MODIFICATIONS OF THE IWG CRITERIA

PET

PET using [^{18}F]fluorodeoxyglucose (FDG), has emerged as a powerful functional imaging tool for staging, restaging, and response assessment of lymphomas.[4-24,25] The advantage of PET over conventional imaging techniques such as computed tomography (CT) or magnetic resonance imaging is its ability to distinguish between viable tumor and necrosis or fibrosis in residual mass(es) often present after treatment.[9,11,26-28] This information may have important clinical consequences. Juweid et al[20] evaluated the impact of integrating PET into the IWG criteria in a retrospective study of 54 patients with diffuse large B-cell NHL who had been treated with an anthracycline-based regimen. PET increased the number of complete remission (CR) patients, eliminated the CRu category, and enhanced the ability to discern the difference in progression-free survival (PFS) between patients experiencing CR and partial remission (PR). Such findings provided rationale for incorporating PET into revised criteria.

However, a number of issues with PET need to be considered. The technique for performing and interpreting PET has only recently been standardized.[29] There is variability among readers and equipment. PET is also associated with false-positive findings due to rebound thymic hyperplasia, infection, inflammation, sarcoidosis, or brown fat. Diffusely increased bone marrow uptake is often observed after treatment or administration of hematopoietic growth factors.[19,29,33,34] There are also false-negative results with PET relating to the resolution of the equipment, technique, and variability of FDG avidity among histologic subtypes.[10,29-32] These and other considerations regarding interpretation of PET scans have recently been addressed.[29]

Recommendations for the use of PET or PET/CT. Current recommendations for the use of PET scans reflect the FDG avidity of the lymphoma subtype, and the relevant end points of the clinical trial (Table 1).

1. PET is strongly recommended before treatment for patients with routinely FDG-avid, potentially curable lymphomas (eg, diffuse large B-cell lymphoma [DLBCL], Hodgkin's lymphoma) to better delineate the extent of disease; however, currently it is not mandated because of limitations imposed by cost and availability. For incurable, routinely FDG-avid, indolent, and aggressive histologies (eg, follicular lymphoma and mantle-cell lymphoma), and for most variably FDG-avid lymphomas, the primary end points for clinical trials generally include PFS, event-free survival, and overall survival. PET is not recommended before treatment unless response rate is a major end point of the trial.

2. Numerous studies have demonstrated that PET performed after one to four cycles of multiagent chemotherapy predicts therapeutic outcome[5-7,21,24,35,36]; however, no currently available data demonstrate improvement in results by altering treatment based on this information. Until such data exist, this practice should be restricted to clinical trials evaluating PET in this context.

3. PET is essential for the post-treatment assessment of DLBCL and Hodgkin's lymphoma because a complete response is required for a curative outcome. However, PET is recommended in the other, incurable histologies only if they were PET positive before treatment and if response rate is a primary end point of a clinical study.

4. Current data are inadequate to recommend routine surveillance PET scans after the restaging study.

Timing of PET scans after therapy. Post-therapy inflammatory changes may persist for up to 2 weeks after chemotherapy alone in lymphoma patients and for up to 2 to 3 months or longer after radiation therapy or chemotherapy plus radiation. To minimize the frequency of these potentially confounding interpretation finding, PET scans should not be performed for at least 3 weeks, and preferably 6 to 8 weeks, after completion of therapy.[29]

Definition of a positive PET scan. Visual assessment currently is considered adequate for determining whether a PET scan is positive, and use of the standardized uptake value is not necessary.[29] A more extensive description of interpretation of PET scans is provided in the consensus guidelines of the Imaging Subcommittee.[29] In brief, a positive scan is defined as focal or diffuse FDG uptake above background in a location incompatible with normal anatomy or physiology, without a specific standardized uptake value cutoff.[29] Other causes of false-positive scans should be ruled out. Exceptions include mild and diffusely increased FDG uptake at the site of moderate- or large-sized masses with an intensity that is lower than or equal to the mediastinal blood pool, hepatic or splenic nodules 1.5 cm with FDG uptake lower than the surrounding liver/spleen

Table 1. Recommended Timing of PET (PET/CT) Scans in Lymphoma Clinical Trials

Histology	Pretreatment	Mid-Treatment	Response Assessment	Post-Treatment Surveillance
Routinely FDG avid				
DLBCL	Yes*	Clinical trial	Yes	No
HL	Yes*	Clinical trial	Yes	No
Follicular NHL	Not	Clinical trial	Not	No
MCL	Not	Clinical trial	Not	No
Variably FDG avid				
Other aggressive NHLs	Not	Clinical trial	Not‡	No
Other indolent NHLs	Not	Clinical trial	Not‡	No

Abbreviations: PET, positron emission tomography; CT, computed tomography; FDG, [^{18}F]fluorodeoxyglucose; DLBCL, diffuse large B-cell lymphoma; HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; MCL, mantle-cell lymphoma; ORR, overall response rate; CR, complete remission.
*Recommended but not required pretreatment.
†Recommended only if ORR/CR is a primary study end point.
‡Recommended only if PET is positive pretreatment.

uptake, and diffusely increased bone marrow uptake within weeks after treatment. Specific criteria for lung nodules based on lesion size have been developed.[29]

Bone Marrow Assessment

Restaging bone marrow examinations are commonly used to assess response to therapy. The determination of involvement may be difficult, given that no universally accepted standards exist. The usual approach to response determination relies on morphologic assessment of the bone marrow biopsy, and clot section if adequate and available, whereas ancillary studies using immunohistochemistry, flow cytometry, and polymerase chain reaction methodology are largely ignored or underused. Moreover, a direct comparison of these studies and their respective sensitivity and specificity for the detection of occult but clinically meaningful involvement are lacking. Thus, recommendations regarding the use of these strategies and their interpretation are largely empiric at this time.

The recommendation for bone marrow response is that histologically normal bone marrows with a small (< 2%) clonal B-cell population detected by flow cytometry should be considered normal, given that definitive clinical studies that demonstrate an inferior outcome are lacking. Immunohistochemistry has a clear role in the assessment of the bone marrow at diagnosis and restaging after therapy. When antibodies are used to detect CD20 and CD3 expression, morphologically normal bone marrows can often be shown to harbor disease. Sensitivity can be increased with the use of subtype-specific antibody panels directed at CD5, cyclin D1, CD23, CD10, DBA44, and kappa and lambda light chains. Less common lymphoma subtypes with occult bone marrow disease are particularly well suited to this approach, including splenic marginal zone B-cell lymphomas and a number of subtypes of DLBCL (ie, intravascular large B-cell lymphoma and HIV-related DLBCL). Indolent B-cell lymphomas and chronic lymphocytic leukemia are more difficult to assess, given that the distinction from reactive lymphoid aggregates and nodular partial remissions in the bone marrow can be difficult to assess because of the frequent admixture of reactive T cells in these diseases. Immunohistochemistry using anti-CD5 and anti-CD23 can be helpful in this setting, as are stains for kappa and lambda light chains that can detect surface membrane immunoglobulin in paraffin sections. Similarly, antibodies to cyclin D1 and CD10 are useful for recognizing subtle bone marrow involvement in mantle-cell lymphoma and follicular lymphoma, respectively. In the future, antibodies to Bcl-6 may improve detection of occult follicular lymphoma in the bone marrow; however, technical problems preclude their general use at this time. In fact, many routinely used immunohistochemical reagents can be difficult to apply consistently to the evaluation of bone marrow samples, largely due to subtleties in fixation methods and decalcification techniques.

Caution is recommended when interpreting biopsies post-therapy for residual disease. The use of rituximab may lead to a false-negative interpretation of residual B-cell disease, despite the fact that the widely used commercial anti-CD20 (L26) recognizes a cytoplasmic epitope of CD20, in contrast to the surface epitope recognized by rituximab. The judicious use of another pan–B-cell antibody, CD79a, is strongly recommended when evaluating post-treatment samples. Similar caution is required when interpreting CD20 flow cytometric data for several months after therapy with rituximab, given that surface epitopes may be blocked. The availability of clot sections allows for immunohistochemical analysis without the influence of decalcification and may be useful for the post-treatment evaluation of bone marrow involvement.

Lastly, the role of molecular genetic analyses in the determination of response to therapy is difficult to resolve. Assay techniques and sensitivity vary enormously between laboratories, making systematic recommendations impossible. Residual clonal disease may exist without morphologic evidence of lymphoma (ie, gastric mucosa-associated lymphoid tissue [MALT] lymphoma after therapy). In aggregate, these data suggest that the disappearance of the molecular clone may lag behind the disappearance of morphologic evidence of disease. Alternatively, these findings may represent the persistence of residual disease or potentially repopulating lymphoma stem cells in biopsies lacking morphologic evidence of lymphoma. These distinctions need to be reconciled before molecular testing can be considered routine, particularly when the findings affect treatment decisions.

Sensitive and sophisticated diagnostic approaches such as flow cytometry and/or molecular genetic analyses should be incorporated into clinical trials to determine their relevance and potential utility for directing therapy. However, for routine practice we do not recommend that clinical decision making be based solely on flow cytometry and/or molecular genetic analyses that indicate a residual small (< 2% of gated or live events) B-cell clone in the absence of other supportive findings from morphology and immunohistochemistry. We strongly encourage investigators to collect these data together with clinical correlative data that might eventually support their routine use for the assessment of response criteria for lymphoid malignancies.

REVISED RESPONSE CRITERIA

CR

The designation of CR requires the following (Table 2):

1. Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy.

2a. Typically FDG-avid lymphoma: in patients with no pretreatment PET scan or when the PET scan was positive before therapy, a post-treatment residual mass of any size is permitted as long as it is PET negative.

2b. Variably FDG-avid lymphomas/FDG avidity unknown: in patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, all lymph nodes and nodal masses must have regressed on CT to normal size (≤ 1.5 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1.0 cm in their short axis before treatment must have decreased to ≤ 1.0 cm in their short axis after treatment.

3. The spleen and/or liver, if considered enlarged before therapy on the basis of a physical examination or CT scan, should not be palpable on physical examination and should be considered normal size by imaging studies, and nodules related to lymphoma should disappear. However, determination of splenic involvement is not always reliable because a spleen considered normal in size may still contain lymphoma, whereas an enlarged spleen may reflect variations in anatomy, blood volume, the use of hematopoietic growth factors, or causes other than lymphoma.

Table 2. Response Definitions for Clinical Trials

Response	Definition	Nodal Masses	Spleen, Liver	Bone Marrow
CR	Disappearance of all evidence of disease	(a) FDG-avid or PET positive prior to therapy; mass of any size permitted if PET negative (b) Variably FDG-avid or PET negative; regression to normal size on CT	Not palpable, nodules disappeared	Infiltrate cleared on repeat biopsy; if indeterminate by morphology, immunohistochemistry should be negative
PR	Regression of measuable disease and no new sites	≥ 50% decrease in SPD of up to 6 largest dominant masses; no increase in size of other nodes (a) FDG-avid or PET positive prior to therapy; one or more PET positive at previously involved site (b) Variably FDG-avid or PET negative; regression on CT	≥ 50% decrease in SPD of nodules (for single nodule in greatest transverse diameter); no increase in size of liver or spleen	Irrelevant if positive prior to therapy; cell type should be specified
SD	Failure to attain CR/PR or PD	(a) FDG-avid or PET positive prior to therapy; PET positive at prior sites of disease and no new sites on CT or PET (b) Variably FDG-avid or PET negative; no change in size of previous lesions on CT		
Relapsed disease or PD	Any new lesion or increase by ≥ 50% of previously involved sites from nadir	Appearance of a new lesion(s) > 1.5 cm in any axis, ≥ 50% increase in SPD of more than one node, or ≥ 50% increase in longest diameter of a previously identifed node > 1 cm in short axis Lesions PET positive if FDG-avid lymphoma or PET positive prior to therapy	> 50% increase from nadir in the SPD of any previous lesions	New or recurrent involvement

Abbreviations: CR, complete remission; FDG, [^{18}F]fluorodeoxyglucose; PET, positron emission tomography; CT, computed tomography; PR, partial remission; SPD, sum of the product of the diameters; SD, stable disease; PD, progressive disease.

4. If the bone marrow was involved by lymphoma before treatment, the infiltrate must have cleared on repeat bone marrow biopsy. The biopsy sample on which this determination is made must be adequate (with a goal of > 20 mm unilateral core). If the sample is indeterminate by morphology, it should be negative by immunohistochemistry. A sample that is negative by immunohistochemistry but that demonstrates a small population of clonal lymphocytes by flow cytometry will be considered a CR until data become available demonstrating a clear difference in patient outcome.

CRu

The use of the above definition for CR and that below for PR eliminates the category of CRu.

PR

The designation of PR requires all of the following:

1. At least a 50% decrease in sum of the product of the diameters (SPD) of up to six of the largest dominant nodes or nodal masses. These nodes or masses should be selected according to all of the following: they should be clearly measurable in at least 2 perpendicular dimensions; if possible they should be from disparate regions of the body; and they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.

2. No increase should be observed in the size of other nodes, liver, or spleen.

3. Splenic and hepatic nodules must regress by ≥ 50% in their SPD or, for single nodules, in the greatest transverse diameter.

4. With the exception of splenic and hepatic nodules, involvement of other organs is usually assessable and no measurable disease should be present.

5. Bone marrow assessment is irrelevant for determination of a PR if the sample was positive before treatment. However, if positive, the cell type should be specified (eg, large-cell lymphoma or small neoplastic B cells). Patients who achieve a CR by the above criteria, but who have persistent morphologic bone marrow involvement will be considered partial responders.

When the bone marrow was involved before therapy and a clinical CR was achieved, but with no bone marrow assessment after treatment, patients should be considered partial responders.

6. No new sites of disease should be observed.

7. Typically FDG-avid lymphoma: for patients with no pretreatment PET scan or if the PET scan was positive before therapy, the post-treatment PET should be positive in at least one previously involved site.

8. Variably FDG-avid lymphomas/FDG-avidity unknown: for patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, CT criteria should be used.

In patients with follicular lymphoma or mantle-cell lymphoma, a PET scan is only indicated with one or at most two residual masses that have regressed by more than 50% on CT; those with more than two residual lesions are unlikely to be PET negative and should be considered partial responders.

Stable Disease

Stable disease (SD) is defined as the following:

1. A patient is considered to have SD when he or she fails to attain the criteria needed for a CR or PR, but does not fulfill those for progressive disease (see Relapsed Disease [after CR]/Progressive Disease [after PR, SD]).

2. Typically FGD-avid lymphomas: the PET should be positive at prior sites of disease with no new areas of involvement on the post-treatment CT or PET.

3. Variably FDG-avid lymphomas/FDG-avidity unknown: for patients without a pretreatment PET scan or if the pretreatment PET was negative, there must be no change in the size of the previous lesions on the post-treatment CT scan.

Relapsed Disease (after CR)/Progressive Disease (after PR, SD)

Lymph nodes should be considered abnormal if the long axis is more than 1.5 cm regardless of the short axis. If a lymph node has a long axis of 1.1 to 1.5 cm, it should only be considered abnormal if its short axis is more than 1.0. Lymph nodes $\leq 1.0 \times \leq 1.0$ cm will not be considered as abnormal for relapse or progressive disease.

1. Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased FDG uptake in a previously unaffected site should only be considered relapsed or progressive disease after confirmation with other modalities. In patients with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.

2. At least a 50% increase from nadir in the SPD of any previously involved nodes, or in a single involved node, or the size of other lesions (eg, splenic or hepatic nodules). To be considered progressive disease, a lymph node with a diameter of the short axis of less than 1.0 cm must increase by $\geq$ 50% and to a size of 1.5 $\times$ 1.5 cm or more than 1.5 cm in the long axis.

3. At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis.

4. Lesions should be PET positive if observed in a typical FDG-avid lymphoma or the lesion was PET positive before therapy unless the lesion is too small to be detected with current PET systems ($<$ 1.5 cm in its long axis by CT).

Measurable extranodal disease should be assessed in a manner similar to that for nodal disease. For these recommendations, the spleen is considered nodal disease. Disease that is only assessable (eg, pleural effusions, bone lesions) will be recorded as present or absent only, unless, while an abnormality is still noted by imaging studies or physical examination, it is found to be histologically negative.

In clinical trials where PET is unavailable to the vast majority of participants, or where PET is not deemed necessary or appropriate for use (eg, a trial in patients with MALT lymphoma), response should be assessed as above, but only using CT scans. However, residual masses should not be assigned CRu status, but should be considered partial responses.

Primary CNS Lymphomas

Recommendations of the International Workshop on Evaluation of Primary Central Nervous System Lymphomas were adopted in their entirety.[37]

Primary Gastric Lymphoma

Evaluation of patients with primary gastric lymphomas, especially MALT lymphomas, is difficult and confounded by the observation that prolonged clinical remissions may be associated with transient histologic and molecular relapses, and persistence of monoclonal B cells after histologic regression.[38,39] Repeated biopsies remain a fundamental follow-up procedure, despite problems with reproducibility.

Interpretation of residual lymphoid infiltrates in post-treatment gastric biopsies can be difficult, with no uniform criteria for the definition of histologic remission. Older assessment systems have not been adopted uniformly.[40,41] A histologic grading system proposed by the Groupe d'Etude des Lymphomes de l'Adulte may be an improvement over prior schemes, but will require additional validation.[42,43]

Follow-Up Evaluation

The manner in which patients are evaluated after completing treatment may vary according to whether treatment was administered in a clinical trial or clinical practice, or whether treatment was delivered with curative or palliative intent. Good clinical judgment and a careful history and physical examination are the most important components of monitoring patients after treatment. Additional testing at follow-up visits should include CBC and serum chemistries, including lactate dehydrogenase and other blood tests and imaging studies for relevant clinical indications. There is no evidence to support regular surveillance CT scans, given that the patient or physician identifies the relapse more than 80% of the time without the need for imaging studies.[44-47] Data with PET are also insufficient to recommend routine procedures at this time.[48]

In a clinical trial, uniformity of reassessment is necessary to ensure comparability among studies with respect to the major end points of event-free survival, disease-free survival, and PFS. It is obvious, for example, that a protocol requiring re-evaluation every 2 months will produce different results compared with one requiring the same testing annually, even if the true times to events are the same. One recommendation has been to assess patients on clinical trials after completion of treatment at a minimum of every 3 months for 2 years, then every 6 months for 3 years, and then annually for at least 5 years.[1] Few recurrences occur beyond that point for patients with diffuse large-cell NHL or Hodgkin's lymphoma. However, the risk of relapse for patients with follicular and other indolent histologies is continuous. These intervals may vary with specific treatments, duration of treatment, protocols, or unique drug characteristics. Recently, the National Comprehensive Cancer Network published recommendations for follow-up of patients with Hodgkin's and NHL:[49,50] for patients with Hodgkin's lymphoma in an initial CR, an interim history and physical examination every 2 to 4 months for 1 to 2 years, then every 3 to 6 months for the next 3 to 5 years, with annual monitoring for late effects after 5 years. For follicular or other indolent histology lymphoma patients in a CR, the recommendation for follow-up was every 3 months for a year then every 3 to 6 months. For diffuse large B-cell NHL, the guidelines proposed follow-up every 3 months for 24 months then every 6 months for 36 months.[49,50]

Patients with a follicular or low-grade NHL who are being managed with a so-called watch and wait approach should be monitored for the development of disease-related symptoms or signs of organ involvement. No consensus regarding the frequency of follow-up of such patients exists and the interval should be specified in the protocol. Otherwise, imaging studies should be individualized based on the location of the disease and informed by the behavior of palpable disease.

END POINTS

The major end points of clinical trials should reflect the histology, clinical situation (eg, initial treatment *v* salvage), and objectives of the study (Table 3). It is important that consistent definitions of end points are used, and we hope that this document will harmonize the use of those definitions.

End points based on tumor measurements are greatly influenced by response criteria. Overall and complete response rates usually can be assessed accurately in single-arm as well as randomized trials.

Table 3. Efficacy End Points

End Point	Patients	Definition	Measured From
Primary			
Overall survival	All	Death as a result of any cause	Entry onto study
Progression-free survival	All	Disease progression or death as a result of any cause	Entry onto study
Secondary			
Event-free survival	All	Failure of treatment or death as a result of any cause	Entry onto study
Time to progression	All	Time to progression or death as a result of lymphoma	Entry onto study
Disease-free survival	in CR	Time to relapse or death as a result of lymphoma or acute toxicity of treatment	Documentation of response
Response duration	In CR or PR	Time to relapse or progression	Documentation of response
Lymphoma-specific survival	All	Time to death as a result of lymphoma	Entry onto study
Time to next treatment	All	Time to new treatment	End of primary treatment

Abbreviations: CR, complete remission; PR, partial remission.

However, response rates do not necessarily influence other measures of overall clinical benefit or outcome in patients with lymphoma,[51] and are not considered as important as other end points. Exceptions are phase II trials of novel new agents, in which identification of biologic activity is of interest. Durable complete responses, if associated with measures of clinical benefit, may also be relevant.

Overall Survival

Overall survival is the least ambiguous end point, although it usually is not optimal to use for a lymphoma clinical trial. Overall survival is defined as the time from entry onto the clinical trial (random assignment in a phase III study) until death as a result of any cause. Survival, as well as other time-dependent variables (PFS, event-free survival) should be measured in a randomized trial because data derived from historical controls are unreliable and subject to bias. Survival should be measured in the intent-to-treat population, including all patients even if they did not fulfill the eligibility criteria. A per-protocol analysis includes all patients who received the treatment to which they were assigned. A treatment-given analysis includes all patients who received a particular treatment. Both of these types of analyses should be interpreted with caution because they are subject to considerable bias.

PFS

PFS is defined as the time from entry onto a study until lymphoma progression or death as a result of any cause. PFS is often considered the preferred end point in lymphoma clinical trials, especially those involving incurable histologic subtypes (eg, follicular, other low-grade lymphoma, or mantle cell lymphoma). PFS reflects tumor growth, and therefore is interpretable earlier than the end point of overall survival. In addition, PFS is not confounded by the administration of subsequent therapy. However, in studies in which failure to respond without progression is considered an indication for another therapy, such patients should be censored at that point for the progression analysis. Whether a prolongation of PFS represents direct clinical benefit or is an acceptable surrogate for clinical benefit depends on the magnitude of the effect and the risk-benefit ratio of the therapy under investigation. Unlike survival, the precise date of progression is generally unknown. It may be defined as the first date of documentation of a new lesion or enlargement of a previous lesion, or the date of the scheduled clinic visit immediately after radiologic assessment has been completed. When there is missing information, censoring of the data may be defined as the last date at which progression status was assessed adequately or the first date of unscheduled new antilymphoma treatment.

Event-Free Survival

Event-free survival (time to treatment failure) is measured from the time from study entry to any treatment failure including disease progression, or discontinuation of treatment for any reason (eg, disease progression, toxicity, patient preference, initiation of new treatment without documented progression, or death). This composite end point is generally not encouraged by regulatory agencies because it combines efficacy, toxicity, and patient withdrawal. However, it may be useful in the evaluation of some therapies such as those that are highly toxic.

Time to Progression

Time to progression (TTP) is defined as the time from study entry until documented lymphoma progression or death as a result of lymphoma. In TTP, deaths from other causes are censored either at the time of death or at an earlier time of assessment, representing a random pattern of loss from the study. TTP is not as useful as PFS unless the majority of deaths on a study are unrelated to the lymphoma due to the toxicity of the treatment and/or prolonged follow-up.

Disease-Free Survival

Disease-free survival is measured from the time of occurrence of disease-free state or attainment of a CR to disease recurrence or death as a result of lymphoma or acute toxicity of treatment. This definition may be complicated by deaths that occur during the follow-up period that are unrelated to the lymphoma, and there is controversy about whether such deaths should be considered as events or censored at the time of occurrence. Although it is often possible to identify those deaths related to the lymphoma, there is the potential for bias in the attribution of deaths.

Response Duration

Response duration is from the time when criteria for response (ie, CR or PR) are met, for which the event is the first documentation of relapse or progression.

Lymphoma-Specific Survival

Lymphoma-specific survival (eg, disease-specific survival, cause-specific survival) is defined as time from study entry to death as a result of lymphoma. This end point is potentially subject to bias because the exact cause of death is not always easy to ascertain. To minimize the risk of bias, the event should be recorded as death as a result of lymphoma, or as a result of toxicity from the drug. Death as a result of unknown causes should be attributed to the therapy.

Time to Next Treatment

For certain trials, time to next lymphoma treatment may be of interest, and is defined as time from the end of primary treatment until the institution of the next therapy.

Clinical Benefit

One of the most important end points for patients as well as for drug approval by regulatory agencies has been evidence of clinical benefit. Clinical benefit may reflect improvement in quality of life, or reduction in patient symptoms, transfusion requirements, frequent infections, or other parameters. Time to reappearance or progression of lymphoma-related symptoms can also be used in this end point.

We hope that these revised guidelines will improve comparability among studies, and facilitate new agent development leading to improved therapies for patients with lymphoma.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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Case Report

T-Cell Large Granular Lymphocyte Leukemia of Donor Origin After Cord Blood Transplantation

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Abstract

We report the first case of T-cell large granular lymphocyte leukemia of donor origin after a second cord blood transplantation for acute myeloid leukemia, and review the literature regarding rare cases of T-cell–origin posttransplantation lymphoproliferative disorders.

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Key words: Bone marrow, Epstein-Barr virus, Polymerase chain reaction, Posttransplantation lymphoproliferative disorders, T-cell receptor

Introduction

T-cell large granular lymphocyte leukemia (LGL; LGLL) is characterized by the monoclonal proliferation of $CD3^+$, and $CD8^+$ LGLs, with abundant cytoplasm and fine or coarse azurophilic granules.[1,2] Reactive expansion of LGL in the peripheral blood has been occasionally reported during viral infection and in recovery phase of allogeneic hematopoietic stem cell transplantation (HSCT).[3,4]

Posttransplantation lymphoproliferative disorder (PTLD) is a characteristic lymphoid proliferation or the development of lymphoma in a setting of decreased T-cell immune surveillance, typically in recipients of solid organ transplantation or allogeneic HSCT. Most reported cases of PTLD are of B-cell origin, in association with Epstein-Barr virus (EBV) infection, which leads to monoclonal or, less frequently, polyclonal proliferation of B cells. Most of the rare cases of T-cell PTLD were reported after solid organ transplantation, with very rare cases after allogeneic HSCT.

In this report, we describe the unique clinical and laboratory findings of a patient with γδ T-cell LGLL of cord donor origin after a second cord blood transplantation for acute myeloid leukemia.

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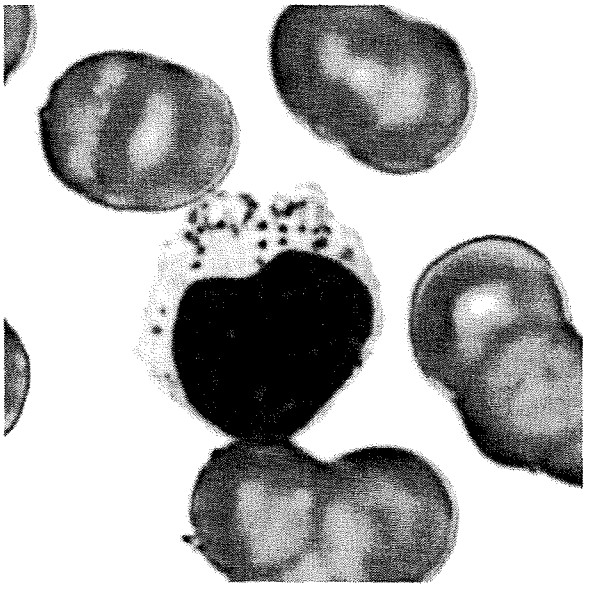
Case Report

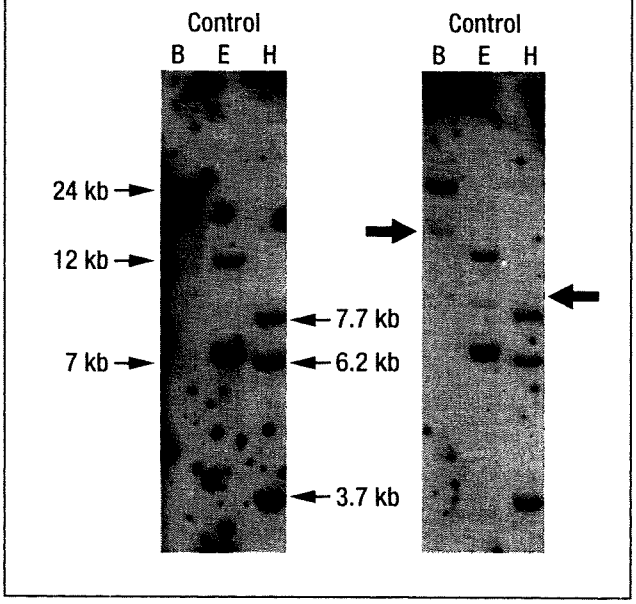
A 58-year-old Japanese man with acute myeloid leukemia (French-American-British classification; M2) in second complete remission received allogeneic HSCT from an unrelated female cord blood donor. The conditioning regimen consisted of total body irradiation of 12 Gy in 6 fractions from day −6 to −4, and cyclophosphamide 60 mg/kg once daily intravenously on days −3 to −2 (total dose, 120 mg/kg). He received human leukocyte antigen–loci mismatched (2 by serology and 2 by DNA typing) unrelated cord blood, which contained 3.03×10^7 nucleated cells/kg in January 2003. Cyclosporine and short-term methotrexate were used as graft-versus-host disease prophylaxis. However, hematologic recovery was not observed up to day 40, and we concluded that this was a case of primary graft failure without leukemia relapse because the results of interphase fluorescence in situ hybridization analysis on days 23, 30, and 37 on bone marrow (BM) samples were negative. Because his condition remained good, we planned a second cord blood transplantation with a reduced-intensity regimen, which consisted of fludarabine 30 mg/kg once daily intravenously from days −8 to −3 (total dose 180 mg/kg), busulfan 4 mg/kg orally on days −6 and −5 (total dose 8 mg/kg), and total body irradiation of 4 Gy in 1 fraction on day −1. Cyclosporine and mycophenolate mofetil 15 mg/kg twice daily were administered. On day 51 of the initial transplantation in March 2003, human leukocyte antigen–loci mismatched (2 by serology and 3 by DNA typing) male cord blood, containing 2.6×10^7/kg nucleated cells, was infused. Neutrophil engraftment was observed by day 33 after second transplantation. Acute and chronic graft-versus-host disease did not develop, and cyclosporine was tapered off in November 2003.

Figure 1 T-Cell Large Granular Lymphocyte Leukemia Stained with May-Giemsa on the Peripheral Blood Smear



The predominant cells were typical of LGLs with abundant cytoplasm and fine or coarse azurophilic granules.
Hematoxylin and eosin stain; original magnification ×1000.

Figure 2 Southern Blots of T-Cell Receptor β-Chain Gene Rearrangements



DNA from BM of this patient was hybridized with a TCR Cβ1 probe.
Arrows indicate rearranged bands.
Abbreviations: B = Bam H1; E = Eco R; H = Hind III

In February 2004, 10 months after the second cord blood transplantation, he developed anorexia, abdominal distention with fluid accumulation, and edema in the lower extremities. A computed tomography scan showed gross ascites and mild pleural effusion but no sign of enlarged lymph nodes or hepatosplenomegaly. The peripheral white blood cell count was 10,300/μL (10.3 × 10^9/L), and 30% of the cells had a morphology of medium to large lymphocytes with abundant azurophilic granules in the cytoplasm, as shown in Figure 1. The hemoglobin level was 8.8 g/dL (88 g/L), and the platelet count was 192 × 10^3/μL (1.92 × 10^9/L).

A retrospective review of the peripheral blood smears disclosed that the appearance of LGL coincided with the tapering off of immunosuppression 3 months before the admission.

Flow cytometry examination of the peripheral blood mononuclear cells showed a homogeneous population of T-cell LGLs positive for CD2, CD3, CD8, CD56, and T-cell receptor (TCR)–γδ, but negative for CD4 and TCR-αβ. The BM biopsy specimen histologically showed 10% of hypocellular gelatinous marrow with diffuse infiltration of medium to large lymphoid cells. Immunoperoxidase studies on sections of BM showed strong expression of T-cell–restricted intracellular antigen-1, partially positive staining of CD8 and granzyme B, but no expression of CD3 or CD20. Southern blot analysis of the BM cells revealed a clonal rearrangement of the TCR-β chain, as shown in Figure 2 and TCR-δ chain (data not shown).

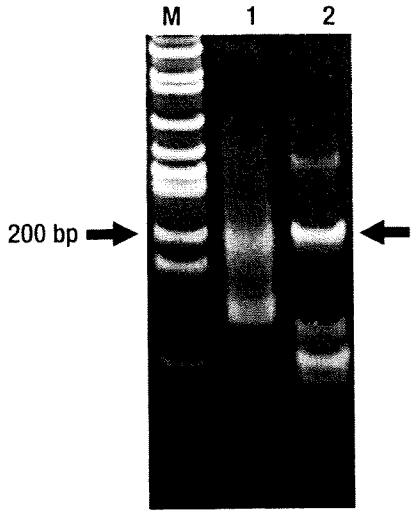
Abdominal paracentesis was performed with milky chylous fluid, and a flow cytometry examination showed results similar to those in the peripheral blood. Multiprimer-based polymerase chain reaction (PCR) analysis of ascitic cells also showed clonal rearrangement of the TCR-δ chain, as shown in Figure 3. The primer sets were used in the following locations: Vδ1, 5'-AAA GTG GTC GCT ATT CTG TC-3'; Vδ2A, 5'-GCA CCA TCA GAG AGA GAT GA-3'; Jδ, 5'- TGG TTC CAC AGT CAC ACG GG-3'; Dδ3B, 5'-TTG TAG CAC CGT GCG TAT CC-3'. The amplified 200 base-pair PCR products of the TCR-δ chain were then cloned into the pCR-TOPO vector. The DNA sequences of 3 clones amplified by vectors were identical and had high homology to TCR-δ chain including a 197 base-pair sequence (data not shown). This sequence also involved the forward and reverse primers Vδ1 and Jδ, respectively, described previously.

The results of all of the previously mentioned studies indicated the clonal expansion of T cells compatible with a diagnosis of T-cell LGLL with γδ T-cell phenotype involving peripheral blood, BM, and ascites.

Donor-recipient DNA chimerism was analyzed by comparing the short tandem repeat findings for the donor blood sample and pretransplantation recipient samples. Eleven short tandem repeat loci were analyzed by PCR using an AmpFlSTR SGM Plus® kit. The peripheral blood sample (containing 30% T-LGL) and the second cord blood sample showed the same peaks at the locus (D16S539), as shown in Figure 4. These results further confirmed that the expanded γδ T-LGL cells were exclusively of second cord blood transplantation donor origin.

Serologic examination showed no evidence of viral infection. Real-time PCR analysis revealed a high load of EBV (7.9 × 10^3 copies/10^6 cells). However, in situ hybridization studies of BM cells did not reveal EBV-encoded small RNA, and Southern blot analysis of BM cells also showed no band for

Figure 3 Polymerase Chain Reaction for T-Cell Receptor δ Gene Rearrangement



(1) Negative control; and (2) patient's sample of frozen neoplastic lymphoid cells in ascites. A clonal band was identified at approximately 200 base pairs.
Abbreviations: bp = base pairs; M = molecular weight marker

clonal EBV genomes. Chromosome analysis demonstrated a normal 46, XY karyotype in all 20 cells examined.

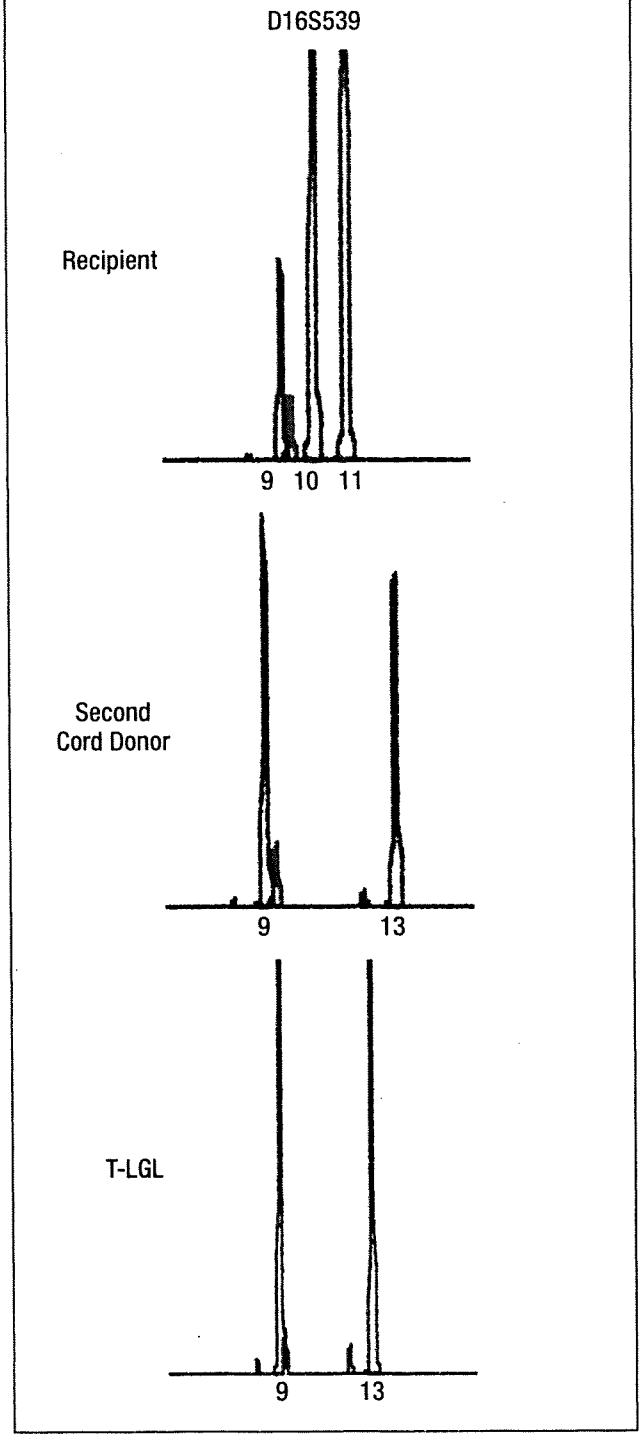
After admission, his abdominal distention and dyspnea with hypoxemia progressed rapidly with spiking fever. A computed tomography scan demonstrated acute respiratory distress syndrome. Because we found no evidence of bacterial or fungal infection or drug-induced pneumonia, cyclosporine and methylprednisolone were started immediately but with no effect, and he died of acute respiratory failure 1 week later. A postmortem lung biopsy showed extensive diffuse alveolar damage without the T-LGL cell's involvement; on the other hand, the leukemic cell involvement in Glisson's sheath was shown by a liver biopsy.

Discussion

In this case, the increase in LGLs developed 7 months after the second cord blood transplantation, and the kinetics of LGLs correlated with the tapering off of immunosuppression, which suggested the possibility that lymphocytosis might have been associated with reactive expansion because of viral infection or an alloimmune reaction. However, our case showed *TCR-β* and *TCR-δ* gene rearrangement by Southern blot analysis and *TCR-δ* gene rearrangement by PCR and cytotoxic T-cell immunophenotype, which were compatible with T-cell LGLL.

Most cases of PTLD, usually of B-cell origin, are associated with EBV infection and represent the EBV-induced monoclonal expansion of B cells in conditions with decreased T-cell immune surveillance.[5,6] Although there have been some reports of EBV-associated PTLD after cord blood transplantation,[7-10] the incidence of PTLD of T-cell origin has been reported to be only 4%-14% with a less frequent association with EBV.[6,11]

Figure 4 Donor-Recipient DNA Chimerism Analysis by Comparing the Short Tandem Repeat



The peripheral blood sample (containing 30% T-LGL) and the second cord blood sample showed the same peaks at the locus (D16S539).

In our case, because a high viral load of EBV was detected by real-time PCR analysis, we initially speculated that γδ T-LGLL was EBV-associated PTLD, but this was later denied based on the results of EBV-encoded small RNA in situ

Table 1A Literature Review of T-Cell Posttransplantation Lymphoproliferative Disorder After Hematopoietic Stem Cell Transplantation[7,13-16]

Study	Case Number	Age/Sex	Donor	Diagnosis	Origin	Involved Organ
Zutter et al[13]	1	14/Male	Sibling*	Lymphoblastic lymphoma	Recipient	Lymph node, BM
Zutter et al[13]	2	9/Male	Sibling*	Lymphoblastic lymphoma	ND	Pericardium, pleura
Zutter et al[13]	3	2/Female	Father	NHL (polymorphic)	Donor	Lung, liver, spleen
Wang et al[14]	4	13/Male	Sibling*	NHL (diffuse large)	Recipient	Lymph node
Sirvent et al[7]	5	ND/ND	ND	LGL ($\alpha\beta$)	ND	PB, BM
Collins et al[15]	6	11/Male	ND	NHL (polymorphic)	ND	Lymph node, brain
Au et al[16]	7	39/Male	Unrelated	LGL	Donor	PB, BM
Our Case	8	58/Male	UCB	LGL ($\gamma\delta$)	Donor	PB, BM, ascites, liver

*Human leukocyte antigen–matched sibling.
Abbreviations: ND = not determined; NHL = non-Hodgkin lymphoma; PB = peripheral blood; UCB = unrelated cord blood

Table 1B Literature Review of T-Cell Posttransplantation Lymphoproliferative Disorder After Hematopoietic Stem Cell Transplantation[7,13-16]

Study	Case Number	Time to PTLD* (Days)	EBER-ISH	Rearrangement	Survival† (Days)
Zutter et al[13]	1	1290	Not determined	TCR-γ (SB)	851
Zutter et al[13]	2	630	Not determined	Not determined	180
Zutter et al[13]	3	39	Not determined	Polyclonal	11
Wang et al[14]	4	601	Negative	TCR-γ (PCR)	> 1170
Sirvent et al[7]	5	300	Negative	TCR-β (SB)	> 690
Collins et al[15]	6	90	Negative	Not determined	29
Au et al[16]	7	180	Negative	TCR-γ (PCR)	134
Our Case	8	330	Negative	TCR-β (SB), TCR-δ (SB, PCR)	30

*Time from transplantation to PTLD.
†Survival time from diagnosis of PTLD.
Abbreviations: EBER-ISH = EBV-encoded small RNA in situ hybridization; SB = Southern blotting

hybridization stains and Southern blot EBV terminal repeat analysis. Therefore, the clinical significance of EBV infection in this case remains undetermined.

Most previously reported cases of T-cell PTLD developed after solid organ transplantation,[12] and there have been only 7 previously documented cases of T-cell PTLD after allogeneic HSCT, as summarized in Table 1.[7,13-16] Posttransplantation lymphoproliferative disorder was of donor origin in 3 of 8 total cases, including our case, of recipient origin in 2, and of undetermined origin in the remaining 3. No correlation has been demonstrated between EBV and T-cell PTLD after HSCT.

Generally, most cases of B-cell posttransplantation lymphoproliferative disorder after HSCT develop within the first 5 months, because the balance between proliferating EBV-infected B cells and cytotoxic T cells cannot be controlled with the unrecovered lymphocyte components.[17] In solid organ transplantation, EBV-positive cases tend to occur earlier than EBV-negative cases, ie, a median interval of 6-10 months compared with 4-5 years.[6,7] Some cases of T-cell PTLD have a longer interval between the day of transplantation and the occurrence of PTLD than in B-cell PTLD. The donor source of transplantation included sibling (3 cases), father (1 case), unrelated (1 case), cord (our case), and not described (2 cases). Therefore, whereas there has been very little experience with cases after cord blood transplantation, all 8 cases of PTLD in the literature are of B-cell origin.[8-11] Our case is the first report of PTLD of T-cell origin after cord blood transplantation and might reflect very intense immunosuppression passing through consecutive cord blood transplantation.

It has been reported that T-cell PTLD has a worse prognosis than B-cell PTLD in a solid organ transplantation setting. In 1 series of 6 cases presenting with T-cell non-Hodgkin lymphoma as PTLD, pulmonary involvement was reported in 5 cases and marrow infiltration in 4 cases. All patients showed aggressive courses.[18] Of importance is that of 8 patients with T-cell PTLD after HSCT: 3 patients who died within 30 days had extranodal involvement in the lung, liver, spleen, brain, and/or ascites.

Conclusion

We have reported an unusual case of EBV-negative, T-cell PTLD as γδ T-cell LGLL of donor origin after a second cord blood transplantation. The occurrence of T-cell PTLD after HSCT is extremely rare, and the efficient accumulation of knowledge and further research are needed to establish the oncogenic mechanism and appropriate therapeutic maneuvers in this disease entity.

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REVIEW ARTICLE

Kensei Tobinai

Proteasome inhibitor, bortezomib, for myeloma and lymphoma

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Abstract Bortezomib, a boronic acid, is a potent and selective proteasome inhibitor. The 20S proteasome is an enzyme complex present in cells, and it degrades many cell-cycle control factors, signal transduction factors, transcription factors, and oncogene and anti-oncogene products, thus controlling cell proliferation, differentiation, and apoptosis. Bortezomib is a novel molecular targeting agent which was designed to exhibit an antitumor effect by selectively inhibiting the 20S proteasome. Multiple myeloma is one of the incurable B-cell malignancies that continues to relapse with current treatment modalities, and the duration to progression becomes shorter in patients who repeatedly receive chemotherapy. There are no available treatment options in which durable efficacy can be expected after relapse; therefore, an effective therapy with a novel mechanism of action has been desired. In this review article, the results of clinical trials of bortezomib for multiple myeloma, including a Japanese phase I/II and pharmacokinetic/pharmacodynamic study, and those for non-Hodgkin lymphoma, especially for mantle cell lymphoma, are summarized. In the Japanese phase I/II study of bortezomib for relapsed multiple myeloma, this agent showed remarkable efficacy, with acceptable toxicities and unique pharmacokinetic/pharmacodynamic profiles, warranting further investigations, including more relevant administration schedules.

Key words Proteasome inhibitor · Bortezomib · Multiple myeloma · Lymphoma

Introduction

Bortezomib, a small-peptide boronic acid, is a potent and selective proteasome inhibitor developed by Millennium Pharmaceuticals (Cambridge, MA, USA) and it is the first anticancer agent having this mechanism of action which has been investigated in clinical settings. The 20S proteasome is an enzyme complex present in cells, and due to its specific and rapid degrading action on ubiquitinized proteins, it degrades many cell-cycle control factors, signal transduction factors, transcription factors, and oncogene and anti-oncogene products, thus controlling cell proliferation, differentiation and apoptosis.[1–6] Bortezomib is a novel molecular-targeting agent which was designed to exhibit an antitumor effect by selectively inhibiting the 20S proteasome, affecting the amount of protein controlling the cell cycle and nuclear factor-κB (NF-κB) activation.[7]

Preclinical investigations by the United States National Cancer Institute have revealed that bortezomib has a potent cytotoxic activity and exhibits a unique cytotoxic pattern compared to approximately 60000 other compounds.[8] It was found that the 20S proteasome inhibition by bortezomib influenced multiple signaling pathways and exhibited an antitumor effect by acting on the tumor microenvironment, including inhibiting tumor angiogenesis, the adhesion of multiple myeloma (MM) cells to bone marrow stromal cells, and interleukin-6 secretion required for the proliferation of MM cells.[9–12]

MM is one of the B-cell malignancies with a poor prognosis, and cure cannot be expected in most patients with current treatment modalities. The peak age of onset of this disease is 65–70 years, and the median survival of patients with MM is approximately 3 years with conventional chemotherapy; the 5-year survival rate is approximately 25% and the 10-year survival rate is less than 5%.[13,14]

In previously untreated patients with MM, melphalan + prednisolone (MP), MP-like regimens, and vincristine + doxorubicin + dexamethasone (VAD) have been frequently applied.[15–18] Several but not all randomized controlled trials have shown that high-dose chemotherapy followed by autologous stem cell transplantation (SCT) is superior to conventional-dose chemotherapy in terms of response rate and progression-free and overall survivals,[19–21] and high-dose chemotherapy is recommended for MM patients 65 years of age or younger as a part of the initial therapy.

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However, there has been no consensus regarding the standard therapy for relapsed MM.[22] MM is an incurable disease that continues to relapse, and the duration to progression becomes shorter in patients who repeatedly receive treatment.[23] There have been no available treatment options in which a durable efficacy can be expected after relapse; therefore, an effective therapy with a novel mechanism of action has been desired.

In this review article, the results of clinical trials of bortezomib for MM and non-Hodgkin lymphoma (NHL) are summarized. Subsequent to the successful development of bortezomib, clinical trials of new proteasome inhibitors have been initiated recently.[24]

Phase I studies of bortezomib

A phase I study of bortezomib in patients with hematologic malignancies was initiated in 1999.[25] Its purpose was to determine the maximum tolerated dose (MTD), dose-limiting toxicity (DLT), and pharmacodynamics (PD) of bortezomib in patients with refractory hematologic malignancies. Twenty-seven patients received bortezomib twice weekly for 4 weeks at either 0.40, 1.04, 1.20, or 1.38 mg/m^2, followed by a 2-week rest. The PD of bortezomib was evaluated by measurement of whole-blood 20S proteasome activity. DLTs at doses above the 1.04 mg/m^2 included thrombocytopenia, hyponatremia, hypokalemia, fatigue, and malaise. In 3 of 10 patients who received additional cycles of bortezomib, serious reversible adverse events (AEs) appeared during cycle 2, including one episode of postural hypotension, one systemic hypersensitivity, and one grade 4 elevation of hepatic transaminase. PD studies revealed that bortezomib induced 20S proteasome inhibition in a time-dependent manner, and this inhibition was related to the doses of bortezomib. Among 9 assessable patients with heavily pretreated plasma cell dyscrasias, there was one complete response (CR) and a reduction in paraprotein levels and/or marrow plasmacytosis in the 8 others. In addition, 1 patient with mantle cell lymphoma (MCL) and another with follicular lymphoma (FL) showed shrinkage of lymphomatous lesions. Bortezomib was well tolerated at 1.04 mg/m^2 on this dose-intensive schedule. Definitive antitumor activity against refractory MM and possible activity against NHL were suggested, and further investigations were regarded as warranted.[25]

Independently from the above-described phase I study for hematologic malignancies, a phase I study for patients with solid tumor was conducted with a different administration schedule; a twice-weekly IV bolus for 2 weeks, followed by a 1-week recovery period.[26] Forty-three heavily pretreated patients received bortezomib in doses ranging from 0.13 to 1.56 mg/m^2 per dose. DLTs on this schedule were diarrhea and sensory neurotoxicity. Other AEs included fatigue, fever, anorexia, nausea, vomiting, rash, pruritus, and headache; however, there were no apparent DLTs. There was one objective response in a patient with refractory non-small cell lung cancer. The authors recommended the schedule used in this trial at 1.56 mg/m^2 per dose for subsequent phase II trials, although they stated that attention should be paid to patients with preexisting neuropathy.[26]

Phase II studies of bortezomib for relapsed or refractory MM

Based on the results of the phase I studies along with the preclinical evidence of antimyeloma activity, phase II studies for relapsed or refractory MM were conducted, including the Study of Uncontrolled Multiple Myeloma Managed with Proteasome Inhibition Therapy (SUMMIT)[27] and the Clinical Response and Efficacy Study of Bortezomib in the Treatment of Relapsing Multiple Myeloma (CREST)[28] trials.

SUMMIT trial

In this multicenter phase II trial, 202 patients with relapsed or refractory MM were enrolled.[27] Patients received bortezomib at 1.3 mg/m^2 twice weekly for 2 weeks, followed by 1-week rest, for up to eight cycles. In patients showing a suboptimal response, oral dexamethasone (20 mg daily) was added. The response was evaluated according to the criteria of the European Group for Blood and Marrow Transplantation (EBMT)[29] and confirmed by an independent review committee. Of 193 assessable patients, 92% had been treated with three or more antimyeloma agents, and in 91%, the disease was refractory to the therapy received most recently. The efficacy rate with bortezomib was 35%, and those showing responses included 7 patients in whom paraprotein became undetectable and 12 in whom paraprotein was detectable only by immunofixation. The median overall survival (OS) time after bortezomib treatment was 16 months, with a median duration of response of 12 months. Grade 3 AEs included thrombocytopenia (28%), fatigue (12%), peripheral neuropathy (12%), and neutropenia (11%). Grade 4 AEs were observed in 14% of patients. Bortezomib was found to be active in patients with relapsed MM that was refractory to conventional chemotherapy.[27]

CREST trial

In another phase II trial, 54 patients with relapsed or refractory MM were randomized to receive IV 1.0 or 1.3 mg/m^2 bortezomib twice weekly for 2 weeks, every 3 weeks for a maximum of eight cycles.[28] Dexamethasone was allowed in patients showing progressive or stable disease after two or four cycles of bortezomib alone, respectively. Responses were evaluated using the modified EBMT criteria.[29] The complete response (CR) + partial response (PR) rate for bortezomib alone was 30% (8/27; 90% confidence interval [CI], 15.7–47.1) and 38% (10/26; 90% CI, 22.6–56.4) in the 1.0 mg/m^2 and 1.3 mg/m^2 groups, respectively. The overall response (CR + PR) rate for patients who received bortezomib alone or bortezomib in combination with dexametha-

sone was 37% and 50% for the 1.0 and 1.3 mg/m^2 cohorts, respectively. Common grade 3 AEs included thrombocytopenia (24%), neutropenia (17%), lymphopenia (11%), and peripheral neuropathy (9%). Grade 4 AEs were observed in 9% (5/54). It was concluded that bortezomib alone or in combination with dexamethasone demonstrated therapeutic activity in patients with relapsed or refractory MM.[28]

Based on the remarkable efficacy observed in these phase II studies,[27,28] the United States Food and Drug Administration (FDA) provided accelerated approval of the use of bortezomib to the pharmaceutical company as a third-line drug for the treatment of MM in 2003.

Additional studies in SUMMIT and CREST trials

Using the database of the SUMMIT and CREST trials, several additional studies were conducted.

Clinical factors predictive of outcome with bortezomib in relapsed or refractory MM

Potential associations between baseline parameters and outcomes with bortezomib treatment were analyzed in 202 patients.[30] Using the EBMT criteria [29], the overall response rate (CR + PR) of bortezomib alone was 27% and was not associated with sex, ethnic group, performance status, immunoglobulin isotype, chromosome 13 deletion, number or type of previous therapies, or concentration of hemoglobin or β2-microglobulin in a univariate analysis. By multivariate analysis, factors associated with lower response rates were age 65 years or older and plasma-cell infiltration in bone marrow greater than 50%. Factors that may be indicative of tumor burden (bone marrow MM cells >50%, hypoalbuminemia, thrombocytopenia) were predictive of OS. Chromosome 13 deletion and elevated β2-microglobulin, generally unfavorable factors in MM, were not predictive of poor outcome with bortezomib in this study, which appeared to be unique.

Patients with impaired renal function

Response rates, safety, and 20S proteasome activity were evaluated in relation to baseline creatinine clearance (Ccr).[31] Of ten patients with Ccr less than or equal to 30 ml/min, seven patients completed eight cycles of the protocol treatment; four at 1.3 mg/m^2 and three at 1.0 mg/m^2. Three of the ten patients responded (2 PRs and 1 minimal response [MR]), a response rate similar to that of the overall treated population. Patients with Ccr more than 80 ml/min (n = 105), 51–80 ml/min (n = 99), and 50 ml/min or less (n = 52) had similar rates of treatment discontinuation and similar AE profiles. Renal function did not appear to affect the 1-h proteasome inhibition or its recovery. The clinical experience in a limited number of patients with impaired renal function suggests that bortezomib provides clinical benefit with acceptable toxicities in this high-risk population.

Risk factors and kinetics of thrombocytopenia associated with bortezomib treatment

Bortezomib treatment was found to be associated with thrombocytopenia; however, its cause and kinetics might be different from those of conventional cytotoxic agents. The frequency, kinetics, and mechanism of thrombocytopenia following treatment with bortezomib 1.3 mg/m^2 were analyzed in 228 patients.[32] The mean platelet count decreased by approximately 60% during treatment but recovered rapidly between treatments in a cyclic fashion. Among responders, the pretreatment platelet count increased significantly during subsequent cycles of therapy. The mean percent reduction in platelets was independent of baseline platelet count, paraprotein concentration, and bone marrow plasmacytosis. Plasma thrombopoietin levels were inversely correlated with platelet counts. Murine studies demonstrated a reduction in platelet counts following a single bortezomib dose, without negative effects on megakaryocytic cellularity, ploidy, or morphology. These results suggested that bortezomib-induced thrombocytopenia was due to a reversible effect on megakaryocytic function rather than a direct cytotoxic effect on megakaryocytes or their progenitors.[32]

Phase III study of bortezomib versus high dose dexamethasone in relapsed or refractory MM

Following these encouraging phase II studies, an international, multicenter phase III study (Assessment of Proteasome Inhibition for Extending Remissions [APEX] Trial) comparing bortezomib and high-dose dexamethasone in patients with relapsed or refractory MM was conducted.[33,34] The scheme of the APEX study is shown in Fig. 1. Six hundred and sixty-nine patients were randomly assigned to receive either an IV bolus of bortezomib (1.3 mg/m^2) on days 1, 4, 8, and 11 for eight 3-week cycles, followed by treatment on days 1, 8, 15, and 22 for three 5-week cycles, or high-dose dexamethasone (40 mg orally) on days 1 through 4, 9 through 12, and 17 through 20 for four 5-week cycles, followed by treatment on days 1 through 4 for five 4-week cycles. Patients assigned to receive dexamethasone were permitted to cross over to receive bortezomib in a companion study after disease progression. The primary endpoint of this phase III study was time to progression (TTP). Patients treated with bortezomib showed higher response rates, a longer TTP, and a longer OS than those with dexamethasone. The overall response rates (ORRs) were 38% for bortezomib and 18% for dexamethasone ($P < 0.001$), and the CR rates were 6% and less than 1%, respectively ($P < 0.001$). Median TTPs in the bortezomib and dexamethasone arms were 6.2 months and 3.5 months, respectively (hazard ratio [HR], 0.55; $P < 0.001$). The 1-year OS rate was 80% among patients taking bortezomib and 66% among patients taking dexamethasone ($P = 0.003$), and the HR for OS with bortezomib was 0.57 ($P = 0.001$). Grade 3 or greater AEs were observed in 75% of patients treated

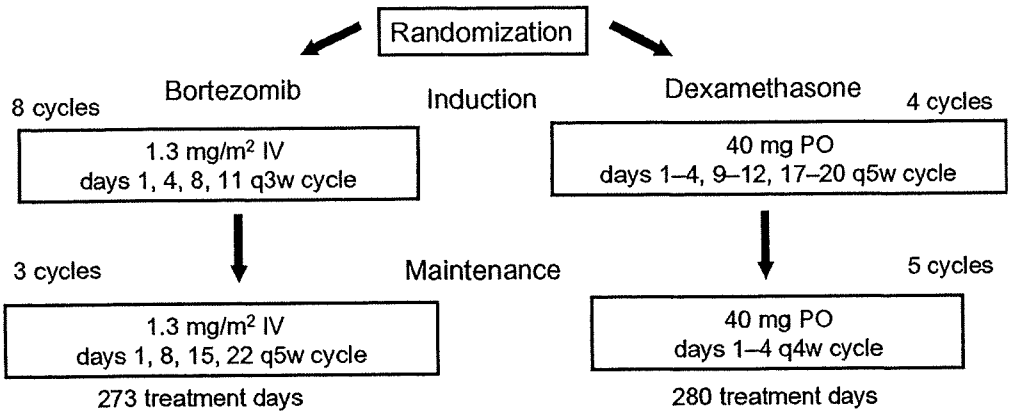

Fig. 1. Scheme of the Assessment of Proteasome Inhibition for Extending Remissions (APEX) Study, a phase III study comparing bortezomib and dexamethasone in relapsed or refractory multiple myeloma. *w*, week (Modified from Richardson et al. [33]N Engl J Med 2005;352:2487–2498)

Table 1. Therapeutic results of a phase III study comparing bortezomib and high-dose dexamethasone in patients with relapsed or refractory multiple myeloma

	Bortezomib	Dexamethasone	*P*
Time to progression (months)	6.2	3.5	<0.0001
Overall response rate (%)	38	18	<0.0001
CR + nCR (%)	13	2	<0.001
1-Year survival (%)	80	66	0.003

Modified from Richardson et al.[33,34]
CR, Complete response; nCR, near CR

with bortezomib and in 60% of those with dexamethasone. These results indicated that bortezomib is superior to high-dose dexamethasone for the treatment of patients with relapsed or refractory MM.[33,34] Table 1 summarizes the main therapeutic results of this phase III study. Based on the results of this phase III study, the United States FDA, in 2005, provided regular approval of bortezomib for the treatment of MM progressing after at least one prior therapy.[35]

After the publication of this landmark APEX Trial [33], an additional study regarding the safety and efficacy of bortezomib in high-risk and elderly populations was conducted.[36] Generally, adverse prognostic factors in MM include older age, number of prior therapies, and higher International Staging System (ISS) stage.[37] The efficacy of bortezomib and dexamethasone was compared in elderly (age >=65 years) and high-risk (>one line of prior therapy; ISS stage II/III; refractory to prior therapy) patients. Bortezomib demonstrated substantial clinical activity in these high-risk populations. The ORR (34%–40% vs 13%–19%), including the CR rate (5%–8% vs 0–1%), was significantly higher with bortezomib than with dexamethasone in all four subgroups. Similarly, median TTP was significantly longer with bortezomib than with dexamethasone, and the 1-year OS rate was significantly higher with bortezomib in all subgroups. As in the total APEX population, rates of grade 3 or greater AEs were higher in bortezomib- than in dexamethasone-treated patients aged 65 years or more and with more than one line of prior therapy, while rates of serious AEs were similar; toxicities were generally manageable. These results suggested that bortezomib should be considered an appropriate treatment for elderly and high-risk patients with relapsed or refractory MM.[36]

Phase II study of bortezomib alone or in combination with dexamethasone for previously untreated MM

Bortezomib was examined as first-line treatment in 32 consecutive patients with untreated symptomatic MM.[38] Patients received bortezomib 1.3 mg/m^2 for a maximum of six 3-week cycles; oral dexamethasone 40 mg was added if a less than PR was achieved after two cycles or a less than CR after four cycles of bortezomib alone. The ORR was 88%, with undetectable paraprotein (CR) in 6%, and detectable paraprotein by immunofixation only in 19%. All 32 patients completed the first two cycles of bortezomib alone, of whom 3% achieved CR, 9% near CR (nCR), and 28% PR. Ten patients received single-agent bortezomib on study, and dexamethasone was added in 22, leading to 15 improved ORRs. The common grade 2 or greater AEs included sensory neuropathy (31%), constipation (28%), myalgia (28%), and fatigue (25%). Sensory neuropathy of grade 2 or 3 was reversible within a median of 3 months in 5 of 10 patients. Bortezomib treatment did not affect stem cell mobilization in 8 patients or autologous SCT in 6 patients. It was concluded that bortezomib alone or in combination with dexamethasone is an effective induction therapy with a high CR rate and manageable toxicities in previously untreated patients with MM.[38]

Clinical trials of bortezomib in combination with anti-myeloma agents

Various kinds of clinical trials regarding bortezomib in combination with other anti-myeloma agents are being conducted. Table 2 summarizes the results of the main clinical trials of bortezomib alone or bortezomib in combination with other agents for relapsed or refractory MM. In addition, an international phase III study (DOXIL-MMY-3001 study) revealed remarkable results.[39] For patients with pre-

Table 2. Summarized results of main clinical trials of bortezomib alone or bortezomib in combination with other antimyeloma agents for relapsed or refractory multiple myeloma

Regimen	Phase	*n*	CR + PR	CR + nCR	Reference
Single-agent bortezomib (APEX)	III	331	43%	16%	Richardson et al.[34]
Plus oral melphalan	I/II	35	47%	15%	Berenson et al.[61]
Plus oral cyclophosphamide	II	50	82%	16%	Kropff et al.[62]
Plus corticosteroids	II	29	62%	6%	Suvannasankha et al.[63]
Plus pegylated liposomal doxorubicin	I	42	73%	36%	Orlowski et al.[40]

n, Number of patients; CR, complete response; PR, partial response; nCR, near CR
[a]Includes only CRs

viously treated MM, the combination of bortezomib and pegylated liposomal doxorubicin (PLD) was compared with bortezomib alone. Six hundred and forty-six patients from 123 centers in 18 countries were randomly assigned either to IV bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11 of every 21-day cycle, or to the same bortezomib regimen with PLD, 30 mg/m^2, on day 4. The dose and schedule of the combination therapy (bortezomib and PLD) were based on the results of the preceding phase I study.[40] Both groups received a median of five cycles of the protocol treatment. The ORR was 43% for bortezomib and 48% for the combination. The median TTP was improved from 6.5 months for bortezomib alone to 9.3 months for the PLD + bortezomib combination ($P < 0.001$), and the median duration of response was increased from 7.0 months to 10.2 months with the combination ($P < 0.001$). Updated OS analysis revealed that PLD + bortezomib significantly improved OS ($P < 0.05$; HR, 1.41, 95% CI, 1.002–1.97). The toxicity profiles of the combination therapy were consistent with the known toxicities of the two agents. Grade 3 or greater AEs were more frequent in the combination therapy, primarily due to the increase in myelosuppression and gastrointestinal toxicities. These results suggest that PLD with bortezomib is superior to bortezomib monotherapy for the treatment of relapsed or refractory MM.[39]

In addition to this landmark study, a multicenter phase III study of bortezomib plus MP compared with MP alone is being conducted for previously untreated patients with MM, for whom SCT is not applicable. If positive results of bortezomib + MP are obtained, the standard therapy for untreated MM may change.

Phase I/II and PK/PD study of bortezomib for relapsed or refractory MM in Japan[41]

The PK profiles of bortezomib in patients with MM were not fully elucidated in the preceding United States studies. The objectives of the Japanese phase I/II study were to characterize PK/PD profiles, DLTs, and the recommended dose of bortezomib for the subsequent phase II part in Japanese patients (phase I part), and to investigate antitumor activity and safety (phase II part). Bortezomib was given as an IV bolus of 0.7, 1.0, and 1.3 mg/m^2 on days 1, 4, 8, and 11 every 21 days for up to six cycles. Thirty-four patients with relapsed or refractory MM were enrolled, and 33 of them were assessable for response. The plasma concentrations of bortezomib were assessed on days 1 and 11 in 16 patients at 0.7, 1.0, and 1.3 mg/m^2 per day (cycle1 of phase I part). Sixty-five percent of the patients were male and the median age was 60 years (range, 34–72 years). The median number of treatment cycles of bortezomib was 4 (range, 1–6). DLT (in cycle 1) of grade 3 febrile neutropenia, observed in 1 of 6 patients at 1.3 mg/m^2, led to this dose level being the recommended dose for the subsequent phase II part. Grade 3 or greater AEs were infrequent; however, 15% of patients had to discontinue bortezomib treatment due to AEs. Grade 3 or greater hematologic AEs included lymphopenia (56%), neutropenia (44%), anemia, and thrombocytopenia (32%). One patient suffered from fatal pulmonary disorder, and the autopsy revealed diffuse alveolar damage. Pleural/pericardial effusion, bronchial wall thickening, and lumen narrowing were also observed. Other non-hematologic AEs were relatively mild. According to the modified EBMT criteria,[29] objective responses were observed in 10 of the 33 patients (30%; 95% CI, 16–49), including 5 immunofixation-positive CRs (15%) and 5 PRs (15%).

The plasma concentration-time profile of bortezomib was not dependent on the dose administered. A biexponential decline was observed after administration of the bolus dose, characterized by a rapid distribution phase and subsequent prolonged elimination phase. The elimination of unchanged drug from plasma on day 11 was slower than that on day 1. The volume of distribution (Vz) value was indicative of extensive distribution into tissues. At all dose levels, the elimination half-life (t1/2) was prolonged and total clearance (CL) was decreased on day 11 as compared to these parameters on day 1. Accordingly, estimated plasma concentration at the end of administration (C_0) and area under the curve (AUC) were increased on day 11. AUC increased dose-dependently despite noticeable interpatient variations, while C_0 did not show apparent dose-dependency. These results, together with the tissue distribution data in animal studies, suggest that bortezomib is rapidly distributed into the extravascular tissues. There were no major differences in the mean observed maximum inhibition of the 20S proteasome activity inhibition rates (E_{20smax}) in whole blood on days 1 and 11, and their times (t_{20smax}) were taken from the effect vs time profiles. No major differences in the relationship between plasma concentration of bortezomib and inhibition of the 20S proteasome were observed between Japanese patients and

non-Japanese patients. This was evident by the comparable maximum effect (E_{max}) and plasma concentrations of bortezomib required to achieve 50% of E_{max} (EC_{50}), calculated using the simple E_{max} model. In conclusion, bortezomib showed remarkable efficacy with acceptable toxicities in Japanese patients with relapsed or refractory MM. The unique PK/PD profiles of bortezomib revealed by this study warrant further investigations, including more relevant administration schedules.[41]

Based on the results of the Japanese phase I/II study[41] and those of the preceding United States studies,[27,33] in 2006, bortezomib was approved by the Ministry of Health, Labor, and Welfare of Japan for the treatment of relapsed or refractory MM.

Pulmonary complications in Japanese patients with MM

In addition to one fatal case in the above-described phase I/II study in Japan,[41] severe pulmonary complications were reported in a retrospective study carried out before the approval of bortezomib for general use in Japan.[42] In this study, 4 of 13 patients with relapsed or refractory MM developed severe pulmonary complications, and 2 of them died of respiratory failure. Considering possible ethnic differences in terms of susceptibility to the pulmonary toxicities of bortezomib, the Japanese Society of Hematology and the Japanese Society of Clinical Hematology sent urgent questionnaires to the members of both societies to explore the details of pulmonary complications.[43] Clinical details were available for 46 patients who had been treated with personally imported bortezomib. Seven patients (15%) were revealed to have developed pulmonary complications, and three of them died of respiratory failure. Of the seven patients who developed pulmonary complications, six had undergone SCT. Multivariate analysis revealed that the concomitant use of corticosteroids might reduce the risk of pulmonary complications and that prior SCT might increase the risk. Because these observations were based on a retrospective analysis of a limited Japanese cohort; however, further investigations are needed. In addition, physicians should be cautious of possible ethnic differences in toxicity profiles of bortezomib, and the use of personally imported anticancer agents during the conduct of a phase I or I/II trial before the official approval for general use is not recommended.

Clinical trials of bortezomib for non-Hodgkin lymphoma (NHL)

In addition to MM, bortezomib has been tested against various subtypes of NHL.

Clinical trials of bortezomib for mantle cell lymphoma (MCL)

MCL accounts for approximately 5%–6% of all NHL cases. It is generally an incurable subtype of B-cell NHL, and has one of the poorest prognoses of all NHLs. Thus, novel therapies have been desired for MCL. MCL is characterized by overexpression of cyclin D1, resulting from the t(11;14)(q13;q32) translocation. NF-κB is constitutively expressed in MCL cells, and increased proteasome degradation of *p27* and *p53* mutations is associated with poor survival in MCL patients. Preclinical studies of bortezomib have demonstrated its activity in MCL cells and MCL xenograft models. Single-center and national multicenter studies have shown the antitumor activity of bortezomib in MCL patients.[44–47] Table 3 summarizes the results of phase II studies of bortezomib alone in patients with relapsed or refractory MCL.

To confirm these results, an international multicenter phase II study was conducted.[48] Bortezomib 1.3mg/m^2 was administered on days 1, 4, 8, and 11 of a 21-day cycle, for up to 17 cycles. Response and progression were determined using International Workshop Response Criteria.[49] In total, 155 patients were treated. The median number of prior therapies was one (range, one to three). ORR in 141 assessable patients was 33%, including 8% CR/unconfirmed CR (CRu). The median duration of response was 9.2 months, and the median TTP was 6.2 months. The median OS has not been reached after a median follow-up of 13.4 months. The toxicity profiles of bortezomib were similar to previous experience in MM trials. The most common AEs of grade 3 or greater were peripheral neuropathy (13%), fatigue (12%), and thrombocytopenia (11%). Death from causes that were considered to be treatment-related was reported for 3% of patients. These results confirm the activity of bortezomib in relapsed or refractory MCL, with predictable and manageable toxicities. Bortezomib provides significant clinical activity in MCL, and may represent a new treatment option for this population with a usually very poor outcome. Studies of bortezomib-based combinations in MCL are underway.

Table 3. Summarized results of phase II studies of bortezomib alone in patients with relapsed or refractory mantle-cell lymphoma

Dose and schedule of bortezomib	*n*	CR/CRu	PR	ORR	Reference
1.5mg/m^2 days 1, 4, 8, 11 q3w	29	6/0	6	41%	Goy et al.[44]
1.5mg/m^2 days 1, 4, 8, 11 q3w	10	0/1	4	50%	O'Connor et al.[45]
1.3mg/m^2 days 1, 4, 8, 11 q3w	24	1/0	6	29%	Strauss et al.[46]
1.3mg/m^2 days 1, 4, 8, 11 q3w	29	0/1	12	46%	Belch et al.[47]
1.3mg/m^2, days 1, 4, 8, 11 q3w	141	9/2	36	33%	Fisher et al.[48]

n, Number of patients; CR, complete response; CRu, CR, unconfirmed; PR, partial response; ORR, overall response rate

Clinical trials of rituximab and bortezomib for follicular lymphoma (FL)

Randomized phase II study of rituximab and bortezomib

Preclinical data with combined rituximab and bortezomib suggest additive cytotoxic activity. This randomized phase II study investigated the ORR, with weekly or twice-weekly administrations of bortezomib, in patients with relapsed or refractory FL, or marginal zone B-cell lymphoma (MZBCL).[50] Patients were randomized to bortezomib 1.3 mg/m^2 twice weekly on days 1, 4, 8, and 11 of a 21-day cycle (arm A) or bortezomib 1.6 mg/m^2 weekly on days 1, 8, 15, and 22 of a 35-day cycle (arm B) for up to 15 weeks (five and three cycles in arms A and B, respectively). Starting from day 1, rituximab at 375 mg/m^2 was administered weekly for 4 weeks in both arms. The scheme of this randomized phase II study is shown in Fig. 2. Seventy-four of the 81 patients who were randomized (35 arm A and 39 arm B) were evaluable for response. Table 4 summarizes the main results. ORR was 51% in arm A, and 54% in arm B. Thirty patients showed progressive disease (17 in arm A and 13 in arm B). The protocol treatment was well tolerated in both arms; grade 3 or greater AEs were seen in 54% in arm A and in 18% in arm B. It was concluded that weekly and twice-weekly bortezomib plus rituximab are active and well-tolerated treatments for patients with relapsed or refractory indolent B-cell NHL. The more convenient weekly regimen offers efficacy similar to that of the twice-weekly regimen, with less toxicity.[50] Based on the results of this randomized phase II study, an international phase III study comparing bortezomib plus rituximab versus rituximab alone for relapsed or refractory indolent B-NHL is underway, using a weekly administration schedule.

Table 4. Summarized results of a randomized phase II study comparing two combination schedules of bortezomib and rituximab in relapsed or refractory patients with follicular lymphoma or marginal zone B-cell lymphoma

Response (%)	Twice-Weekly, bortezomib + rituximab (arm A)	Once-Weekly, bortezomib + rituximab (arm B)
n	35	39
Overall response	51	54
CR/CRu	12	13
Partial response	40	41
Stable disease	31	33
Progressive disease	17	13
Grade 3/4 toxicity	54	18

n, Number of patients; CR, complete response; CRu, CR, unconfirmed

Clinical trials for diffuse large B-cell lymphoma (DLBCL)

Several phase III studies indicate that rituximab plus CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) (R-CHOP) is a new standard in the treatment of previously untreated DLBCL.[51-54] The results of the following phase I/II study of R-CHOP plus bortezomib for untreated DLBCL were presented at the annual meeting of the American Society of Clinical Oncology (ASCO) in 2007.

Phase I/II study of R-CHOP plus bortezomib for untreated DLBCL[55]

Patients with previously untreated DLBCL ($n = 40$) received CHOP chemotherapy every 3 weeks, rituximab (375 mg/m^2 each cycle of CHOP) plus bortezomib at 0.7 mg/m^2 (arm 0; $n = 4$), 1.0 mg/m^2 (arm 1; $n = 8$), or 1.3 mg/m^2 (arm 2; $n = 28$ including phase I and all phase II) on days 1 and 4 of each cycle. Median age ($n = 40$) was 58 years (range, 21–86 years), and 35 subjects (88%) had stage III/IV disease at study entry, and 29 (73%) had elevated serum lactate dehydrogenase (LDH). Patients generally had unfavorable baseline international prognostic index (IPI) scores of 2 in 16 subjects (40%) and 3–5 in 19 subjects (48%). Median follow-up was 21 months (range, 9–35 months). The protocol treatment was generally well tolerated. Peripheral neuropathy occurred in 22 subjects (55%), with 45% grade 1, 5% grade 2, and 5% grade 3. Grade 4 hematologic toxicity included thrombocytopenia (15%) and leukopenia (15%). Four subjects (3 over age 75 years and all with high-risk IPI) died prior to the first response assessment. Intent to treat (ITT) ORR ($n = 40$) was 90%, with 68% CR/CRu. For the evaluable subset ($n = 36$), ORR was 100%, with CR/CRu 75%. The Kaplan-Meier estimate ($n = 40$) of 2-year progression-free survival was 72%. Of all 19 enrolled (ITT) patients in the high-intermediate or high-risk IPI groups, 14 (74%) were alive without progression at last assessment. The authors concluded that bortezomib can be administered with acceptable toxicity profiles in conjunction with R-CHOP chemotherapy, and that efficacy findings with this combination regimen in newly-diagnosed DLBCL are encouraging and warrant further studies.[55]

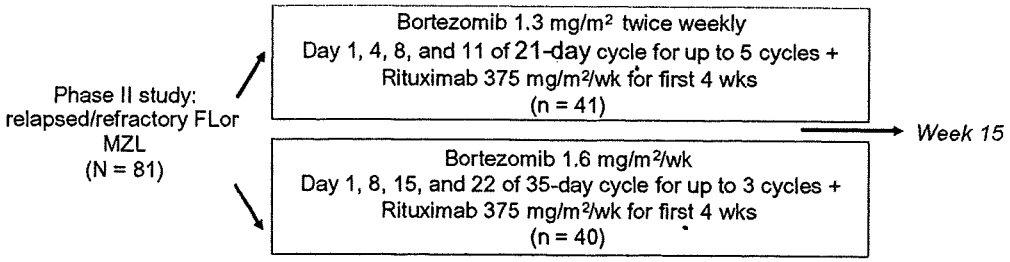

Fig. 2. Scheme of a randomized phase II study regarding the combination schedule of bortezomib plus rituximab in relapsed or refractory follicular lymphoma (*FL*). *MZL*, marginal zone lymphoma. (Modified from de Vos et al. [50] Proc ASH 2005, no.17)

In addition to the above-described United States phase I/II study, the results of a randomized phase II study of bortezomib plus R-CHOP for untreated B-cell NHL including DLBCL, using a different dose and schedule of bortezomib, conducted by the Groupe d'Etude des Lymphomes de l'Adulte (GELA), were presented at the ASCO meeting in 2007.[56] The authors concluded that R-CHOP plus bortezomib is a highly effective regimen for untreated B-NHL. Because the higher doses of bortezomib in combination with R-CHOP led to severe neuropathy, however, the combination of bortezomib with vinca alkaloids should be cautiously evaluated in future trials, in view of overlapping neurotoxicity.

Bortezomib for peripheral T-cell malignancies

At the annual meeting of the American Society of Hematology in 2006, Zinzani and colleagues[57] reported the preliminary results of a phase II study of bortezomib in relapsed or refractory T-cell malignancies. The target populations of this phase II study were previously pretreated patients with peripheral T-cell lymphoma (PTCL) with only skin involvement and cutaneous T-cell lymphoma (CTCL). Bortezomib was given at 1.3 mg/m^2 IV on days 1, 4, 8, and 11 every 21 days for up to six cycles. Fifteen patients were enrolled and 12 (10 CTCL and 2 PTCL) were evaluable for response. ORR was 67% (2 CR + 6 PR), and the responding patients were 7 with CTCL and 1 with PTCL. These preliminary data suggest that bortezomib is active for CTCL and PTCL with skin involvement.[57] Considering the promising results of preclinical studies of bortezomib for adult T-cell leukemia-lymphoma,[58,59] further investigations are warranted, to clarify the role of bortezomib in the treatment of peripheral T-cell malignancies.

Bortezomib for solid tumors and other proteasome inhibitors

In addition to hematologic malignancies, many clinical trials are underway of bortezomib in combination with other anticancer agents against solid tumors.[60] Considering the remarkable therapeutic results of bortezomib as a single agent or in combination with other anticancer agents, and the encouraging results of carfilzomib (PR-171),[24] the use of proteasome inhibitors will continue to be one of the important development strategies in clinical oncology.

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