemotherapy regimen and fludarabine plus mitoxantrone vs. CHOP conventional chemotherapy regimen) to evaluate the effectiveness of fludarabine regimens for untreated indolent lymphoma patients. Concerning our *Italian Co-operative Study Group* trial, 12 Italian centers are randomizing patients for a comparative study of fludarabine plus mitoxantrone (FM) versus CHOP chemotherapy with the addition of rituximab in selected cases. To be eligible, patients are required to have a histologically proven diagnosis of CD20-positive lymphoma according to the REAL classification and a positive polymerase chain-reaction (PCR) analysis (bone marrow and/or peripheral blood) for Bcl-2/IgH, age 15 to 70 years, stage II-IV and an ECOG performance status of 0-2. After randomization, patients are allocated to the FM arm (fludarabine 25 mg/m^2/day IV on days 1 to 3 and mitoxantrone 10 mg/m^2 IV on day 1) or CHOP arm (doxorubicin 50 mg/m^2 IV on day 1, cyclophosphamide 750 mg/m^2 IV on day 1, vincristine 1.4 mg/m^2 IV on day 1 and prednisone 100 mg/day orally on days 1 to 5). Patients in both arms are assigned to receive 6 cycles of chemotherapy. Thereafter, to be eligible for rituximab treatment, the patients had to have had a partial or complete clinical response (PR or CR) and still be PCR-positive in the bone marrow and/or peripheral blood in two molecular analyses performed 4 and 6 weeks after the sixth cycle. These patients were elected to receive 4 weekly IV doses of rituximab (375 mg/m^2). So far, 150 patients have been enrolled.

From the Institute of Hematology and Medical Oncology "Seràgnoli", University of Bologna, Italy

Correspondence: Pier Luigi Zinzani, Institute of Hematology and Medical Oncology "Seràgnoli", University of Bologna, Italy.

Myeloablative radiochemotherapy followed by stem cell transplantation versus interferon-maintenance therapy for treatment in remission after cytoreductive chemotherapy in follicular lymphomas. Results of a randomized study by the German low grade Lymphoma Study Group (GLSG)

W. Hiddemann, M. Unterhalt, M. Dreyling for the GLSG

Little progress has been achieved over the past decades in the treatment of follicular lymphomas (FL) and the prognosis of patients with this disease has literally remained unchanged. New treatment options, however, justify the hope for improvements in the near future. These perspectives comprise the addition of rituximab to combination chemotherapy for remission induction, maintenance with rituximab and particularly myeloablative radiochemotherapy with subsequent stem cell transplantation. The last approach is based on the radiosensitivity of lymphoma cells and has revealed promising results in a series of phase II studies. In April 1996 the GLSG embarked on a prospective, randomized comparison of myeloablative radiochemotherapy with subsequent stem cell transplantation versus interferon-α maintenance that was considered standard treatment. Initially therapy comprised a randomized comparison between CHOP (cyclophosphamide, adriamycin, vincristine, prednisone) versus MCP (mitoxantrone, chlorambucil and prednisone) or CHOP versus rituximab + CHOP.

A total of 664 patients (571 FL) entered this trial between July 1996 and February 2003. Of these patients, 405 (355 FL) who achieved a complete or partial remission after initial treatment were eligible for subsequent randomization for myeloablative radiochemotherapy with stem cell transplantation (SCT) versus interferon-α maintenance. This randomization was balanced for histology, the type of initial cytoreductive therapy, risk factors and the rapidity of response to initial chemotherapy. At the last evaluation in February 2003 a highly significant advantage in favor of myeloablative radiochemotherapy with stem cell transplantion was observed for event-free interval (estimated median of 28 months (26 for FL) after end of initial therapy for the IFN-group while the median was not reached in the SCT group or any of its subgroups after 6 years of observation, $p<0.0005$). Stem cell transplantion was feasible with peripheral blood SCT-related death occurring in 2.7% (95%-CI: 0.9%-6.2%) of patients. As of today no differences have been observed in overall survival, this being 80% (86% for FL) at 4.5 years for all patients.

These data indicate marked anti-lymphoma activity of myeloablative radiochemotherapy followed by stem cell transplantation and a significant improvement of the event-free interval. Further follow-up is needed to judge the impact of this approach on overall survival.

From the Klinikum Großhadern, Medizinische Klinik III, Marchioninistr. 15, 81377 München, Germany.

Correspondence: Prof. Dr. Wolfgang Hiddemann, Klinikum Großhadern, Medizinische Klinik III, Marchioninistr. 15, 81377 München, Germany. Phone: international +49.89.70952550. Fax: international +49:89.70958875. E-mail: sekrmed3@med3.med.uni-muenchen.de