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IN
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Management of Relapsed/Refractory Multiple Myeloma with Bendamustine-Bortezomib-Dexamethasone

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A ZIA RENATA

(Dedicated to my aunt, Renata Croce, 24/03/1930 – 15/04/2016)

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1. Introduction

1.1 Background

Multiple myeloma (MM) is a malignant neoplasm of plasma cells that accumulate in bone marrow, leading to bone destruction and marrow failure. It accounts for approximately 1.8% of all hematologic and solid cancers and slightly > 15% of hematologic malignancies in the United States[1]. MM is most frequently diagnosed among people aged 65 to 74 years (median age 69 years)[2]. During 2016, the American Cancer Society estimated that 30.330 new cancer cases occurred in USA, with 12.650 deaths[1]. Over the past decade, statistical analysis show that the rates for new MM cases have been increasing an average of 0.8% each year[2]. However, these analysis also reveal that death rates have been declining an average of 0.8% each year (period 2004-2013) thanks to the availability of newer and more effective treatment options[2]. MM is typically sensitive to different classes of cytotoxic drugs, both as frontline treatment and as treatment for relapsed disease. Unfortunately, even if responses are typically durable, nowadays MM is not considered curable with current approaches. However, treatment of MM has been rapidly evolving, due to the introduction of new classes of drugs, such as proteasome inhibitors, immunomodulatory drugs (IMIDs), histone deacetylase inhibitors and monoclonal antibodies and new indications for old classes of drugs, such as alkylating agents[3-5]. Moreover, there is increasing understanding of MM tumor biology, creating the rational for new combinations of drugs and new therapies development[6,7]. Discover of the associated cytogenetic abnormalities confirm the hypothesis that MM is a heterogeneous disease, suggesting that risk-adapted therapies and individualizing treatment will further help to improve patient management. Bendamustine is a molecule largely adopted in the past as effective chemotherapeutic agent in several types of hematological and non-hematological malignancies. Its unique mechanism of action, both as alkylating agent and antimetabolite, probably accounts for its wide efficacy profile also in the treatment of relapsing/refractory multiple myeloma. In this specific clinical setting, in which patients experience several therapy lines and have poor prognosis, bendamustine combined with bortezomib and dexamethasone, is emerging as an effective salvage therapy, also in the era of new drugs. In fact, despite the introduction of so-called novel agents, such as second generation proteasome inhibitors (carfilzomib) or third generation immunomodulatory drugs (pomalidomide), many trials have demonstrated that bendamustine in

combination with other agents is also a valid therapeutic option as these mentioned above. This retrospective, observational study aimed to evaluate, in a real-life setting, a cohort of heavily pre-treated patients affected by relapsing/refractory multiple myeloma, whose salvage therapy consisted in courses of bendamustine-bortezomib-dexamethasone. Efficacy and safety data were evaluated, focusing especially on effectiveness of this regimen on previously bortezomib-refractory patients and on its tolerability. Data on efficacy and safety of our real-life experience were highly comparable to those of major trials adopting the same regimen in the same clinical setting, demonstrating how it is a feasible salvage therapeutic option, in a context of poor treatment choices. Moreover, our data revealed how bendamustine addition could overcome a previous pharmacological refractoriness to bortezomib, leading to clinical response also those patients already treated with this proteasome inhibitor.

1.2 Initial Diagnostic Workup

Initial diagnostic workup in all patients should include detailed history and physical examination and baseline blood studies and biological assessments to differentiate symptomatic and asymptomatic MM: complete blood count with differential and platelet counts, evaluation of kidney function and serum electrolytes, lactate dehydrogenase (LDH), serum calcium, albumin, and beta-2 microglobulin. Increased creatinine levels and blood urea nitrogen levels indicate decreased kidney function, whereas increased LDH and beta-2 microglobulin levels reflect tumor cell burden.

The monoclonal protein (M-protein) components in serum and urine are evaluated by the urine and serum analyses. Urine analysis as a part of the initial diagnostic workup should include evaluating urine protein electrophoresis, urine immunofixation electrophoresis and 24-hour urine for total protein. Serum analysis should include quantitative immunoglobulin levels (IgG, IgA, and IgM), serum protein electrophoresis, and serum immunofixation electrophoresis in order to obtain more specific information about the type of M-protein present. Evaluating changes and proportions of proteins, in particular the M-protein, helps track disease progression and response to treatments. Serum free light chain (FLC) assay along with serum protein electrophoresis and serum immunofixation electrophoresis yields high sensitivity while screening for MM and related plasma cell disorders[8]. Serum FLC assay also has prognostic value in plasma cell disorders, including monoclonal gammopathy of undetermined significance (MGUS),

smoldering myeloma, active myeloma, immunoglobulin light chain amyloidosis, and solitary plasmacytoma[8,9]. The serum FLC assay is also important for quantitative monitoring of patients with oligosecretory myeloma and light chain amyloidosis. In particular, the FLC ratio is mandatory for documenting stringent complete response (CR), according to the International Myeloma Working Group (IMWG) Uniform Response Criteria[10]. The FLC assay cannot replace the 24-hour urine protein electrophoresis for monitoring patients with measurable urinary M-proteins.

Most patients present serum M-protein with or without associated urinary M-protein. In the Mayo Clinic review of 1,027 MM newly diagnosed patients, 20% had secretory urinary M-proteins; however, 3% had neither serum nor urine M-protein and therefore had nonsecretory myeloma[11].Serum FLC assay is useful to monitor disease response and progression in a proportion of patients with nonsecretory myeloma. After the MM or M-protein is quantified, it is very important to use the same test for serial studies to ensure accurate relative quantification. At diagnosis, bone marrow aspiration and biopsy is recommended to evaluate bone marrow plasma cell infiltration and to detect quantitative and/or qualitative abnormalities of bone marrow plasma cells. To evaluate osteolysis (lytic bone lesions), full skeleton radiographic survey (Rx) or whole-body, low-dose CT is recommended, but also the importance of role of FDG PET/CT scan is increasing.

Although MM may be morphologically similar, cytogenetic and molecular biology differences confirm that several subtypes of the disease can be identified. Bone marrow aspiration, performed at initial diagnosis, should include chromosome analysis by metaphase cytogenetics and FISH (fluorescence in situ hybridization) should be performed with the plasma cells obtained from the bone marrow aspiration. Specific chromosomal abnormalities have been identified in patients with MM involving translocations, deletions, or amplifications.

In particular, deletion of 17p13 (the locus for the tumor-suppressor gene, p53) leads to loss of heterozygosity of TP53 and is considered a high-risk feature in MM[12-14]. Other high-risk chromosomal aberrations are characterized by structural changes that include specific rearrangements involving the IGH gene (encoding immunoglobulin heavy chain) located at 14q32, whose alteration identifies several subgroups of patients. The MM main translocations are t(11;14) (q13;q32), t(4;14) (p16;q32), and t(14;16) (q32;q23). In particular, t(4;14) and t(14;16) have a poor prognosis, although t(11;14) seems to impart no increased risk[15-17]. Del13q is a common abnormality observed on FISH studies, but is can be considered a negative prognostic factor only when observed on metaphase cytogenetics.

Abnormalities of chromosome 1 are also among the frequent chromosomal alterations in MM[18]; the long arm is most often associated with amplifications while short arm is most often associated with deletions[19]. Gains/amplification of 1q21 increases the risk of progression of disease and amplification incidence is higher in relapsed patients than in newly diagnosed disease[18,20].

The important of patients' stratification into various risk groups, based on chromosomal markers, is important for prognostic evaluation, selection, and sequencing of therapy approaches[21,22].

In addition to prognostic role of cytogenetic analysis, biological factors or gene expression signatures may help for discerning prognosis and rational therapeutic decisions[23,24]. In particular, the application of high-throughput genomic tools such as gene expression profiling (GEP) helps to understand the molecular subtypes of MM [25]. Thanks to the novel agents for treatment approaches, a majority of patients can now anticipate long-term disease control. However, high-risk disease patients do not receive the same benefit from certain approaches as low-risk patients and need alternative treatments. Gene expression profiling is a fast and powerful with the potential to provide additional prognostic value to improve risk stratification and to help therapeutic decisions. Recently,15-gene, 70-gene, and 92-gene models based on GEP signatures of MM cells have been identified and developed [26-28]. It has been demonstrated that patients in the high-risk group based on the 15-gene[26], 70-gene[27], or 92-gene[28] models had shorter survival compared with the low-risk group. GEP is not currently routinely used in clinical practice during diagnostic workup, even if it can be considered a useful tool and may be helpful in selected patients to estimate disease aggressiveness and individualize treatment.

Bone marrow immunohistochemistry may be useful in some cases to confirm presence of monoclonal plasma cells and to more accurately quantify plasma cell involvement; bone marrow flow cytometry can help in certain situations.

1.3 Additional Diagnostic Tests

Active MM is positive on PET scan[31,32]. FDG PET/CT and MRI scans are more sensitive than total body Rx and are particularly indicated when symptomatic areas show no abnormality on routine radiographs. FDG PET/CT results after induction therapy and autologous stem cell transplant (Auto-SCT) help in predicting the prognosis of patients with symptomatic MM[33,34].

To confirm the presence of plasmacytomas a tissue biopsy may also be necessary. Plasma cell proliferation assays may be helpful to identify the fraction of proliferating myeloma cell population[35]. Also, if amyloidosis is suspected, bone marrow and fat pad staining for the presence of amyloid should be considered and serum viscosity should be evaluated, particularly in those with high levels of M-protein. Considered that bisphosphonate therapy is a possibility in supportive care of patients with MM, a baseline bone densitometry test may be recommended.

In selected MM patients, allogeneic stem cells transplantation (allo-SCT) may be considered. In this approach, myeloablative or nonmyeloablative/reduced-intensity therapy is administered with an infusion of stem cells (peripheral blood or bone marrow) obtained from a donor, preferably an HLA-identical sibling. In such cases, the patient will need to be HLA-typed.

1.4 Diagnostic Categories

As seen above, patients are initially classified as either having smoldering/asymptomatic disease or active/symptomatic disease.

In addition to existing requirements of CRAB features, IMWG recently updated the disease definition of MM to include biomarkers[36]; the CRAB criteria include hypercalcemia (>11.5 mg/dL), renal insufficiency (creatinine >2 mg/dL or creatinine clearance <40 mL/min), anemia (hemoglobin <10 g/dL or 2 g/dL < normal), and the presence of bone lesions. The IMWG has also clarified that presence of ≥1 osteolytic lesions seen on skeletal radiography, whole-body MRI, or whole-body PET/CT fulfills the criteria for bone disease[36]. The MM-defining biomarkers identified by the IMWG include ≥1 of the following: ≥60% clonal plasmacells in the bone marrow; involved/uninvolved FLC ratio of ≥100 with the involved FLC being ≥100 mg/L; or MRI with ≥1 focal lesion (involving bone or bone marrow)[36].

The IMWG criteria for a diagnosis of smoldering (asymptomatic) myeloma include serum M-protein (IgG or IgA) ≥30 g/L or 3.0 g/dL and/or clonal bone marrow plasma cells 10% to 60% and absence of myeloma-defining events or amyloidosis[36]. The updated IMWG diagnostic criteria allow initiation of therapy before end-organ damage on the basis of specific biomarkers, and also allow the use of sensitive imaging criteria to diagnose MM, including PET/CT and MRI[36].

Active myeloma can be staged using either the Durie-Salmon staging system or the International Staging System (ISS) (Table 1)[37]. The ISS is based on easily obtained laboratory measures

(serum beta-2 microglobulin and serum albumin) and is easier to use than the Durie-Salmon staging system for patients with previously untreated MM. The ISS has been recently revised to incorporate the serum LDH and high-risk FISH abnormalities [t(4;14), t(14;16), 17p13 deletion][38].

Table 1: International Staging System (ISS)

Stage	International Staging System (ISS)	Revised-ISS (R-ISS)
I	Serum beta-2 microglobulin <3.5 mg/L, Serum albumin ≥3.5 g/dL	ISS stage I and standard-risk chromosomal abnormalities by iFISH[2] and Serum LDH ≤ the upper limit of normal
II	Not ISS stage I or III	Not R-ISS stage I or III
III	Serum beta-2 microglobulin ≥5.5 mg/L	ISS stage III and either high-risk chromosomal abnormalities by iFISH[2] or Serum LDH > the upper limit of normal

1.5 Response Criteria

Assessing the response to treatment is a key determinant of MM. The IMWG response criteria were developed from the EBMT (European Society for Blood and Marrow Transplantation)/IBMTR (International Bone Marrow Transplant Registry)/ABMTR (Autologous Blood and Marrow Transplant Registry) response criteria[39], with revisions and improvements to help uniform reporting.

The updated IMWG response criteria definitions[10,40-43] for stringent CR, immunophenotypic CR, molecular CR, CR, VGPR (very good partial response), PR (partial response), MR (minimal response) for relapsed/refractory myeloma, SD (stable disease), and PD (progressive disease) are outlined on table 2[42,43]. In order to include measures of minimal residual disease (MRD) assessments, the response criteria has recently been updated, and it is recommended that the IMWG uniform response criteria should be used in future clinical trials.

Table 2: Updated IMWG Response Criteria

RESPONSE CRITERIA FOR MULTIPLE MYELOMA
(Revised based on the new criteria by International Myeloma Working Group [IMWG])

IMWG criteria for response assessment including criteria for minimal residual disease (MRD)	
Response Category	Response Criteria
IMWG MRD criteria (requires a complete response as defined below)	
Sustained MRD-negative	MRD negativity in the marrow (next-generation flow [NGF], next-generation sequencing [NGS], or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years)†
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF‡ on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using a validated equivalent method with a minimum sensitivity of 1 in 10^5 nucleated cells§ or higher
Imaging plus MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool standardized uptake value (SUV) or decrease to less than that of surrounding normal tissue¶
Standard IMWG response criteria‖	
Stringent complete response	Complete response as defined below plus normal FLC ratio** and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells)††
Complete response	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h
Partial response	≥50% reduction of serum M-protein plus reduction in 24-h urinary M-protein by ≥90% or to <200 mg per 24 h; If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria; If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (sum of the products of the maximal perpendicular diameters [SPD] of measured lesions)§§ of soft tissue plasmacytomas is also required
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50%–89%. In addition to the above listed criteria, if present at baseline, a ≥50% reduction in SPD§§ of soft tissue plasmacytomas is also required

RESPONSE CRITERIA FOR MULTIPLE MYELOMA
(Revised based on the new criteria by International Myeloma Working Group [IMWG])

Response Category	Response Criteria
Stable disease	Not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates. Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease
Progressive disease[¶¶,IIII]	Any one or more of the following criteria: Increase of 25% from lowest confirmed response value in one or more of the following criteria: Serum M-protein (absolute increase must be ≥0.5 g/dL); Serum M-protein increase ≥1 g/dL, if the lowest M component was ≥5 g/dL; Urine M-protein (absolute increase must be ≥200 mg/24 h); In patients without measurable serum and urine M-protein levels, the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg/dL); In patients without measurable serum and urine M-protein levels and without measurable involved FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥10%); Appearance of a new lesion(s), ≥50% increase from nadir in SPD[§§] of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis; ≥50% increase in circulating plasma cells (minimum of 200 cells per μL) if this is the only measure of disease
Clinical relapse	Clinical relapse requires one or more of the following criteria: Direct indicators of increasing disease and/or end organ dysfunction (calcium elevation, renal failure, anemia, lytic bone lesions [CRAB features]) related to the underlying clonal plasma-cell proliferative disorder. It is not used in calculation of time to progression or progression-free survival but is listed as something that can be reported optionally or for use in clinical practice; Development of new soft tissue plasmacytomas or bone lesions (osteoporotic fractures do not constitute progression); Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and ≥1 cm) increase as measured serially by the SPD[§§] of the measurable lesion; Hypercalcemia (>11 mg/dL); Decrease in hemoglobin of ≥2 g/dL not related to therapy or other non-myeloma-related conditions; Rise in serum creatinine by 2 mg/dL or more from the start of the therapy and attributable to myeloma; Hyperviscosity related to serum paraprotein
Relapse from complete response (to be used only if the endpoint is disease-free survival)	Any one or more of the following criteria: Reappearance of serum or urine M-protein by immunofixation or electrophoresis; Development of ≥5% plasma cells in the bone marrow; Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcemia) (see above)
Relapse from MRD negative (to be used only if the endpoint is disease-free survival)	Any one or more of the following criteria: Loss of MRD negative state (evidence of clonal plasma cells on NGF or NGS, or positive imaging study for recurrence of myeloma); Reappearance of serum or urine M-protein by immunofixation or electrophoresis; Development of ≥5% clonal plasma cells in the bone marrow; Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcemia)

2. Multiple Myeloma Treatment

2.1 Frontline treatment

MM patients presenting with active/symptomatic disease are initially treated with frontline treatment and, in selected patients, it is followed by high-dose chemotherapy with autologous stem cell transplantation. The recent aims are to improve the response rates and depth of response in both transplant and non-transplant candidates. It should be important to assess for response to frontline therapy after 1 to 2 cycles of therapy.

Stem cell toxins, such as alkylating or nitrosoureas agents, may compromise stem cell reserve, and regimens with these agents (in particular melphalan) should be avoided in patients who are potential candidates for SCT. In patients with advanced MM is really important to determine whether they are candidates for high-dose therapy and transplant, based on age and co-morbidities: advanced age and renal dysfunction are not absolute contraindications to transplant. All patients also need careful attention to supportive care: 80% of patients have bone disease and up to 33% have renal compromise. Frontline proteosome inhibitor–based regimens may be indicated in patients with renal failure and in those with certain adverse cytogenetic features[44].

Appropriate adjunctive measures should be used in order to manage renal dysfunction, bone disease, and other complications such as hypercalcemia, hyperviscosity, and coagulation/thrombosis. So, supportive care is critical to avoid early complications that may compromise seriously therapeutic outcome.

3-drug regimens over 2-drug regimens is actually preferred as the standard of care for primary treatment of MM. This is based on improved response rates, depth of response, and rates of progression-free survival (PFS) and overall survival (OS) seen with 3-drug regimens in clinical trials. However, the panel notes that doublets could be used in elderly and/or frail patients, who could be unable to tolerate a 3-drug regimen.

In patients receiving an IMiD-based therapy, prophylaxis with full-dose aspirin is recommended and an anticoagulation agent is recommended for patients receiving an IMiD-based therapy and who are at high risk for thrombosis.

In patients receiving proteosome inhibitor–based therapies, prophylactic antiviral therapy is recommended[45-46], because of risk of reactivation of herpes simplex infection or herpes zoster, due to impaired lymphocyte function that results from MM and/or its treatment-related myelosuppression[46-49].

Regarding carfilzomib, second generation proteosome inhibitor, careful assessment should be performed before initiating treatment close monitoring during treatment is recommendedbecause it can potentially cause cardiac and pulmonary toxicities[50].

2.2 Therapy Regimens for newly-diagnosed Transplant Candidates

Bortezomib is the first proteosome inhibitor (PI) active in MM (Figure 1). Bortezomib-based 3-drug regimens have been listed as preferred primary therapy options for patients who are SCT eligible; these regiments include bortezomib/thalidomide/dexamethasone, bortezomib/lenalidomide/dexamethasone, bortezomib/doxorubicin/dexamethasone, and bortezomib/cyclophosphamide/dexamethasone.

Considering results ofthe MMY-3021 trial[51], subcutaneous administration is the preferred route for bortezomib.

In this trial, a randomization of 222 patients to single-agent bortezomib administered either by the conventional intravenous route or by subcutaneous route was performed, and it was demonstrated non-inferior efficacy with subcutaneous versus intravenous bortezomib with regard to the primary end point (ORR, overall response rate after 4 cycles of single-agent bortezomib). Moreover, consistent results were shown with regard to secondary end points, with a significant reduction in peripheral neuropathy, and there were no significant differences in terms of time to progression or in one-year OS between groups[51-52]. Herpes prophylaxis in patients receiving bortezomib therapy should be mandatory. During frontline triplet treatment, it is recommended to harvest peripheral blood early in the course of primary treatment, preferably after 3 to 4 cycles of initial therapy.

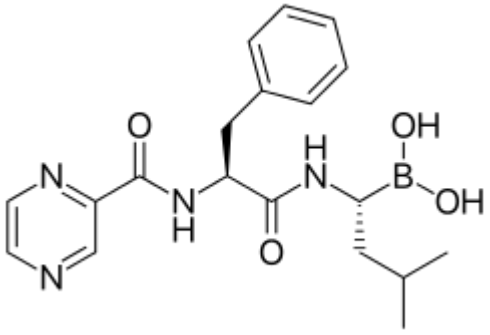

Figure 1: Bortezomib structure. Bortezomib is a modified dipeptide boronic acid from leucine and phenylalanine

2.2.1 Bortezomib/Thalidomide/Dexamethasone

Thalidomide has an important role in treatment of MM, thanks to its efficacy in attacking multiple targets in the microenvironment of the myeloma cell, producing apoptosis, inhibition of angiogenesis, and cytokine circuits, among others (Figure 2).

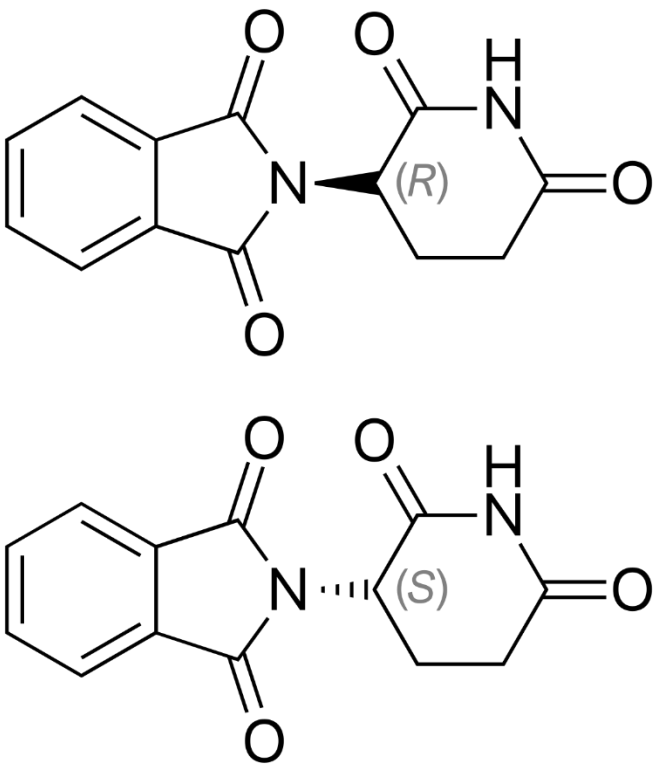

Figure 2: Thalidomide structure

The most important phase III trial regarding a comparison between bortezomib, thalidomide, and dexamethasone (n=241) versus thalidomide and dexamethasone (n=239) as frontline therapy followed by tandem autologous SCT with high-dose melphalan and then consolidation therapy with the same primary regimen was presented by GIMEMA Italian Multiple Myeloma Network[53]. ORR significantly improved after primary treatment, thanks to the addition of bortezomib to thalidomide and dexamethasone. In fact, after frontline therapy, CR/nCR (near CR) was achieved in 73 patients (31%; 95% CI, 25.0–36.8) receiving bortezomib/thalidomide/dexamethasone, and 27 patients (11%; 95% CI, 7.3–15.4) on thalidomide/dexamethasone[53]. Rates of CR/nCR and VGPR or better continued to be significantly higher in the VTD group than in the TD group after the first and second autologous

SCT, and subsequent consolidation treatment. However, patients receiving the bortezomib-containing regimen experienced grade 3/4 PN.

A single-institution retrospective study shows similar data to the interim data from the GIMEMA trial[54]. However, the findings of this analysis confirm that ORR after primary therapy with bortezomib, thalidomide, and dexamethasone was 94% of the patients (32 of 34 patients showed some response, including a VGPR rate ≥56%)[54].

The Spanish Myeloma Group (PETHEMA/GEM) also demonstrated in a phase III trial a significantly higher CR rate with bortezomib/thalidomide/dexamethasone as primary therapy overall (35% vs 14%; P=.001) and in patients with high-risk cytogenetics (35% vs 0%; P=.002)[55]. In particular, the CR rate continued to be significantly higher after autologous SCT (46% vs 24%) in patients treated with VTD versus TD as primary therapy[55].

The phase III IFM 2013-04 trial is comparing 4 cycles of CyBorD versus 4 cycles of VTD as induction therapy before autologous SCT in newly diagnosed MM patients (N=340)[56]. The preliminary results show that patients who received VTD as induction therapy experienced higher ORR (92.3%) compared with those who received CyBorD (84%). In particular, those who received VTD had significantly greater VGPR (P=.04) and PR (P=.02) rates[56].

Moreover, the hematologic toxicity was greater in CyBorD arm, however, in the VTD arm higher rates of PN were reported[56].

Nowadays, in Italy, VTD is the preferred regimen for newly diagnosed MM patients eligible to autologous SCT, with a recommendation of appropriate thromboprophylaxis.

2.2.2 Bortezomib/Dexamethasone

In the IFM cooperative group trial, 482 transplant-eligible patients were randomized to one of the 4 frontline treatment arms: 121 in VAD alone, 121 in VAD plus consolidation therapy with DCEP (dexamethasone/cyclophosphamide/etoposide/cisplatin); 121 in bortezomib/dexamethasone, and 119 in bortezomib/dexamethasone plus consolidation with DCEP[57]. Primary end-point was to assess response rate after frontline treatment, according to modified EBMT criteria[39], including nCR (CR but immunofixation-positive)[58] and VGPR (serum M-protein reduction ≥90%; urine light chain <100 mg/24 hours)[10]. After frontline treatment, ORR (78.5% vs 62.8%) and rates of CR/nCR (14.8% vs 6.4%) and VGPR (37.7% vs 15.1%) were significantly higher with VD versus VAD[57]. At a median follow-up of 32.2 months, median PFS was modestly, but not statistically significantly, prolonged compared with VAD (36.0 vs

29.7 months)[57]. Use of DCEP as consolidation therapy after primary therapy did not have a significant impact on the rates of response[57]. Bortezomib/dexamethasone regimen was equally effective in patients with high-risk MM, including those with ISS stage III disease and poor-risk cytogenetic abnormalities. Severe adverse events incidence was similar between the 2 groups. Hematologic toxicity and deaths related toxicity were more frequent with VAD versus VD. Bortezomib/dexamethasone showed a rate of PN during induction through first transplantation significantly higher than VAD[57].

A phase III randomized trial, conducted by IFM, compared bortezomib/dexamethasone with a combination of reduced doses of bortezomib and thalidomide plus dexamethasone[59]. Response rates in this study match those described in previous trials comparing VAD with bortezomib and dexamethasone[57].

High-risk patients [with either t(4;14) or del(17p)] are known to have a short EFS (event-free survival) and OS. A trial analyzed a large series of newly diagnosed transplant-eligible MM patients (aged <65 years) with and t(4;14) or del(17p) treated with bortezomib/dexamethasone versus VAD as frontline treatment[58-59]. The analysis demonstrated that bortezomib, compared with patients treated with VAD primary therapy, improves the prognosis (in terms of both EFS and OS; p<.001 and p<.001, respectively) of patients with t(4;14).

2.2.3 Lenalidomide/Dexamethasone

Lenalidomide is a potent analogue of thalidomide, believed to attack multiple targets in the microenvironment of the myeloma cell, producing apoptosis and inhibition of angiogenesis and cytokine circuits. It was approved from the FDA for the treatment of relapsed/refractory MM in combination with dexamethasone (Figure 3).

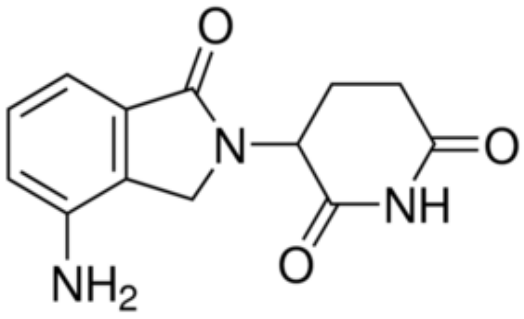

Figure 3: Lenalidomide structure.

However, lenalidomide and dexamethasone have more recently also been investigated as frontline treatment. S0232, by SWOG, a phase III randomized controlled study, compared dexamethasone single agent with dexamethasone plus lenalidomide for newly diagnosed MM patients[60]. At interim analysis, this trial was halted and patients on dexamethasone alone arm were allowed to switch to lenalidomide/dexamethasone arm. Moreover, based on the preliminary results from the ECOG phase III study (E4A03), the SWOG data and safety monitoring committee based its recommendation to permanently close enrollment[61]. At the end of first year, the time the SWOG trial was halted, lenalidomide plus dexamethasone arm showed improved CR rate compared with dexamethasone alone (22.1% vs 3.8%)[60].

In another open-label trial, 445 newly diagnosed MM patients were randomly assigned to high-dose or low-dose regimens. High-dose dexamethasone showed a superior response, with 169 (79%) of 214 patients receiving high-dose therapy and 142 (68%) of 205 patients on low-dose therapy with a CR or PR within 4 cycles[62]. However, the higher response rates did not result in statistical superior time to progression, PFS, or OS compared with low-dose dexamethasone, and this trial was stopped after 1 year. Considered that the OS rate was significantly higher in low-dose arm, patients on high-dose therapy were allowed to cross-over. The OS rate in the low-dose dexamethasone group was 96% compared with 87% in the high-dose group (P=.0002), at 1-year interim analysis; 2-year OS was 87% versus 75%, respectively.

Inferior OS with high-dose dexamethasone seemed to be related to increased deaths caused by toxicity. 52% of patients on the high-dose had grade 3-4 toxic effects in the first 4 months, versus 35% on the low-dose regimen, including DVT (26% vs 12%), infections including pneumonia (16% vs 9%), and fatigue (15% vs 9%). Patients who had received 4 cycles of primary treatment with either dose followed by autologous SCT showed a 3-year OS rate of 92%, suggesting that lenalidomide and dexamethasone is a reasonable option for primary therapy before auto-SCT. However, a limit of this trial was that the choice to proceed to SCT was not randomized but based on physician and patient preference.

Extra-hematologic adverse events showed an incidence of DVT lower with single-agent lenalidomide or lenalidomide plus low-dose dexamethasone than when it is combined with high-dose dexamethasone. Recently, a report showed that patients treated with lenalidomide and high-dose dexamethasone that developed during therapy a venous thromboembolism did not experience shorter OS or time to progression[63]. Prophylactic anticoagulation is recommended in patients receiving this therapy[45,64].

However, it has been reported a decrease in CD34-positive cells collected after prolonged lenalidomide treatment has been reported[65,66]. IMWG guidelines suggest that patients managed with lenalidomide and dexamethasone should have their stem cells collected within the first 4 cycles of therapy[67]. It has also been demonstrated that chemomobilization could overcome this inability to collect stem cells[68]. In particular, there are data showing the efficacy of addition of plerixafor when conventional mobilization methods fails for successful stem cell harvest[69,70].
Lenalidomide/dexamethasone can be considered one of the best options in frontline treatment, with a recommendation together with an appropriate thromboprophylaxis.

2.2.4 Bortezomib/Cyclophosphamide/Dexamethasone (CyBorD)

CyBorD as primary treatment was evaluated in three phase II studies involving newly diagnosed MM, demonstrating high response rates[71-73].
In particular, Reeder et al (USA and Canada)[72] showed an ORR of 88%, including rates of VGPR or greater of 61% and CR/near CR of 39%. The depth of response seen after frontline treatment was maintained, in those who underwent transplantation, after transplant, with 70% rates of CR/near CR; rate of at least VGPR or better was 74%. According to the long-term follow-up analysis, the 5-year PFS and OS rates were 42% (95% CI, 31–57) and 70% (95% CI, 59–82)[74].
German DSMM XIa study also demonstrated high responses with frontline CyBorD (ORR, 84%; PR, 74%; CR, 10%), with high response rates also in patients with unfavorable cytogenetics[73]. In updated results of EVOLUTION study, frontline treatment with CyBorD demonstrated an ORR of 75% (CR, 22%; ≥VGPR, 41%) and the 1-year PFS rate was 93%[71].
Twice-weekly bortezomib can be associated with toxicities that may limit efficacy caused by treatment delays or discontinuation. So, Reeder et al[75] modified the regimen to a once-weekly schedule. In the study, patients treated with weekly bortezomib achieved responses similar to the twice-weekly schedule (ORR, 93% vs 88%; VGPR, 60% vs 61%, respectively), showing fewer grade 3/4 adverse events (37%/3% vs 48%/12%). Fewer dose reductions of bortezomib/dexamethasone were required in the modified schedule and neuropathy rates were the same in both cohorts, even though the total bortezomib dose per cycle was higher in the weekly versus the twice-weekly schedule (6.0 mg/m2 vs 5.2 mg/m2)[75].

2.2.5 Bortezomib/Doxorubicin/Dexamethasone (PAD)

HOVON-65/GMMG-HD4 group phase III trial of newly diagnosed patients with stage II/III MM update results demonstrated high response rates after primary therapy with bortezomib/doxorubicin/dexamethasone (PAD) versus VAD. This superior response rate (CR + near CR, 31% vs 15%; P<.001) was also maintained after SCT, with a significantly higher ORR[76]. Del(13q) did not have a significant impact on response and no unexpected toxicities occurred. In particular, response rates improved with bortezomib maintenance (34% vs 49%; P<.001)[76]. PFS in patients treated with PAD as frontline therapy followed by SCT and bortezomib maintenance, after a median follow-up of 41 months, was 35 versus 28 months in patients treated with VAD followed by SCT and maintenance with thalidomide. Patients treated with PAD had also a significantly better PFS (HR, 0.75; 95% CI, 0.62–0.90; P=.002), and OS was also found to be improved in PAD arm (HR, 0.77; 95% CI, 0.60–1.00; P=.049). In high-risk patients, presenting with increased creatinine >2 mg/dL, bortezomib-containing regimen significantly improved PFS from a median of 13 to 30 months (HR, 0.45; 95% CI, 0.26–0.78; P=.004) and OS from a median of 21 to 54 months (HR, 0.33; 95% CI, 0.16–0.65; P<.001).
In patients with deletion of 17p13 also a benefit in terms of increased PFS was evaluated[76]. Peripheral neuropathy (PN)-rate (grade 2 to 4) was higher in those treated with the bortezomib-containing regimen versus VAD (40% vs 18%). Moreover, newly developed grade 3 to 4 PN occurred in 8% of patients during thalidomide maintenance and 5% of patients during bortezomib maintenance[76].

2.2.6 Bortezomib/Lenalidomide/Dexamethasone (VRD)

Bortezomib/lenalidomide/dexamethasone is active and well-tolerated in all newly diagnosed patients with MM, transplant eligible, and transplant ineligible, as demonstrated in phase II and III studies[71,77,78].
In particular, the first phase I/II prospective study of bortezomib/lenalidomide/dexamethasone in newly diagnosed MM patients, the rate of ≥ PR was 100%, with 74% VGPR or better and 52% CR/nCR[77]. Trials of phase II EVOLUTION[71] and phase II IFM2008 trial[78] also confirmed the benefits of bortezomib/lenalidomide/dexamethasone as frontline treatment. In IFM2008 trial, patients received bortezomib/lenalidomide/dexamethasone as induction therapy followed by SCT[78], and they subsequently received 2 cycles of bortezomib/lenalidomide/dexamethasone as

consolidation cycles and 1-year lenalidomide maintenance. Responses ≥ VGPR at the completion of induction was 58%, while after transplantation and consolidation therapy it was 70% and 87%, respectively[78].

EVOLUTION was a really interesting trial, designed to assess the efficacy and tolerability of combining bortezomib/cyclophosphamide/lenalidomide/dexamethasone versus bortezomib/lenalidomide/dexamethasone versus bortezomib/cyclophosphamide/dexamethasone (CyBorD) in a randomized multicenter setting[71]. The ORR after primary treatment with bortezomib/lenalidomide/dexamethasone followed by maintenance with bortezomib was 85% (51% ≥VGPR; 24% CR) and corresponding 1-year PFS was 83% in the bortezomib/lenalidomide/dexamethasone arm[71].

Bortezomib/lenalidomide/dexamethasone triplet was compared to lenalidomide and dexamethasone in SWOG S077, a multicenter phase III[79], where 525 previously untreated MM patients were randomly assigned to receive 6 months of induction therapy with either bortezomib/lenalidomide/dexamethasone versus lenalidomide/dexamethasone each followed by maintenance therapy with lenalidomide/dexamethasone until progression or unacceptable toxicity. At a median follow-up of 55 months, treatment with bortezomib/lenalidomide/dexamethasone compared with lenalidomide/dexamethasone resulted in higher rates of ORR (82% vs 72%) and CR (16% vs 8%), superior median PFS (median, 43 vs 30 months; hazard ratio [HR], 0.71; 95% CI, 0.56–0.91), and improved OS (median, 75 vs 64 months; HR, 0.71; 95% CI, 0.52–0.97). As expected, grade 3 or higher neuropathy was more frequent in the bortezomib-containing arm (24% vs 5%; P<.0001).

Nowadays, considering these data, bortezomib/lenalidomide/dexamethasone regimen can be considered the preferred primary treatment of transplant-eligible patients with newly-diagnosed MM, even if this regimen is not yet available in Italy.

2.2.7 Carfilzomib/Lenalidomide/Dexamethasone

Carfilzomib is a second-generation proteasome inhibitor that binds highly selectively and irreversibly to the proteasome (Figure 4).

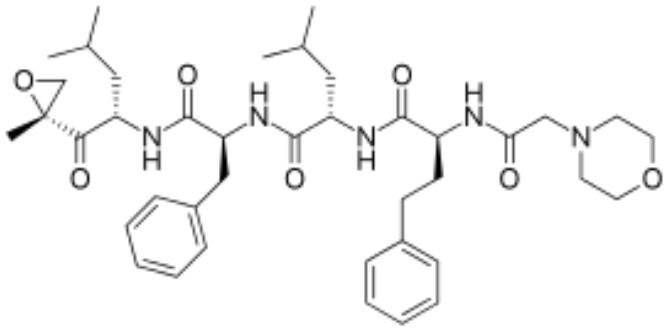

Figure 4: Chemical structure of Carfilzomib

It is administered intravenously. Moreover, preclinical studies with carfilzomib have shown lack of neurodegeneration in vitro[80] and less neurotoxicity in animal studies[81]. Moreover, Carfilzomib has demonstrated important antimyeloma activity in patients with relapsed and/or refractory MM with an acceptable tolerability profile, including limited neuropathy after prolonged treatment[82-84].

In particular, the efficacy and safety of carfilzomib in combination with lenalidomide and dexamethasone, as primary therapy for MM patients, were evaluated in 2 single-arm trials.

First, the combination of carfilzomib/lenalidomide/dexamethasone (KRD) in newly diagnosed MM patients was evaluated in a multicenter phase I/II trial[85]. In this trial, patients (n=53) received KRD, and, after 4 cycles, stem cells were collected from eligible patients (n=35)[85], 7 of whom proceeded to autologous SCT, while the remainder continued with KRD[85]. With median follow-up of 13 months, 24-month PFS was estimated at 92%. Considering safety, the most common grade 3 and 4 toxicities in ≥10% of patients included hypophosphatemia (25%), hyperglycemia (23%), anemia (21%), thrombocytopenia (17%), and neutropenia (17%). In particular, PN was limited to grade 1/2 (23%)[85].

The second phase II trial also KRD in newly diagnosed MM patients (n=45). After 8 cycles of treatment, patients with SD (stable disease) received up to 24 cycles of lenalidomide, 10 mg/day (days 1 to 21)[86]; with 38 patients who were evaluable for response and toxicity. PFS was 83.3%, after a median follow-up of 10 months. A total of 25 patients completed 8 cycles of KRD, of which 24 continued to lenalidomide therapy and 1 patient opted to exit the study after initial therapy. Considering safety, the most common extra-hematologic and hematologic toxicities (≥grade 3) in more than 10% of patients included electrolyte disturbances (18%), liver function test elevation (13%), rash/pruritus (11%), fatigue (11%), lymphopenia (63%), anemia (16%), leukopenia (13%), and thrombocytopenia (11%)[87].

Based on the above data, nowadays, in USA, KRD is an option for frontline primary treatment of MM transplant-eligible patients. However, it is not yet available in Italy in this setting.

2.2.8 Ixazomib/Lenalidomide/Dexamethasone

Ixazomib is a new-generation oral proteosome inhibitor that was approved by the FDA in combination with lenalidomide and dexamethasone (IRD) for MM patients with who have received at least one prior therapy (Figure 5).

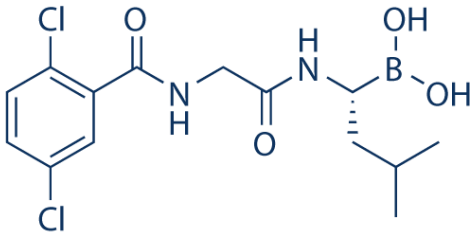

Figure 5: Ixazomib structure

However, in a phase I/II trial, Kumar et al[88] studied an all oral combination of ixazomib/lenalidomide/dexamethasone in newly diagnosed MM patients. This trial showed that IRD was well tolerated and active in the study population. In particular, of the 64 patients in whom the response could be evaluated, 37 (58%; 95% CI, 45–70) had a VGPR or better. Considering safety, grade 3 or higher adverse events related to any drug in the combination were reported in 41 (63%) patients. These included extrahematological toxicity, skin and subcutaneous tissue disorders (11 patients, 17%), and hematological toxicity including neutropenia (8 patients, 12%), and thrombocytopenia (5 patients, 8%); drug-related PN of grade 3 or higher occurred in only 4 (6%) patients.

Based on these phase II results and the efficacy of the combination of other proteosome inhibitors (bortezomib or carfilzomib) in combination with lenalidomide/dexamethasone as primary therapy in newly diagnosed MM[56,85-87,89], IRD could be considered an option (at the moment not yet in Italy) for the treatment of newly diagnosed MM patients.

2.3 Preferred Primary Therapy Regimens for Non-Transplant Candidates

Many of the described regimens for transplant candidates are also options for non-transplant candidates. As in transplant-eligible patients, three-drug regimens are preferred because have been shown to induce higher response rates and better depth of response in clinical trials.
However, the two-drug regimens are reserved for elderly and/or frail patients. In particular, the best options for non-transplant candidates include bortezomib/cyclophosphamide/dexamethasone, bortezomib/lenalidomide/dexamethasone, and lenalidomide/low-dose dexamethasone.
Melphalan-containing regimens should be no longer considered the standard of care in this setting in the era of novel agents.

2.3.1 Bortezomib/Melphalan/Prednisone

The role of bortezomib/melphalan/prednisone (VMP) as frontline treatment for inelegible-SCT MM patients was studied in a phase III trial, evaluating nine 6-week cycles of VMP versus MP, randomly assigning 682 patients[90]. The median age of patients in this study was 71 years, and patients with serum creatinine higher than 2 mg/dL were excluded. ORR (at least PR) was 71% in VMP versus 35% in MP, with CR 30% versus 4%, respectively. The median time to progression was 24 months versus 16.6 months, and median duration of response was 19.9 months versus 13.1 months. Considering safety, grade 3 adverse events were higher in the bortezomib group than in the control group (53% versus 44%), but there were no significant differences in grade 4 events (28% versus 27%, respectively), or treatment-related deaths (1% and 2%).
Considering these findings, VMP can be considered as a primary therapy option for newly diagnosed myeloma who are inelegible for high-dose therapy.

2.3.2 Bortezomib/Cyclophosphamide/Dexamethasone

The role of CyBorD as frontline treatment for inelegible-SCT MM patients was studied in a small phase II trial (n=20)[91]. The median age of patients in this study was 76 years (range, 66–90 years). After a median of 5 cycles, the ORR was 95%, with 70% of patients achieving a VGPR or better. Considering safety, 6 patients experienced non-hematologic grade 3/4 adverse events

(20%), including muscle weakness, sepsis, and pneumonia. Neutropenia and thrombocytopenia were seen in 2 patients (10%)[91].

Considering these findings[91], together with the earlier described results from the EVOLUTION trial[54] and the phase II trials[72,73], CyBorD can be considered as a primary therapy option for non-transplant candidates.

2.3.3 Lenalidomide/Low-Dose Dexamethasone

The results of two trials: SWOG SO232[60], which included transplant-ineligible patients, and the ECOG E4A03[92], which included elderly MM patients, demonstrate that lenalidomide in combination with low-dose dexamethasone is a effective and well-tolerated regimen for these patients. In the ECOG E4A03 trial, OS rate was significantly higher in the lenalidomide plus low-dose dexamethasone arm compared with the lenalidomide plus high-dose dexamethasone arm[62]. The inferior survival outcome seen with high-dose dexamethasone was greatest in patients aged ≥65 years. However, at 2 years, patients who did not proceed to transplant had an OS rate of 91% with lenalidomide and low-dose dexamethasone[62].

The FIRST trial, an international, multicenter trial, evaluated efficacy and safety of lenalidomide/dexamethasone given continuously or for 72 weeks compared with melphalan/prednisone/thalidomide (MPT) in elderly (n=1,623) transplantation-ineligible newly diagnosed MM patients[93].

The primary end point was PFS, while secondary end points were OS and safety, including the incidence of secondary malignancies. After a median of 37 months of follow-up, the risk of progression or death was reduced by 28% in patients receiving continuous lenalidomide/dexamethasone versus MPT (HR, 0.72; 95% CI, 0.61–0.85; P<.001)[93]. Moreover, continuous lenalidomide/dexamethasone also reduced the risk of progression or death compared with 18 cycles of lenalidomide/dexamethasone (HR, 0.70; 95% CI, 0.89–1.20; P=.70). In particular, in the interim analysis, also an OS benefit was seen in the lenalidomide/dexamethasone arm versus MPT (HR, 0.78; CI, 0.64–0.96; P=.02)[93].

During last years, several reports have shown higher incidences of secondary malignancies when lenalidomide is used as a maintenance therapy post-transplantation or in a melphalan-containing regimen[94-97]. In the FIRST trial, the overall incidence of secondary malignancies, including solid tumours and hematologic malignancies, was lower in the continuous lenalidomide/dexamethasone arm. In particular, the overall rates of second primary cancers were

3.0% in the continuous lenalidomide/dexamethasone arm, 6.0% in the arm receiving 18 cycles of lenalidomide/dexamethasone, and 5.0% in the MPT arm[93]. Moreover, regarding renal impairment, in an analysis based on renal function of patients enrolled in the FIRST trial, continuous lenalidomide/low-dose dexamethasone compared with MPT reduced the risk of progression or death in patients with normal, mild, and moderate renal impairment by 33%, 30%, and 35%, respectively[98].

So, lenalidomide/low-dose dexamethasone is considered a category one of the best options for transplant-ineligible patients with MM, with appropriate thromboprophylaxis for patients receiving this therapy.

Moreover, based on the results of the FIRST trial[93], continuous lenalidomide/dexamethasone until disease progression should be considered for patients who are not eligible for transplant.

2.3.4 Bortezomib/Dexamethasone

UP-FRONT, a US community-based, randomized, open-label, multicenter phase IIIb, trial compared safety and efficacy of 3 highly active bortezomib-based regimens in previously untreated elderly ineligible for SCT MM patients[99]. The patients with symptomatic, measurable MM were randomized (1:1:1) to one of the following regimens: VTD (n=167); bortezomib/dexamethasone (n=168); or melphalan/prednisone/bortezomib (n=167) followed by maintenance therapy with bortezomib. The primary end point was PFS; secondary end points included ORR, CR/nCR and VGPR rates, OS, and safety. All three induction regimens exhibited substantial activity, with ORR of 80% (VTD), 73% (bortezomib/dexamethasone), and 69% (melphalan/prednisone/bortezomib) during the treatment period[99]. However, after a median follow-up of 21.8 months, no significant difference in PFS was observed between the treatment arms[99]. Response rates, including CR and VGPR or better, improved after bortezomib maintenance, with no concomitant increase in the incidence of PN.

So, bortezomib/dexamethasone can be considered as option for frontline treatment for MM patients who are ineligible for transplant.

2.3.5 Bortezomib/Lenalidomide/Dexamethasone

Phase II study results, earlier discussed, have shown that primary therapy with VRD is active and well tolerated in all newly diagnosed MM patients regardless of autologous SCT status[77].

The earlier discussed randomized phase III SWOG S0777 trial, comparing VRD versus RD as induction therapy without an intent of immediate transplantation, reported superior results with the three-drug regimen[79]. So, VRD can be considered one of the best options for MM patients who are ineligible for SCT. However, it is not yet available in Italy.

2.3.6 Ixazomib/Lenalidomide/Dexamethasone

A earlier discussed phase I/II study evaluated the safety and efficacy of the all-oral combination of ixazomib with lenalidomide and dexamethasone in newly diagnosed MM patients[88]. Both efficacy and tolerability of this regimen in older patients (≥65 years) was similar to that in younger patients in this study. So, also IRD can be considered as option for frontline treatment for all patients with newly diagnosed MM, including those who are not eligible for SCT. However, it is not yet available in Italy.

2.3.7 Carfilzomib/Lenalidomide/Dexamethasone

The results of a earlier discussed phase I/II trial demonstrated that KRD is well-tolerated and is also effective in all patients with newly diagnosed MM[85]. An updated follow-up analysis of the subset of elderly patients (23 patients, age ≥65 years) showed that use of KRD regimen for an extended period resulted in deep and durable responses. In particular, all patients experienced at least a PR, with a median follow-up of 30.5 months. Moreover, the reported PFS rate was 79.6% (95% CI: 53.5–92.0) and OS was 100%[89].

The phase II trial by Korde et al[87] also showed that treatment with KRD regimen results in high rates of deep remission and no MRD. The results were very similar across age groups, with the oldest patient on the trial being 88 years of age[87], and the regimen was found to be effective also in individuals with high-risk disease[100].

Based on these phase II studies that did not exclude transplant ineligible patients, KRD can be considered as an important option for the treatment of all patients with newly diagnosed MM, including those who are not eligible for SCT. The only problem is that carfilzomib can potentially cause cardiac and pulmonary toxicities in elderly patients[101], and that's why it should be recommended adequate monitoring of these patients.

3. Stem Cell Transplantation in Multiple Myeloma

3.1 Monitoring After Primary Myeloma Therapy of Both Transplant and Non-Transplant Candidates

Patients on treatment should be monitored not only for response to primary therapy but also for symptoms related to disease and/or treatment. In particular, it should be recommended to re-evaluate (after 1–2 cycles) with the laboratory tests, skeletal survey, and bone marrow aspiration and biopsy only if indicated, to determine treatment response or whether the primary disease is progressive. Patient potentially transplant candidates must undergo a stem cell harvest after 4 to 6 cycles of therapy, collecting at that enough stem cells for 2 transplants (depending on the intended number of transplants and age) in anticipation of a tandem transplant or a second transplant as subsequent therapy. Alternatively, all patients may consider continuation of primary therapy until the best response is reached. The optimal duration of primary therapy after achieving maximal response is actually unknown; hence, maintenance therapy or observation could be considered beyond maximal response.

Follow-up tests after frontline myeloma treatment include those used for initial diagnosis: a complete blood count with differential and platelet counts; renal function corrected serum calcium; and quantification of M-protein and immunoglobulins. The serum FLC may be assessed as clinically indicated, and they have a role especially in patients with oligosecretory or nonsecretory MM. Response should be assessed using the IMWG criteria[10].

Other tests, such as skeletal survey, MRI, and PET/CT scan, bone marrow aspiration and biopsy, may be performed as indicated by symptoms to detect disease progression. Patients eligible for SCT should be referred for evaluation by SCT team and stem cells should be harvested.

3.2 Stem Cell Transplants

High-dose therapy with stem cell support is a critical component in the treatment plan of newly diagnosed eligible MM patients. There are different types of SCT: single autologous SCT, a tandem SCT (a planned second course of high-dose therapy and SCT within 6 months of the first course), or an allogeneic SCT. Moreover, an allogeneic SCT can be performed or after prior myeloablative therapy or after non-myeloablative therapy. Non-myeloablative therapy, or “mini

transplant," has been investigated as a technique to decrease toxicity of the allotransplant while preserving the alloimmune GvM (graft-versus-myeloma) effect[102,103]. It is important to note that non-myeloablative allogeneic transplant by itself is not adequate therapy and it should be usually performed following maximal tumor control through adequate induction therapy or an autologous SCT. An allogeneic SCT may also follow an autologous SCT.

All candidates for pre-SCT high-dose chemotherapy must have sufficient liver, renal, pulmonary, and cardiac function. However, renal dysfunction is not an absolute contraindication to transplant. Earlier studies of autologous transplant included TBI (total body irradiation) as a component of the preparative regimen. Chemotherapy regimens have only recently been shown to have equivalent efficacy and less toxicity than TBI, whose regimens have now been abandoned[104], but newer, potentially less toxic radiation techniques aimed to deliver total marrow irradiation while reducing toxicities to non-target organs are currently undergoing evaluation in clinical trials[105].

3.2.1 Autologous SCTs

Autologous SCT results in high response rates and remains the standard of care after frontline treatment for eligible patients. Results of the first randomized trial were reported in 1996, demonstrating that autologous SCT is associated with statistically significant higher response rates and increased OS and EFS when compared with the response of similar patients treated with conventional therapy[106]. In 2003, a second trial comparing high-dose therapy with standard therapy showed an increase in the CR rate and an improvement in OS, 54 months in the high-dose group vs 42 months for standard therapy[107], with a more pronounced benefit was for higher-risk patients. Barlogie et al[108] reported on the results of arandomized American trial comparing 510 patients to receive high-dose therapy with autologous stem cell support or standard therapy: with a median follow-up of 76 months, no differences were seen in response rates, PFS, or OS between the groups. The reasons for the discrepant results are not clear, but may be related to differences in the specific high-dose and conventional regimens between the American and French study. For example, TBI was included as part of the high-dose regimen in the American study; then TBI has subsequently been found to be inferior to high-dose melphalan[104].

Another important trial included 190 patients, median age 55 to 65 years, randomized to standard or high-dose therapy[109]. This study was specifically designed to include older patients, with a median age in this trial of 61 years compared with the median age of the participants in other

trials which ranged from 54 to 57 years. No significant difference was seen in OS, although a trend was seen toward improved EFS in the high-dose group (P=.7), after 120 months of follow-up. Moreover, in the high-dose group the period without symptoms, treatment, or treatment toxicity was significantly longer. The study showed that the equivalent survival suggests that the treatment choice between high-dose and conventional-dose chemotherapy should be based on personal choice in older patients. For example, an early transplant may be favored because patients can enjoy a longer interval of symptom-free time. However, this study[110] showed also that a transplant performed at relapse has a similar OS compared with an early transplant. A randomized French trial examined the choice of early versus late transplant and the results in both arms are comparable with respect to OS[111]. However, early SCT was shown to be superior in terms of quality of life, assessed as time without symptoms and side effects from therapy[111].

All randomized studies of autologous SCT after primary therapy were designed and implemented before the availability of thalidomide, lenalidomide, or bortezomib. Therefore, the role of transplant may evolve in the future. The results of the PETHEMA trial strongly support, even in the era of novel agents, the use of upfront autologous SCT for MM[55], with evaluation of responses after induction therapy and after autologous SCT. Considering patients who actually underwent the autologous SCT, the CR rates increased in the group treated with VTD from 35% pretransplant to 57% posttransplant as induction therapy, and, in the group treated with thalidomide and dexamethasone as induction therapy, from 14% to 40%, respectively[55].

A recent phase III study compared high-dose melphalan followed by autologous SCT with MPR (melphalan/prednisone/lenalidomide): patients (n=402) were randomly assigned, in a 1:1:1:1 ratio, to 1 of 4 groups: high-dose therapy and SCT followed by maintenance with lenalidomide; high-dose therapy and SCT alone; primary therapy with MPR followed by lenalidomide; and primary therapy with lenalidomide alone. The primary study end point was PFS, secondary end points included ORR, OS, the time to a response, and safety[112]. High-dose melphalan therapy followed by SCT was associated with a significant reduction in the risk of progression or death (HR, 0.44) and prolonged OS (HR for death, 0.55)[112].

Results from the IFM 2005-01 study of symptomatic myeloma patients receiving frontline treatment with bortezomib and dexamethasone versus VAD showed a marked improvement in ORR with bortezomib and dexamethasone over VAD[57]. Evaluation of responses was performed after primary treatment and post-autologous SCT. After the first autologous SCT, CR/nCR rates were 35.0% in the bortezomib plus dexamethasone arm, compared with 18.4% in the VAD arm[57], VGPR rates were 54.3% versus 37.2%. and median PFS was 36.0 months versus 29.7

months (P=.064) with bortezomib plus dexamethasone versus VAD after a median follow-up of 32.2 months[57]. Moreover, PFS was also significantly longer in the patients achieving greater than or equal to a VGPR after primary treatment than in patients achieving a less than VGPR (median, 36 vs 29.7 months)[57].

In another trial, 474 patients were randomized to frontline treatment with VTD (n=236) versus TD (n=238) before double autologous SCT[113]. The three-drug regimen yielded high response rates compared with the two-drug regimen, with a CR rate of 19% vs 5% and greater than or equal to VGPR of 62% vs 31%. After SCT, improved incremental responses were still seen with VTD compared with TD. Considered together, these studies suggest that improved responses with the primary regimen result in improved outcomes after SCT.

Trials have found that PD emerging after frontline treatment does not preclude a good response to autologous SCT[108,114,115]. In particular, Kumar et al[115] considered a case series of 50 patients with primary progressive MM receiving an autologous SCT, comparing results with those of 100 patients with responsive disease undergoing autologous SCT. The 1-year PFS from the time of SCT was 70% in the primary progressive group compared with 83% in the chemosensitive group.

Nowadays, autologous SCT is the best option after frontline treatment and for treatment of primary progressive or refractory disease after primary treatment.

3.2.2 Tandem SCTs

Tandem SCT is defined as a planned second course of high-dose therapy and SCT within 6 months of the first course. Several randomized trials have evaluated the role of planned tandem transplants. In particular, the IFM94 randomized trial reported by Attal et al[116] compared newly diagnosed MM patients to single or tandem autologous transplants. In tandem transplant group, a total of 78% of patients received the second transplant at a median time of 2.5 months after the first. There were a variety of options for relapsed disease treatment: for example, patients with relapsed disease in either group underwent either no therapy, additional conventional therapy, or another SCT. EFS 7 years after diagnosis was 10% in the single transplant group versus 20% in the double transplant group.

Stadtmauer[117], in accompanying editorial, questions whether the promising results might be related to type of regimens used, rather than to the effect of 2 courses of high-dose therapy. For example, patients received 140 mg/m^2 of melphalan plus TBI in the single transplant arm, while

the same dose without TBI for the initial transplant and with TBI for the second transplant in the tandem arm. As earlier noted, TBI has shown more toxicity without providing additional benefit. Based on this, Stadtmauer suggests that the increased survival in IFM94's tandem arm may have resulted from greater cumulative exposure to melphalan, 280 vs 140 mg/m^2.

In a subset analysis, the patients who did not achieve a CR or a VGPR within 3 months after the first SCT appeared to benefit the most from a second SCT. The investigators suggested that the improvement associated with tandem transplant is related not to improved response rates but rather to longer durations of response. Four other randomized trials have compared single versus tandem SCT[109,118-120], with no one showing a significant improvement in OS. However, considering that the median follow-up in these trials ranged from 42 to 53 months, it is not surprising the lack of significant improvement. Cavo et al[118] found that patients not in CR or nCR after the first SCT benefited the most from a second SCT, confirming the observations of the IFM94 trial using non-TBI-based high-dose regimens.

In both the Italian and French trials, the benefit of a second autologous SCT was seen in patients who do not achieve a CR or VGPR, more than 90% reduction in M-protein level, with the first procedure. However, these two trials were not adequately powered to evaluate the equivalence of one versus two SCT in patients achieving a CR or VGPR after the first transplantation.

Barlogie et al[121] found in a review of long-term outcomes of several trials of autologous transplantation that tandem SCT were superior to both single SCT and standard therapies. Also, when EFS was sustained for at least 3.5 years after tandem SCT, post-relapse survival was longer. However, at diagnosis, in all eligible patients it's recommended to collect enough stem cells for two transplants. A tandem SCT with or without maintenance therapy can be considered for all patients who are eligible for SCT, and it is an option for patients who do not achieve at least a VGPR after the first autologous SCT. Palumbo et al[112] support for use of lenalidomide maintenance therapy after autologous transplantation: although it is associated with more frequent grade 3 or 4 neutropenia and infections, lenalidomide maintenance, compared with no maintenance, was found to significantly reduce risk of disease progression or death (HR, 0.47) after both single and tandem transplantation[112].

The benefit from the second SCT in patients who have CR or VGPR, or in those who achieve less than a VGPR after the first SCT, should preferably be determined in a randomized clinical trial. In fact, such a randomized prospective Intergroup- and NIH-supported trial is currently ongoing. For this group of patients, the other options include observation or maintenance therapy.

A retrospective case-matched control trial was performed comparing patients who underwent a second autologous SCT versus those treated with conventional chemotherapy for relapsed MM[122]. This retrospective analysis demonstrated that a second autologous SCT is associated with superior relapse-associated mortality compared with conventional chemotherapy, 68% vs 78%, along with improved OS, 32% vs 22%, at 4 years, as seen in other published smaller studies[123-125]. In this trial, factors associated with improved OS and PFS included younger age (<55 years), beta-2 microglobulin level <2.5 mg/L at diagnosis, a remission duration of more than 9 months, and better than a PR to their first autologous SCT. So, a second autologous SCT, for relapsed or progressive MM, may be an option for carefully selected patients. Some of these patients can achieve durable CR or PR[125,126].

A multicenter, randomized phase III trial compared high-dose melphalan plussecond autologous SCT with cyclophosphamide in relapsed MM patients who had received autologous SCT as frontline treatment[127]. The patients included in the study needed treatment for progressive or relapsed disease at least 18 months after a previous autologous SCT, and first received PAD induction therapy. Then, patients with adequately harvested stem cells were after induction randomized to high-dose melphalan plus second autologous SCT (n=89) or oral cyclophosphamide (n=85). The primary end-point was time to disease progression[127]. After a median follow-up of 31 months, median time to progression in patients who underwent second autologous SCT after induction was 19 months versus 11 months for those treated with cyclophosphamide (HR, 0.36; 95% CI, 0.25–0.53; P<.0001). Considering hematologic toxicity, grade 3/4 neutropenia (76% vs 13%) and thrombocytopenia (51% vs 5%) were higher in autologous SCT versus cyclophosphamide[127].

Moreover, StaMINA trial indicated that a tandem autologous SCT followed by lenalidomide maintenance has similar outcomes to a single autologous SCT followed by lenalidomide maintenance in frontline treatment of MM[128]. EMN02/HO95 MM trial, an intergroup, multicenter, phase III study, suggests that in newly diagnosed MM tandem autologous SCT, compared with single autologous SCT after induction therapy with a bortezomib-based regimen, appears to be superior in extending PFS[129].

So, second autologous SCT for relapsed disease may be considered either on or off clinical trial considering the interval between the first SCT and documented progression[130-133] and 2 to 3 years is the minimum length of remission for considering second autologous SCT for relapsed disease.

3.2.3 Allogeneic SCT

Allogeneic SCT includes either myeloablative or non-myeloablative (mini transplant) transplants. Allogeneic SCT can be considered as an alternative to autologous SCT to avoid the contamination of reinfused autologous tumor cells, but also to take advantage of the beneficial graft-versus-tumor (GvT) effect associated with allogeneic transplants. However, older MM population, lack of a suitable donor and increased morbidity has limited this approach in MM. Non-myeloablative transplants are designed to decrease the morbidity of the high-dose chemotherapy but preserve the beneficial GvT effect. Moreover, the principal difference between myeloablative and non-myeloablative transplants relates to the chemotherapy regimen used. Considered the small candidate pool, it should be not surprising that no randomized clinical trials comparing myeloablative allogeneic to autologous SCT have ever been performed, but multiple case series have been published describing allogeneic SCT as an initial or as therapy for relapsed/refractory MM. Kyle in a review[134] reported a mortality rate of 25% within 100 days and overall transplant-related mortality of approximately 40% and few patients were cured. In all reports it has also reported increased morbidity without convincing proof of improved survival[114,135]. However, SWOG randomized trial of autologous transplant versus conventional chemotherapy showed interesting data[108]. At start, the original trial had an ablative, allogeneic transplant group consisting of patients with HLA identical siblings; the allogeneic arm was then closed because 36 patients received allograftswith a 6-month mortality of 45%. With 7 years of follow-up the OS of the conventional chemotherapy, autologous, and allogeneic arms were all identical at 39%. However, the autologous and conventional chemotherapy arms do not demonstrate a plateau, while the allogenic curve was flat at 39% and this suggests that a proportion of these patients are long-term survivors. So, there is on-going interest in myeloablative allogeneic SCT, particularly given the lack of a significant cure rate for single or tandem autologous SCT.

Today, also in era of novel agent, allogeneic SCT should be considered an option, preferably in a clinical trial in patients whose disease responds to primary therapy, with primary PD, or with PD after an initial autologous SCT.

Another possibility is first autologous SCT followed by a mini-allogeneic transplant: Bruno et al[136] showed in a prospective trial that, among patients <65 years with HLA-matched siblings who received an autograft-allograft regimen, the CR rate after allografting was 55% compared with 26% after double autograft in patients without HLA-matched siblings. Median OS was

higher, 80 vs 54 months. In the prospective PETHEMA trial, in patients who did not achieve at least a nCR with a first autologous SCT, no significant difference was seen in OS after double autologous SCT versus autologous SCT followed by mini-allogeneic transplant. However, a trend toward a longer PFS was observed in the group treated with autologous SCT followed by mini-allogeneic transplant[137]. IFM99-03 trial by Garban et al[138] and the BMT CTN 0102 trial[139] reportedin patients with high risk, in contrast, no OS or PFS advantage with autologous transplant followed by allogeneic transplant.

In a prospective trial of newly diagnosed MM patients, based on the availability of an HLA-identical sibling, were selected for treatment with autologous SCT followed by reduced-intensity conditioning allogeneic SCT or autologous SCT[140]. Induction chemotherapy consisted of the chemotherapy that was standard at that time, VAD or VAD-like regimen. After 60 months, in the group treated with autologous SCT followed by reduced-intensity conditioning allogeneic SCT the incidence of relapse/progression was 49% versus 78% in the autologous SCT group. At 60 months, the OS and CR rates were 65% and 51%, respectively, for patients treated with autologous SCT followed by reduced-intensity conditioning allogeneic SCT compared with 58% and 41%, respectively, for those treated with autologous SCT. Based on these results, patients who have an HLA-identical sibling as part of their frontline treatment may be considered candidates for reduced-intensity allogeneic SCT.

Mini-allogeneic transplants have also been investigated as therapy for relapsed/refractory MM thanks to its graft-versus-myeloma effect. Younger age and response to prior SCT are associated with better response and OS rates[102-143]. In a case series report, patients with previously treated relapsed disease or PD (n = 54) were treated with an autologous SCT, followed by a mini-allogeneic transplant[141].

At a median of 552 days after the mini-allogeneic transplant, OS rate was 78%, with a 57% CR rate and an ORR of 83%. This trial concluded that this approach reduced the toxicities of a myeloablative allogeneic SCT, while preserving antitumor activity.

The largest case series was reported by the EBMT[142], heterogeneous population of 229 patients, where the 3-year OS and PFS were 41% and 21%, respectively. Chemoresistant disease was associated to adverse OS, while more than 1 prior transplant, was associated to improved OS with graft-versus-host disease (GVHD), thus confirming the importance of a GvT effect[144]. This trial concluded that mini-allogeneic transplantation is an option in relapsed/refractory patients, but that heavily pretreated and patients with PD are unlikely to benefit.

Donor lymphocyte infusions can be received to stimulate a beneficial GvT effect for patients whose disease either did not respond to relapses after allogeneic stem cell or other myeloma therapies[145-152].

3.2.4 Follow-Up After SCT

Follow-up is the same of other myeloma treatments, in addition, MRD assessment is increasingly being incorporated into post-treatment assessments, and it has been identified as an important prognostic factor. A prospective trial of newly diagnosed MM patients evaluated MRD in bone marrow samples and showed that MRD negativity after autologous SCT translated to significantly improved PFS and OS rates, at a median follow-up of 57 months[153] and, also in another study, MRD negativity post-autologous SCT was predictive of favorable PFS and OS[154]. In the allogeneic SCT setting, similar results have also been reported: the presence of MRD post allogeneic SCT has been associated with a significantly adverse PFS and OS[144].

4. Maintenance Therapy

4.1 Lenalidomide as Maintenance Therapy After Autologous SCT

Two independent randomized phase III trials evaluated the role oflenalidomide as maintenance therapy after autologous transplantation[94,95].

In the first, CALGB 100104, patients were randomized to maintenance treatment with lenalidomide (n=231) versus placebo (n=229) after autologous SCT[95]. 37% of the patients receiving lenalidomide versus 58% receiving placebo had disease progression or died, at a median follow-up of 34 months. The median time to progression was 46 months in the lenalidomide group versus 27 months in the placebo group (P<.001). Second primary cancers were revealed in 18 patients who received lenalidomide (8%) and in 6 patients who received placebo (3%)[95].

IFM2005-02, international, randomized, double-blind phase III trial[94] (n=614) showed that patients treated with consolidation therapy with lenalidomide after an autologous SCT followed by lenalidomide as maintenance therapy had upgraded responses. Patients enrolled in the trial were 614:307 were randomly assigned to lenalidomide maintenance therapy and 307 to placebo arm. Maintenance therapy was continued until unacceptable toxic effects occurred, the disease progressed, orthe patient withdrew consent. After a median follow-up of 30 months, the final analysis of the IFM2005-02 trial was performed and 264 patients had disease progression, 104 in the lenalidomide arm and 160 in the placebo arm, with a median PFS of 41 months in the lenalidomide arm compared with 23 months in the placebo arm (HR, 0.50; P<.001; median follow-up period was 30 months). The probability of surviving without progression for 3 years after randomization was 59% in lenalidomide arm and 35% in placebo arm, confirming the benefit of lenalidomide maintenance therapy. In particular, this benefit was observed not only in patients who had a VGPR at randomization (64% vs 49%; P=.006) but also in those who did not (51% vs 18%; P<.001). However, an increased incidence of second primary cancers was observed in the lenalidomide arm, with 32 second primary cancers in the lenalidomide arm versus 12 in the placebo group[94].

In a phase II trial by the IFM group, maintenance with lenalidomide was shown to upgrade responses seen after induction therapy with lenalidomide/bortezomib/dexamethasone followed by autologous transplant in 27% of patients (8 of 31 patients)[78].

Palumbo et al[112] showed that, although maintenance therapy with lenalidomide is associated with more frequent grade 3/4 neutropenia and infections, it significantly reduced risk of disease progression or death (HR, 0.47), compared with no maintenance[112].

HOVON 76 trial indicated that lenalidomide maintenance may not be a feasible option after mini-allogeneic SCT[155]. However, another recently reported trial has shown in patients with high-risk MM the feasibility of maintenance therapy with low-dose lenalidomide after allogeneic SCT[156].

4.2 Lenalidomide as Maintenance Therapy After Non-Transplant Active Primary Treatment

MM-015 the phase III trial showed that lenalidomide maintenance after primary therapy with MPR significantly reduced the risk of disease progression, increasing PFS[157]. In this trial, newly diagnosed MM patients (n=459), aged ≥65 years, were randomized to receive MP followed by placebo, MPR, or MPR followed by lenalidomide until progression. Lenalidomide maintenance significantly prolonged PFS and the PFS of patients treated with MPR followed by maintenance lenalidomide, compared with the other 2 arms, was significantly prolonged (n=152; median, 31 months): MPR (n =153; median, 14 months; HR, 0.49; P<.001) or MP (n=154; median, 13 months; HR, 0.40; P<.001). Lenalidomide maintenance, compared with placebo, improved PFS by 66% regardless of age[157]. In the FIRST trial, use of lenalidomide indefinitely until progression was associated with a superior PFS, compared with a fixed duration of 18 months.

Considering the results of the phase III trials[94,95,157], lenalidomide is one of the preferred maintenance regimens, having a better efficacy and lacking the neurologic toxicity seen with thalidomide. However, the only problem seems to be an increased risk for secondary cancers, especially post-transplantation[94-96] or after treatment with a melphalan-containing regimen[97]. However, in FIRST trial, in continuous treatment lenalidomide/dexamethasone arm, the absence of the alkylating agent melphalan seems to be more effective not only in terms of improving PFS but also of lowering incidence of second malignancies[93].

An important meta-analysis of four randomized controlled trials, comparing patients treated with lenalidomide maintenance versus those treated without maintenance or with placebo in both the transplant and non-transplant settings[158], showed that patients treated with lenalidomide maintenance had significantly improved PFS (HR, 0.49; P<.001) and a trend toward OS (HR,

0.77; P=.071) versus no maintenance or placebo[158]. Moreover, with the use of lenalidomide there was more grade 3/4 neutropenia and a two-fold increased risk of secondary malignancies.

4.3 Bortezomib as Maintenance Therapy After Autologous SCT

HOVON study showed that maintenance with single-agent bortezomib after autologous SCT is is associated with improvement of ORRand well tolerated[76]. In this trial, patients were randomly assigned to one of the two arms, consisting of either primary treatment with VAD followed by autologous SCT and maintenance with thalidomide, or with PAD followed by autologous SCT and bortezomib as maintenance therapy, for 2 years, with high nCR/CR rates after frontline treatment with the bortezomib-based regimen. So, bortezomib as maintenance treatmentwas associated with additional improvement of response rates and was well tolerated[76]. A multicenter phase III trial in newly diagnosed MM patients showed that improved PFS due to consolidation with bortezomib after autologous SCT only in patients not achieving at least a VGPR after autologous SCT[159]: no difference in PFS was seen in patients with a VGPR or better after autologous SCT.

4.4 Bortezomib as Maintenance Therapy After Non-Transplant Active Primary Treatment

The preliminary results of the UPFRONT randomized phase III trial also show that maintenance with single-agent bortezomib is well-tolerated when administered after treatment with bortezomib-based primary therapy[160]. Newly diagnosed MM patients, ineligible for high-dose therapy and SCT, enrolled in the UPFRONT trial, were randomized (1:1:1) and treated with one of the following bortezomib-based primary regimens, followed by maintenance treatment with bortezomib: bortezomib and dexamethasone; bortezomib in combination with thalidomide and dexamethasone; or bortezomib with melphalan and prednisone. The response rates, including CR and VGPR or better, improved after bortezomib maintenance in all arms, without concomitant increase in the incidence of PN[160].
So, considering these results, bortezomib can play a role in mantenance treatment of MM.

5. Therapy for relapsed/refractory and progressive Myeloma

Nowadays, several therapies are nowadays as options for previously treated Multiple Myeloma, in different clinical situations, such as patients in relapse after allogenic or autologous SCT or with primary PD after initial autologous or allogenic SCT and patients who are ineligible for SCT with progressive or relapsing disease after primary therapy.
In particular, if the relapse occurse in less than six months after the end of the therapy, patients could be retreated with the same regimen.
The first possibility is the addition of dexamethasone to bortezomib in rrMM, who had progressive disease during bortezomib monotherapy: this can give an improvement of response in 18-34% of patients[161-163].

5.1 Lenalidomide/Dexamethasone

Lenalidomide has been approved by FDA as treatment for MM who had received at least one prior treatment, thanks to two important studies of a total of 692 patients randomized in two arms: dexamethasone with or without lenalidomide. The primary endpoint was the time to progression (TTP), in both the studies. The median TTP was longer in lenalidomide arm versus dexamethasone group, as confirmed in a pre-planned interim analysis[164,165]. The updated data from MM-009, the North American phase III trial, in 353 previously treated MM patients showed increased OS and median TTP in lenalidomide plus dexamethasone versus dexamethasone plus placebo[165], as confirmed also by another international trial, MM-010[164]: in both the trials the patients had been heavily pretreated before enrollment, and many had more than three prior lines of therapies, and more than half of patients had also undergone SCT[164,165].
Considering safety, most adverse events and grade 3/4 adverse events were more frequent in lenalidomide plus dexamethasone arm, with great prevalence of thrombocytopenia (61.5%) and neutropenia (58.8%).
However, lenalidomide/dexamethasone is an important opportunity in relapsed/refractory MM patients[165], while lenalidomide monotherapy is a possibility for steroid-intolerant patients.

5.2 Carfilzomib/Lenalidomide/Dexamethasone

A randomized, multicenter trial of phase III, ASPIRE, enrolling 792 rrMM patients, compared the combination of lenalidomide and dexamethasone with or without carfilzomib in MM patients who had received one to three prior lines of treatment. The primary endpoint was PFS, significantly improved in carfilzomib arm, by 8.7 months (26.3 months in carfilzomib arm versus 17.6 months in len-dexa arm; HR for progression or death, 0.69; 95% CI, 0.57-0.83; P=.0001). Considering safety, the incidence of PN was nearly identical in both arms (17.1% carfilzomib arm versus 17% len-dexa arm), and non-hematologic adverse-events of at least grade 3 which were higher in carfilzomib arm included dyspnea, 2.8% vs 1.8%, cardiac failure, 3.8% vs 1.8%, and hypertension, 4.3% vs 1.8%.

In particular, in carfilzomib arm there were fewer discontinuations due to side effects, 15.3% vs 17.7%, and, in this arm, it was reported superior health-related quality of life[166].

So, nowadays, carfilzomib/lenalidomide/dexamethasone (KRD) is considered one of the best options in rrMM, and it has been recently approved in Italy for this setting of patients.

5.3 Carfilzomib/Dexamethasone

An important multicenter, randomized, phase III trial, ENDEVOR, compared carfilzomib/dexamethasone (KD) to bortezomib/dexamethasone (VD) in rrMM, treated with multiple prior lines of therapy. It was shown a two-fold improvement in median PFS in KD arm (18.7 months vs 9.4 months; HR=0.53; P < .0001)[167]. ORR was 77% in KD arm versus 63% in VD group (with rates of CR or better which were 13% versus 6% and VGPR 42% versus 22%, respectively).

Median duration of response was 21.3 months in KD arm versus 10.4 months in VD arm. Adverse events of grade 3 or higher were hypertension (6% versus 3%), dyspnea (5% versus 2%). PN of grade 2 or higher was 6% in KD arm versus 32% in VD arm[167].

Considering these data, KD can be considering a very good option for rrMM. However, it is not yet available in Italy.

5.4 Pomalidomide/Dexamethasone

Pomalidomide is a new generation IMID, analogue of thalidomide like lenalidomide, and it has been demonstrated its potent anti-myeloma and immunomodulatory activities (Figure 6, Figure 7)[168].

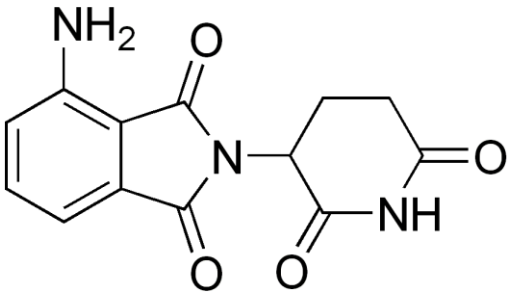

Figure 6: Pomalidomide structure

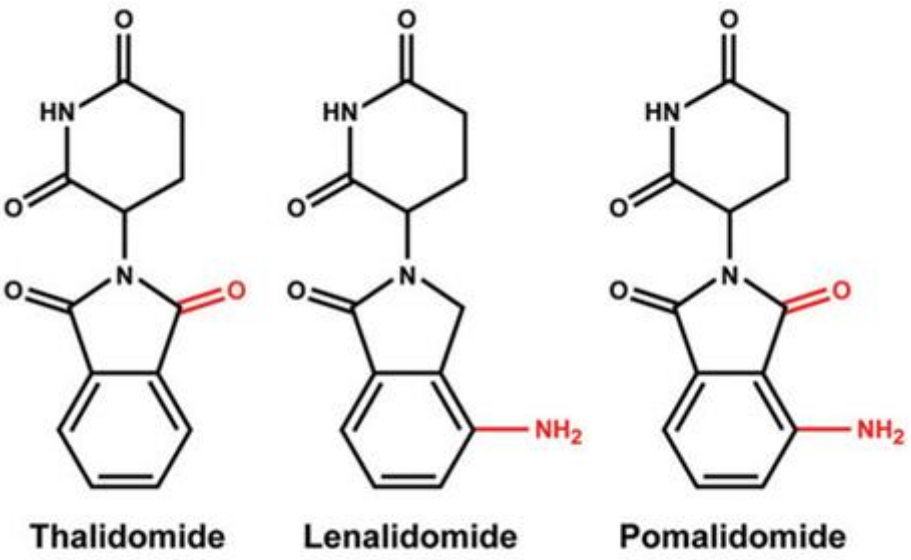

Figure 7: Chemical stuctures of IMIDs: Thalidomide, Lenalidomide and Pomalidomide

A ranzomized multicenter phase I trial compared pomalidomide (standard schedule: 4mg orally on days 1-21 of each 28-day cycle), with or without dexamethasone (40mg/week), showing efficacy and manageable toxicity in rrMM patients, including also those refractoty to both lenalidomide and bortezomib[169]. Then, a randomized open-label phase II trial compared

pomalidomide and low-dose dexamethasone versus single-agent pomalidomide in rrMM patients who had previously received a trial of lenalidomide and bortezomib[170]. This trial evaluated 221 patients, who, after a median follow-up of 14.2 months, showed a median PFS of 4.2 months in pomalidomide plus low-dose dexamethasone arm, compared with 2.7 months in patients treated with pomalidomide alone (HR, 0.68; P=0.003)[171] and median OS was 16.5 months in pom-dexa arm compared to 13.6 months with pomalidomide alone[171]. Considering safety, grade 3-4 neutropenia occurred in 41% in pomalidomide plus low-dose dexamethasone versus 48% in pomalidomide monotherapy arm. No grade 3 to 4 PN was reported.

Then, MM-003, a phase III, multicenter, randomized, open-label trial, was conducted in Europe, comparing the efficacy and safety of pomalidomide and low-dose dexamethasone (n=302) versus high-dose dexamethasone (n=153) in rrMM who were refractory to both bortezomib and lenalidomide[172]. Primary endpoint of the study was PFS, which, after a median follow-up of 10 months, was significantly longer in pomalidomide and low-dose dexamethasone arm compared with high-dose dexamethasone (4.0 vs 19 months; HR, 0.45; P<0.0001)[173], and also median OS was significantly longer in pomalidomide and low-dose dexamethasone (12.7 months vs 8.1 months; HR=0.74; P=0.0285)[173]. Considering safety, the most common grade 3 and 4 hematologic adverse events found to be higher in pom-dexa arm were neutropenia and pneumonia[173]. Other phase III trials of pomalidomide plus low-dose dexamethasone in combination with other agents (eg. bortezomib NCT01734928) are currently ongoing. Moreover, a European multicenter, single arm, open-label phase IIIb trial, which evaluated the safety and efficacy of pomalidamide and low-dose dexamethasone in a large international patient population (N=604)[174], showed a median PFS of 4.2 months and a OS of 11.9 month. PFS, OR, and ORR were similar whether the patients had previously received lenalidomide or bortezomib[174]: these results are consistent with those observed in the pivotal MM-003 trial.

Then, several complementary phase II trials have been published evaluating pomalidomide and dexamethasone in rrMM patients, refractory to lenalidomide and/or bortezomib. A phase II trial compared two different dose schedules of pom-dexa in 84 rrMM patients. Pomalidomide was given orally at standard dose on days 1 to 21 or continuously over a 28-day cycle, and dexamethasone 40mg was given orally once weekly[175]. ORR was 35% and 34% for patients in the 21-day and 28-day arms, respectively. Median follow-up was 23 months, and median duration of response, PFS, and OS were 7.3, 4.6, and 14.9 months across both groups, respectively. Adverse events were similar in both groups, primarly due to myelosuppression[175]. Another multicenter phase II trial compared two dose-schedules of pomalidomide (2 versus 4

mg/day) with dexamethasone 40 mg weekly in heavily pre-treated rrMM patients (n=35)[176]. ORR was 49% in the 2-mg arm versus 43% in the 4-mg, respectively, and myelosuppression was the most common adverse event[176].

So, pomalidomide was approved by FDA for MM patients who have previously received at least two therapies, including lenalidomide and bortezomib, and have shown disease progression on or within 60 days of completion of the last therapy. The recommended schedule and dose of pomalidomide is 4 mg orally on days 1 to 21 of 28-day cycles, repeated until disease progression of unacceptable adverse events, monitoring patients for hematologic toxicities, especially neutropenia.

So, pomalidomide plus dexamethasone is an option in patients who have received at least two prior therapies, including an immunomodulatory agent and bortezomib, and have demonstrated disease progression on or within 60 days of completion of the last therapy and, for steroid-intolerant individuals, pomalidomide monotherapy can be considered.

5.5 Elotuzumab/Lenalidomide/Dexamethasone

Elotuzumab is a humanized monoclonal antibody specific for signaling lymphocytic activation molecule-F7 (SLAMF7). SLAMF7, also called CS1 (cell-surface glycoprotein CD2 subset 1) or CD319 or CRACC, is a glycoprotein expressed on myeloma and natural killer (NK) cells but not on normal tissues (Figure 8)[177].

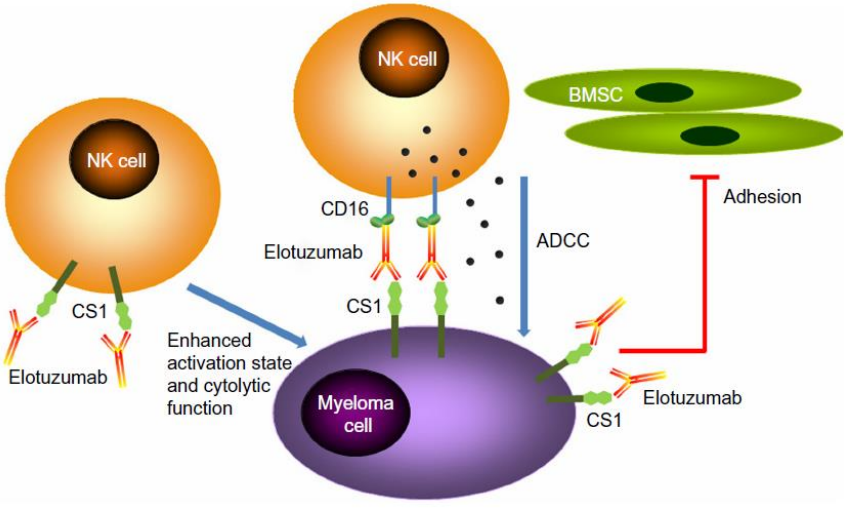

Figure 8: Elotuzumab - Mechanism of action

Elotuzumab in combination with lenalidomide and dexamethasone was approved by the FDA for the treatment of rrMM patients, who have received one to three prior therapies.

This approvation was due to the encouraging results of the multicenter phase III trial, ELOQUENT-2, which randomized 646 patients (1:1) to receive either elotuzumab in combination with lenalidomide and dexamethasone (ERD) or lenalidomide and dexamethasone alone[178].

Considering PFS rates at the end of 1 and 2 years, they were higher for those receiving ERD (68% at 1 year and 41% at 2 year), and PFS was 19.4 months in ERD arm versus 14.9 months in those receiving lenalidomide and dexamethasone alone (HR for progression or death in the elotuzuamab group. 0.70; 95% CI, 0.57-0.85; $p<0.001$), indicating a relative reduction of 30% in the risk of disease progression or death[178]. In both arms of the trial, common grade 3 or 4 adverse events were lymphocytopenia, neutropenia, fatigue, and pneumonia.

Infusion reactions were not common: they occurred in 33 patients (10%) in the ERD arm, and were grade 1 or 2 in 19 patients[178].

The subset analyses of extended 3-year follow-up confirmed these results, with median duration of response reported with ERD which was 20.3 months versus 16.6 months of lenalidomide-dexamethasone arm, showing that PFS benefit with the Elo-based triplet was durable over time[179].

FDA approved elotuzumab in combination with lenalidomide and dexamethasone in previously treated MM, and, thanks to a novel mechanism of action added to immunomodulation, this is one of the best options which is available in rrMM.

5.6 Ixazomib/Lenalidomide/Dexamethasone

As previously discussed, ixazomib is an oral, new-generation, proteosome inhibitor. A double-blind, placebo-controlled, randomized, phase III trial, TOURMALINE MM1, randomized 722 patients with rrMM, comparing a combination of ixazomib, lenalidomide and dexamethasone (IRD) versus lenalidomide and dexamethasone alone (control group). TOURMALINE MM1 was designed thanks to the promising results of a phase I/II trial, previously discussed[180].

This trial has shown a significant improvement in PFS with IRD: after a median follow-up of almost 15 months, a 35% improvement in PFS was seen in IRD, compared with the control

group (HR, 0.742; P=0.012). Median PFS was 20.6 months in IRD versus 14.7 months in Len-Dexa. Moreover, in IRD, the ORR (78.3% vs 71.5%., P=0.035) and CR (11.7% vs 6.6%, P=0.019) were also significantly improved. In particular, high-risk cytogenetics patients enrolled in the trial had a similar HR for PFS as the entire study population (HR, 0.596 and 0.543, respectively). Considering safety, grade ≥3 adverse events were 68% in IRD versus 61% in control group, and these included neutropenia (19% with ixazomib/lenalidomide/dexamethasone vs 16% with lenalidomide/dexamethasone), anemia (9% vs 13%), thrombocytopenia (13% vs 5%), and pneumonia (6% vs 8%). Serious adverse events were reported in 40% in IRD and 44% in Len-Dexa[180].

Based on these results, ixazomib/lenalidomide/dexamethasone was approved by FDA for rrMM, and can be considered a very good option in this setting of patients, in particular for unfit ones, thanks to the oral formulation. It is available in Italy thanks to compassionate use program.

5.7 Daratumumab single agent

Daratumumab is a innovative human IgG kappa monoclonal antibody that targets the CD38 surface protein on myeloma cells (Figure 9)[181], recently approved by FDA, based on the surprising results of a phase I/II trial, for the treatment of rrMM patients, who have received at least three prior lines of therapy including a PI and a immunomodulatory agent, or who are double refractory to PI and IMIDs.

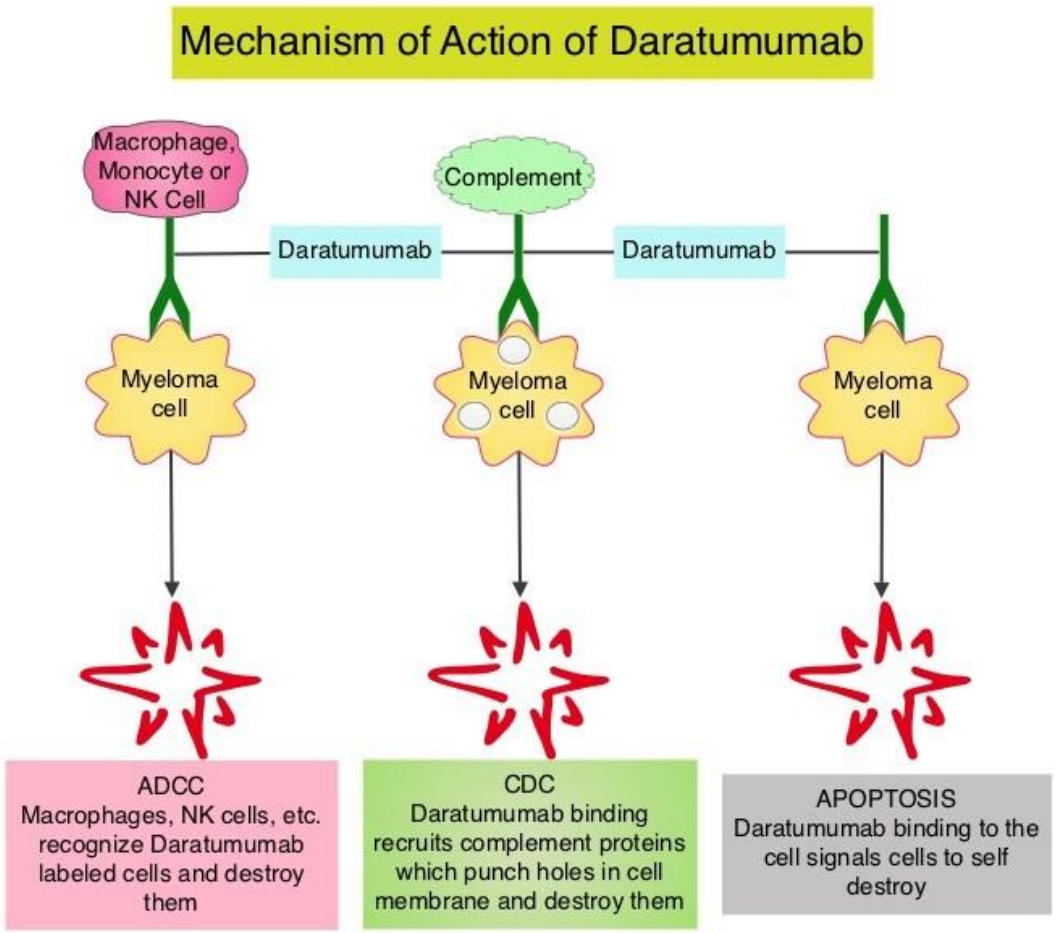

Figure 9: Daratumumab – Mechanism of action

In the phase I/II trial, patients who had previously received more than 3 lines of therapy including an IMID and a PI or were double refractory to a PI and IMID were randomized to two different doses of daratumumab (8mg/kg versus 16 mg/kg). ORR was 29.2% (with 3sCR, 10 VGPR, and 18PR). Median duration of response was 7.4 months and median time to progression was 3.7 months. The estimated 1-year OS rate was 65%[182]. Reported adverse events were fatigue (39.6%), anemia (33.0%), nausea (29.2%), and thrombocytopenia (25.5%). Grade 1/2 infusion-related reactions were quite common, seen in 42.5% of patients, mainly during first infusion, but, however, no patients discontinued the study due to infusion-related reactions[182].

So, daratumumab single agent can be considered as an option for rrMM patients who have received at least three prior lines of therapy including a PI and an IMID or who are double refractory to a proteasome inhibitor and immunomodulatory agent.

5.8 Daratumumab/Bortezomib/Dexamethasone (CASTOR)

CASTOR is a randomized, multicenter, phase III trial evaluating the combination of daratumumab, bortezomib and dexamethasone (DVD) in rrMM[183]. This regimen markedly improved outcomes for rrMM patients. Patients (n=498) were randomized to receive DVD versus VD. The ORR was 82.9% in DVD arm, compared to 63.2% in the control arm (P<0.001). Moreover, the rates of VGPR and CR were double in DVD arm compared to the control arm (59.2% vs 29.1%, P<0.001 and 19.2% vs 9.0%, P=0.001. respectively), and the 12-month estimated rate of PFS was significantly higher in the DVD arm compared to the control (60.7% vs 26.9%). The most common grade 3/4 adverse events reported in daratumumab and control groups were thrombocytopenia (45.3% and 32.9%, respectively), anemia (14.4% and 16.0%, respectively), and neutropenia (12.8% and 4.2% respectively). Grade 1 or 2 infusion-related reactions associated with daratumumab were reported in 45.3% of the patients in the DVD and grade 3 in only 8.6% of the patients: these reaction rates are consistent with findings from previous trials of daratumumab[181,182].

Daratumumab/bortezomib/dexamethasone was approved by FDA for rrMM and can be considered one of the best options in this setting of patients.

5.9 Daratumumab/Lenalidomide/Dexamethasone (POLLUX)

POLLUX is a randomized, multicenter, phase III trial evaluating the combination of daratumumab, lenalidomide and dexamethasone (DRD) in rrMM[184]. This regimen markedly improved outcomes for rrMM patients. Patients (n=569) were randomized to receive DRD versus RD.

At a median follow-up of 13.5 months, 12 months-PFS was 83.2% in DRD arm versus 60.1% in the control group, and it was shown also a significantly higher ORR, 92.9% in DRD versus 76.4% in RD, with a rate of CR or better of 43.1% versus 19.2% (p<0.001).

The most common grade 3/4 adverse events reported in daratumumab and control groups were neutropenia (51.9% and 37% respectively), thrombocytopenia (12.7% and 13.5%, respectively), anemia (12.4% and 19.6%, respectively). Grade 1 or 2 infusion-related reactions associated with daratumumab were reported in 47.7% of the patients in the DRD: these reaction rates are consistent with findings from previous trials of daratumumab[181,182].

Daratumumab/lenalidomide/dexamethasone was approved by FDA for rrMM and can be considered, considering these data, the best option in this setting of patients.

5.10 Bortezomib/Liposomal Doxorubicin

Bortezomib combined with liposomal doxorubicin was approved by FDA as option for rrMM who have not previously received bortezomib and have received at least 1 prior therapy. The FDA approval was based on a priority review of data from an international phase III study (n=646), showing that use of bortezomib plus liposomal doxorubicin significantly extended the median time to disease progression compared with bortezomib alone (9.3 vs 6.5 months)[185]. In particular, median duration of response was increased from 7.0 months to 10.2 months. So, bortezomib plus liposomal doxorubicin can be considered another effective option in rrMM.

5.11 Bendamustine single agent

Bendamustine is an alkylating agent, with a demonstrated activity in many hematological malignancies (Figure 10).

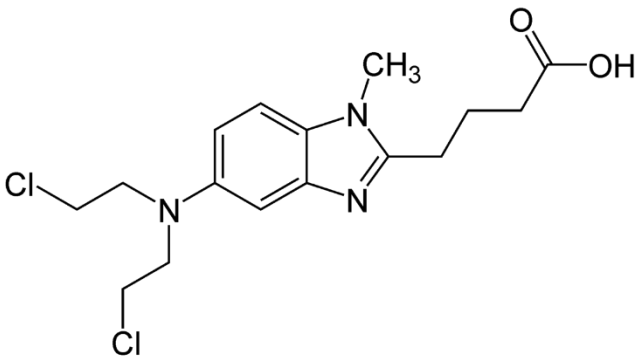

Figure 10: Bendamustine structure

Knop et al evaluated 31 patients, who had experienced relapse after autologous transplation, and received increasing doses of bendamustine[186]. ORR was 55%, with a median PFS of 26 weeks for all patients, and 36 weeks for patients treated with higher doses (90-100 mg/m^2). Considering safety, toxicity was mild and mainly hematologic. Then, a retrospective analysis of 39 rrMM patients has reported that bendamustine is effective and tolerable in this setting of advanced MM,

with an ORR of 36%[187]. Bendamustine single agent can be considered one of the options in rrMM.

5.12 Bendamustine/Lenalidomide/Dexamethasone

The combination of bendamustine, lenalidomide, and dexamethasone (BRD) was evaluated in a multicenter phase I/II trial, as treatment for rrMM patients (n=29)[188]. Considering results, PR rate was 52% (n=13) of patients, VGPR 24% (n=6). The median PFS was 6.1 months (95% CI, 3.7-9.4 months), and the one-year PFS rate was 20% (95% CI, 6%-41%)[189].
BRD can be considered one of the approved options for rrMM, not refractory to lenalidomide.

5.13 Bendamustine/Bortezomib/Dexamethasone

Bendamustine/bortezomib/dexamethasone (BVD) was evaluated in a phase II trial. BVD was administered over six 28-day cycles and then every 56 days for six more cycles in patients (n=75; median age 68 years) with rrMM treated with multiple prior therapies and not refractory to bortezomib. Considering results, ORR was 71.5% (16% CR, 18.5% VGPR, 37% PR). At 12-month follow-up, median time to progression was 16.5 months and 1-year OS was 78%[189].
Based on these results, BVD can be considered one of the approved options for rrMM, not refractory to bortezomib.

5.14 Bortezomib/Lenalidomide/Dexamethasone

It has been demonstrated in preclinical studies that lenalidomide sensitizes myeloma cells to bortezomib and dexamethasone. In particular, phase I and phase II trials have demonstrated that bortezomib/lenalidomide/dexamethasone (VRD) is a well-tolerated and active regimen in rrMM, with durable responses in heavily pretreated patients, also those previously treated with bortezomib, lenalidomide, thalidomide and SCT[189,190]. Updated data after over two years of follow-up show a median PFS of 9.5 months with a median OS of 26 months, together with a 12- and 24-months OS rates of 86% and 55%, respectively[193].
Bortezomib/Lenalidomide/Dexamethasone, is a very good option for rrMM. However, it is not yet approved in Italy.

5.15 Bortezomib/Cyclophosphamide/Dexamethasone

Another option for rrMM is the addition of an alkylating agent, such as cyclophosphamide, and a novel agent, such as bortezomib or lenalidomide, to dexamethasone. Bortezomib/cyclophosphamide/dexamethasone (VCD) is an effective regimen, with an acceptable toxicity profile, in rrMM[191].

5.16 Pomalidomide/Bortezomib/Dexamethasone

Pomalidomide/bortezomib/dexamethasone has been evaluated in rrMM patients in early phase I/II studies[192,193]. Considering the particularly encouraging ORRs observed in these trials, this triplet is being currently evaluated in an ongoing phase III trial[194].

5.17 Pomalidomide/Carfilzomib/Dexamethasone

Encouraging results of a phase I trial[195], were the basis for a phase II trial aiming to evaluate the safety and efficacy of pomalidomide/carfilzomib/dexamethasone in rrMM patients refractory to lenalidomide and proteosome inhibitor-naive or sensitive. Median follow-up was of 7.2 cycles (range= 0.6-27.1 cycles), after which PR reported was 84%, MR 91%, VGPR 26%, and CR/nCR was 12%[196]. Moreover, after a median follow-upof 18 months (r. 1-39 months), median PFS for all 55 patients was 12.9 months and the estimated 18-month OS was 86.5%[196].
So, considering the promising results, pomalidomide/carfilzomib/dexamethasone could be a potential option in rrMM patients who have previously received at least two therapies, including an IMID and bortezomib, and who have demonstrated disease progression on or within 60 days of completion of the last therapy.

5.18 Lenalidomide/Cyclophosphamide/Dexamethasone

The efficacy of lenalidomide in combination with cyclophosphamide and dexamethasone was assessed thanks to a retrospective analysis which showed that this regimen is effective in heavily pre-treated patients with manageable toxicity profile[197].

5.19 Ixazomib/Dexamethasone

Ixazomib as single-agent, in combination with dexamethasone, has been evaluated in two phase I trials for rrMM, which estabilished the maximun tolerated dose of ixazomib to be 2.0 mg/m^2 on a twice-weekly schedule and 2.97 mg/m^2 on a weekly schedule[198,199]. The enrolled patients had multiple prior lines of therapy, with a median of 4 prior lines of therapy in both studies. In the trial with the weekly schedule[198], the rate of PR or better (≥PR) was 27%. In the twice-weekly schedule trial, ≥PR rate was 15%[199]. Considering safety, adverse events, grade ≥3, were reported in 78% on the twice weekly schedule[198], and they were considered drug-related in 62%, and 65% on the weekly schedule[199]. These were particularly related to hematological toxicity, and included thrombocytopenia (37%), neutropenia (17%), and skin and subcutaneous tissue disorders (8%) on the twice-weekly schedule, and thrombocytopenia (33%), neutropenia (18%), and diarrhea (17%) on the weekly schedule. PN was reported in 17%, drug-related in 12%, with no grade 3 events, on the twice-weekly schedule[199]. On the weekly schedule drug-related peripheral neuropathy was reported in 20% of patients, with 2% grade 3[198].

Then, phase II trials were designed to evaluate the role of ixazomib with or without dexamethasone in rrMM patients, who have limited prior exposure to bortezomib[200,201]. In one trial, 33 patients with rrMM received weekly ixazomib 5.5 mg and had dexamethasone added for suboptimal response or disease progression (67% of patients). After the addition of dexamethasone, 6 additional patients achieved a PR[200]. Patients were responders if they achieved at least PR, so, reported ORR (≥PR), with or without the addition of dexamethasone, was 34%[200]. The most common adverse events observed included thrombocytopenia, fatigue, nausea, and diarrhea, and grade ≥3 were reported in 78%[200].

Another phase II trial evaluated the efficacy of two doses of weekly ixazomib (arm A, 4 mg and arm B, 5.5 mg) plus weekly dexamethasone (40 mg), in rrMM patients, who had not been previously treated with a proteasome inhibitor, including bortezomib, or had received less than 6 cycles of therapy with bortezomib and had at least PR and no progression at the time of bortezomib-discontinuation[201]. The ORRs were 31% in arm A (95% CI: 17-49) and 51% (95% CI: 34-69) in arm B, and, in particular, the response rates were 38% for arm A and 52% for arm B among the patients with no prior bortezomib exposure. The most common toxicities were fatigue, thrombocytopenia, diarrhea, and nausea (with more grade 3 toxicities among arm B). PN, possibly related to ixazomib, was seen in arm A in 55%, only grade 1 or 2, and in arm B in 43% (2 patients with grade 3)[201].

So, combination ixazomib/dexamethasone is another treatment opinion for rrMM, who have received at least one prior therapy, available in Italy in compassionate-use program.

5.20 Elotuzumab/Bortezomib/Dexamethasone

Elotuzumab is a humanized monoclonal antibody specific for SLAMF7, previously described. Numerous randomized trials have shown that triplets combinations have been shown to be consistently more effective than 2-drug combinations for rrMM. An interesting phase II trial evaluated the effect of addition of elotuzumab to bortezomib and dexamethasone in rrMM patients[202].

At the moment, interim analysis results have demonstrated a 28% reduction in risk of disease progression or death for triple-drug arm compared to bortezomib/dexamethasone (HR, 0.72; 70% CI, 0.59-0.88). In the elotuzumab-conteining arm, median PFS was significantly higher, 9.7 months vs 6.9 months. After 2 year of follow-up, the addition of elotuzumab continued to show an efficacy benefit, compared to bortezomib/dexamethasone alone, with a 24% relative risk reduction in PFS (HR, 0.76; 70% CI, 0.63-0.91)[202].

Elotuzumab/bortezomib/dexamethasone can be considered as treatment option for patients with rrMM, who have received at least one prior therapy.

5.21 Panobinostat/Carfilzomib

Panobinostat is a pan-deacetylase inhibitor, a new mechanism of action in MM, that epigenetically modulates class I and II HDAC enzymes (Figure 11).

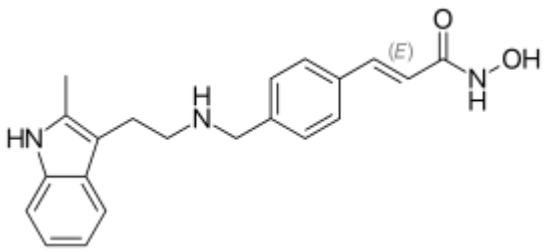

Figure 11: Chemical structure of Panobinostat

A multicenter, phase I/II trial evaluated the efficacy and safety of the combination of panobinostat and carfilzomib in rrMM, who had relapsed after at least one prior treatment[203,204].
The phase I aimed to determine the maximum tolerable dose of panobinostat plus carfilzomib, while the primary endpoint of the phase II was ORR.
In the phase I trial, no dose-limiting toxicities were observed at any of the planned dose levels. In phase II, of the 42 evaluable patients, ORR was 67% and the clinical benefit rate was 79%[203]. Moreover, ORR was 67% for patients who are refractory to prior PI tratment and 75% for patients refractory to prior immnune-modulating drug treatment. Median PFS was 7.7 months, at a median follow-up of 17 months[203,204]. Grade 3/4 treatment-related adverse events included 38% thrombocytopenia, 21% neutropenia, 11% fatigue, 9% anemia, 9% hypertension, and 7% diarrhea.
The MDT of carfilzomib plus panobinostat was not reached with the 4 dosing schedules in the first phase I study, and so two additional dosing schedules were evaluated. The maximum planned dose from the first trial was panobinostat 30 mg plus carfilzomib 20/45 mg/mg^2. The dose of carfilzomib was escalated to 20/56 mg/mg^2 in this study[205]. Considering safety, the most common adverse events grade ≥3 were thrombocytopenia (31%), fatigue (4%), and diarrhea (4%). Considering efficacy, ORR was 82%, with 34% ≥VGPR and 48% PR and the clinical benefit rate was 91%.
Panobinostat in combination with carfilzomib could be considered another interesting option in rrMM.

5.22 Panobinostat/Bortezomib/Dexamethasone

Panobinostat in combination with bortezomib and dexamethasone was recently approved by FDA for rrMM patients who have previously had at least two prior therapies with regimens containing an immunomodulatory agent and bortezomib.
This approval was based on the results of PANORAMA-1, a randomized placebo-controlled phase III study. This trial randomized 768 rrMM patients, who had received prior treatment with an immunomodulatory agent and bortezomib, to receive bortezomib and dexamethasone along with either panobinostat or placebo. Panobinostat-containing regimen showed an improved median PFS (11.99 months [95% CI; 10.33-12.94 months] vs 8.08 months [95% CI; 7.56-9.23

months]; HR, 0.63;95% CI, 0.52-0.76; P<0.0001) along an increased depth of repsonse[206-207], while the final OS data from this study are not yet available.

The regimen containing panobinostat is associated with significant toxicity. Serious adverse events were reported in 228 (60%) of 381 patients in the panobinostat group and 157 (42%) of 377 patients in the placebo group. Common grade 3-4 laboratory abnormalities and adverse events were more in the panobinostat group versus the control group including thrombocytopenia (67% vs 31%), lymphopenia (53% vs 40%), diarrhea (26% vs 8%), fatigue (4ç% vs 2%), and peripheral neuropathy (18% vs 5%).

A phase II, single-arm, multicenter trial, PANORAMA-2, evaluated the combination of panobinostat with bortezomib and dexamethasone in patients who had rrMM, refractory to bortezomib (N=55)[207]. ORR was 34.5% with the panobinostat-containing regimen, and median PFS was 5.4 months, while OS had not been reached at a median follow-up of 8.3 months[207]. Considering safety, common grade 3/4 adverse events included thrombocytopenia (63.6%), fatigue (20.2%), and diarrhea (20.0%).

Thanks to the results of these trials, panobinostat plus bortezomib and dexamethasone can be considered an option in rrMM patients who have received at least two prior therapies, including an IMID and bortezomib. However, it is not yet approved in Italy.

5.23 Pomalidomide/Cyclophosphamide/Dexamethasone

The combination of pomalidomide/cyclophosphamide/dexamethasone was compared to pomalidomide/dexamethasone in a phase II trial rrMM patients (n=70), who had received more than 2 prior therapies[208].

The triplet significantly improved the ORR, with ≥PR in 64.7% versus 39.9% in pom-dexa arm (P=0.0355), while median PFS was 9.5 months versus 4.4 months. Considering safety, there were no significant differences in AE reports between the two arms: grade 3 and 4 anemia, neutropenia, and thrombocytopenia, respectively, were reported in 11%, 31% and 6% of patients treated with pom-dexa and 24%, 52%, and 15% of patients treated with pomalidomide/cyclophosphamide/dexamethasone[209]. A single center retrospective study[209] of rrMM patients (n = 20) who received pomalidomide/cyclophosphamide/dexamethasone until SCT or disease progression, reported similar results, with ORR of 63%, and with 42% of patients

responding after 1 cycle with a median time to response of 3 cycles. Morevoer, 1-year median PFS was 80.7% and 65% of patients were relapse-free[210].
Pomalidomide/cyclophosphamide/dexamethasone is another treatment option rrMM patients who have received at least one prior therapy.

5.24 High-dose Cyclophosphamide

The ECOG group (Eastern Cooperative Oncology Group) evaluated high-dose cyclophosphamide treatment in poor-risk myeloma patients, who had disease refractory to prior chemotherapy[211]. ORR was 43%, with 29% response rate in patients who are refractory to prior therapy with cyclophosphamide. Also high-dose cyclophosphamide can be considered as option in rrMM.

5.25 Pegylated liposomial doxorubicin/cyclophosphamide/dexamethasone (CED regimen)

Since 2009, in our Institution, some patients affected by Multiple Myeloma, relapsed and refractory to most of the available therapeutic options (2-7), have been treated with courses of pegylated liposomial doxorubicin (35 mg/sqm, day 1), cyclophosphamide (800 mg/sqm day 1) and dexamethasone (20 mg days 1-4), with pegfilgrastimat day +4, every 28 days (CED regimen), until progression of disease.
So far, 31 patients (16 women, 12 men), with median age 63.4 years (range: 43-84) affected by relapsed and progressive multiple myeloma, whose median number of previous treatments was 6 (range 2-11) have been treated with CED schedule (mediannumber of courses: 4.3, range: 2-17). Available results refer to 31 patients completing at least two courses of CED, while 4 patients who received only 1 cycle were excluded from analysis. Tolerability profile of CED was satisfactory: hematological toxicity was present in all patients, but grade 3 transfusion-dependent anemia or neutropenia was verified in 37% and 46% of cases, without necessity of hospitalization. No severe extrahematologic toxicity was observed: grade 1 gastrointestinal side effect (nausea) in the majority of patients, and two grade 3 extra-hematological events: acute renal failure in a patient and bradycardia in another patient, both of them not requiring hospitalization. According to IMWG response criteria, after a median follow-up of 6 months of

treatment (r.2-17+), ORR was 51% (2 CR, 2 VGPR, 8 PR, 4 MR) with 10 disease progressions and 5 patients in stable disease. Median OS from start of CED was 5.9 months (range 2-17). These effects appear impressive, in patients so far lacking available therapeutic options. Together to Romano's results[212,213], our observations underline the efficacy of pegylated liposomial doxorubicin, that seems to give a contribute in a particularly severe setting of patients, without significant side effects.

6. Supportive care in MM

Recently, important advances have been made in supportive care of patients with MM. This involves careful patient education about the possibility of side effects of each drug, the informations about drug combinations being used, and the supportive care measures required. In fact, supportive care in MM, can be categorized into those measures that address specific drugs and those required for all patients.

6.1 Bone disease and bisphosphonates

Bone disease of myeloma develops in 85% of patients, in the form of osteolytic lesions and/or diffuse osteopenia, and the related complications can be considered as the major cause of limitations in performance status and in quality of life in patients with MM. A double-blind, large, randomized trial has shown, in patients with Durie-Salmon stage III MM and at least one lytic lesion, that monthly use of intravenous bisphosphonate pamidronate can decrease pain and bone-related complications, improving performance status and preserving quality of life[214,215]. Equivalent benefits were demonstrated with zoledronic acid[216]. Zervas et al[217] showed with zoledronic acid compared with pamidronate a 9.5-fold greater risk for the development of osteonecrosis of the jaw and so, they should have a dental examination before the start of bisphosphonate therapy and be monitored for osteo-necrosis of the jaw. Moreover, it's mandatory to monitor accurately renal function patients who are on bisphosphonates treatment.

A multicenter, international trial, MRC Myeloma IX, compared effects of zoledronic acid versus clodronate, a bisphosphonate not currently FDA-approved, in MM patients initiating chemotherapy regardless of bone disease. The enrolled patients were randomized to receive zoledronic acid (n=981) or clodronic acid (n=979). Zoledronic acid was previously reported to reduce mortality and significantly improve PFS[218]. Considering safety, patients on both arms had similar occurrence of acute renal failure and treatment-related serious adverse events, even if zoledronic acid was associated with significantly higher rates of confirmed osteonecrosis of the jaw[218-220].

Recently, the study reanalyzed and reported survival outcomes, and after an extended follow-up of a median of 5.9 years, in addition to PFS, also the OS was significantly improved (52 vs 46 months; HR, 0.86; P=.01) in zolendronic acid arm, compared with clodronic acid[221]. However,

also the long-term rates of osteonecrosis of the jaw were higher with zoledronic acid (3.7% vs 0.5%; P=.0001)[221].

A recent interesting meta-analysis of 20 randomized controlled trials of comparing bisphosphonates with either placebo or a different bisphosphonate as a comparator concluded that adding bisphosphonates to MM treatment reduces vertebral fractures and probably reduces pain. However, whether zoledronate is superior to pamidronate and other bisphosphonates remains to be determined[222]. Bisphosphonates should be recommended for all patients receiving myeloma therapy for symptomatic disease regardless of documented bone disease, while, in patients with smoldering or stage I MM, they could be considered but preferably in a clinical trial. For monitoring these patients, a skeletal survey annually or as clinically indicated is recommended, while bone densitometry or other metabolic studies should be reserved for clinical trials.

6.2 Radiotherapy

Even in the era of novel agents, radiotherapy has an important role in MM, in particular for palliative treatment. In particular, low-dose radiation therapy (10–30 Gy) is used for the palliative treatment of uncontrolled pain, impending pathologic fracture, or impending spinal cord compression[223]. To limit the effect of irradiation on stem cell harvest or its effect on potential future treatments, limited involved fields should be used, and, moreover, the radiation doses administered should not preclude stem cells collection in potential candidates for high-dose therapy and SCT. However, orthopedic consultation should be obtained for impending bone compression of the spinal cord, or vertebral column instability or actual fractures in weight-bearing bones, and, in particular, either vertebroplasty or kyphoplasty should be considered for symptomatic vertebral compression fractures.

6.3 Hypercalcemia

In MM, excess bone resorption from MM bone disease can lead to excessive release of calcium into the blood, and this could contribute to hypercalcemia. Its symptoms include gastrointestinal disturbances and polyuria, with progressive dehydration and decreases in glomerular filtration

rate. Hypercalcemia treatment can be performed with hydration and furosemide, steroids, bisphosphonates (better zolendronic acid), and/or calcitonin[219,224,225].

6.4 Plasmapheresis, ESAs, Infections and Thrombosis

In MM, plasmapheresis should be used as adjunctive therapy for symptomatic hyperviscosity, and also for adjunctive treatment of renal dysfunction[226].

Erythropoietin therapy should be considered for MM anemic patients, especially for those with renal failure, and in these patients to measure endogenous erythropoietin levels may help in treatment planning[225,226].

Many strategies are helpful to prevent infection in MM, and, in particular, intravenous immunoglobulin (IVIg) therapy should be considered for recurrent, life-threatening infections; pneumococcal and influenza vaccine could also be considered; and Pneumocystis carinii pneumonia (PCP), herpes, and antifungal prophylaxis is recommended if a high-dose regimen is used. In particular, as previously described, bortezomib treatment has been associated with an incidence of herpes zoster, and in these patients herpes prophylaxis is recommended[48,49].

Thrombosis is relatively common when IMIDs (thalidomide or lenalidomide) are used with steroids, and it can be particularly frequent when treating newly diagnosed patients. As previously described, when IMiDs are used in combination therapy during induction, the use of prophylactic anticoagulation agents should be recommended[64,229,230].

Moreover, hydration should be maintained while non-steroidal anti-inflammatory drugs (NSAIDs) should be avoided to decrease the chances of renal dysfunction.

7. Aim of the study

Despite the context of several promising therapeutic advances, there are still few effective and durable treatment options for rrMM, even if the last decade has been signed by a reawaking of bendamustine, a molecule already largely adopted in hematological malignancies, as effective agent for Hodgkin lymphoma, non-Hodgkin lymphoma and chronic lymphatic leukemia treatment[231,232]. Bendamustine is a chemotherapeutic agent with unique properties of alkylating agent that differs from the other major compounds of this category, such as cyclophosphamide, melphalan and chlorambucil[233]. Several mechanisms of action are supposed to be the key of efficacy of bendamustine in inducing an extensive and long-lasting DNA damage: stress response to DNA damage and apoptosis via p53-pathway, inhibition of mitotic checkpoints, and induction of mitotic catastrophe[233,234]. Moreover, due to its chemical structure, besides its peculiar alkylating agent activity, it is supposed that bendamustine has also antimetabolite properties, resulting in a bi-functional chemotherapy agent, with a unique mechanism of actions among alkylating agents[234]. It is not yet clear whether bendamustine can overcome the resistance to melphalan or have a synergic mechanism of action with this molecule, but its multiple actions explain its lack of cross-resistance with other DNA-damaging agents previously adopted in the same patient[235,236]. Bendamustine was evaluated in patients with MM as single agent or combined with IMIDs (thalidomide and lenalidomide) or bortezomib, giving promising results as upfront therapy and, especially, in pre-treated patients, also with renal impairment[237]. According to a study conducted by Pönisch et al. in 2010, combination of bendamustine-prednisone was approved in Europe as first-line treatment for patient older than 65 years not ASCT eligible and with a diagnosis of peripheral neuropathy impeding the use of bortezomib or thalidomide-based regimens[238]. However, given the well know evidence of effectiveness of various bortezomib-based regimens as salvage treatment for rrMM[164,239,240] and according to pre-clinical studies[241], bendamustine combined with bortezomib and dexamethasone (BVD) could be a feasible therapeutic option, also for heavily pre-treated patients[242]. Starting from preliminary data about efficacy and safety on BVD regimen for rrMM elderly patients reported in an abstract by Hrusovsky I et al in 2007 with an overall response rate (ORR) of 72.5%[243], several other trials have demonstrated or are still underway to demonstrate the effectiveness of this regimen in rrMM setting. Pönisch et al. adopted bendamustine/prednisone/bortezomib (BPV) regimen in patients with rrMM, obtaining an ORR of 69% with no severe toxicity[244]. A phase II italian study by Offidani et al. showed an ORR of 61.5% with BVD in rrMM patients who underwent to

more than two prior line of treatment[189]. Another phase II study by Ludwig et al. gave as results an ORR of 60.8 %, but in patients with a median of two prior therapy lines before BVD[245]. The aim of this observational retrospective study is to evaluate efficacy and safety of BVD regimen in patients with rrMM in a real-life setting and to compare it with the results of the major studies.

8. Methods

From October 2014 to December 2016, 56 patients, affected by rrMM, and referred as outpatients to our institutions (Hematology, University Hospital Policlinico Federico II and Hematology, AORN Cardarelli Hospital, Naples, Italy), previously treated with several lines of therapy, were assigned to receive BVD as salvage treatment. All patients had become refractory to previous therapies and had received at least two previous lines of treatment (median n. 6, range 2-11). All patients had been treated with bortezomib-based regimen, such as bortezomib-dexamethasone (VD), bortezomib-thalidomide-dexamethasone (VTD), bortezomib-melphalan-prednisone (VMP) and with lenalidomide-based regimens, such as lenalidomide-dexamethasone (RD) or bortezomib-lenalidomide-dexamethasone (VRD). Particularly, it has to be underlined that 100% of patients had developed resistance to a bortezomib-containing regimen, *i.e.* VD, VTD, VMP and VRD. Thirthy-eight young patients had received ASCT (68%); particularly, 28 (50%) had received one ASCT and 10 (18%) double ASCT. Also patients who underwent to two prior ASCT, at relapse, were treated with at least one bortezomib-based regimen. Finally, of notice, some patients had also received and subsequently became refractory to a novel agent-containing regimen: in details, two patients (3.5%) had been treated with pomalidomide-dexamethasone and three patients (5.3%) with carfilzomib-lenalidomide-dexamethasone. Characteristics of the patients are summarized in Table 3.

Table 3: Baseline characteristics of patients

Characteristic	**No.**
Number of patients	56
Sex	
Male/Female	31/25
Median age, years (range)	
At diagnosis	57 (45-73)
At start of BVD	61.8(37-83)
Median previous regimens (range)	6 (2-11)
FISH analysis	12/56
Negative	10/56
del13q	1/56
t(11;14)	1/56
Previous therapies, no of patients/(%)	
Bortezomib	56(100%)
First and second generation IMIDs (thalidomide and lenalidomide)	56(100%)
Carfilzomib	3(5.3%)
Pomalidomide	2(3.5%)
Autologus SCT	38 (68%)
First	28 (50%)
Second	10 (18%)

Elderly patients and young patients no more ASCT-eligible were treated at the clinical or biochemical relapse according to IMWG criteria: onset of new or worsening of pre-existent CRAB symptoms, appearing of a new bone or soft-tissue lesion (plasmocytoma), doubling of monoclonal component. BVD treatment schedule adopted was: bendamustine (B) 90 mg/sqm i.v. days 1, 2; Bortezomib (Vel) 1/1.3 mg/sqm s.c. days 1, 4, 8, 11; Dexamethasone (Dexa) 20 mg, p.o., days 1, 2, 4, 5, 8, 9, 11, 12. Cycles were planned to be repeated every 28 days, until progression of disease or unacceptable toxicity, but were actually administered with median of 32 days (range 28-39) due to possible delaying adverse events. Associated supportive care to prevent anemia and neutropenia was administered. In particular, G-CSF analogue pegfilgrastim 6 mg s.c. was given in day + 4 of treatment and epoietin alfa 40.000 I.U. s.c. was indicated if required (Hemoglobin < 10 g/dl). Prophylactic antimicrobial therapy was prescribed: levofloxacin 500 mg/d, p.o., days 9-21 and fluconazole 100 mg/d days 9-21. Antiemetic drugs and allopurinol if required were given. All patients were evaluated for response to therapy according to uniform IWMG response criteria[10]. Primary endpoint was to evaluate ORR (≥MR) and ORR2 (≥SD) in our population of rrMM patients treated with BVD regimen. With adoption of ORR2 as one of primary endpoints, we can better define the treatment response in a cohort of patients whose outcome is significantly poor due to a heavily pre-treated advanced disease. Secondary endpoints were progression-free survival (PFS), overall survival (OS), time to response (TTR) and safety in the same population. Regarding safety data, despite there are no biological parameters that can be used to evaluate the peculiar damage of a given drug on the bone marrow, we aimed to distinguish the hematological adverse events (AEs) in two separate toxicity profiles for each patient: pre-BVD toxicity profile (Safety 1) and post-BVD toxicity profile (Safety 2). Moreover, all these parameters were evaluated in a retrospective real-life context of our two institutions, focusing also on aspects as: feasibility of a BVD re-challenging in patients heavily pre-treated, G-CSF supportive care effectiveness and differences between drug-induced toxicity and pre-existing toxicity on bone marrow. Response evaluation was routinely performed every month with physical examination, complete blood count, biochemical serum and urine analysis and, when necessary, bone marrow examination, PET/CT or MRI. Due to the observational/retrospective nature of the study and anonymous data collection, informed consent was not necessary.

9. Results

Median follow-up of patients was 14 months (range 2-36+). Analysis on all rrMM patients treated with BVD as salvage line showed the outstanding values of 67.8 % and 89.2%, for ORR and ORR2 respectively, after a median of 6 cycles of BVD. In particular, according to IMWG criteria, one patient (1.8%) achieved a sCR, 3 patients (5.3%) achieved a CR, 7 patients (12.5%) achieved a VGPR, 18 patients (32.1%) achieved a PR, 9 patients (16%) achieved a MR, 12 patients (21.4%) achieved a SD, and 8 patients (14.3%) were in PD (Table 4).

Table 4: Response rates according to IMWG criteria. sCR: stringent complete response, CR: complete response, VGPR: very good partial response, PR: partial response, MR: minimal response, SD: stable disease, PD: progressive disease, ORR: overall response rate (≥MR), ORR2: overall response rate 2 (≥SD), OS: overall survival, PFS: progression-free survival and TTR: time to response.Response rates are expressed as percentage with n. of patients in brackets; OS, PFS and TTR are expressed in median number of months with range in brackets.

Response evaluation	
ORR	67.8%
ORR2	89.2%
sCR	1.8% (1/56)
CR	5.3% (3/56)
VGPR	12.5% (7/56)
PR	32.1% (18/56)
MR	16% (9/56)
SD	21.4% (12/56)
PD	14.3% (8/56)
OS from diagnosis	62.7(6-151)
OS from start BVD	9.8(2-36)
PFS	8.5(7-25)
TTR	1.2(1-3)

Interesting additional results in terms of ORR (obtaining at least a PR) were also achieved in particular settings of patients. Concerning efficacy of BVD in patients refractory to previous bortezomib-based lines of therapy, in 21 patients (37.4%) refractoriness to bortezomib was overtaken by synergistic action of bendamustine with this proteasome inhibitor, obtaining at least a PR. BVD regimen was also active in a small, but important group of patients who previously had received a novel agent-based therapy: in particular, 2/56 patients (3.5%) previously treated with pomalidomide-dexamethasone achieved at least a PR and 3/56 patients (5.3%) previously treated with carfilzomib-dexamethasone regimen obtained a PR. Bendamustine was also

effective as *"bridge to transplant"* both for second ASCT or alloSCT, in 11 patients (19%) and 2 patients (3.5%), respectively. Finally, this treatment was also effective, after previous efficacy, as re-treatment effective strategy post-ASCT in 1 patient (1.8%)[242]. Regarding secondary endpoints, an OS of 62.7 months from diagnosis (Figure 12) and of 9.8 from BVD start were achieved (Figure 13), and a median PFS of 8.5 months (Figure 14) with a TTR of 1.2 months were observed (Figure 15).

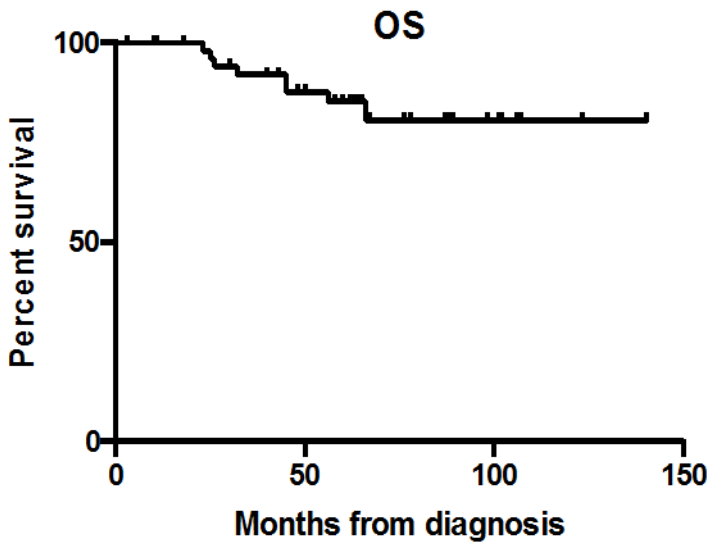

Figure 12: OS from diagnosis

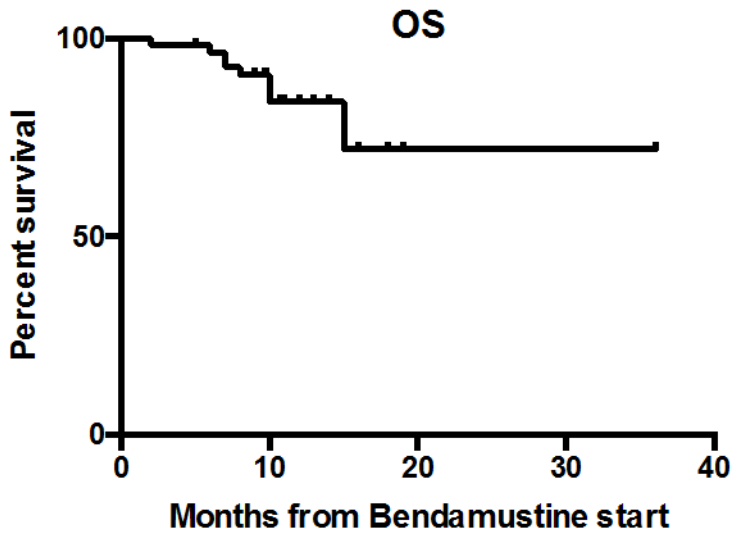

Figure 13: OS from start therapy with BVD

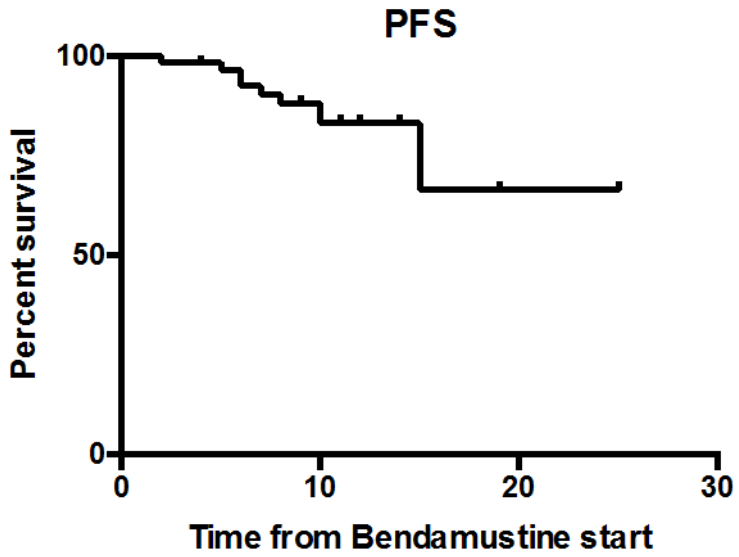

Figure 14: Progression Free Survival (PFS)

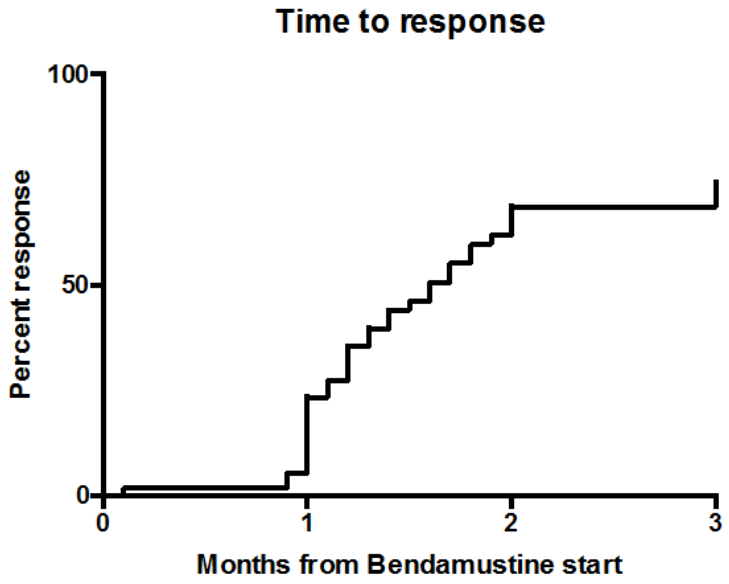

Figure 15: Time to Response (TTR)

9.1 Safety

Safety 1 profile showed no grade 4 hematologic AEs, 46% of grade 2-3 anemia, 14 of grade 3 neutropenia and 43% of thrombocytopenia. In the Safety 2 profile, it was not encountered any grade 4 hematological AE, grade 3 anemia in 41 % and grade 2 in 52% of patients (ESA largely required). Grade 2-3 neutropenia and thrombocytopenia in 37% and 34% were present, respectively. No severe extra-hematological AEs requiring hospitalization were noted. Safety 1 and Safety 2 profiles are summarized in Table 5 and 6.

Table 5: Safety 1 profile

Safety 1 profile - Adverse events	**%/No.**
Anemia	
Grade 4 (transfusion-dependent)	0
Grade 3 (transfusion-dependent)	8 (14%)
Grade 2 (ESAs-dependent)	18 (32%)
Grade 1/No Anemia	30 (53%)
Neutropenia	
Grade 4	0
Grade 3	8 (14%)
Thrombocytopenia	
Grade 4	0
Grade 2 and 3	24 (43%)
Grade 1/No Thrombocytopenia	32 (57%)
Peripheral Neuropathy	
Any grade	18 (32%)
≥ grade 3	3 (5%)

Table 6: Safety 2 profile

Safety 2 profile - Adverse events	**%/No.**
Anemia	
Grade 4 (transfusion-dependent)	0
Grade 3 (transfusion-dependent)	23 (41%)
Grade 2 (ESAs-dependent)	29 (52%)
Grade 1/No Anemia	4 (7%)
Neutropenia	
Grade 4	0
Grade 2-3	21 (37%)
Grade 1	11 (19%)
Thrombocytopenia	
Grade 4	0
Grade 2 and 3	19 (56%)
Grade 1/No Thrombocytopenia	37 (66%)
Peripheral Neuropathy	
Any grade	23 (41%)
≥ grade 3	5 (9%)

10. Discussion

Our aim is to compare our real-life retrospective data with other previously conducted trials. In an early dose-escalation study of 2007 by Fenk et al.[247], 7 rrMM patients treated with triplet combination of BVD (B 50-100 mg/m^2 d 1, 8 + Vel 1.3 mg/m^2 d 1, 4, 8, 11 + Dexa 40 mg d 1, 4, 8, 11 every 28 d), had an ORR of 86%. A phase I/II trial by Berenson et al.[248], involving 40 patients, aimed to evaluate maximum tolerated dose (MTD), efficacy and toxicity of bendamustine-bortezomib without dexametasone, with the following schedule: B 90 mg/m^2 d 1,4 + Vel 1.0 mg/m^2 d 1, 4, 8, 11 every 28 days). Maximum Tolerated Dose was established at 90 mg/m^2, and an ORR of 48% with a median OS of 13.3 months clearly demonstrated the synergistic action of dexamethasone with bendamustine, because its exclusion from the treatment schedule can notably affect the effectiveness of therapy. Pönisch et al.[244], in a retrospective analysis of 78 patients treated with BPV (B 60 up to 120 mg/m^2 d 1, 2 + PDN 100 mg d 1, 2, 4, 8, 11 + Vel 1.3 mg/m^2 d 1, 4, 8, 11 every 28 days), distinguishes between cases without and with severe hematological toxicities, due to previous treatment, reporting no differences in ORR (69%) but, statistical differences in median PFS (50 and 5 months, respectively) and in median PFS (11 and 3 months, respectively). Complete and accurate data on efficacy and safety of BVD in rrMM were reported in a phase 2 trial by Offidani et al.[189], whose schedule treatment was: B 70mg/m^2 d 1, 8 + Vel 1.3 mg/m^2 d 1, 4, 8, 11 + Dexa 20 mg d 1, 2, 4, 5, 8, 9, 11, 12 every 28 days for 2 cycles, subsequently B 70 mg/m^2 d 1, 8 + Vel 1.3 mg/m^2 d 1, 8, 15, 22 + Dexa 20 mg days 1, 8, 15, 22 every 28 days. For 75 rrMM patients the ORR reported was 75%, whereas PFS and OS were 12.5 months and 24 months, respectively. Univariate analysis showed that previous lines of treatment containing both lenalidomide and bortezomib affected negatively ORR and time to progression. Phase 2 trial involving 79 patients carried out by Ludwig et al.[245] reported valid data of BVD activity and safety in rrMM patients. The algorithm of treatment consisted in B 70 mg/m^2 d 1, 4 + Vel 1.3 mg/m^2 d 1, 4, 8, 11 + Dexa 20 mg d 1, 4, 8, 11 every 28 days for maximum 8 cycles. The ORR measured was 60.9%, median OS was 25.6 months and median PFS was 9.7 months. Pre-treatment with bortezomib, lenalidomide or bortezomib-lenalidomide did not affect significantly ORR and, furthermore, a PR or better response was reported for 5 of 8 patients previously exposed to 2 or 3 bortezomib lines. In a French multicenter trial, Rodon et al.[249] reported an ORR of 69.8%, an OS of 23 months and a PFS of 10.8 months in 73 patients, with median age slightly higher than in other trials (76 years), adopting the sequent schedule: B 70 mg/m^2 d 1, 8 + Vel 1.3 mg/m^2 d 1, 8, 15,

22 + Dexa 20 mg d 1, 8, 15, 22 every 28 days; responder patients received also a maintenance of 6 cycles given 1 month out of 2. Finally, regarding BVD regimen efficacy given to previously untreated patients, Mateos et al.[250] adopted this schedule for 60 MM patients (42 of them candidate to ASCT) as front-line treatment: B 90 mg/m^2 d 1, 4 + Vel 1.3 mg/m^2 1, 4, 8, 11, 22, 25, 29, 32 + prednisone 60 mg/m^2 d 1-4 for the first cycle, then B 90 mg/m^2 d 1, 8 + Vel 1.3 mg/m^2 d 1, 8, 15, 22 + prednisone 60 mg/m^2 d 1-4. ORR in all population was 84%, whereas the 2-years OS and 2-years PFS were 86% and 62% respectively. Data obtained by our cohort showed a remarkable ORR of 67.8% for all patients enrolled in the BVD salvage protocol. This is an impressive result and comparable with ORR obtained by Ludwig et al. and Offidani et al.[189,245]. However, in the aforementioned trials, the median number of prior therapy lines was 2 (range 1-6) and 1 (range 1-4) respectively, while in patients enrolled by our institution the median number of preceding lines was 6 (median 2-11). Moreover, our study was focused on a cohort of patients that were all previously exposed and became refractory to bortezomib, whereas the percentage of this type of patients were 63.3% in Ludwig trial and 46.5% in Offidani population. Higher number of prior lines of therapy and higher proportion of bortezomib-refractory patients in our real-life setting make the obtained ORR value of 67.8% an excellent response rate for a salvage therapy. These results are also strengthened by the observation that, in our cohort, 2 patients (3.5%) previously treated with and became refractory to pomalidomide-dexamethasone and 3 patients (5.3%) to carfilzomib-lenalidomide-dexamethasone responded to BVD when, showing an impressive capability of bendamustine, when associated to bortezomib, to overcome several therapy resistances. Furthermore, if we consider as "responders" also patients who achieve a SD (ORR2), the result of 89.2% in our population, is a valid parameter to assess responses rates in this particular setting of frail and poor responder patients. Regarding safety profile of bendamustine, in our study we should highlight the cumulative toxicity effect of prior lines on bone marrow, that unavoidably impaired marrow function, creating a "*background*" of hematological toxicity in the patient. As nowadays there is a survival improvement of patients affected with MM thanks to novel agents' introduction and improvement of supportive care[4], it is increasingly common that clinician specialists may face long-survivor patients with pre-existing poor and frail bone marrow function due to toxicity of prior therapy lines applied. Therefore, to assess the safety profile of a salvage line of therapy in a heavily pre-treated cohort of patients, such as rrMM patients, whose possible hematological AEs incidence and severity are clearly affected by previously damaged bone marrow function, we distinguished two safety profiles both by chronological and biological

criteria. Safety 1, in fact, is the cumulative result of all previously chemotherapy lines who patient underwent, and it is disease-related, host-related, and treatment-related[251] and it was supposed to be the baseline bone marrow function prior to BVD. On the other side, Safety 2 profile is related to the mere effect of bendamustine or, possibly, of bortezomib and dexamethasone when combined with bendamustine, on the bone marrow at the moment of starting BVD regimen. All our data on hematological toxicity showed, despite high dose of bendamustine used in our schedule (90 mg/m^2) and the median number of 6 prior lines of therapy, the level of bone marrow function impairment was highly manageable, for both anemia and neutropenia. Adoption of Epoietin alfa, usually in a weekly dose of 40.000 I.U., until hemoglobin values of 10 g/dl were obtained, was sufficient to avoid blood transfusion in more than 50% of patients. Regarding febrile neutropenia management, the risk was effectively reduced with adequate use of a long-acting GCSF (pegfilgrastim 6 mg) given by subcutaneous route, as suggested by EORTC international guidelines[252]. Moreover, according to our previous published experience, is clearly demonstrated that pegfilgrastim is more effective for reducing febrile neutropenia episodes than filgrastim, in patient undergoing BVD[253]. In fact, the cohort of patients whose neutropenia was managed with pegfilgrastim had fewer febrile neutropenia-related chemotherapy delay than those with filgrastim (8.3% and 17.3% respectively) and also fewer days of hospitalization due to febrile neutropenia complications (median of 0 and 15 days, respectively). These data account for the favorable hematological safety profile, with low rates of chemotherapy-related infectious episodes, despite we adopted a treatment schedule with high dose of bendamustine in a frail population of patients. Moreover, pegfilgrastim has the advantage of mono-administration, with fewer GCSF-related side effects as bone pain, easily manageable by paracetamol. Regarding extra-hematological toxicity profile of BVD, grade 1 gastro-intestinal impairment (nausea, vomiting, diarrhea, etc) reported in 55% of patients, easily manageable with common anti-emetic prophylaxis and treatment. Regarding peripheral neuropathy (PN) rate, probably the most clinically relevant extra-hematological AE, is clearly affected by the re-treatment with a bortezomib-based regimen (BVD, in this case) in patients previously exposed to bortezomib. Thus, it can be expected a certain grade of PN in these patients, but as data revealed, also this AE was acceptable in terms or rates and grade of severity. Particularly, at baseline, 32% (n=18) any grade PN was noted, 6% (n=3) of ≥ grade 3 cases. After a median of 6 cycles, overall PN rate was 41% (n=23), with 9% (n=5) of ≥ grade 3. Neither at baseline nor after BVD treatment grade 4 of PN were detected. Therefore, the overall safety profile results very acceptable, also in frail patients with a poor outcome, as rrMM patients.

In the rrMM setting, an old and well known drug as bendamustine, combined in the BVD regimen employed in patients also heavily pre-treated with and resistant to bortezomib, can be an effective and safe treatment option, despite the increasing availability of new generation therapeutic molecules. Data emerging from this real-life retrospective evaluation have been demonstrated to be highly comparable to major clinical trials adopting this combination in the same setting, in terms of ORR, OS, PFS and safety profile in rrMM patients. Moreover, the observation that in our cohort of patients with high median number of previous therapy lines and all refractory to bortezomib, shows that bendamustine combined with bortezomib breakthroughs the acquired pharmacological resistance. Furthermore, bendamustine appears as a molecule capable to induce response also in a patient with a background of severe pre-existing bone marrow impairment due to previous therapies. In conclusion, a so-called “old” chemotherapeutic molecule, already adopted for long time in other clinical settings, can be a feasible, effective and safe treatment option for rrMM patients with no other therapeutic options.

11. References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7–30.
2. Cancer Stat Facts: Myeloma. National Cancer Institute Surveillane, Epidemiology, and End Results Program Web site. Avilable at http://seer. cancer.gov/statfacts/html/mulmy.html. Accessed January 24, 2017.
3. Brenner H, Gondos A, Pulte D. Recent major improvement in long-term survival of younger patients with multiple myeloma. Blood 2008; 111: 2521– 2526.
4. Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood 2008; 111: 2516–2520.
5. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med 2011; 364: 1046–1060.
6. Anderson KC. Oncogenomics to target myeloma in the bone marrow microenvironment. Clin Cancer Res 2011; 17: 1225–1233.
7. Hideshima T, Anderson K. Molecular mechanisms of novel therapeutic approaches for multiple myeloma. Nat Rev Cancer 2002; 2: 927–937.
8. Dispenzieri A, Kyle R, Merlini G, et al. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. Leukemia 2009; 23: 215–224.
9. Kuhnemund A, Liebisch P, Bauchmuller K, et al. 'Light-chain escape- multiple myeloma'- an escape phenomenon from plateau phase: report of the largest patient series using LC-monitoring. J Cancer Res Clin Oncol 2009; 135: 477–484.
10. Durie BG, Harousseau JL, Miguel JS, et al. International uniform response criteria for multiple myeloma. Leukemia 2006; 20: 1467–1473.
11. Kyle RA, Gertz MA, Witzig TE, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc 2003; 78: 21–33.
12. Xiong W, Wu X, Starnes S, et al. An analysis of the clinical and biologic significance of TP53 loss and the identification of potential novel transcriptional targets of TP53 in multiple myeloma. Blood 2008; 112: 4235–4246.
13. Drach J, Ackermann J, Fritz E, et al. Presence of a p53 gene deletion in patients with multiple myeloma predicts for short survival after conventional-dose chemotherapy. Blood 1998; 92: 802–809.

14. Avet-Loiseau H, Attal M, Moreau P, et al. Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myelome. Blood 2007; 109: 3489–3495.
15. Gertz MA, Lacy MQ, Dispenzieri A, et al. Clinical implications of t(11;14) (q13;q32), t(4;14)(p16.3;q32), and -17p13 in myeloma patients treated with high-dose therapy. Blood 2005; 106: 2837–2840.
16. Gutierrez NC, Castellanos MV, Martin ML, et al. Prognostic and biological implications of genetic abnormalities in multiple myeloma undergoing autologous stem cell transplantation: t(4;14) is the most relevant adverse prognostic factor, whereas RB deletion as a unique abnormality is not associated with adverse prognosis. Leukemia 2007; 21: 143–150.
17. Ross FM, Avet-Loiseau H, Ameye G, et al. Report from the European Myeloma Network on interphase FISH in multiple myeloma and related disorders. Haematologica 2012; 97: 1272–1277.
18. Hanamura I, Stewart JP, Huang Y, et al. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. Blood 2006; 108: 1724–1732.
19. Carrasco DR, Tonon G, Huang Y, et al. High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. Cancer Cell 2006; 9: 313–325.
20. Rosinol L, Carrio A, Blade J, et al. Comparative genomic hybridisation identifies two variants of smoldering multiple myeloma. Br J Haematol 2005; 130: 729–732.
21. Dispenzieri A, Rajkumar SV, Gertz MA, et al. Treatment of newly diagnosed multiple myeloma based on Mayo Stratification of Myeloma and Risk-adapted Therapy (mSMART): consensus statement. Mayo Clin Proc 2007; 82: 323–341.
22. Kumar SK, Mikhael JR, Buadi FK, et al. Management of newly diagnosed symptomatic multiple myeloma: updated Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) consensus guidelines. Mayo Clin Proc 2009; 84: 1095–1110.
23. Moreau P, Attal M, Garban F, et al. Heterogeneity of t(4;14) in multiple myeloma. Long-term follow-up of 100 cases treated with tandem transplantation in IFM99 trials. Leukemia 2007; 21: 2020–2024.

24. Fonseca R, Bergsagel PL, Drach J, et al. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. Leukemia 2009; 23: 2210–2221.
25. Zhou Y, Barlogie B, Shaughnessy JD Jr. The molecular characterization and clinical management of multiple myeloma in the post-genome era. Leukemia 2009; 23: 1941–1956.
26. Decaux O, Lode L, Magrangeas F, et al. Prediction of survival in multiple myeloma based on gene expression profiles reveals cell cycle and chromosomal instability signatures in high-risk patients and hyperdiploid signatures in low-risk patients: a study of the Intergroupe Francophone du Myelome. J Clin Oncol 2008; 26: 4798–4805.
27. Shaughnessy JD Jr, Zhan F, Burington BE, et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood 2007; 109: 2276–2284.
28. Kuiper R, Broyl A, de Knegt Y, et al. A gene expression signature for high- risk multiple myeloma. Leukemia 2012; 26: 2406–2413.
29. Moulopoulos LA, Dimopoulos MA, Weber D, et al. Magnetic resonance imaging in the staging of solitary plasmacytoma of bone. J Clin Oncol 1993; 11: 1311–1315.
30. Zamagni E, Cavo M. The role of imaging techniques in the management of multiple myeloma. Br J Haematol 2012; 159: 499–513.
31. Durie B, Waxman A, D'Agnolo A, Williams CM. Whole-body (18) F-FDG PET identifies high-risk myeloma. J Nucl Med 2002; 43: 1457–1463.
32. Schirrmeister H, Bommer M, Buck AK, et al. Initial results in the assessment of multiple myeloma using 18F-FDG PET. Eur J Nucl Med Mol Imaging 2002; 29: 361–366.
33. Zamagni E, Patriarca F, Nanni C, et al. Prognostic relevance of 18-F FDG PET/CT in newly diagnosed multiple myeloma patients treated with up- front autologous transplantation. Blood 2011; 118: 5989–5995.
34. Nanni C, Zamagni E, Celli M, et al. The value of 18F-FDG PET/CT after autologous stem cell transplantation (ASCT) in patients affected by multiple myeloma (MM): experience with 77 patients. Clin Nucl Med 2013; 38: e74–79.
35. Greipp PR, Lust JA, O'Fallon WM, et al. Plasma cell labeling index and beta 2-microglobulin predict survival independent of thymidine kinase and C-reactive protein in multiple myeloma. Blood 1993; 81: 3382–3387.
36. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014; 15: e538–548.

37. Greipp PR, San Miguel J, Durie BGM, et al. International Staging System for multiple myeloma. J Clin Oncol 2005; 23: 3412–3420.
38. Palumbo A, Avet-Loiseau H, Oliva S, et al. Revised International Staging System for Multiple Myeloma: a report from International Myeloma Working Group. J Clin Oncol 2015; 33: 2863–2869.
39. Blade J, Samson D, Reece D, et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. Br J Haematol 1998; 102: 1115–1123.
40. Rajkumar SV. Multiple myeloma: 2011 update on diagnosis, risk- stratification, and management. Am J Hematol 2011; 86: 57–65.
41. Palumbo A, Rajkumar SV, San Miguel JF, et al. International Myeloma Working Group consensus statement for the management, treatment, and supportive care of patients with myeloma not eligible for standard autologous stem-cell transplantation. J Clin Oncol 2014; 32: 587–600.
42. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol 2016; 17: e328–346.
43. Kumar SK, Callander NS, Alsina M, et al. Multiple Myeloma, Version 3.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2017 Feb; 15(2): 230-269.
44. Avet-Loiseau H, Leleu X, Roussel M, et al. Bortezomib plus dexamethasone induction improves outcome of patients With t(4;14) myeloma but not outcome of patients with del(17p). J Clin Oncol 2010; 28: 4630–4634.
45. Mateos MV. Management of treatment-related adverse events in patients with multiple myeloma. Cancer Treat Rev 2010;36(Suppl 2): S24–32.
46. Siegel D, Martin T, Nooka A, et al. Integrated safety profile of single-agent carfilzomib: experience from 526 patients enrolled in 4 phase II clinical studies. Haematologica 2013; 98:1753–1761.
47. Chanan-Khan A, Sonneveld P, Schuster M, et al. Analysis of herpes zoster events among bortezomib-treated patients in the phase III APEX study. J Clin Oncol 2008; 26: 4784–4790.

48. Mateos M, Hernandez J, Hernandez M, et al. Bortezomib plus melphalan and prednisone in elderly untreated patients with multiple myeloma: results of a multicenter phase 1/2 study. Blood 2006; 108: 2165–2172.
49. Richardson P, Sonneveld P, Schuster M, et al. Bortezomib or high- dose dexamethasone for relapsed multiple myeloma. N Engl J Med 2005; 352: 2487–2498.
50. Kyprolis (carfilzomib) for injection. FDA Web site. Available at: http:// www.fda.gov/safety/medwatch/safetyinformation/ucm441458.htm. Accessed January 24, 2017.
51. Moreau P, Pylypenko H, Grosicki S, et al. Subcutaneous versus intravenous administration of bortezomib in patients with relapsed multiple myeloma: a randomised, phase 3, non-inferiority study. Lancet Oncol 2011; 12: 431– 440.
52. Arnulf B, Pylypenko H, Grosicki S, et al. Updated survival analysis of a randomized, phase 3 study of subcutaneous versus intravenous bortezomib in patients with relapsed multiple myeloma. Haematologica 2012; 97: 1925–1928.
53. Cavo M, Tacchetti P, Patriarca F, et al. Bortezomib with thalidomide plus dexamethasone compared with thalidomide plus dexamethasone as induction therapy before, and consolidation therapy after, double autologous stem-cell transplantation in newly diagnosed multiple myeloma: a randomised phase 3 study. Lancet 2010; 376: 2075–2085.
54. Kaufman JL, Nooka A, Vrana M, et al. Bortezomib, thalidomide, and dexamethasone as induction therapy for patients with symptomatic multiple myeloma: a retrospective study. Cancer 2010; 116: 3143–3151.
55. Rosinol L, Oriol A, Teruel AI, et al. Superiority of bortezomib, thalidomide, and dexamethasone (VTD) as induction pretransplantation therapy in multiple myeloma: a randomized phase 3 PETHEMA/GEM study. Blood 2012; 120: 1589–1596.
56. Moreau P, Hulin C, MACRO M, et al. Bortezomib, thalidomide and dexamethasone (VTD) is superior to bortezomib, cyclophosphamide and dexamethasone (VCD) prior to autologous stem cell transplantation for patients with de novo multiple myeloma. Results of the prospective IFM 2013-04 trial. Blood 2015; 126: 393–393.
57. Harousseau JL, Attal M, Avet-Loiseau H, et al. Bortezomib plus dexamethasone is superior to vincristine plus doxorubicin plus dexamethasone as induction treatment prior to autologous stem-cell transplantation in newly diagnosed multiple myeloma: results of the IFM 2005-01 phase III trial. J Clin Oncol 2010; 28: 4621–4629.

58. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med 2003; 348: 2609–2617.
59. Moreau P, Avet-Loiseau H, Facon T, et al. Bortezomib plus dexamethasone versus reduced-dose bortezomib, thalidomide plus dexamethasone as induction treatment before autologous stem cell transplantation in newly diagnosed multiple myeloma. Blood 2011; 118: 5752–5758.
60. Zonder JA, Crowley J, Hussein MA, et al. Superiority of lenalidomide (Len) plus high-dose dexamethasone (HD) compared to HD alone as treatment of newly-diagnosed multiple myeloma (NDMM): results of the randomized, double-blinded, placebo-controlled SWOG Trial S0232 [abstract]. Blood 2007; 110: Abstract 77.
61. Rajkumar SV, Jacobus S, Callander N, et al. A randomized trial of lenalidomide plus high-dose dexamethasone (RD) versus lenalidomide plus low-dose dexamethasone (Rd) in newly diagnosed multiple myeloma (E4A03): a trial coordinated by the Eastern Cooperative Oncology Group [abstract]. Blood 2007; 110: Abstract 74.
62. Rajkumar SV, Jacobus S, Callander NS, et al. Lenalidomide plus high- dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial. Lancet Oncol 2010; 11: 29–37.
63. Zangari M, Tricot G, Polavaram L, et al. Survival effect of venous thromboembolism in patients with multiple myeloma treated with lenalidomide and high-dose dexamethasone. J Clin Oncol 2010; 28: 132–135.
64. Palumbo A, Rajkumar SV, Dimopoulos MA, et al. Prevention of thalidomide- and lenalidomide-associated thrombosis in myeloma. Leukemia 2008; 22: 414–423.
65. Kumar S, Dispenzieri A, Lacy MQ, et al. Impact of lenalidomide therapy on stem cell mobilization and engraftment post-peripheral blood stem cell transplantation in patients with newly diagnosed myeloma. Leukemia 2007; 21: 2035–2042.
66. Paripati H, Stewart AK, Cabou S, et al. Compromised stem cell mobilization following induction therapy with lenalidomide in myeloma. Leukemia 2008; 22: 1282–1284.
67. Kumar S, Giralt S, Stadtmauer EA, et al. Mobilization in myeloma revisited: IMWG consensus perspectives on stem cell collection following initial therapy with thalidomide-, lenalidomide-, or bortezomib-containing regimens. Blood 2009; 114: 1729–1735.

68. Mark T, Stern J, Furst JR, et al. Stem cell mobilization with cyclophosphamide overcomes the suppressive effect of lenalidomide therapy on stem cell collection in multiple myeloma. Biol Blood Marrow Transplant 2008; 14: 795–798.
69. Nademanee AP, DiPersio JF, Maziarz RT, et al. Plerixafor plus granulocyte colony-stimulating factor versus placebo plus granulocyte colony- stimulating factor for mobilization of CD34(+) hematopoietic stem cells in patients with multiple myeloma and low peripheral blood CD34(+) cell count: results of a subset analysis of a randomized trial. Biol Blood Marrow Transplant 2012; 18: 1564–1572.
70. Duarte RF, Shaw BE, Marin P, et al. Plerixafor plus granulocyte CSF can mobilize hematopoietic stem cells from multiple myeloma and lymphoma patients failing previous mobilization attempts: EU compassionate use data. Bone Marrow Transplant 2011; 46: 52–58.
71. Kumar S, Flinn I, Richardson PG, et al. Randomized, multicenter, phase 2 study (EVOLUTION) of combinations of bortezomib, dexamethasone, cyclophosphamide, and lenalidomide in previously untreated multiple myeloma. Blood 2012; 119: 4375–4382.
72. Reeder CB, Reece DE, Kukreti V, et al. Cyclophosphamide, bortezomib and dexamethasone induction for newly diagnosed multiple myeloma: high response rates in a phase II clinical trial. Leukemia 2009; 23: 1337–1341.
73. Einsele H, Liebisch P, Langer C, et al. Velcade, intravenous cyclophosphamide and dexamethasone (VCD) induction for previously untreated multiple myeloma (German DSMM XIa Trial) [abstract]. Blood 2009;114: Abstract 131.
74. Reeder CB, Reece DE, Kukreti V, et al. Long-term survival with cyclophosphamide, bortezomib and dexamethasone induction therapy in patients with newly diagnosed multiple myeloma. Br J Haematol 2014; 167: 563–565.
75. Reeder CB, Reece DE, Kukreti V, et al. Once- versus twice-weekly bortezomib induction therapy with CyBorD in newly diagnosed multiple myeloma. Blood 2010; 115: 3416–3417.
76. Sonneveld P, Schmidt-Wolf IG, van der Holt B, et al. Bortezomib induction and maintenance treatment in patients with newly diagnosed multiple myeloma: results of the randomized phase III HOVON-65/ GMMG-HD4 trial. J Clin Oncol 2012; 30: 2946–2955.
77. Richardson PG, Weller E, Lonial S, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood 2010; 116: 679–686.

78. Roussel M, Lauwers-Cances V, Robillard N, et al. Front-line transplantation program with lenalidomide, bortezomib, and dexamethasone combination as induction and consolidation followed by lenalidomide maintenance in patients with multiple myeloma: a phase II study by the Intergroupe Francophone du Myelome. J Clin Oncol 2014; 32: 2712–2717.
79. Durie B, Hoering A, Rajkumar SV, et al. Bortezomib, lenalidomide and dexamethasone vs. lenalidomide and dexamethasone in patients (Pts) with previously untreated multiple myeloma without an intent for immediate autologous stem cell transplant (ASCT): results of the randomized phase III trial SWOG S0777 [abtract]. Blood 2015; 126: Abstract 25.
80. Arastu-Kapur S, Anderl JL, Kraus M, et al. Nonproteasomal targets of the proteasome inhibitors bortezomib and carfilzomib: a link to clinical adverse events. Clin Cancer Res 2011; 17: 2734–2743.
81. Kirk CJ, Jiang J, Muchamuel T, et al. The selective proteasome inhibitor carfilzomib is well tolerated in experimental animals with dose intensive administration [abstract]. Blood 2008; 112: Abstract 2765.
82. Siegel DS, Martin T, Wang M, et al. A phase 2 study of single-agent carfilzomib (PX-171-003-A1) in patients with relapsed and refractory multiple myeloma. Blood 2012; 120: 2817–2825.
83. Vij R, Wang M, Kaufman JL, et al. An open-label, single-arm, phase 2 (PX- 171-004) study of single-agent carfilzomib in bortezomib-naive patients with relapsed and/or refractory multiple myeloma. Blood 2012; 119: 5661–5670.
84. Vij R, Wang M, Orlowski R, et al. Initial results of PX-171-003, an open-label, single-arm, phase II study of carfilzomib (CFZ) in patients with relapsed and refractory multiple myeloma (MM) [abstract]. Blood 2008; 112: Abstract 865.
85. Jakubowiak AJ, Dytfeld D, Griffith KA, et al. A phase 1/2 study of carfilzomib in combination with lenalidomide and low-dose dexamethasone as a frontline treatment for multiple myeloma. Blood 2012; 120: 1801–1809.
86. Korde N, Zingone A, Kwok M, et al. Phase II clinical and correlative study of carfilzomib, lenalidomide, and dexamethasone (CRd) in newly diagnosed Multiple Myeloma (MM) patients [abstract]. Blood 2012; 120: Abstract 732.
87. Korde N, Zingone A, Kwok M, et al. Phase II clinical and correlative study of carfilzomib, lenalidomide, and dexamethasone followed by lenalidomide extended dosing (CRD-R) induces high rates of MRD negativity in newly diagnosed multiple myeloma (MM) patients [abstract]. Blood 2013; 122: Abstract 538.

88. Kumar SK, Berdeja JG, Niesvizky R, et al. Safety and tolerability of ixazomib, an oral proteasome inhibitor, in combination with lenalidomide and dexamethasone in patients with previously untreated multiple myeloma: an open-label phase 1/2 study. Lancet Oncol 2014; 15: 1503–1512.
89. Dytfeld D, Jasielec J, Griffith KA, et al. Carfilzomib, lenalidomide, and low-dose dexamethasone in elderly patients with newly diagnosed multiple myeloma. Haematologica 2014; 99: e162–164.
90. San Miguel JF, Schlag R, Khuageva NK, for the VISTA Trial Investigators*: Bortezomib plus Melphalan and Prednisone for Initial Treatment of Multiple Myeloma. N Engl J Med 2008; 359: 906-917.
91. Zepeda J, H. V, Duggan P, et al. Cyclophosphamide, bortezomib and dexamethasone (CyBORD) is a feasible and active regimen for non-transplant eligible multiple myeloma patients [abstract]. Blood 2014; 124: Abstract 5751.
92. Rajkumar SV, Jacobus S, Callander N, et al. A randomized phase III trial of lenalidomide pus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone in newly diagnosed multiple myeloma (E4A03): a trial coordinated by the eastern Cooperative Oncology Group [abstract]. Blood 2006; 108: Abstract 799.
93. Benboubker L, Dimopoulos MA, Dispenzieri A, et al. Lenalidomide and dexamethasone in transplant-ineligible patients with myeloma. N Engl J Med 2014; 371: 906–917.
94. Attal M, Lauwers-Cances V, Marit G, et al. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. N Engl J Med 2012; 366: 1782–1791.
95. McCarthy PL, Owzar K, Hofmeister CC, et al. Lenalidomide after stem-cell transplantation for multiple myeloma. N Engl J Med 2012; 366: 1770–1781.
96. Usmani SZ, Sexton R, Hoering A, et al. Second malignancies in total therapy 2 and 3 for newly diagnosed multiple myeloma: influence of thalidomide and lenalidomide during maintenance. Blood 2012; 120: 1597–1600.
97. Palumbo A, Bringhen S, Kumar SK, et al. Second primary malignancies with lenalidomide therapy for newly diagnosed myeloma: a meta-analysis of individual patient data. Lancet Oncol 2014; 15: 333–342.
98. Dimopoulos MA, Cheung MC, Roussel M, et al. Impact of renal impairment on outcomes with lenalidomide and dexamethasone treatment in the FIRST trial, a randomized, open-label phase 3 trial in transplant- ineligible patients with multiple myeloma. Haematologica 2016; 101: 363– 370.

99. Niesvizky R, Flinn IW, Rifkin R, et al. Efficacy and safety of three bortezomib-based combinations in elderly, newly diagnosed multiple myeloma patients: results from all randomized patients in the community-based, phase 3b UPFRONT study [abstract]. Blood 2011; 118: Abstract 478.
100. Korde N, Roschewski M, Zingone A, et al. Treatment with carfilzomib- lenalidomide-dexamethasone with lenalidomide extension in patients with smoldering or newly diagnosed multiple myeloma. JAMA Oncol 2015; 1:746–754.
101. Srikanth M, Davies FE, Wu P, et al. Survival and outcome of blastoid variant myeloma following treatment with the novel thalidomide containing regime DT-PACE. Eur J Haematol 2008; 81: 432–436.
102. Badros A, Barlogie B, Morris C, et al. High response rate in refractory and poor-risk multiple myeloma after allotransplantation using a nonmyeloablative conditioning regimen and donor lymphocyte infusions. Blood 2001; 97: 2574–2579.
103. Kroger N, Sayer HG, Schwerdtfeger R, et al. Unrelated stem cell transplantation in multiple myeloma after a reduced-intensity conditioning with pretransplantation antithymocyte globulin is highly effective with low transplantation-related mortality. Blood 2002; 100: 3919–3924.
104. Moreau P, Facon T, Attal M, et al. Comparison of 200 mg/mq melphalan and 8 Gy total body irradiation plus 140 mg/mq melphalan as conditioning regimens for peripheral blood stem cell transplantation in patients with newly diagnosed multiple myeloma: final analysis of the Intergroupe Francophone du Myelome 9502 randomized trial. Blood 2002; 99: 731–735.
105. Somlo G, Spielberger R, Frankel P, et al. Total marrow irradiation: a new ablative regimen as part of tandem autologous stem cell transplantation for patients with multiple myeloma. Clin Cancer Res 2011; 17: 174–182.
106. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. N Engl J Med 1996; 335: 91–97.
107. Child J, Morgan G, Davies F, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J Med 2003; 348: 1875–1883.
108. Barlogie B, Kyle R, Anderson K, et al. Standard chemotherapy compared with high-dose chemoradiotherapy for multiple myeloma: final results of phase III US Intergroup Trial S9321. J Clin Oncol 2006; 24: 929–936.

109. Fermand J, Katsahian S, Divine M, et al. High-dose therapy and autologous blood stem-cell transplantation compared with conventional treatment in myeloma patients aged 55 to 65 years: long-term results of a randomized control trial from the Group Myelome-Autogreffe. J Clin Oncol 2005; 23: 9227–9233.
110. Cavo M, Tacchetti P, Patriarca F, et al. Superior complete response rate and progression-free survival after autologous transplantation with up-front velcade-thalidomide-dexamethasone compared with thalidomide-dexamethasone in newly diagnosed multiple myeloma [abstract]. Blood 2008; 112: Abstract 158.
111. Fermand JP, Ravaud P, Chevret S, et al. High-dose therapy and autologous peripheral blood stem cell transplantation in multiple myeloma: up-front or rescue treatment? Results of a multicenter sequential randomized clinical trial. Blood 1998; 92: 3131–3136.
112. Palumbo A, Cavallo F, Gay F, et al. Autologous transplantation and maintenance therapy in multiple myeloma. N Engl J Med 2014; 371: 895– 905.
113. Cavo M, Tacchetti P, Patriarca F, et al. A phase III study of double autotransplantation incorporating bortezomib-thalidomide-dexamethasone (VTD) or thalidomide-dexamethasone (TD) for multiple myeloma: superior clinical outcomes with VTD compared to TD [abstract]. Blood 2009; 114: Abstract 351.
114. Hahn T, Wingard J, Anderson K, et al. The role of cytotoxic therapy with hematopoietic stem cell transplantation in the therapy of multiple myeloma: an evidence-based review. Biol Blood Marrow Transplant 2003; 9:4–37.
115. Kumar S, Lacy MQ, Dispenzieri A, et al. High-dose therapy and autologous stem cell transplantation for multiple myeloma poorly responsive to initial therapy. Bone Marrow Transplant 2004; 34: 161–167.
116. Attal M, Harousseau J, Facon T, et al. Single versus double autologous stem- cell transplantation for multiple myeloma. N Engl J Med 2003; 349: 2495–2502.
117. Stadtmauer EA. Multiple myeloma, 2004—one or two transplants? N Engl J Med 2003; 349: 2551–2553.
118. Cavo M, Tosi P, Zamagni E, et al. Prospective, randomized study of single compared with double autologous stem-cell transplantation for multiple myeloma: Bologna 96 clinical study. J Clin Oncol 2007; 25: 2434–2441.
119. Sonneveld P, van der Holt B, Segeren C, et al. Intensive versus double intensive therapy in untreated multiple myeloma: Updated analysis of the randomized phase III study HOVON 24 MM [abstract]. Blood 2004; 104: Abstract 948.

120. Goldschmidt H. Single vs double high-dose therapy in multiple myeloma: second analysis of the GMMG-HD2 trial [abstract]. Haematologica 2005; 90(s1): Abstract 38.
121. Barlogie B, Attal M, Crowley J, et al. Long-term follow-up of autotransplantation trials for multiple myeloma: update of protocols conducted by the Intergroupe Francophone du Myelome, Southwest Oncology Group, and University of Arkansas for Medical Sciences. J Clin Oncol 2010; 28: 1209–1214.
122. Cook G, Liakopoulou E, Pearce R, et al. Factors influencing the outcome of a second autologous stem cell transplant (ASCT) in relapsed multiple myeloma: a study from the British Society of Blood and Marrow Transplantation Registry. Biol Blood Marrow Transplant 2011; 17: 1638–1645.
123. Olin RL, Vogl DT, Porter DL, et al. Second auto-SCT is safe and effective salvage therapy for relapsed multiple myeloma. Bone Marrow Transplant 2009; 43: 417–422.
124. Burzynski JA, Toro JJ, Patel RC, et al. Toxicity of a second autologous peripheral blood stem cell transplant in patients with relapsed or recurrent multiple myeloma. Leuk Lymphoma 2009; 50: 1442–1447.
125. Alvares CL, Davies FE, Horton C, et al. The role of second autografts in the management of myeloma at first relapse. Haematologica 2006; 91: 141–142.
126. Fenk R, Liese V, Neubauer F, et al. Predictive factors for successful salvage high-dose therapy in patients with multiple myeloma relapsing after autologous blood stem cell transplantation. Leuk Lymphoma 2011; 52: 1455–1462.
127. Cook G, Williams C, Brown JM, et al. High-dose chemotherapy plus autologous stem-cell transplantation as consolidation therapy in patients with relapsed multiple myeloma after previous autologous stem- cell transplantation (NCRI Myeloma X Relapse [Intensive trial]): a randomised, open-label, phase 3 trial. Lancet Oncol 2014; 15: 874–885.
128. Stadtmauer A, Pasquini M, Blackwell B, et al. Comparison of autologous hematopoietic cell transplant (autoHCT), bortezomib, lenalidomide (Len) and dexamethasone (RVD) consolidation with len maintenance (ACM), tandem autohct with len maintenance (TAM) and autohct with len maintenance (AM) for up-front treatment of patients with multiple myeloma (MM): primary results from the randomized phase III trial of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN 0702 – StaMINA trial) [abstract]. Blood 2016; 128: Abstract LBA-1.
129. Cavo M, Petrucci MT, Di Raimondo F, et al. Upfront single versus double autologous stem cell transplantation for newly diagnosed multiple myeloma: an intergroup, multicenter,

phase III study of the European Myeloma Network (EMN02/HO95 MM trial). Presented at the 58th Annual Meeting & Exposition; December 3–6, 2016; San Diego, California.

130. Auner HW, Szydlo R, Rone A, et al. Salvage autologous stem cell transplantation for multiple myeloma relapsing or progressing after up- front autologous transplantation. Leuk Lymphoma 2013; 54: 2200–2204.
131. Jimenez-Zepeda VH, Mikhael J, Winter A, et al. Second autologous stem cell transplantation as salvage therapy for multiple myeloma: Impact on progression-free and overall survival. Biol Blood Marrow Transplant 2012; 18: 773–779.
132. Sellner L, Heiss C, Benner A, et al. Autologous retransplantation for patients with recurrent multiple myeloma: A single-center experience with 200 patients. Cancer 2013; 119: 2438–2446.
133. Shah N, Ahmed F, Bashir Q, et al. Durable remission with salvage second autotransplants in patients with multiple myeloma. Cancer 2012; 118: 3549–3555.
134. Kyle RA. High-dose therapy in multiple myeloma and primary amyloidosis: an overview. Semin Oncol 1999; 26: 74–83.
135. Kumar A, Loughran T, Alsina M, et al. Management of multiple myeloma: a systematic review and critical appraisal of published studies. Lancet Oncol 2003; 4: 293–304.
136. Bruno B, Rotta M, Patriarca F, et al. A comparison of allografting with autografting for newly diagnosed myeloma. N Engl J Med 2007; 356: 1110–1120.
137. Rosinol L, Perez-Simon JA, Sureda A, et al. A prospective PETHEMA study of tandem autologous transplantation versus autograft followed by reduced-intensity conditioning allogeneic transplantation in newly diagnosed multiple myeloma. Blood 2008; 112: 3591–3593.
138. Garban F, Attal M, Michallet M, et al. Prospective comparison of autologous stem cell transplantation followed by dose-reduced allograft (IFM99-03 trial) with tandem autologous stem cell transplantation (IFM99-04 trial) in high-risk de novo multiple myeloma. Blood 2006; 107: 3474–3480.
139. Krishnan A, Pasquini MC, Logan B, et al. Autologous haemopoietic stem- cell transplantation followed by allogeneic or autologous haemopoietic stem-cell transplantation in patients with multiple myeloma (BMT CTN 0102): a phase 3 biological assignment trial. Lancet Oncol 2011; 12: 1195–1203.

140. Bjorkstrand B, Iacobelli S, Hegenbart U, et al. Tandem autologous/ reduced-intensity conditioning allogeneic stem-cell transplantation versus autologous transplantation in myeloma: long-term follow-up. J Clin Oncol 2011; 29: 3016–3022.
141. Maloney D, Molina A, Sahebi F, et al. Allografting with nonmyeloablative conditioning following cytoreductive autografts for the treatment of patients with multiple myeloma. Blood 2003; 102: 3447–3454.
142. Crawley C, Lalancette M, Szydlo R, et al. Outcomes for reduced-intensity allogeneic transplantation for multiple myeloma: an analysis of prognostic factors from the Chronic Leukaemia Working Party of the EBMT. Blood 2005; 105: 4532–4539.
143. de Lavallade H, El-Cheikh J, Faucher C, et al. Reduced-intensity conditioning allogeneic SCT as salvage treatment for relapsed multiple myeloma. Bone Marrow Transplant 2008; 41: 953–960.
144. Putkonen M, Kairisto V, Juvonen V, et al. Depth of response assessed by quantitative ASO-PCR predicts the outcome after stem cell transplantation in multiple myeloma. Eur J Haematol 2010; 85: 416–423.
145. Zeiser R, Bertz H, Spyridonidis A, et al. Donor lymphocyte infusions for multiple myeloma: clinical results and novel perspectives. Bone Marrow Transplant 2004; 34: 923–928.
146. van de Donk NW, Kroger N, Hegenbart U, et al. Prognostic factors for donor lymphocyte infusions following non-myeloablative allogeneic stem cell transplantation in multiple myeloma. Bone Marrow Transplant 2006; 37: 1135–1141.
147. Lokhorst HM, Wu K, Verdonck LF, et al. The occurrence of graft-versus-host disease is the major predictive factor for response to donor lymphocyte infusions in multiple myeloma. Blood 2004; 103: 4362–4364.
148. Lokhorst HM, Schattenberg A, Cornelissen JJ, et al. Donor lymphocyte infusions for relapsed multiple myeloma after allogeneic stem-cell transplantation: predictive factors for response and long-term outcome. J Clin Oncol 2000; 18: 3031–3037.
149. Lokhorst HM, Schattenberg A, Cornelissen JJ, et al. Donor leukocyte infusions are effective in relapsed multiple myeloma after allogeneic bone marrow transplantation. Blood 1997; 90: 4206–4211.
150. Salama M, Nevill T, Marcellus D, et al. Donor leukocyte infusions for multiple myeloma. Bone Marrow Transplant 2000; 26: 1179–1184.

151. Tricot G, Vesole DH, Jagannath S, et al. Graft-versus-myeloma effect: proof of principle. Blood 1996; 87: 1196–1198.
152. Ayuk F, Shimoni A, Nagler A, et al. Efficacy and toxicity of low-dose escalating donor lymphocyte infusion given after reduced intensity conditioning allograft for multiple myeloma. Leukemia 2004; 18: 659–662.
153. Paiva B, Vidriales MB, Cervero J, et al. Multiparameter flow cytometric remission is the most relevant prognostic factor for multiple myeloma patients who undergo autologous stem cell transplantation. Blood 2008; 112: 4017–4023.
154. Rawstron AC, Child JA, de Tute RM, et al. Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: impact on outcome in the Medical Research Council Myeloma IX Study. J Clin Oncol 2013; 31: 2540–2547.
155. Kneppers E, van der Holt B, Kersten MJ, et al. Lenalidomide maintenance following non-myeloablative allogeneic stem cell transplantation in multiple myeloma is not feasible: results of the HOVON 76 trial. Blood 2011; 118: 2413–2419.
156. Alsina M, Becker PS, Zhong X, et al. Lenalidomide maintenance for high- risk multiple myeloma after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2014; 20: 1183–1189.
157. Palumbo A, Hajek R, Delforge M, et al. Continuous lenalidomide treatment for newly diagnosed multiple myeloma. N Engl J Med 2012; 366: 1759–1769.
158. Singh PP, Kumar SK, LaPlant BR, et al. Lenalidomide maintenance therapy in multiple myeloma: a meta-analysis of randomized trials [asbtract]. Blood 2013; 22: Abstract 407.
159. Mellqvist UH, Gimsing P, Hjertner O, et al. Bortezomib consolidation after autologous stem cell transplantation in multiple myeloma: a Nordic Myeloma Study Group randomized phase 3 trial. Blood 2013; 121: 4647–4654.
160. Niesvizky R, Flinn IW, Rifkin RM, et al. Phase 3b UPFRONT study: safety and efficacy of weekly bortezomib maintenance therapy after bortezomib- based induction regimens in elderly, newly diagnosed multiple myeloma patients [abstract]. Blood 2010; 116: Abstract 619.
161. Mikhael JR, Belch AR, Prince HM, et al. High response rate to bortezomib with or without dexametasone in patients with relapsed or refractory multiple myeloma: result of a global phase 3b expanded access program. Br J Haematol 2009; 144: 169-175.
162. Jagannath S, Barlogie B, Beenson J, et al. A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br J Haematol 2004; 127: 165-172.

163. Jagannath S, Richardson PG, Barlogie B, et al. Bortezomib in combination with dexamethasone for the traetment of patients with relapsed and/or refractory multiple myeloma with less than optimal response to bortezomib alone. Haematologica 2006; 91: 929-934.
164. Dimopoulos M, Spencer A, Attal M, et al. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. N Engl J Med 2007; 357: 2123-2132.
165. Weber DM, Chen C, Niesvizky R, et al. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. N Engl J Med 2007; 357: 2133-2142.
166. Kropff M, Bisping G, Schuck E, et al. Bortezomib in combination with intermediate-dose dexamethasone and continuous low-dose oral cyclophosphamide for relapsed mutiple myeloma. Br J Haematol 2007; 138: 330-337.
167. Stewart AK, Rajkumar SV, Dimopoulos MA, et al. Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. N Engl J Med 2015; 372: 142-152.
168. Dimopoulos MA, Moreau P, Palumbo A, et al. Carfilzomib and dexamethasone versus bortezomib and dexamethasone for patients with relapsed or refractory multiple myeloma (ENDEAVOR): a randomised, phase 3, open-labeled, multicentre study. Lancet Oncol 2016; 17: 27-38
169. Gorgun G, Calabrese E, Soydan E, et al. Immunomodulatory effects of lenalidomide and pomalidomide on interaction of tumor and bone marrow accessory cells in multiple myeloma. Blood 2010; 116: 3227-3237.
170. Richardson PG, Siegel DS, Baz R, et al. Phase I study of pomalidomide MTD, safety and efficacy in patients with refractory multiple myeloma who have received lenalidomide and bortezomib. Blood 2012; 121: 1961-1967.
171. Richardson PG, Siegel DS, Vij R, et al. Randomized, open laber phase 1/2 study of pomalidomide (POM) alone or in combination with low-dose dexamethasone (LoDex) in patients (Pts) with relapsed and refractory multiple myeloma who have received prior treatment that includes lenalidomide (LEN) and bortezomib (BORT): phase 2 results. Blood 2011; 118: Abstract 634.
172. Richardson PG, Siegel DS, Vij R, et al. Pomalidomide alone or in combination with low-dose dexamethasone in relapsed and refractory multiple myeloma: a randomized phase 2 study. Blood 2014; 123: 1826-1832.
173. Dimopoulos MA, Lacy MQ, Moreau P, et al. Pomalidomide in combination with low-dose dexamethasone: demonstrates a significant progression free survival and overall survival

advantage, in relapsed/refractory MM: A phase 3, multicenter, randomized, open-label study. Blood 2012; 120: LBA-6.

174. San Miguel J, Weisel K, Moreau P, et al. Pomalidomide plus low-dose dexamethasone versus high-dose dexamethasone alone for patients with relapsed and refractory multiple myeloma (MM-003): a randomised, open-label, phase 3 trial. Lancet Oncol 2013; 14: 1055-1066.
175. Dimopoulos MA, Palumbo A, Weisel K, et al. Safety and efficacy in the stratus (MM-010) trial, a single-arm phase 3b study evaluating pomalidomide + low-dose dexamethasone in patients with refractory or relapsed and refractory multiple myeloma. Vol. 124; 2014: 80-80.
176. Leleu X, Attal M, Arnulf B, et al. Pomalidomide plus low dose dexamethasone is active and well tolerated in bortezomib and lenalidomide refractory multiple myeloma: IFM 2009-02. Blood 2013; 121: 1968-1975.
177. Hsi ED, Steinle R, Balasa B, et al. CS1, a potential new therapeutic antibody target for the treatment of multiple myeloma. Clin Cancer Res 2008; 14: 2775-2784.
178. Lonial S, Dimopoulos M, Palumbo A, et al. Elotuzumab therapy for relapsed or refractory multiple myeloma. N Engl J Med 2015; 373: 621-631.
179. Lonial S, Richardson PG, Mateos M-V, et al. ELOQUENT-2 update: Phase III study of elotuzumab plus lenalidomide/dexamethasone (ELd) vs Ld in relapsed/refractory multiple myeloma (RRMM) - Identifying responders by subset analysis. J Clin Oncol 2016;34 no. 15_suppl 8037.
180. Moreau P, Masszi T, Grzasko N, et al. Ixazomib, an investigational oral proteasome inhibitor (PI), in combination with lenalidomide and dexamethasone (IRd), significantly extends progression-free survival (PFS) for patients (Pts) with relapsed/refractory multiple myeloma (RRMM): The phase 3 Tourmaline-MM1 study. Blood 2015; 126: 727-727.
181. Lokhorst HM, Plesner T, Laubach JP, et al. Targeting CD38 with daratumumab monotherapy in multiple myeloma. N Engl J Med 2015; 373: 1207-1219.
182. Lonial S, Weiss BM, Usmani SZ, et al. Daratumumab monotherapy in patients with treatment-refractory multiple myeloma (SIRIUS): an open-label, randomised, phase 2 trial. Lancet 2016; 387: 1551-1560.
183. Lazzarino M, Corso A, Barbarano L, et al. DCEP (dexamethasone, cyclophosphamide, etoposide, and cisplatin) is an effective regimen for peripheral blood stem cell collection in multiple myeloma. Bone Marrow Transplant 2001; 28: 835-839.

184. Dimopoulos MA, Orio A, Nahi H, et al. Daratumumab, Lenalidomide, and Dexamethasone for Multiple Myeloma. New Engl J Med 2016, Oct 6; 375 (14): 1319-1331.
185. Orlowski R, Nagler A, Sonneveld P, et al. Randomized phase III study of pegylated liposomal doxorubicin plus bortezomib compared with bortezomib alone in relapsed or refractory multiple myeloma: combination therapy improves time to progression. J Clin Oncol 2007; 25: 3892-3901.
186. Knop S, Straka C, Haen M, et al. The efficacy and toxicity of bendamustine in recurrent multiple myeloma after high-dose chemotherapy. Haematologica 2005; 90: 1287-1288.
187. Michael M, Bruns I, Bolke E, et al. Bendamustine in patients with relapsed or refractory multiple myeloma. Eur J Med Red 2010; 15: 13-19.
188. Lentzsch S, O'Sullivan A, Kennedy RC, et al. Combination of bendamustine, lenalidomide, and dexamethasone (BLD) in patients with relapsed or refractory multiple myeloma is feasible and highly effective: results of phase 1/2 open-label, dose escalation study. Blood 2012; 119: 4608-4613.
189. Offidani M, Corvatta L, Maracci L, et al. Efficacy and tolerability of bendamustine, bortezomib and dexamethasone in patients with relapsed-refractory multiple myeloma: a phase II study. Blood Cancer J 2013; 3: e162.
190. Anderson KC, Jagannath S, Jakubowiak A, et al. Lenalidomide, bortezomib, and dexamethasone in relapsed/refractory multiple myeloma (MM): Encouraging outcomes and tolerability in a phase II study. J Clin Oncol 2009; 27: Abstract 8536.
191. Richardson PG, Jagannath S, Jakubowiak AJ, et al. Phase II trial of lenalidomide, bortezomib and dexamethasone in patients (pts) with relapsed and relapsed/refractory multiple myeloma (MM): updated efficacy and safety data after >2 years of follow-up. Blood 2010; 116: Abstract 3049.
192. Davies FE, Wu P, Jenner M, et al. The combination of cyclophosphamide, velcade and dexamethasone induces high response rates with comparable toxicity to velcade alone and velcade plus dexamethasone. Haematologica 2007; 92: 1149-1150.
193. Lacy MQ, LaPlant BR, Laumann KM, et al. Pomalidomide, bortezomib, and dexamethasone for patients with relapsed lenalidomide refractory multiple myeloma. 2014; Volume 124, Issue 21; Abstract 304.
194. Richardson PG, Hofmeister C, Raje NS, et al. A Phase 1, Multicenter study of pomalidomide, bortezomib, and low-dose dexamethasone in patients with proteasome

inhibitor exposed and lenalidomide-refractory myeloma (Trial MM-005). Blood 2015; 126: Abstract 3036.

195. Richardson P, Bensmaine A, Doerr T, et al. MM-007: A phase 3 trial comparing the efficacy and safety of pomalidomide (POM), bortezomib (BORT), and low-dose dexamethasone (LoDEX [PVD]) versus BORT and LoDEX (VD) in subjects with relapsed or refractory multiple myeloma (RRMM). J Clin Oncol 33, 2015 (suppl; Abstract TPS8610).
196. Shah JJ, Stadtmauer EA, Abonour R, et al. Carfilzomib, pomalidomide, and dexamethasone for relapsed or refractory myeloma. Blood 2015; 126: 2284-2290.
197. Rosenbaum CA, Stephens LA, Kukreti V, et al. Phase 1/2 study of carfilzomib, pomalidomide, and dexamethasone (KPd) in patients (Pts) with relapsed/refractory multiple myeloma (RRMM): A Multiple Myeloma Research Consortium multicenter study. ASCO Meeting Abstract 2016; 34: 8007.
198. Morgan GJ, Schey SA, Wu P, et al. Lenalidomide (Revlimid) in combination with cyclophosphamide and dexamethasone (RCD), is an effective and tolerated regimen for myeloma patients. Br J Haematol 2007; 137: 268-269.
199. Kumar SK, Bensinger WI, Zimmerman TM, et al. Phase 1 study of weekly dosing with the investigational oral proteasome inhibitor ixazomib in relapsed/refractory multiple myeloma. Blood 2014; 124: 1047-1055.
200. Richardson PG, Baz R, Wang M, et al. Phase 1 study of twice-weekly ixazomib, an oral proteasome inhibitor, in relapsed/refractory multiple myeloma patients. Blood 2014; 124: 1038-1046.
201. Kumar SK, LaPlant B, Roy V, et al. Phase 2 trial of ixazomib in patients with relapsed multiple myeloma not refractory to bortezomib. Blood Cancer J 2015; 5: e338.
202. Kumar SK, LaPlant B, Reeder CB, et al. Randomized phase 2 trial of two different doses of ixazomib in patients with relapsed multiple myeloma not refractory to bortezomib. Blood 2015; 126: 3050-3050.
203. Jakubowiak A, Offidani M, Pegourie B, et al. Randomized phase 2 study: elotuzumab plus bortezomib/dexamethasone vs bortezomib/dexamethasone for relapsed/refractory MM. Blood 2016; 127: 2833-2840.
204. Berdeja JG, Hart LL, Mace JR, et al. Phase I/II study of the combination of panobinostat and carfilzomib in patients with relapsed/refractory multiple myeloma. Haematologica 2015; 100: 670-676.

205. Berdeja JG, Gregory TK, Matous J, et al. Phase I/II study of the combination of panobinostat (PAN) and carfilzomib (CFZ) in patients (pts) with relapsed or relapsed/refractory multiple myeloma (MM). ASCO Meeting 2015; 33: Abstract 8513.
206. Atadja P. Development of the pan-DAC inhibitor panobinostat (LBH589): successes and challenges. Cancer Lett 2009; 280: 233-241.
207. San-Miguel JF, Hungria VT, Yoon SS, et al. Panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma: a multicentre, randomised, double-blind phase 3 trial. Lancet Oncol 2014; 15: 1195-1206.
208. Richardson PG, Schlossman RL, Alsina M, et al. PANORAMA 2: panobinostat in combination with bortezomib and dexamethasone in patients with relapsed and bortezomib-refractory myeloma. Blood 2013; 122: 2331-2337.
209. Baz RC, Martin TG, 3rd, Lin HY, et al. Randomized multicenter phase 2 study of pomalidomide, cyclophosphamide, and dexamethasone in relapsed refractory myeloma. Blood 2016; 127: 2561-2568.
210. Garderet L, Guelongo OOJ, Beohou E, et al. Pomalidomide, Cyclophosphamide and Dexamethasone for relapsed/refractory multiple myeloma: a retrospective single center experience. Poster presented at 57th Annual Meeting and Exposition of the American Society of Hematology (ASH); December 5-8, 2015.
211. Lenhard RE, Jr., Oken MM, Barnes JM, et al. High-dose cyclophosphamide. An effective treatment for advanced refractory multiple myeloma. Cancer 1984; 53: 1456-1460.
212. Romano A, Chiarenza A, Conticello C, et al. Salvage therapy with pegylatedliposomaldoxorubicin, bortezomib, cyclophosphamide, and dexamethasone in relapsed/refractory myeloma patients.Eur J Haematol. 2014 Sep; 93(3): 207-13. doi: 10.1111/ejh.12325. Epub 2014 Apr 18.
213. Cerchione C, Lucignano M, Pane F, Catalano L. Salvage therapy with pegylated liposomial doxorubicin-based regimen in relapsed/refractory multiple myeloma: comments to the article by Romano et al. Eur J Haematol. 2016 May; 96(5): 544.
214. Berenson JR, Lichtenstein A, Porter L, et al. Long-term pamidronate tr-eatment of advanced multiple myeloma patients reduces skeletal events. Myeloma Aredia Study Group. J Clin Oncol 1998; 16: 593–602.

215. Berenson JR, Lichtenstein A, Porter L, et al. Efficacy of pamidronate in reducing skeletal events in patients with advanced multiple myeloma. Myeloma Aredia Study Group. N Engl J Med 1996; 334 :488–493.
216. Major P, Lortholary A, Hon J, et al. Zoledronic acid is superior to pamidronate in the treatment of hypercalcemia of malignancy: a pooled analysis of two randomized, controlled clinical trials. J Clin Oncol 2001; 19: 558–567.
217. Zervas K, Verrou E, Teleioudis Z, et al. Incidence, risk factors and management of osteonecrosis of the jaw in patients with multiple myeloma: a single-centre experience in 303 patients. Br J Haematol 2006; 134: 620–623.
218. Morgan GJ, Davies FE, Gregory WM, et al. First-line treatment with zoledronic acid as compared with clodronic acid in multiple myeloma (MRC Myeloma IX): a randomised controlled trial. Lancet 2010; 376: 1989–1999.
219. Boyd K, Morgan G, Davies F, et al. Does zoledronic acid (ZOL) reduce skeletal-related events (SREs) and improve progression-free survival (PFS) in patients (Pts) with multiple myeloma (MM) with or without bone disease? MRC myeloma IX study results [abstract]. J Clin Oncol 2011; 29: Abstract 8010.
220. Morgan GJ, Davies F, Gregory W, et al. Defining the biological subgroup of multiple myeloma patients which benefits maximally from the overall survival (OS) benefit associated with treatment with zoledronic acid (ZOL) [abstract]. J Clin Oncol 2011; 29: Abstract 8083.
221. Morgan GJ, Davies FE, Gregory WM, et al. Long-term follow-up of MRC Myeloma IX trial: Survival outcomes with bisphosphonate and thalidomide treatment. Clin Cancer Res 2013; 19: 6030–6038.
222. Mhaskar R, Redzepovic J, Wheatley K, et al. Bisphosphonates in multiple myeloma: a network meta-analysis. Cochrane Database Syst Rev 2012;5:CD003188.
223. Hu K, Yahalom J. Radiotherapy in the management of plasma cell tumors. Oncology (Williston Park) 2000; 14: 101–108.
224. Major PP, Coleman RE. Zoledronic acid in the treatment of hypercalcemia of malignancy: results of the international clinical development program. Semin Oncol 2001; 28:17–24.
225. Pecherstorfer M, Steinhauer EU, Rizzoli R, et al. Efficacy and safety of ibandronate in the treatment of hypercalcemia of malignancy: a randomized multicentric comparison to pamidronate. Support Care Cancer 2003; 11: 539–547.

226. Lindsley H, Teller D, Noonan B, et al. Hyperviscosity syndrome in multiple myeloma. A reversible, concentration-dependent aggregation of the myeloma protein. Am J Med 1973; 54: 682–688.
227. Ludwig H, Fritz E, Kotzmann H, et al. Erythropoietin treatment of anemia associated with multiple myeloma. N Engl J Med 1990; 322: 1693–1699.
228. Osterborg A, Boogaerts MA, Cimino R, et al. Recombinant human erythropoietin in transfusion-dependent anemic patients with multiple myeloma and non-Hodgkin's lymphoma—a randomized multicenter study. The European Study Group of Erythropoietin (Epoetin Beta) Treatment in Multiple Myeloma and Non-Hodgkin's Lymphoma. Blood 1996; 87: 2675– 2682.
229. Ikhlaque N, Seshadri V, Kathula S, Baumann M. Efficacy of prophylactic warfarin for prevention of thalidomide-related deep venous thrombosis. Am J Hematol 2006; 81: 420–422.
230. Baz R, Li L, Kottke-Marchant K, et al. The role of aspirin in the prevention of thrombotic complications of thalidomide and anthracycline-based chemotherapy for multiple myeloma. Mayo Clin Proc 2005; 80: 1568–1574.
231. Cheson BD, Rummel MJ. Bendamustine: rebirth of an old drug. J. Clin. Oncol. 2009 Mar 20; 27(9): 1492-501.
232. Kalaycio M. Bendamustine: a new look at an old drug. Clin Cancer Res. 2008 Jan 1; 14(1): 309-17.
233. Leoni LM, Bailey B, Reifert J et al. Bendamustine (Treanda) displays a distinct pattern of cytotoxicity and unique mechanistic features compared with other alkylating agents. Clin Cancer Res. 2008 Jan 1; 14(1): 309-17.
234. Leoni LM, Hartley JA. Mechanism of action: the unique pattern of bendamustine-induced cytotoxicity. Semin Hematol. 2011 Apr; 48 Suppl 1: S12-23.
235. Cives M, Ciavarella S, Rizzo FM, De Matteo M, Dammacco F, Silvestris F. Bendamustine overcomes resistance to melphalan in myeloma cell lines by inducing cell death through mitotic catastrophe. Cell Signal. 2013 May; 25(5): 1108-17.
236. Surget S, Lemieux-Blanchard E, Maïga S et al. Bendamustine and melphalan kill myeloma cells similarly through reactive oxygen species production and activation of the p53 pathway and do not overcome resistance to each other. Leuk Lymphoma. 2014 Sep; 55(9): 2165-73.

237. Gentile M, Vigna E, Recchia AG et al. Bendamustine in multiple myeloma. Eur J Haematol. 2015 Nov; 95(5): 377-88.
238. Pönisch W, Mitrou PS, Merkle K et al. Treatment of bendamustine and prednisone in patients with newly diagnosed multiple myeloma results in superior complete response rate, prolonged time to treatment failure and improved quality of life compared to treatment with melphalan and prednisone--a randomized phase III study of the East German Study Group of Hematology and Oncology (OSHO). J Cancer Res Clin Oncol. 2006 Apr; 132(4): 205-12.
239. Lee SS, Suh C, Kim BS et al. Bortezomib, doxorubicin, and dexamethasone combination therapy followed by thalidomide and dexamethasone consolidation as a salvage treatment for relapsed or refractory multiple myeloma: analysis of efficacy and safety. Ann Hematol. 2010 Sep; 89(9): 905-12.
240. Terpos E, Kastritis E, Roussou M et al. The combination of bortezomib, melphalan, dexamethasone and intermittent thalidomide is an effective regimen for relapsed/refractory myeloma and is associated with improvement of abnormal bone metabolism and angiogenesis. Leukemia. 2008 Dec; 22(12): 2247-56.
241. Zhang S, Wang X, Chen L et al. Synergistic Activity of Bendamustine in Combination with Doxorubicin and Bortezomib in Multiple Myeloma Cells. Blood 2008 112: 5171.
242. Cerchione C, Nappi D, Di Perna M et al. Retreatment with Bendamustine-Bortezomib-Dexamethasone in a Patient with Relapsed/Refractory Multiple Myeloma.Case Rep Hematol 2016; 2016: 6745286.
243. Hrusovsky I, Heidtmann HH. Combination therapy of bortezomib with bendamustine in elderly patients with advanced multiple myeloma [abstract]. Blood. 2007; 110(11). Abstract 4851.
244. Pönisch W, Bourgeois M, Moll B et al. Combined bendamustine, prednisone and bortezomib (BPV) in patients with relapsed or refractory multiple myeloma. J Cancer Res Clin Oncol. 2013 Mar; 139(3): 499-508.
245. Ludwig H, Kasparu H, Leitgeb C et al. Bendamustine-bortezomib-dexamethasone is an active and well-tolerated regimen in patients with relapsed or refractory multiple myeloma. Blood. 2014 Feb 13; 123(7): 985-91.
246. Durie BG, Harousseau JL, Miguel JS et al. International uniform response criteria for multiple myeloma. Leukemia 2006 Sep; 20(9): 1467-73.

247. Fenk R, Michael M, Zohren F et al. Escalation therapy with bortezomib, dexamethasone and bendamustine for patients with relapsed or refractory multiple myeloma. Leuk Lymphoma. 2007 Dec; 48(12): 2345-51.
248. Berenson JR, Yellin O, Bessudo A et al. Phase I/II trial assessing bendamustine plus bortezomib combination therapy for the treatment of patients with relapsed or refractory multiple myeloma. Br J Haematol. 2013 Feb; 160(3): 321-30.
249. Rodon P, Hulin C, Pegourie B et al. Phase II study of bendamustine, bortezomib and dexamethasone as second-line treatment for elderly patients with multiple myeloma: the Intergroupe Francophone du Myelome 2009-01 trial. Haematologica. 2015 Feb; 100(2): e56-9.
250. Mateos MV, Oriol A, Rosiñol L et al. Bendamustine, bortezomib and prednisone for the treatment of patients with newly diagnosed multiple myeloma: results of a prospective phase 2 Spanish/PETHEMA trial. Haematologica. 2015 Aug; 100(8): 1096-102.
251. Kurtin S. Myeloid toxicity of cancer treatment. J Adv Pract Oncol. 2012 Jul;3(4): 209-24.
252. Aapro MS, Bohlius J, Cameron DA et al. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. Eur J Cancer. 2011 Jan; 47(1): 8-32.
253. Cerchione C, Catalano L, Peluso I et al. Managing neutropenia by pegfilgrastim in patients affected by relapsed/refractory multiple myeloma treated with bendamustine-bortezomib-dexamethasone. Support Care Cancer. 2016 Dec; 24(12): 4835-4837.

12. Publications in extenso

1. **Cerchione C**, Fabbricini R, Pane F, Luciano L.
Vitiligo-like lesions in an adult patient treated with Imatinib Mesylate
Leuk Res.2009 Aug; 33(8): e104-5.

2. Pane F, Quintarelli F, Esposito N, Izzo B, De Angelis B, Peluso A, Muccioli Casadei G, Cosenza M, **Cerchione C**, Camera A, Luciano L.
Acute and Chronic Ph+ Leukemias: differential leukemogenesis pathways translate into different clinical needs
Hematologica, Vol.94 Supplement n°4, October 2009.

3. **Cerchione C**, Catalano L, Cerciello G, Avilia S, Picardi M, Risitano AM, Pisano I, Alfinito F, Pane F.
Role of Lenalidomide in the management of myelodysplastic syndromes with del(5q) associated with pure red cell aplasia (PRCA)
Ann Hematol. 2015 Mar; 94(3): 531-4.

4. **Cerchione C**, Catalano L, Pareto AE, Picardi M, Pane F.
Pegfilgrastim in primary prophylaxis of febrile neutropenia during chemotherapy of relapsed and refractory multiple myeloma: a real-life experience
Support Care Cancer. 2015 Feb; 23(2): 301-2.

5. Fonti R, Pace L, **Cerchione C**, Catalano L, Salvatore B, De Luca S, Pane F, Salvatore M, Del Vecchio S.
18-FDG PET/CT, 99mTc-MIBI, and MRI in the prediction of outcome of patients with Multiple Myeloma: a comparative study
Clin Nucl Med 2015 Apr; 40(4): 303-8

6. **Cerchione C**, Lucignano M, Pane F, Catalano L.
Salvage therapy with pegylated liposomial doxorubicin-based regimen in relapsed/refractory multiple myeloma: comments to the article by Romano et al.
Eur J Haematol. 2016 May; 96(5): 544.

7. Cerchione C, Catalano L, Peluso I, Nappi D, Di Perna M, Salvatore D, Migliaccio I, Picardi M, Pane F.
Managing neutropenia by pegfilgrastim in patients affected by relapsed/refractory multiple myeloma treated with bendamustine-bortezomib-dexamethasone
Support Care Cancer. 2016 Dec; 24(12): 4835-4837.

8. **Cerchione C**, De Renzo A, Di Perna M, Della Pepa R, Pugliese N, Catalano L, Pane F, Picardi M.
Pegfilgrastim in primary prophylaxis of febrile neutropenia following front-line bendamustine plus rituximab treatment in patients with indolent non Hodgkin lymphoma: a single center, real-life experience
Support Care Cancer. 2017 Mar; 25(3): 839-845.

9. **Cerchione C**, Nappi D, Di Perna M, Zacheo I, Pareto AE, Picardi M, Catalano L, Pane F.
Retreatment with Bendamustine-Bortezomib-Dexamethasone in a patient with relapsed/refractory multiple myeloma
Case Rep Hematol. 2016; 2016: 6745286.

10. **Cerchione C**, Cerciello G, Avilia S, Della Pepa R, Pugliese N, Picardi M, Pane F, Catalano L.
Management of iron overload in myelodysplastic syndromes: combined deferasirox and deferoxamine in a patient with liver disease
Blood Transfus. 2016 Oct 24; 1-4.

11. Della Pepa R, Pagano L, **Cerchione C**, Pugliese N, Pane F, Picardi M.
Reply to the letter to the editor "chronic disseminated candidiasis" by Kenneth Rolston
Support Care Cancer. 2017 Apr; 25(4): 1045-1046.

12. Pugliese N, Di Perna M, Cozzolino I, Ciancia G, Pettinato G, Zeppa P, Varone V, Masone S, **Cerchione C**, Della Pepa R, Simeone L, Salvatore C, Pane F, Picardi M.
Randomized comparison of power Doppler ultrasonography-guided core-needle biopsy with open surgical biopsy for the characterization of lymphadenopathies in patients with suspected lymphoma
Ann Hematol. 2017 Apr; 96(4): 627-637.

13. **Cerchione C**, Peluso I, Nappi D, Pareto AE, Picardi M, Martinelli V, Pane F.
Ruxolitinib rechallenge in combination with hydroxyurea is effective in reverting cachexia and reducing blood transfusion demand and splenomegaly symptoms in a patient with primary myelofibrosis
Ann Hematol. 2017 Apr; 96(4): 697-699.

14. **Cerchione C**, Nappi D, Di Perna M, Zacheo I, Migliaccio I, Salvatore D, Picardi M, Pane F, Catalano L.
A case of efficacy of bendamustine in heavily pretreated multiple myeloma, refractory to pomalidomide
Clinical Case reports, Vol 5, Issue 4, April 2017 Pages 505-507.

15. **Cerchione C**, Maraolo AE, Marano L, Pugliese N, Nappi D, Tosone G, Cimmino I, Cozzolino I, Martinelli V, Pane F, Picardi M.
Secondary syphilis mimicking malignancy: A case report and review of literature
Journal of infection and chemotherapy - In press.

16. Della Pepa R, **Cerchione C**, Pugliese N, Colicchio R, Salvatore P, Sirignano C, Soscia E, Pagano L, Sanguinetti M, Pane F, Picardi M.
1,3-beta-D-glucan antigenemia and multiphasic contrast-enhanced computed tomography as crucial tools for early diagnostic chronic disseminated candidiasis in patients with acute leukemia: three case reports and review of literature
Supportive Care in Cancer - In press.

17. Pugliese N, Salvatore P, Iula VD, Catania MR, Chiurazzi F, Raimondo M, Beneduce G, **Cerchione C**, Della Pepa R, Giordano C, Simeone L, Pane F, Picardi M.
Ultrasonography-driven tigecycline-based antibiotic therapy significantly increases survival among patients with neutropenic enterocolitis following cytarabine-containing chemotherapy for the remission induction of acute myeloid leukemia
Cancer Medicine - In press.

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Letter to the Editor

Vitiligo-like lesions in an adult patient treated with Imatinib mesylate

1. Introduction

Imatinib mesylate (Gleveec) is at present the first line treatment for CML and GIST.

The best known side effects of Imatinib mesylate are periorbital edema, fluid retention, nausea, emesis, diarrhea and myelosuppression [1]. In addition, a number of dermatological side effects have been documented, such as a pruritic maculopapular exanthema, follicular mucinosis, erythroderma, graft-versus-host-like-disease, a mycosis fungoides-like reaction, small vessels vasculitis, generalized exanthematous pustolosis, Stevens–Johnson syndrome, a pityriasis rosea-like eruption, Sweet syndrome, and a lichenoid eruption. In some cases, it can also induce local or generalized hyperpigmentation, and hair repigmentation [2–4].

We describe here a case of CML patient treated with Imatinib who developed vitiligo-like lesions.

Vitiligo is an acquired progressive disorder that results in selective disappearance of epidermal and follicular melanocytes, leaving depigmented spots of the skin, with a consequent disfigurement and possible impact on the quality of life.

Indeed, patients who have face involvement abhor having two skin colours; they may accept depigmentation of the remaining pigmented skin as a reasonable treatment, when repigmentation of involved skin is not possible.

2. Case report

A 38-year-old Caucasian man was first seen in January 2006. Physical examination showed splenomegaly and hepatomegaly. A blood count showed leukocytes of 260.930 μL^{-1} with the presence of myeloid precursors at various stages of differentiation in peripheral blood.

Cytogenetic and molecular analyses showed the presence of the Ph chromosome and of the hybrid bcr/abl gene. With a diagnosis of chronic myeloid leukaemia, the patient was initially treated with hydroxyurea at conventional dosage, because of high white blood counts.

After 2 months, his leukocytes were 50,020 μL^{-1}, with reduced splenomegaly and hepatomegaly.

Imatinib at the dosage of 400 mg/day was introduced, and after 1 month the dosage was increased to 800 mg/day because the patient was enrolled in an Italian trial for high Sokal index patients.

1 month later, the patient developed diarrhea, muscular and joint pain, fluid retention and xerostomia. These side effects spontaneously disappeared.

After 6 months of treatment, he developed patches of hypopigmentation on his face and on his body.

A diagnosis of a vitiligo-like syndrome was made, leading to a 3 month local treatment with corticosteroids.

After 9 months of treatment the dosage was reduced to 400 mg/day for persistence of skin toxicity and other side effects (diarrhea, muscular and joint pain, fluid retention and xerostomia). With dose reduction, even the skin side effect disappeared rapidly.

In April 2008, at 26 months from diagnosis, a bone marrow aspirate showed loss of cytogenetic response, and Imatinib therapy was substituted with 2nd generation tyrosine kinase inhibitors.

3. Discussion

Imatinib is a selective inhibitor of several tyrosine kinases: it inhibits not only the tyrosine kinase BCR-ABL, but also platelet-derived growth factor receptors (PDGFRs) and c-KIT receptor tyrosine kinases [5]. It is thought that the side effects of Imatinib treatment is due to its action on other tyrosine kinases, especially on PDGFRs and c-KIT. Indeed, mutations in the c-kit gene have been associated with hypopigmentary disorders, such as vitiligo and piebaldism [6–9].

The inhibition of melanocyte c-kit receptor tyrosine kinase by imatinib could lead to generalized hypopigmentation [10]. It has been demonstrated that c-kit has an important role in melanogenesis, melanocyte homeostasis and violet B-induced skin depigmentation [11]. A prolonged c-kit inhibition may lead to melanocyte apoptosis, and this provides in vitro evidence of a critical role for SCF/KIT in the homeostasis and survival of human melanocytes [12].

The receptor KIT and its ligand SCF play a very important role not only in the development but also in the maintenance of human melanocytes. In fact, during embryogenesis, SCF and KIT direct melanoblast migration from the neural tube to the skin of the embryo and, in the post-natal period, maintain the survival of melanocytes [1,12].

Even if the exact mechanism by which this occurs is unclear, melanocyte homeostasis and differentiation also have been attributed to SCF/KIT pathway.

Imatinib could decrease skin pigmentation by two mechanisms: a direct one, through inhibition of tyrosine kinase activity, probably blocking the c-kit pathway, and an indirect one, decreasing the stem cell factor secretion, due to the inhibition of fibroblast proliferation [13].

In our case skin depigmentation was dose-related and reversible after Imatinib dose reduction. Hypopigmentation appeared only after high dose of Imatinib.

In conclusion, even a vitiligo-like lesion may be included among the side effects due to Imatinib treatment. Hypopigmentation can be considered as a marker for susceptibility of patients to drug toxicity, but its presence does not seem to influence the clinical outcome.

Fortunately, this side effect seems dose-related and reversible; dose reduction or change towards other tyrosine kinase inhibitors may reconvert the skin to a normal pigmentation.

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Conflict of interest

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References

[1] Tsao AS, Kantarjian H, Cortes J, et al. Imatinib mesylate causes hypopigmentation in the skin. Cancer 2003;98:2483–7.
[2] Alexandrescu DT, Dasanu CA, Farzanmehr H, Kauffman L. Persistent cutaneous hyperpigmentation after tyrosine kinase inhibition with imatinib for GIST. Dermatol Online J 2008;14:7.
[3] Ayirookuzhi SJ, Ma L, Ramshesh P, Mills G. Imatinib-induced Sweet syndrome in a patient with chronic myeloid leukemia. Arch Dermatol 2005;141:368–70.
[4] Pascual JC, Matarredona J, Miralles J, Conesa V, Borras-Blasco J. Oral and cutaneous lichenoid reaction secondary to imatinib: report of two cases. Int J Dermatol 2006;45:1471–3.
[5] Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ, et al. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. J Pharmacol Exp Ther 2000;295:139–45.
[6] Dippel E, Haas N, Grabbe J, Schadendorf D, Hamann K, Czarnetzki BM. Expression of the c-kit receptor in hypomelanosis: a comparative study between piebaldism, naevus depigmentosus and vitiligo. Br J Dermatol 1995;132:182–9.
[7] Grichnik JM, Burch JA, Burchette J, et al. The SCF-KIT pathway plays a critical role in the control of normal human melanocyte homeostasis. J Invest Dermatol 1998;111:233–8.
[8] Nomura K, Hatayama I, Narita T, Kaneko T, Shiraishi M. A novel KIT gene missense mutation in a Japanese family with piebaldism. J Invest Dermatol 1998;111:337–8.
[9] Richards KA, Fukai K, Oiso N, Paller AS. A novel KIT mutation results in piebaldism with progressive depigmentation. J Am Acad Dermatol 2001;44:288–92.
[10] Sharma A, Vora A, Bhutani M. Generalized hypopigmentation due to imatinib: a fairness boon? Indian J Dermatol Venereol Leprol 2005;71:45–6.
[11] McPartlin S, Leach M. Loss of skin pigment caused by imatinib therapy. Br J Haematol 2005;129:448.
[12] Longley BJ, Carter El. SCF-KIT pathway in human epidermal melanocyte homeostasis. J Invest Dermatol 1999;113:139–40.
[13] Cario-André M, Ardilouze L, Pain C, Gauthier Y, Mahon FX, Taieb A. Imatinib mesilate inhibits melanogenesis in vitro. Br J Dermatol 2006;155:493–4.

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tissues: report of clinical advisory committee meeting – Airlie House,Virginia, November 1997.J Clin Oncol 1999;17:3835–49
5. Malcovati L, Germing U, Kuendgen A, et al. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol. 2007 10;25(23):3503-10
6. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. International Vidaza High-Risk MDS Survival Study Group. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009;10(3): 223-32.
7. de Witte T, Hermans J, Vossen J, Bacigalupo A, et al. Haematopoietic stem cell transplantation for patients with myelodysplastic syndromes and secondary acute myeloid leukaemias: A report on behalf of the Chronic Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Br J Haematol 2000;110:620-30.
8. Sierra J, Perez WS, Rozman C ,et al. Bone marrow transplantation from HLA-identical siblings as treatment for myelodysplasia. Blood 2002;100:1997–2004
9. Alessandrino EP, Della Porta MG, Bacigalupo A, et al. WHO classification and WPSS predict posttransplantation outcome in patients with myelodysplastic syndrome: a study from the Gruppo Italiano Trapianto di Midollo Osseo (GITMO) Blood 2008;112:895-902
10. Castro-Malaspina H, Harris RE, Gajewski J, et al. Unrelated donor marrow transplantation for myelodysplastic syndromes: outcome analysis in 510 transplants facilitated by the National Marrow Donor Program. Blood 2002;99:1943–1951.
11. Runde V, de Witte T, Arnold R, Gratwohl A, et al. Bone marrow transplantation from HLA-identical siblings as first-line treatment in patients with myelodysplastic syndromes: early transplantation is associated with improved outcome. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation Bone Marrow Transplant 1998;21(3):255-61
12. de Witte T, Brand R, van Biezen A, Mufti G, Ruutu T, Finke J, et al; Allogeneic stem cell transplantation for patients with refractory anaemia with matched related and unrelated donors: delay of the transplant is associated with inferior survival, the European Blood and Marrow Transplantation Group (EBMT), Chronic Leukemia Working Party (CLWP)-MDS subcommittee. Br J Haematol. 2009 Jul 14. [Epub ahead of print]
13. Cutler CS, Lee S, Greenberg P, Deeg J, Perez WS, Anasetti C, et al. A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved out come. Blood 2004;104:579–85.
14. Della Porta MG, Malcovati L, Boveri E, Travaglino E, Pietra D, Pascutto C, et al. Clinical relevance of bone marrow fibrosis and CD34-positive cell clusters in primary myelodysplastic syndromes. J Clin Oncol. 2009;27(5):754-62. Epub 2008 Dec 22
15. E P Alessandrino, MG Della Porta, A. Bacigalupo, et al. Prognostic impact of pretransplantation transfusion history and secondary iron overload in patients with myelodysplastic syndrome undergoing allogeneic stem cell transplantation: a study from the Gruppo Italiano Trapianto di Midollo Osseo. Haematologica (accepted for publication: 1-09-2009).
16. Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Alyea EP, et al. Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem cell transplantation. Blood 2007; 109:4586-8.
17. De Lima M, Couriel D, Thall PF, et al. Once-daily intravenous busulfan and ?udarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. Blood 2004;104:857–64.
18. De Lima M, Anagnostopoulos A, Munsell M, et al. Non-ablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. Blood 2004;04: 865–72
19. Scott BL, Storer B, Loken MR, et al. Pretransplantation induction chemotherapy and posttransplantation relapse in patients with advanced myelodysplastic syndrome. Biology of Blood and Marrow Transplantation 2005;11:65–73.
20. Nakai K, Kanda Y, Fukuhara S, Sakamaki H, Okamoto S, Kodera Y, et al. Value of chemotherapy before allogeneic hemopoietic stem cell transplantation from an HLA identical sibling donor for myelodysplastic syndrome. Leukemia 2005;19:396-401.
21. Kröger N. Epigenetic modulation and other options to improve outcome of stem cell transplantation in MDS. Hematology Am Soc Hematol Educ Program. 2008:60-7. Review.
22. Anderson JE, Appelbaum FR, Schoch G, et al. Allogeneic marrow transplantation for refractory anemia: a comparison of two preparative regimens and analysis of prognostic factors. Blood 1996;87:51-8
23. Deeg HJ, Shulman HM, Anderson JE, Bryant EM, Gooley TA, Slattery JT, et al: Allogeneic and syngeneic marrow transplantation for myelodysplastic syndrome in patients 55 to 66 years of age. Blood 2000;95:1188-94
24. Deeg HJ, Storer B, Slattery JT, et al. Conditioning with targeted Busulfan and cyclophosphamide for hemopoietic stem cell transplantation from related and unrelated donors in patients with myelodysplastic syndrome. Blood 2002;100:1201–7
25. Alessandrino E.P., Malcovati L., La Nasa G, et al. for GITMO. Reduced Intensity Conditioning with Thiotepa and Fludarabine for Allogeneic Transplantation: Evidence for Low Toxicity and Long-Lasting Disease Control in MDS with Low/Intermediate-1 IPSS Score and in AML from MDS in Complete Remission. Blood (ASH Annual Meeting Abstracts), Nov 2008; 112: 3285
26. Castro-Malaspina H, Jabubowski AA, Papadopoulos EB, Boulad F, Young JW, Kernan NA, et al. Transplantation in remission improves the disease-free survival of patients with advanced myelodysplastic syndromes treated with myeloablative T cell-depleted stem cell transplants from HLA-identical siblings. Biol Blood Marrow Transplant. 2008;14(4):458-68.
27. Russell JA, Tran HT, Quinlan D, et al. Once-daily intravenous busulfan given with ?udarabine as conditioning for allogeneic stem cell transplantation: study of pharmacokinetics and early clinical outcomes. Biology of Blood and Marrow Transplantation 2002; 8: 468–47
28. Ho AY, Pagliuca A, Kenyon M, et al. Reduced-intensity allogeneic hematopoietic stem cell transplantation for myelodysplastic syndrome and acute myeloid leukemia with multilineage dysplasia using ?udarabine, busulphan, and alemtuzumab (FBC) conditioning. Blood 2004;104: 1616–23.
29. Schmid C, Schleuning M, Ledderose G, Tischer J, Kolb HJ. Sequential regimen of chemotherapy, reduced-intensity conditioning for allogeneic stem-cell transplantation, and prophylactic donor lymphocyte transfusion in high-risk acute myeloid leukemia and myelodysplastic syndrome. J Clin Oncol. 2005;23(24):5675-87
30. Martino R, Iacobelli S, Brand R, et al. Retrospective comparison of reduced-intensity conditioning and conventional high-dose conditioning for allogeneic hematopoietic stem cell transplantation using HLA-identical sibling donors in myelodysplastic syndromes. Blood 2006;108: 836–46
31. Scott BL, Sandmaier BM, Storer B, et al. Myeloablative vs nonmyeloablative allogeneic transplantation for patients with myelodysplastic syndrome or acute myelogenous leukemia with multilineage dysplasia: a retrospective analysis. Leukemia 2006;20:128–13
32. Luger S, Ringdem O, Perez SW, et al. Similar outcomes using myeloablative versus reduced intensity and non-myeloablative allogeneic transplant preparative regimens for AML OR MDS: From the center for international blood and marrow transplant research. Blood (ASH annual meeting abstrac) 2008; 112; 348.
33. Warlick ED, Cioc A, Defor T, Dolan M, Weisdorf D. Allogeneic stem cell transplantation for adults with myelodysplastic syndromes: importance of pretransplant disease burden.Biol Blood Marrow Transplant. 2009;15(1):30-8
34. Ooi J: The ef?cacy of unrelated cord blood transplantation for adult myelodysplastic syndrome. Leuk Lymphoma 2006;47:599-602.
35. Popat U, de Lima M, Activitavas T, Rondon G, de Padua S, Koca E, et al. Allogeneic Transplantation (HCT) for Myelodysplastic Syndrome: Recent MDACC Experience. Blood (ASH Annual Meeting Abstracts) 2008;112:1131
36. Ruutu T, Volin V, Beelen D, Trenschel R, et al. Reduced Toxicity Conditioning with Treosulfan and Fludarabine in Allogeneic Hematopoietic Stem Cell Transplantation for Myelodysplastic Syndromes: Results of an International Prospective Phase II Trial.
37. Kröger N, Bornhäuser M, Ehninger G, et al. for the German Cooperative Transplant Study Group. Allogeneic stem cell transplantation after a fludarabine/busulfan-based reduced-intensity conditioning in patients with myelodysplastic syndrome or secondary acute myeloid leukemia. Ann Hematol. 2003;82(6):336-42
38. Lim ZY, Ho AY, Ingram W, Kenyon M, et al. Outcomes of alemtuzumab-based reduced intensity conditioning stem cell transplantation using unrelated donors for myelodysplastic syndrome. Br J Haematol. 2006;135(2):201-9

ACUTE AND CHRONIC PH+ LEUKEMIAS: DIFFERENTIAL LEUKEMOGENESIS PATHWAYS TRANSLATE INTO DIFFERENT CLINICAL NEEDS

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Philadelphia (Ph) chromosome - a balanced, reciprocal translocation involving the long arms of chromosomes 9 and 22 is the hallmark of two types of leukemias. Virtually 100% chronic myeloid leukemia (CML) patients are characterized by the presence of Ph chromosome or, in patients with complex or masked translocations and consequently lack the classical Ph chromosome, by the BCR/ABL gene. The Ph chromosomal aberration is not restricted to the CML, but a sizeable subset of acute lymphoid leukemia (ALL) patients may have this abnormality. Hence, despite the presence of a consistent genetic abnormality Ph leukemias display considerable clinical and hematological heterogeneity, the basis of which is only partially understood.

For various reasons, CML is, between the two types of Ph leukemias, probably one of the most comprehensively studied human malignancies. CML was the first human cancer to be associated with a consistent chro-

mosomal abnormality - the Ph chromosome. CML is characterized by distinct clinical phases: most patients present in chronic phase (CP), a phase in which mature granulocytes are still produced, but patients have an increased number of myeloid progenitor cells in the peripheral blood. As the disease progresses, patients enter an accelerated phase (AP) followed by blast crisis (BC), in which hematopoietic differentiation has become arrested and immature blasts accumulate in the bone marrow (BM) and spill into the circulation. The CP is relatively long-lasting, so researchers have the opportunity to study malignant cells with an 'indolent' behavior and to identify the changes associated with transformation to the 'aggressive' phenotype of blast crisis. Furthermore, CML is unusual in that a single genetic lesion occurring in a hematopoietic stem cell generates a fusion oncogene, BCR-ABL, which encodes a protein tyrosine kinase that is necessary and sufficient for cell transformation. The cytoplasmic location of the BCR-ABL oncoprotein allows access to many cellular substrates that are unavailable to the predominantly nuclear ABL protein, determining their phosphorylation and therefore the activation of proliferation and survival pathways. Finally, CML was the first hematological malignancy for which a program of rational drug design yielded an effective targeted molecular therapy (imatinib mesylate), that is now considered the precursor of a new family of anticancer drugs, the tyrosine-kinase inhibitors (TKIs).

The introduction of TKIs for the treatment of chronic myeloid leukaemia has had a profound and beneficial effect on this disease, which previously had a median survival of 5–7 years. Imatinib moved rapidly from phase I and II trials to a phase III randomised controlled trial (International Randomised Study of Interferon versus ST1571 [IRIS]) in which it was compared with interferon alfa plus cytosine arabinoside (IFN-ara-C). Complete haematological responses and complete cytogenetic remissions were 95% and 94%, respectively, for imatinib, compared with 55% and 8.5% for IFN-ara-C. Progression-free survival at 18 months was 96.7% for imatinib compared with 91.5% for IFN-ara-C. These early findings led to accelerated regulatory approval of imatinib for all phases of CML and the drug became first-line treatment for most patients. At 60 months, the estimated rate of event-free survival was 83% in imatinib recipients, and 93% had not progressed to accelerated/blast-phase CML. The efficacy of imatinib has been confirmed in a recent intention-to-treat analysis of 204 consecutive patients with newly diagnosed CML-CP who received standard-dose imatinib for a median of 38 months, most of them outside of the IRIS trial setting. Follow-up was available for all patients, giving a cumulative CCyR rate of 77% and a projected five-year event-free survival rate of 81% (de Lavallade et al, J Clin Oncol 2008) .

Imatinib also induces responses in a significant percentage of patients with AP or BP CML, although these tend to be transient. In phase II studies in patients with AP (600 mg/day) or BP (majority of patients [86%] received 600 mg/day), sustained CHR rates were 37% and 15%, MCyR rates were 28% and 16%, and CCyR rates were 19% and 7%, respectively. These clinical results support the notion that BCR-ABL must be important for the continued maintenance of the neoplastic phenotype, even in the advanced phase, and that a selection pressure favors the continued activity of the oncoprotein. In fact, increased BCR-ABL expression is likely to contribute to the phenotype of advanced phase disease, as studies using cell line models of CP and BC CML indicate that the oncoprotein exerts dose-dependent effects on growth factor dependence, clonogenicity, migration and the rate at which cells develop resistance to imatinib. Other factors, however, that could affect differentiation arrest and the inappropriate reactivation of self-renewal capacity are important in disease evolution in CML. CML is a good example of a cancer in which the transition from mature, terminally differentiated cells to immature, undifferentiated cells can be observed in the malignant clone. This differentiation arrest implies pathological interference with differentiation 'programmes' involving the targeted activation of tissue-specific genes by transcription factors. Such interference may be activated by oncogene products, as has been demonstrated for the suppression of the transcription factor CEBP alfa by BCR-ABL, or by mutations or gene translocations that result in the formation of dominant-negative transcription factors, such as AML1–EVI1 or NUP98–HOXA9 fusion genes, which have been described in a few isolated cases of myeloid BC. Another aspect of the maturation arrest in BC is the question of whether the transformed subclone originates from a cell that is at a distinct differentiation stage from that which gives rise to CP. Thus, disease progression in CML may originate in more committed precursors than had been previously supposed, as myeloid BC has been reported to involve the granulocyte-macrophage progenitor (GMP) 'pool' rather than the haematopoietic stem cell pool. The self-renewal of GMPs requires the activation of the _-catenin pathway.

One of the most remarkable and intriguing results of the treatment of CML in chronic phase with imatinib is the low rate of progression to advanced phase or BC (2% at 5 years follow-up) in patients who achieve and maintain a complete cytogenetic response within the first 12–18 months after starting the drug treatment. The biological basis of this phenomenon is unknown, but is a matter of great interest and speculation. Genomic instability may be based on alterations of the mechanisms involved in genome surveying for DNA damages and of those responsible for repairing these lesions; it is likely that similar failures of genome surveillance and DNA repair may contribute to the genomic instability of all human cancers. It has been proposed that BCR-ABL induces mutations in genes responsible for maintaining genomic integrity, and that such mutations function as "amplifiers of a genetically unstable phenotype". This could explain the occurrence of the non-random chromosomal abnormalities that characterize CML progression. The most frequent are trisomy 8 (33%), an additional Ph chromosome (30%), isochromosome 17 (20%), trisomy 19 (12%), loss of the Y chromosome (8% of males), trisomy 21 (7%) and monosomy 7 (5%)51. These changes have been used as markers of disease progression, but may not necessarily be causal agents of transformation.

As BCR-ABL increases the level of genomic instability, continuous inhibition of its kinase activity by imatinib should lead to a decreased risk of mutations in general, including in genes that can trigger the blast crisis process. Moreover, although imatinib may be unable to kill the leukemic stem cell, it may drastically reduce its rate of proliferation and self-renewal, driving it to a deep and prolonged quiescence where the chances of DNA breaks and mis-repair are lower in the absence of DNA replication. Another possible mechanism relies on the evidence that the cell of origin of BC may not be a 'true' stem cell, but rather a more committed granulocyte-macrophage progenitor (GMP), and it has been shown that imatinib can eliminate a large proportion of these cells, reducing the population at risk of blastic transformation. An additional aspect of the 5-year clinical trial follow-up study was the observation that the rate of disease progression is not only low, but seems to decrease with time under successful treatment, and the reasons for this are not entirely clear. As the emergence of a subclone of leukemic cells with mutations in the kinase domain of BCR-ABL is the main cause of relapse in patients treated with imatinib, it is reasonable to suppose that the chances of this happening at any time during treatment depend largely on the size of the mutant sub-clone at the start of therapy and on its proliferation rate. Therefore, assuming that both the original (non-mutated) and the mutant BCR-ABL clones have a similar doubling time, it could be predicted that the highest risk for a mutant clone to become dominant and lead to relapse and disease progression occurs within the first years of therapy. A longer follow-up of chronic phase patients treated up-front with imatinib should confirm whether such a trend for a continuous decrease in the risk of disease evolution is statistically significant.

Close molecular monitoring of the blood of patients treated with imatinib or other TKIs demonstrated that a 3- to 4-log reduction in BCR-ABL expression strongly correlated with the ability to achieve long-term remissions. However, most of the patients have low-level persistent BCR-ABL transcripts and relapse on discontinuation of the drug, thus indicating that treatment spares a Ph cell sub-fraction with long term repopulating capability, that may be able to originate the progression of disease. Human cells with long-term engraftment potential were enriched for CD34+ primitive HCS characterized by CD90 expression. Closer analysis of transplantable and long-term culture initiating Ph+ cell subpopulations of CD34+ cells demonstrated predominance of quiescent G0 cells. Phenotypically primitive CD34+CD38- cells from the long-term culture initiating cells of chronic phase CML patients had a propensity to differentiate along the myeloid lineage on long-term engraftment in immunocompromised mice and probably constitute the leukemia-initiating cells thought to be resistant to chemotherapy and targeted therapy. Some studies indicate that specific molecular factors such as promyelocytic leukaemia protein (PML) tumour suppressor protein may have a critical role in haematopoietic stem cell maintenance of CML patients. More recently, it has been shown that constitutively active Smoothened, an essential component of the of Hedgehog (Hh) signalling pathway, augments CML stem cell number. Therefore, Hh pathway activity is required for maintenance of normal and neoplastic stem

cells of the haematopoietic system and raise the possibility that the drug resistance and disease recurrence after imatinib treatment of CML might be avoided by targeting this essential stem cell maintenance pathway. As already mentioned, the more common causes of TKI resistance have been clonal evolution present in up to a quarter of patients on disease progression and mutations of the BCR-ABL. The cells harboring previously mentioned resistance mechanisms are less likely to arise in early chronic phase of the disease and may have preexisted at a more advanced diagnostic stage, but more potent TKIs failed to act on the signaling or the behavior of these CML progenitors. Taken together, these observations suggest that new strategies will be needed to eliminate CML.

The Ph chromosome encodes defines a subgroup of ALL with a particularly unfavorable prognosis. The reasons for the aggressive nature of Ph ALL are still under investigation and have not yet been elucidated. In a large survey recently completed by the GIMEMA group on a large cohort of acute Ph leukemias, 75% of ALL patients and 66% of lymphoid blast crisis CML patients, but none of the patients with myeloid blast crisis, showed homozygous or heterozygous deletions in the IKZF1 gene. IKZF1 encodes the Ikaros protein, a ZnF transcription factor, required for lymphoid lineage differentiation, proliferation, and function. Ikaros contains two separate regions with Zinc Finger domains. Isoforms that lack the N-terminal ZnFs are unable to bind transcriptional targets normally but retain the Carboxy-terminal ZnFs and the ability to dimerise and act as dominant negative inhibitors of Ikaros function. Ikaros transgenic and mutant mouse models have clearly demonstrated the important role of Ikaros in both normal hematopoiesis and tumor suppression. An elevated frequency of genomic aberrations could be directly caused by an abnormally high incidence of DNA double-strand breaks. In normal cells, DNA lesions are detected and repaired by sophisticated physiologic machinery and a system of cell cycle checkpoints, preventing cells that have sustained DNA damage from proliferating further. In Ph cells from ALL patients, however, the already mentioned role of the BCR-ABL oncoprotein to promote DNA damages may be one of the factors involved in the onset of these deletions at the IKZF1 gene. However, sophisticated DNA analysis along the breakpoint cluster regions suggesting that IKZF1 deletions could arise from aberrant RAG-mediated recombination. Consistent with this hypothesis, cells from ALL or lymphoid blast crisis patients, but not those from chronic phase CML patients, were found to contain high levels of activation-induced cytidine deaminase (AID). Indeed, ALL cells are derived from B cell precursors in most cases and typically carry rearranged immunoglobulin heavy chain (IGH) variable (V) region genes devoid of somatic mutations. Somatic hypermutation is restricted to mature germinal center B cells and depends on AID. It was also demonstrated that AID expression in CML cells promotes overall genetic instability by hypermutation of tumor suppressor (including IKZF1) and DNA repair genes. Importantly, these finndings uncover a causative role of AID activity in the acquisition of BCR-ABL mutations leading to Imatinib resistance, thus providing a rationale for the rapid development of drug resistance and disease progression in ALL and lymphoid blast crisis patients.

In conclusion, therapeutic development in CML represents a success story for modern medicine. Patients diagnosed with CML today are expected to have a substantially longer survival than patients diagnosed 20 or even 10 years ago. Despite this, disease eradication and further improvements in prognosis of CML remain high on the agenda. On the other hand, treatment of the advanced phase of Ph leukemias including ALL still remain problematic despite the central role of BCR/ABL oncoprotein in the pathogenesis and the possibility to target its activity by first and second generation TKIs. However, additional factors play important roles in sustaining viability and growth of the Ph cells in the advanced phases and causing the observed rapid loss of TKI treatment response in these patients. Hopefully, the new molecular findings might open the way to the discovery of new and more effective treatment strategies also for these patients.

MYELODISPLASTIC SYNDROMES/MYELOPROLIFERATIVE NEOPLASMS: FROM BIOLOGY TO CLINICAL ASPECTS

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Myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPNs) are rare myeloid malignancies characterized by the presence of both dysplastic and proliferative features [Table 1]. They have been recognized as a separate group of diseases for the first time in 2001 by the WHO classification of Tumors of the Hematopoietic and Lymphoid Tissues.[1] This new category incorporated chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), atypical chronic myeloid leukemia, *BCR/ABL1*-negative (aCML), together with the less well defined unclassifiable forms of MDS/MPNs (MDS/MPN-U), including refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) as a provisional entity.

Because no significant progresses have been made over the last few years in understanding molecular pathogenesis of MDS/MPNs, the 2008 revision of the WHO classification of myeloid neoplasms contained little changes with regard to this disease category.[2] In particular, some cases of CMML with eosinophilia were relocated to the "myeloid/lymphoid neoplasms with eosinophilia and platelet-derived growth factor receptor-, (PDGFRB) rearrangement" category, while for RARS-T the platelet threshold to define thrombocytosis was lowered from 600 to 450 x 10^9/L and the presence of proliferating large megakaryocytes in the marrow resembling those in ET or PMF was added as a required diagnostic criteria.

Taken together, MDS/MPNs represent disease entities with high heterogeneity of clinical and hematologic features, varying from predominantly myelodysplastic to predominantly myeloproliferative forms, which entail different prognosis and demand different medical management. As it has been well expounded in a recent review by Orazi and Germing, the lack of known distinctive genetic features brings about the diagnosis of specific MDS/MPN subtypes to be ascertained by the integration of bone marrow and peripheral blood morphology with other laboratory and clinical findings.[3]

Among MDS/MPNs, CMML and JMML represent the main disorders, respectively, in elderly and in childhood age.

Chronic myelomonocytic leukemia (CMML)

The WHO classification did not make any significant changes in the original FAB criteria for the diagnosis of CMML, which are listed in Table 1. In addition, because in this disease entity a higher proportion of blasts has always been unanimously recognized as being associated to a more unfavorable prognosis, the WHO separated CMML into 2 prognostic subcategories, CMML-1 and CMML-2, depending on the number of blasts in the blood and bone marrow (Table 1).[1]

Having classified CMML among MDS/MPNs, the WHO virtually abolished the distinction between a "dysplastic" (MD-CMML) and a "proliferative" (MP-CMML) variant of the disease, originally proposed by the FAB group in 1994 on the basis of the arbitrary chosen threshold of 13x10^9/L WBC in the blood4. Nonetheless, individual patients necessitate different treatments according to the clinically predominating dysplastic or proliferative manifestations; moreover, MP-CMML are most often related to aberrancies in the RAS/MAPK signalling pathways or, in a minority of cases, to the presence of the $JAK2^{V617F}$ activating mutation, whereas MD-CMML are characterized by a higher frequency of cytogenetic abnormalities. Therefore, even though different etiology and pathogenesis have not been demonstrated so far, from a practical point of view the MD- versus MP- distinction represents a meaningful tool both for clinical management and for further scientific investigations.

The natural course of CMML is highly heterogeneous, with patient life expectancy varying from a few months to numerous years. Besides marrow blasts, several disease- and patient-related variables have been recognized as having significant association with survival length in various retrospective studies, and a few scoring systems have been proposed to estimate the risk of death in patients with CMML. The MD Anderson Prognostic Score (MDAPS), which was developed from the analysis of a large cohort of well-defined patients with CMML, included hemoglobin <12 g/dL, the presence of circulating immature WBCs, absolute blood

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LETTER TO THE EDITOR

Role of lenalidomide in the management of myelodysplastic syndromes with del(5q) associated with pure red cell aplasia (PRCA)

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Dear Editor,

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders of haematopoietic stem cell disorders, characterized by ineffective haematopoiesis that results in peripheral cytopenias and hypercellular bone marrow, with increased propensity to progression into acute myeloid leukaemia (AML) [1].

In a minority of cases, very few (less than 5 %) erythroid precursors are found, whereas the other lineages maintain their dysplastic characteristics. This entity is called MDS associated with pure red cell aplasia (PRCA).

MDS associated with PRCA is a rare condition characterized by severe anaemia, transfusion dependence, reticulocytopenia, reduction of erythroid precursors and multilineage dysplasia [2]. In PRCA, erythroid precursors are nearly absent, while megakaryocytes and granulocytic precursors are usually present at normal levels [3]. Damage of erythroid progenitors appears to be immune mediated; in about 10 % of cases, AML may finally develop. Conventional immunotherapy is ineffective while alemtuzumab combined with cyclosporine A (CyA) seems to be a valid choice [4].

Lenalidomide is an immunomodulatory drug (IMiD), which is well established and approved in the treatment of 5q-myelodysplastic syndromes (MDS). The mode of action includes immune modulation, anti-angiogenetic, anti-inflammatory and anti-proliferative effects. It could be useful to investigate its role in the treatment of PRCA/MDS.

In a large series of 360 patients with MDS, 1.6 % of them were found to have a PRCA [5]. At least 25 cases of MDS with PRCA have been described until today, and 5 of them were associated with del (5) (q14q34) [6]. WHO classification in 2008 has defined MDS with isolated del (5q) as a syndrome characterized by bone marrow blast count <5 %, isolated del (5q) and absence of Auer rods [7]. We report three cases of severe transfusion-dependent macrocytic anaemia with low reticulocyte counts (<1 %) in which del (5) (q14q34) was associated with erythroblastopenia and myelodysplasia (Table 1).

A 61-year-old Caucasian man, with a diagnosis of MDS (refractory anaemia, RA) non-responsive to erythropoiesis-stimulating agents (ESAs) treatment, regularly transfused with 4 blood packages/month, received diagnosis of PRCA and underwent 12 cycles of alemtuzumab+CyA. During 3 years of treatment, transient remissions from transfusion dependence were followed by relapses. During the third year of follow-up, del (5q) was found in bone marrow, with marked dysplastic features: lenalidomide was started, but after a few months, AML emerged with fatal evolution. Evolution towards AML and acquisition of chromosomal abnormalities could have been induced by immunosuppressive therapy, and, in this case, the 5q abnormality might be less responsive to lenalidomide (Fig. 1).

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Table 1 Characteristics of patients with diagnosis of MDS associated with PRCA

Sex/age	Hb	Blood/month (units)	ELC (%)	KD	Cytogenetic alterations	Therapies	Best response	TI
F/35	5.2	2	6	46 (XX)	del 5q (+24 months from diagnosis)	Corticosteroids–alemtuzumab+cyclosporine–lenalidomide	Lenalidomide	2nd course
M/61	5.5	4	8	46 (XY)	del 5q (+36 months from diagnosis)	Alemtuzumab+cyclosporine–ARA-C+doxorubicine+VP16	Alemtuzumab+CyA	7th course
M/65	4.6	4	8	del 5q	del 5q	Alemtuzumab–lenalidomide	None	No

ELC Erythroid-lineage cells (bone marrow at diagnosis), *KD* Karyotype at diagnosis, *TI* Transfusion independence

A 35-year-old Caucasian woman, with diagnosis of MDS (RA) non-responder to ESAs treatment, received diagnosis of MDS/PRCA after 1 year of transfusion dependence (2 units/month). She underwent three courses of CyA and alemtuzumab with short transient periods of transfusion independence: a second bone marrow investigation, performed after 1 year, showed del (5q). Lenalidomide therapy was started; transfusion independence was obtained after 2 months and she maintains the response until now (Fig. 1).

A 65-year-old Caucasian man, with a diagnosis of MDS (RA) non-responder to ESAs treatment, and a transfusion need of 4 blood packages/month, received diagnosis of PRCA and was treated with a single course of alemtuzumab and CyA without any result. Cytogenetic revision of bone marrow highlighted the presence of del (5q), and treatment with lenalidomide was started 3 months after diagnosis. No haematological improvement was observed and therapy was stopped after nine courses. The patient is now transfusion-dependent, 21 months after diagnosis (Fig. 1).

Discussion

In MDS, PRCA could be a consequence of extreme apoptosis of the red cell progenitors leading to their total destruction. Programmed cell death is maximal in advanced RA, RARS, and RAEB. This correlates with the findings that erythroblastopenia is diagnosed mainly in MDS with less than 10 % of blasts, as in our cases. Excessive cell death could be due to cytokines like TNF-α. The EPO action is related to its anti-apoptotic action and has been reported to be efficient in PRCA with myelodysplasia that did not respond to corticotherapy [6].

The association of PRCA with lymphoproliferative disorders and a good response to immunosuppressive therapy point to the fact that erythroblastopenia can be mediated by an autoimmune T cell subset. In vitro inhibition of erythropoiesis in marrow culture systems from patients with PRCA by T cells was demonstrated by Abkhowitz et al. [7]. A study has reported a good erythroid response after cyclosporin A and antithymocyte globulin in hypoplastic myelodysplastic syndrome [8].

Alemtuzumab has also an important role, in combination with CyA, in the treatment of bone marrow failure syndromes [9].

Lenalidomide has a role in the treatment of MDS with del (5q) [10] and trials in low-risk MDS without del (5q) are ongoing, but no reports are available about its role in this setting of patients.

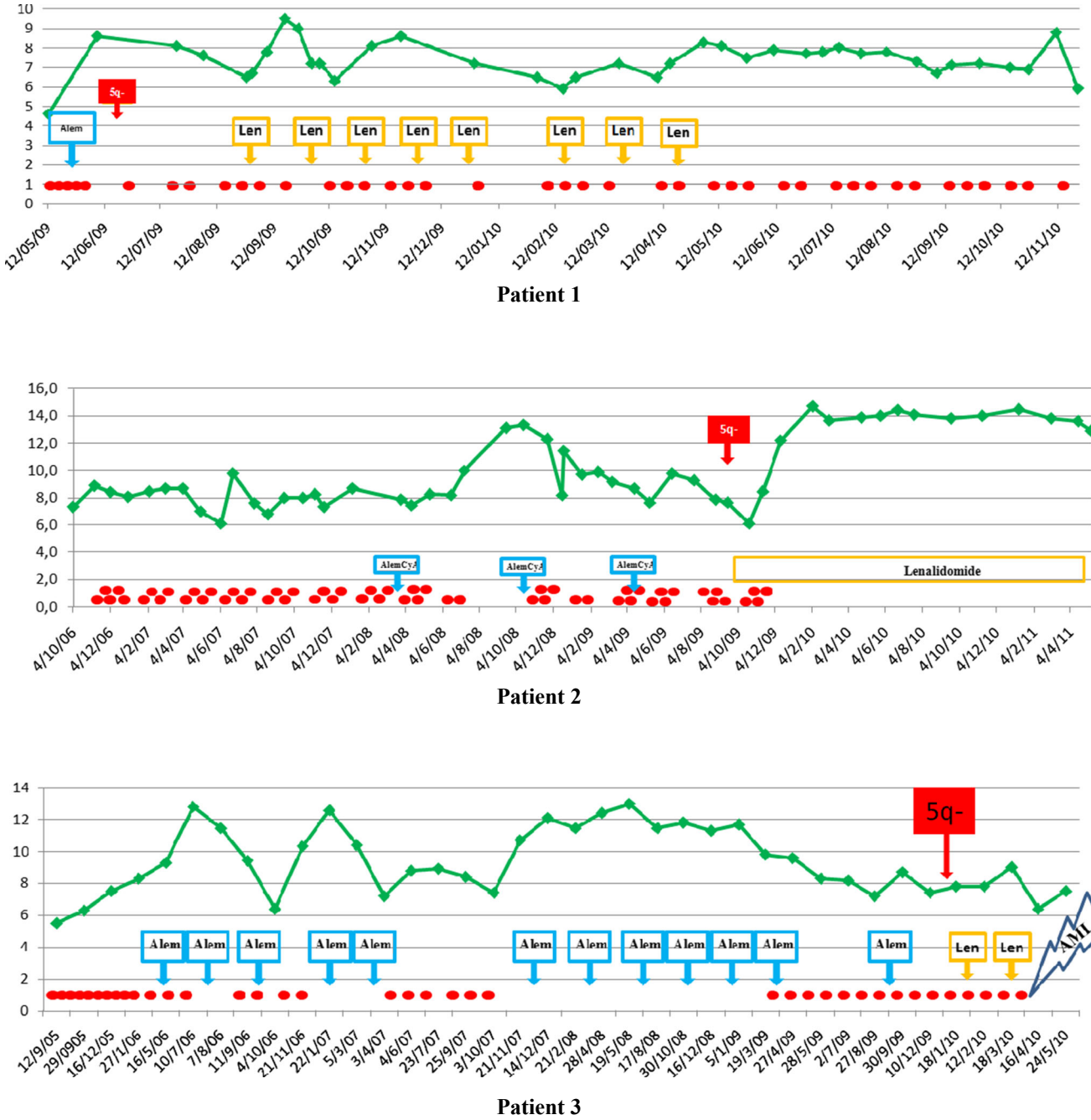

Fig. 1 Patients' clinical history (*Len*, lenalidomide treatment; *Alem*, alemtuzumab treatment; *AML*, progression in AML; *red point*, transfusion; *5q*, discovery of del5q)

In conclusion, here we stress the difficulty of diagnosing PRCA within myelodysplastic syndromes and focus on the relationship among MDS with erythroid aplasia and del (5q), with speculation on the role that lenalidomide could play in such entities.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Tefferi A et al (2009) Myelodysplastic syndromes. N Engl J Med 361:1872–1885
2. Fisch P et al (2000) Pure red cell aplasia. Br J Haematol 111:1010–1022
3. Ammus SS et al (1987) Acquired pure red cell aplasia. Am J Hematol 24:311–326
4. Sawada K et al (2008) Acquired pure red cell aplasia : updated review of treatment. Br J Haematol 142(4):505–14

5. Williamson PJ et al (1991) Red cell aplasia in myelodysplastic syndrome. J Clin Pathol 44:431–432
6. Garcia-Suarez J et al (1998) Myelodysplastic syndrome with erythroid hypoplasia/aplasia : a case report and review of the literature. Am J Hematol 58:316–325
7. Abkhowitz JL et al (1986) Pure red cell aplasia : lymphocyte inhibition of erythropoiesis. Br J Heamatol 63:59–62
8. Biesma DH et al (1997) Immunosuppressive therapy for hypoplastic myelodysplastic syndrome. Cancer 79:1548–1551
9. Kim H et al (2009) A pilot dose-escalating study of alemtuzumab plus cyclosporine for patients with bone marrow failure syndrome. Leuk Res 33(2):222–31
10. List A et al (2006) Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 355(14):1456–65

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LETTER TO THE EDITOR

Pegfilgrastim in primary prophylaxis of febrile neutropenia during chemotherapy of relapsed and refractory multiple myeloma: a real-life experience

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Dear Editor,

Febrile neutropenia (FN) is a serious side effect of chemotherapy, and even when it does not result in significant morbidity, mortality and costs, it normally leads to a delay in subsequent chemotherapy treatments [1]. FN is also associated with suboptimal delivery of chemotherapy and reduced relative dose intensity (RDI), which adversely affects long-term cancer outcome and survival [2]. FN is a surrogate marker for infection during chemotherapy and is characterized by an absolute neutrophil count (ANC) <1000/mm^3 and a single temperature of >38.3 °C (101 °F) or a sustained temperature of ≥38 °C (100.4 °F) for more than 1 h [1, 3]. Risk of FN is dependent on both patient-specific factors (e.g. type of cancer, disease stage, co-morbid conditions and age) and the myelotoxicity of the chemotherapy regimen [1]. Once an episode of FN occurs, the risk of FN increases in subsequent chemotherapy cycles [4].

The American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) recommend the use of granulocyte colony-stimulating factors (G-CSFs) as primary prophylaxis (PP) when the overall FN risk is greater than 20 % following myelosuppressive chemotherapy, and secondary prophylaxis (SP) following FN or a dose-limiting neutropenic event [4, 5].

Recombinant granulocyte colony-stimulating factors (G-CSFs) have been developed to stimulate proliferation and differentiation of neutrophils in patients receiving chemotherapy. Pegfilgrastim is a pegylated long-acting recombinant form of G-CSF which extends the half-life, requiring less frequent dosing than non-pegylated G-CSF [6]. It is indicated to decrease the incidence of infection, as manifested by FN, in patients with non-myeloid malignancies receiving myelosuppressive chemotherapy associated with a clinically significant incidence of FN [5]. Pegfilgrastim is cleared via a neutrophil-mediated system and requires only a single dose administered subcutaneously once per chemotherapy cycle [6–8].

Multiple myeloma (MM) in advanced phases of disease may be managed by regimens combining agents not frequently employed in early phases of treatment [9] (e.g. anthracyclines, alkylating agents, etc.), but myelotoxicity is the main expected side effect [10]. In this context, G-CSFs are often necessary to warrant an effective chemotherapy, counteracting the risks of febrile neutropenia: their use is bound to frequent evaluation of neutrophil counts which may not be frequently performed by patients in home-care. Avoiding severe neutropenia by prophylactic pegfilgrastim seems particularly useful in these cases, where treatment is performed with palliative intent and prolonging life in the best possible conditions is the aim.

The objective of this observational study was to evaluate the efficacy and safety of pegfilgrastim in patients affected by multiple myeloma in an advanced phase of disease, in order to determine whether a single subcutaneous injection of pegfilgrastim is as effective as daily injections of standard filgrastim, in terms of haematological toxicity, febrile neutropenic episodes, antibiotic usage and hospitalization duration.

We have considered 41 patients (22 male and 19 female) with a median age of 63.8 years (range 39–82) affected by multiple myeloma, all relapsed and refractory to a median of six lines of therapy (range 4–8), all previously exposed to bortezomib, lenalidomide and melphalan and all relapsed after auBMT, which have been treated with different chemotherapy regimens combining bortezomib, lenalidomide, bendamustine, melphalan and doxorubicin.

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Since first course, received in our outpatient unit, patients performed blood counts twice weekly and received, from day +8 to day +19 (considering "day +1" the day in which the chemotherapy protocol starts), prophylactic oral quinolones and anti-fungal drugs. During neutropenia after first cycle of chemotherapy, filgrastim (5 μg/kg/day for 3 days) was given if neutrophils count was $<1000\times 10^9$ cells/L. Median number of filgrastim administrations was 4.7 (r. 3–6); nadir neutropenia was registered after a median of 11.3 days (r. 8–14); median of nadir neutrophil count was 1.16×10^9 cells/L (range $0.4–1.8\times10^9$ cells/L), with maximum duration of 13 days.

From the second course of chemotherapy, all patients switched to prophylactic therapy with pegfilgrastim (6 mg), injected subcutaneously with a single administration on day + 3. Primary end point of this study was the duration of neutropenia (neutrophil count $<1.5\times10^9$ cells/L), comparing pegfilgrastim and filgrastim. During pegfilgrastim, neutropenia was never longer than 8 days, with a consequent reduction of neutropenia-related infections. Median nadir neutrophil count, evaluated for every patients for at least three courses of therapy (r. 3–6) registered at day +11, was 1.628 (range $0.93–2.25\times10^9$ cells/L); four patients (9.7 %) needed, after pegfilgrastim administration, a supplement of three administrations of filgrastim. During pegfilgrastim prophylaxis, neutropenia, when present, was shorter than during filgrastim treatment (median of 4 days, range 3–7). Apart from the advantage of the mono-administration, pegfilgrastim was well tolerated in all patients: main side effects in our patients were mild fever and bone pain (5/41 patients, 12 %). Moreover, no hospitalization was needed during pegfilgrastim treatment versus two hospitalizations for FN during filgrastim. During the observation period, no patient died during filgrastim or pegfilgrastim supportive treatment.

The reduction of the days of administration and of the days spent in the hospital make pegfilgrastim an advantageous option in most cases both in terms of quality of life and of cost-effectiveness.

In conclusions, in patients affected by MM exposed to myelosuppressive agents in advanced phases of myeloma disease, pegfilgrastim seems to reduce the incidence of neutropenia, is better tolerated and may increase the possibility to maintain the scheduled time of treatment.

Conflicts of interest No conflicts of interest.

References

1. Crawford J, Dale DC, Lyman GH (2004) Chemotherapy-induced neutropenia: risks, consequences, and new directions for its management. Cancer 100:228–237
2. Pettengell R, Schwenkglenks M, Leonard R et al (2008) Neutropenia occurrence and predictors of reduced chemotherapy delivery: results from the INC-EU prospective observational European neutropenia study. Support Care Cancer 16:1299–1309
3. United States Department of Health & Human Services, National Institutes of Health, National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE). Published: May 28, 2009 (v4.03: June 14, 2010)
4. Smith TJ, Khatcheressian J, Lyman GH et al (2006) 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. J Clin Oncol 24:3187–3205
5. National Comprehensive Cancer Network (NCCN), Inc. Clinical Practice Guidelines in Oncology: Myeloid Growth Factors Version 1.2012. Available from: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#supportive. Accessed 1 Aug 2012
6. Amgen, Inc.(2011) Neulasta (pegfilgrastim) [prescribing information]. Thousand Oaks, CA: Amgen, Inc. http://pi.amgen.com/united_states/neulasta/neulasta_pi_hcp_english.pdf. Accessed Aug 28 2011
7. Green MD, Koelbl H, Baselga J et al (2003) A randomized double-blind multicenter phase III study of fixed-dose single-administration pegfilgrastim versus daily filgrastim in patients receiving myelosuppressive chemotherapy. Ann Oncol 14:29–35
8. Balducci L et al (2007) Elderly cancer patients receiving chemotherapy benefit from first-cycle pegfilgrastim. Oncologist 12:1416–1424
9. Ludwig H et al. (2014) European perspective on multiple myeloma treatment strategies in 2014. Oncol
10. Cömert M et al (2013) Quality of life and supportive care in multiple myeloma. Turk J Haematol 30(3):234–246

^{18}F-FDG PET/CT, ^{99m}Tc-MIBI, and MRI in the Prediction of Outcome of Patients With Multiple Myeloma

A Comparative Study

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Purpose: The aim of this study was to compare the relative contribution of ^{18}F-FDG PET/CT, ^{99m}Tc-MIBI, and MRI in predicting progression-free survival (PFS) and overall survival (OS) in multiple myeloma (MM) patients.

Patients and Methods: Thirty-three newly diagnosed MM patients had been evaluated in a previous study by ^{18}F-FDG PET/CT, ^{99m}Tc-MIBI, and spine and pelvis MRI reporting focal lesions and diffuse bone marrow involvement. Twenty-seven patients were then subjected to a mean follow-up period of 58 months, whereas 6 patients were lost.

Results: ^{18}F-FDG PET/CT, ^{99m}Tc-MIBI, and MRI were positive in 26, 24, and 22 patients, respectively, showing diffuse bone marrow involvement in 12, 21, and 17 patients and a total of 185, 56, and 39 focal lesions, respectively. At follow-up, 18 patients showed complete or partial remission, whereas 9 patients developed progressive disease, 7 of which died of myeloma. Univariate and subsequent multivariate analysis showed that ^{18}F-FDG PET/CT focal uptake and ^{99m}Tc-MIBI focal and diffuse uptake predicted PFS (P = 0.0006), whereas ^{18}F-FDG PET/CT focal uptake and ^{99m}Tc-MIBI focal uptake predicted OS (P = 0.0010). Although MRI diffuse pattern predicted PFS at univariate analysis (P = 0.0376), it was not retained in the model at multivariate analysis. Receiver operating characteristic curve analysis showed that the number of focal lesions best discriminating for PFS and OS prediction was 4 and 11 for ^{18}F-FDG PET/CT and 2 in both cases for ^{99m}Tc-MIBI, respectively. By Kaplan-Meier analysis and log-rank testing, PFS and OS at follow-up were significantly better in patients showing a number of focal lesions at ^{18}F-FDG PET/CT or ^{99m}Tc-MIBI lower than the respective cutoff (P = 0.03, P = 0.004, and P < 0.0001, respectively). Finally, PFS was significantly better in patients showing absent/faint diffuse ^{99m}Tc-MIBI uptake than in those having moderate/intense diffuse uptake (P = 0.0012).

Conclusions: ^{18}F-FDG PET/CT and ^{99m}Tc-MIBI may be useful in predicting PFS and OS in myeloma patients.

Key Words: multiple myeloma, ^{18}F-FDG PET/CT, ^{99m}Tc-MIBI, MRI, prognosis

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Multiple myeloma (MM) is a malignant hematologic disorder characterized by proliferation of clonal plasma cells in the bone marrow. Disease expression is very heterogeneous including diffuse bone marrow infiltration, focal bone lesions, or extramedullary lesions.[1] As a consequence, the clinical course and outcome of disease can be quite variable ranging from few months up to 10 years also due to treatment improvements achieved in the last decades.[2] In this, setting is crucial to identify reliable prognostic factors to estimate the individual patient's outcome. A variety of clinical and hematological parameters, imaging findings, and more recently cytogenetic tests have been used in the effort to obtain a reliable evaluation of clinical status and prognosis of patients with MM.[3] The majority of these parameters have been included in the main 3 staging systems that are currently used in the management and prognostic assessment of MM, namely, Durie and Salmon, Durie and Salmon PLUS, and International Staging System (ISS).[4] Although ISS is based exclusively on the values of serum albumin and β2-microglobilin, in the other 2 systems, imaging plays a very important role because it is used to evaluate the extent of disease at diagnosis, which represents one of the most important prognostic factors in MM patients.[5] In the Durie and Salmon system, osteolytic bone lesions are detected by skeletal radiography. However, the prognostic value of this imaging methodology may be controversial because it can significantly underestimate the extent of bone and bone marrow involvement, especially at the onset of disease.[6] Therefore, the system has been updated in Durie and Salmon PLUS by adding more advanced imaging modalities such as whole-body ^{18}F-FDG PET/CT and/or MRI of the spine and pelvis in the effort to improve the assessment of the extent and severity of disease and also the prognostic stratification of patients. In fact, these techniques have proved to be reliable for predicting the outcome in various series of MM patients, both at diagnosis and after treatment.[7–9]

A further imaging methodology, ^{99m}Tc-MIBI scintigraphy, has been successfully used in the evaluation of MM extent showing high sensitivity and specificity in detecting sites of active disease and bone lesions at diagnosis or follow-up.[10–17] Moreover, focal or combined focal/diffuse patterns of ^{99m}Tc-MIBI uptake have been shown to indicate significantly worse prognosis with shorter overall survival (OS) than normal or diffuse tracer patterns.[18–21]

Therefore, the aim of our study was to evaluate the role and compare the relative contribution of whole-body ^{18}F-FDG PET/CT, whole-body ^{99m}Tc-MIBI, and MRI of the spine and pelvis in the prediction of progression-free survival (PFS) and OS of myeloma patients.

PATIENTS AND METHODS

Patients

We evaluated the PFS and OS in 27 MM patients (7 women, 20 men; mean age, 62 ± 11 years), belonging to a series of 33 newly diagnosed untreated MM patients formerly studied by our group, that had been prospectively evaluated by whole-body ^{18}F-FDG PET/CT, whole-body ^{99m}Tc-MIBI, and spine and pelvis MRI within a period of 10 days.[22] The 27 patients included in the present study were then subjected to follow-up for a mean period of 58 months (range, 1–104 months; median, 66 months). The 6 remaining patients of the original series were lost at follow-up as was not possible to collect

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any further clinical data about them. The study has been approved by the institutional review board, and all subjects signed a written informed consent form before their inclusion in the study.

Clinical characteristics of the 27 patients studied such as age, sex, type of myeloma, bone lesions, performance status, Durie and Salmon stage, and ISS (available on 22 patients) are reported in Table 1. All patients were treated according to therapeutic regimens containing novel agents such as thalidomide, lenalidomide, or bortezomib and/or conventional agents and followed, in 12 of them, by autologous bone marrow transplantation. At reevaluation, patients were considered to have progressive disease when showing a greater than or equal to 25% increase in diffuse bone marrow plasma cell infiltration and/or in the M-protein or new bone lesions; patients were considered to have partial remission when the M-protein decreased more than 50% and complete remission when the percentage of diffuse bone marrow plasma cell infiltration was less than 5% and M-protein was absent.[3]

Imaging Studies

^{18}F-FDG PET/CT, ^{99m}Tc-MIBI, and MRI scans were performed in MM patients at diagnosis as described in the previous study.[22]

Briefly, whole-body ^{18}F-FDG PET/CT scans were acquired after fasting for 8 hours 60 to 90 minutes after IV injection of ^{18}F-FDG (370 MBq) in patients with glucose level less than 120 mg/dL by using a combined PET/CT Discovery LS scanner (GE Healthcare).

^{99m}Tc-MIBI imaging studies were performed by acquiring planar anterior and posterior whole-body scans 10 minutes after IV injection of ^{99m}Tc-MIBI (555 MBq) by using a dual-head γ-camera (ECAM, Siemens).

MRI studies were performed at 1.5 T (Achieva, Philips Medical Systems, the Netherlands) along sagittal planes covering the whole spine with 3 partly overlapping slabs and along coronal planes for the study of the pelvis. The sequence parameters (TR/TE/echo train length) used for the spine were as follows: 477/13/4 for T1-weighted images, 3500/120/43 for T2-weighted images. The sequence parameters used for the pelvis were as follows: 550/14/5 for T1-weighted images, 3500/120/43 for T2-weighted images with spectral pre-saturation with inversion-recovery fat suppression.

TABLE 1. Patients Characteristics

Characteristics	Value
Age (range), y	62 ± 11 (32–84)
Sex	
Female	7 (26%)
Male	20 (74%)
Type of myeloma	
IgG	20 (74%)
IgA	6 (22%)
Nonsecretory	1 (4%)
Bone lesions	
<4 lesions	11 (41%)
≥4 lesions	16 (59%)
Performance status	
≤70%	9 (33%)
80%	8 (30%)
90%	10 (37%)
Stage	
IA	8 (30%)
IIA	4 (15%)
IIIA	15 (55%)
ISS	
I	14 (64%)
II	5 (23%)
III	3 (13%)

TABLE 2. Imaging Findings of Whole-Body ^{18}F-FDG PET/CT, Whole-Body ^{99m}Tc-MIBI, and MRI of the Spine and Pelvis Performed on 27 MM Patients at Diagnosis, According to the Presence of Normal, Diffuse, Focal, or Focal and Diffuse Pattern of Bone Marrow Involvement

	Normal	Diffuse	Focal	Focal + Diffuse
Whole-body ^{18}F-FDG PET/CT	1 (3%)	2 (7%)	14 (51%)	10 (37%)
Whole-body ^{99m}Tc-MIBI	3 (11%)	8 (29%)	3 (11%)	13 (48%)
MRI of the spine and pelvis	5 (18%)	10 (37%)	5 (18%)	7 (25%)

^{18}F-FDG PET/CT, ^{99m}Tc-MIBI, and MRI were read and interpreted blindly by 2 independent nuclear medicine physicians and/or 2 independent radiologists who were unaware of the imaging results. The number of eventual focal lesions and/or the presence or absence of diffuse bone marrow involvement assessed by each imaging methodology were recorded by analyzing the imaging findings of ^{18}F-FDG PET/CT, ^{99m}Tc-MIBI, and MRI as previously described.[10,22,23]

Statistical Analysis

Statistical analysis was performed using SPSS software (IBM SPSS Inc, Chicago, Ill). Univariate and multivariate analyses of clinical and imaging variables were performed using Cox proportional hazards regression. Variables that were found to be predictive of PFS and OS by univariate analysis were included in the multivariate analysis. Receiver operating characteristic (ROC) curve analysis was performed to estimate the best discriminative value of independent prognostic variables between dead and survivors as well as between patients with and without progression. Survival analysis was carried out using Kaplan-Meier method and log-rank tests. Survivors were censored at the time of the last clinical control. A probability value $P < 0.05$ was considered statistically significant.

RESULTS

Imaging findings obtained at diagnosis in the 27 MM patients simultaneously analyzed with ^{18}F-FDG PET/CT, ^{99m}Tc-MIBI, and MRI in the previous study are reported in Table 2. Briefly, whole-body ^{18}F-FDG PET/CT was positive in 26 patients (14 had focal uptake, 2 had diffuse uptake, and 10 combined focal and diffuse uptake). Whole-body ^{99m}Tc-MIBI resulted positive in 24 patients (3 had focal uptake, 8 diffuse uptake, and 13 combined focal and diffuse uptake). Spine and pelvis MRI was positive in 22 patients (5 showed focal pattern, 10 diffuse pattern, and 7 combined focal and diffuse pattern of distribution). By analyzing the number and sites of focal lesions detected, we found a total of 185 focal lesions by ^{18}F-FDG PET/CT—70 in the spine and pelvis (34 and 36, respectively) and 115 in other districts, whereas ^{99m}Tc-MIBI visualized a total of 56 focal lesions—1 in the spine, 6 in the pelvis, and 49 in other districts. Fourteen of the focal lesions visualized by ^{18}F-FDG PET/CT and 3 of those detected by ^{99m}Tc-MIBI were localized in soft tissues. Finally, MRI detected a total of 39 focal lesions, 28 in the spine and 11 in the pelvis.

After a mean follow-up period of 58 months, 9 patients had progressive disease (7 of which died of MM), 10 showed partial remission, and 9 showed complete remission. For survival analysis, patients showing progressive disease or subsequently dead (n = 9) were compared with patients in complete or partial remission (n = 18). Similarly, patients showing progressive disease, partial remission, or complete remission were grouped as survivors (n = 20) and compared with those who were dead (n = 7).

Univariate analysis showed that ^{18}F-FDG PET/CT focal uptake ($\chi^2 = 8.773$; $P = 0.0031$), ^{99m}Tc-MIBI focal uptake ($\chi^2 = 4.633$; $P = 0.0314$), ^{99m}Tc-MIBI diffuse uptake ($\chi^2 = 7.368$; $P = 0.0066$), MRI diffuse distribution ($\chi^2 = 4.321$; $P = 0.0376$), hemoglobin ($\chi^2 = 8.007$; $P = 0.0047$), β2-microglobulin ($\chi^2 = 4.468$; $P = 0.0345$), and stage ($\chi^2 = 5.532$; $P = 0.0187$) were all predictive of PFS (Table 3). When these variables were entered in the multiple regression model, only 3 of them were retained, one derived form ^{18}F-FDG PET/CT (ie, focal uptake) and 2 derived from ^{99m}Tc-MIBI (ie, focal and diffuse uptake) ($\chi^2 = 17.205$; $P = 0.0006$). Receiver operating characteristic curve analysis showed that the number of focal lesions that best discriminate between patients in progression and remission was 4 (area under the curve [AUC], 0.799) by ^{18}F-FDG PET/CT scan and 2 (AUC, 0.716) by ^{99m}Tc-MIBI scintigraphy, respectively. Progression-free survival curve estimated by Kaplan-Meier method and log-rank test, in fact, was significantly prolonged in patients with 4 lesions or fewer compared with patients with more than 4 lesions on ^{18}F-FDG PET/CT ($\chi^2 = 4.6684$; $P = 0.0307$) as well as in patients with 2 lesions or more compared with patients with more than 2 lesions on ^{99m}Tc-MIBI ($\chi^2 = 20.4759$; $P < 0.0001$) (Fig. 1). In addition, PFS curve by Kaplan-Meier method and log-rank test was significantly prolonged in patients showing absent or faint diffuse ^{99m}Tc-MIBI uptake than in those having moderate or intense diffuse uptake ($\chi^2 = 10.5020$; $P = 0.0012$; Fig. 2).

Regarding OS prediction, univariate analysis showed that ^{18}F-FDG PET/CT focal uptake ($\chi^2 = 8.654$; $P = 0.0033$), ^{99m}Tc-MIBI focal uptake ($\chi^2 = 7.596$; $P = 0.0058$), ^{99m}Tc-MIBI diffuse uptake ($\chi^2 = 5.109$; $P = 0.0238$), hemoglobin ($\chi^2 = 4.122$; $P = 0.0423$), and bone marrow transplantation ($\chi^2 = 4.647$; $P = 0.0311$) were all predictive of OS. Moreover, β2-microglobulin ($\chi^2 = 3.772$; $P = 0.0521$) was nearly significant in OS prediction (Table 3). When all these variables were entered in the multiple regression model, only 2 of them were retained, namely, ^{18}F-FDG PET/CT focal uptake and ^{99m}Tc-MIBI focal uptake ($\chi^2 = 13.892$; $P = 0.0010$). ROC curve analysis showed that the number of focal lesions that best discriminate between patients who had died and survivors was 11 (AUC, 0.807) by ^{18}F-FDG PET/CT and 2 (AUC, 0.857) by ^{99m}Tc-MIBI, respectively. Overall survival curve estimated by Kaplan-Meier method and log-rank test, in fact, was significantly prolonged in patients with 11 lesions or fewer compared with patients with more than 11 lesions on ^{18}F-FDG PET/CT ($\chi^2 = 7.9696$; $P = 0.0048$) as well as in patients with 2 lesions or fewer compared with patients with more than 2 lesions on ^{99m}Tc-MIBI ($\chi^2 = 21.2430$; $P < 0.0001$) (Fig. 3). Representative images of focal and diffuse tracer uptake by whole-body ^{18}F-FDG PET/CT and ^{99m}Tc-MIBI scans performed on the same MM patient are shown in Figure 4.

TABLE 3. Predictors of PFS and OS by Univariate Analysis Based on Clinical and Imaging Parameters

	Univariate Analysis			
	PFS		OS	
Variable	χ^2	*P*	χ^2	*P*
Age, y	0.229	0.6322	0.084	0.7710
Hemoglobin	8.007	0.0047	4.122	0.0423
Plasma cell concentration	0.522	0.4702	0.988	0.3203
M-protein	1.435	0.2309	0.695	0.4043
Albumin	3.396	0.0654	1.022	0.3122
β2-microglobulin	4.468	0.0345	3.772	0.0521
Performance status	0.984	0.3211	0.219	0.6401
Stage	5.532	0.0187	2.983	0.0842
ISS	2.207	0.1374	1.344	0.2463
Bone marrow transplantation	1.929	0.1648	4.647	0.0311
^{18}F-FDG PET/CT focal uptake	8.773	0.0031	8.654	0.0033
^{18}F-FDG PET/CT diffuse uptake	0.639	0.4242	0.444	0.5054
^{99m}Tc-MIBI focal uptake	4.633	0.0314	7.596	0.0058
^{99m}Tc-MIBI diffuse uptake	7.368	0.0066	5.109	0.0238
MRI focal pattern	0.679	0.4099	0.228	0.6327
MRI diffuse pattern	4.321	0.0376	2.728	0.0986

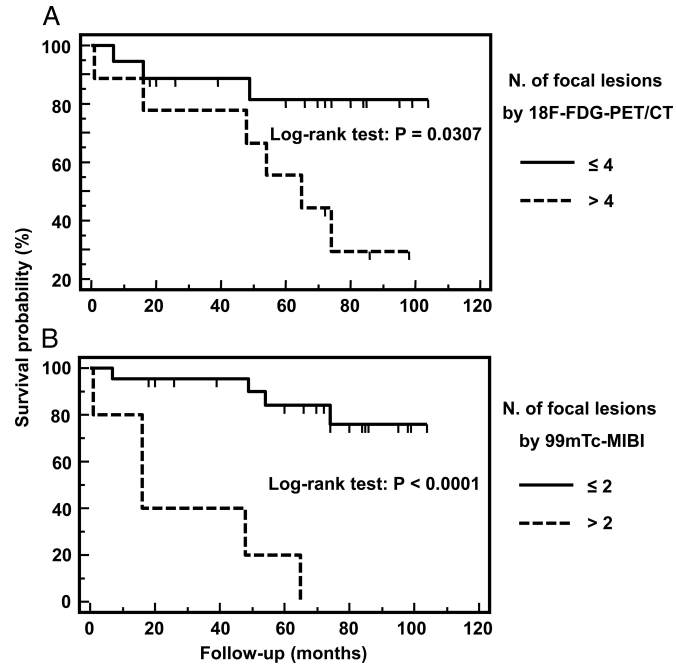

FIGURE 1. Progression-free survival by Kaplan-Meier analysis and log-rank test showing significant difference at 104 months follow-up between MM patients according to the number of focal lesions. **A**, Patients with 4 focal lesions or fewer or 4 focal lesions or more on ^{18}F-FDG PET/CT as assessed by ROC curve analysis ($P = 0.0307$). **B**, Patients with 2 focal lesions or fewer or 2 focal lesions or more on ^{99m}Tc-MIBI as assessed by ROC curve analysis ($P < 0.00001$).

DISCUSSION

The results of our comparative study show that the number of focal lesions detected by ^{18}F-FDG PET/CT or ^{99m}Tc-MIBI is a predictor of disease progression and death in patients with MM independently from other conventional prognostic factors, whereas diffuse ^{99m}Tc-MIBI uptake is an independent predictor of disease progression only.

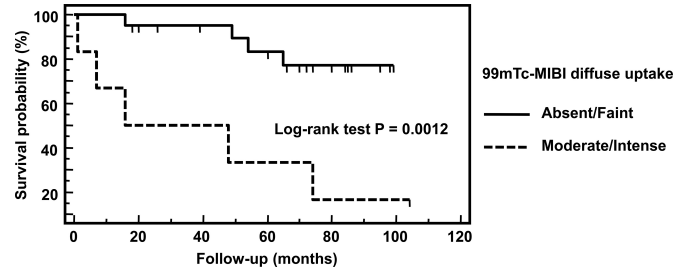

FIGURE 2. Progression-free survival by Kaplan-Meier analysis and log-rank test showing significant difference at 104 months follow-up between MM patients with absent-to-faint as compared with those with moderate-to-intense diffuse ^{99m}Tc-MIBI uptake ($P = 0.0012$).

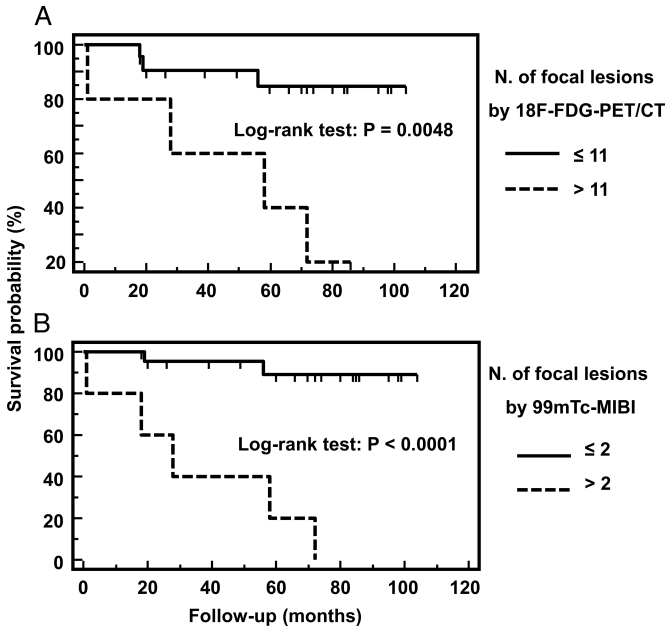

FIGURE 3. OS by Kaplan-Meier analysis and log-rank test showing significant difference at 104 months follow-up between MM patients according to the number of focal lesions. **A**, Patients with 11 focal lesions or fewer or 11 focal lesions or more on ^{18}F-FDG PET/CT as assessed by ROC curve analysis ($P = 0.0048$). **B**, Patients with 2 focal lesions or fewer or 2 focal lesions or more on ^{99m}Tc-MIBI as assessed by ROC curve analysis ($P < 0.00001$).

Multiple myeloma is a heterogeneous disease with respect to clinical presentation and course, biologic characteristics, responsiveness to therapy, and long-term survival.[3] Therefore, various patient-related, disease-related, and therapy-related characteristics have been proposed in the effort to predict disease course and outcome.[2,3]

The definition of prognosis in MM patients is commonly based on stage. The most widely used staging system for MM, the Durie and Salmon system, is considered a reliable and reproducible method to estimate MM tumor burden. In this system, the extent of bone lesions is determined by radiographic bone survey.[6,24] Bone disease is the hallmark of MM, occurring in approximately two thirds of patients at diagnosis and in up to 90% of patients during the course of disease. Despite the substantial advances in MM therapy over the last decades, bone disease remains a major cause of morbidity and mortality in MM patients. Moreover, the presence of lytic bone lesions may have prognostic relevance.[9,25] From these considerations comes the need to estimate as accurately as possible the extent of bone disease, yet conventional skeletal survey is able to detect lytic lesions only when more than 30% of bone substance has been lost leading to a significant underestimation of bone disease.[4] Therefore newer imaging techniques such as ^{18}F-FDG PET/CT and MRI of the spine and pelvis were included in the Durie and Salmon PLUS staging system. In fact, these advanced imaging methodologies can detect intramedullary focal lesions before anatomic changes occur and are also able to detect the diffuse bone marrow infiltration component of MM.[26,27]

In particular, ^{18}F-FDG PET/CT allows to explore the whole body including the skeleton in a single examination session detecting myelomatous lesions with a sensitivity in the order of 90%.[1] A previous study by our group showed that ^{18}F-FDG PET/CT can give a significant contribution in the evaluation of MM patients by detecting more focal lesions than MRI of the spine and pelvis due to the consistent number of lesions outside this anatomic district.[22] In the present study, we

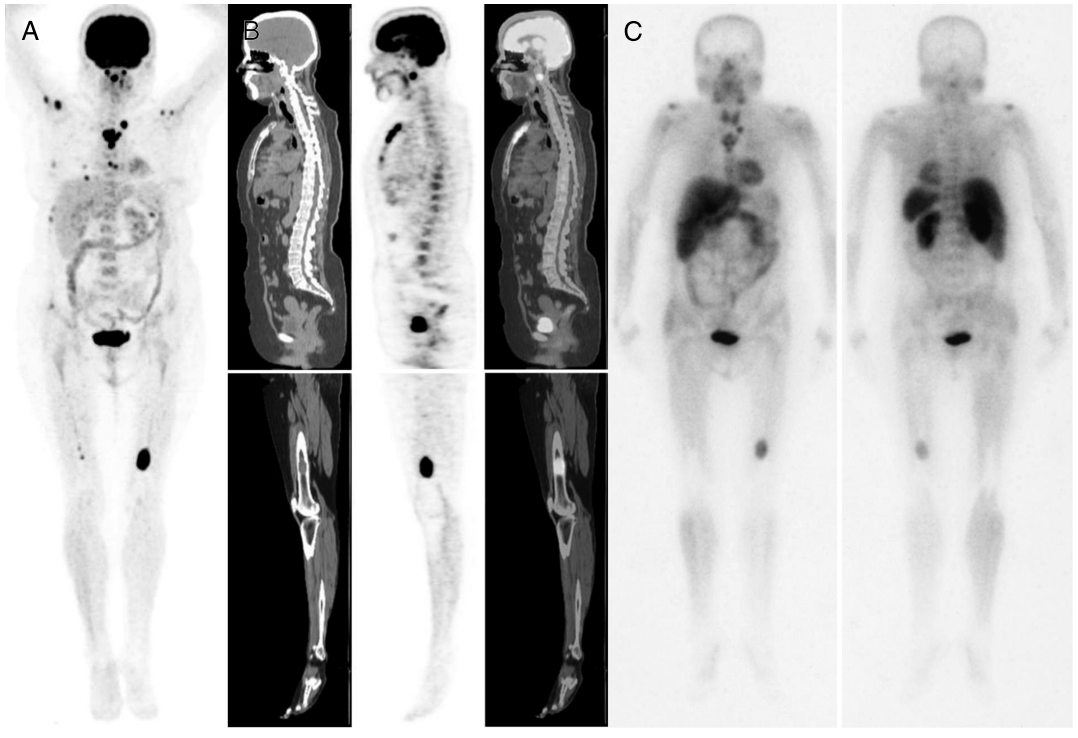

FIGURE 4. Whole-body ^{18}F-FDG PET/CT and ^{99m}Tc-MIBI scans performed on the same MM patient showing diffuse tracer uptake and focal lesions in the axis, acromion processes, humeri, left clavicle, sternum, ribs, right ilium, and femuri. **A**, MIP view. **B**, Sagittal CT, PET, and fusion images of whole-body ^{18}F-FDG PET/CT. **C**, Anterior and posterior whole-body ^{99m}Tc-MIBI.

showed that ^{18}F-FDG PET/CT can also play a role in the prognosis of MM patients. Several studies showed that imaging parameters related to tumor burden such as the number of focal lesions, the intensity of tracer activity by the lesions, the value of metabolic tumor volume, or the presence of extramedullary disease affect survival outcomes.[9,28,29] In particular, the presence of more than 3 FDG-avid focal lesions on baseline examination was the leading independent parameter adversely affecting PFS and OS.[28] Also in our study, among all the variables tested, the number of focal lesions on ^{18}F-FDG PET/CT was shown to be one of the independent factors predicting survival. In fact, the presence of more than 4 and 11 focal lesions was associated with worst PFS and OS, respectively. Conversely, the number of focal lesions on MRI was not predictive of PFS or OS. In a previous study, the presence of more than 7 focal lesions on the initial MRI was associated with inferior outcome.[30] This apparent discrepancy could be explained by the different characteristics of patient population showing at MRI a mean number of focal lesions per patient of 9.9 as compared with 1.4 in our series.

Previous studies by our and other groups showed that focal ^{99m}Tc-MIBI uptake in MM patients indicates active and advanced disease stage[10–16] and affects prognosis.[18,19,21] In our study, the prognostic cutoff threshold for ^{99m}Tc-MIBI was lower as compared with that of ^{18}F-FDG PET/CT; in fact, the presence of few lesions at ^{99m}Tc-MIBI (ie, >2) was able to predict both disease progression and death despite the fact that the number of focal lesions detected by ^{99m}Tc-MIBI scan was less than one third of those detected by ^{18}F-FDG PET/CT. Notably, both ^{99m}Tc-MIBI and ^{18}F-FDG PET/CT focal uptake are independent predictors of PFS and OS therefore providing complementary prognostic information.

In a former study by our group, diffuse ^{99m}Tc-MIBI uptake was graded as low, moderate, or intense according to extension and intensity criteria.[10] Using this score, diffuse uptake correlated with both clinical status and disease stage, and intense diffuse uptake indicates high tumor burden and poor prognosis.[10,21] Accordingly, the present study showed that PFS was significantly prolonged in patients with absent or faint diffuse ^{99m}Tc-MIBI uptake as compared with those having moderate or intense diffuse uptake. Conversely, MRI diffuse bone marrow involvement, despite its significance at univariate analysis in PFS prediction, was not retained in the model at multivariate analysis, although previous studies reported its influence on the prognosis of MM when used as the sole imaging modality.[31,32] Furthermore, in agreements with other studies,[33] our results indicate that ^{18}F-FDG PET/CT diffuse bone marrow uptake in MM patients does not provide useful information for prognosis in our series of patients.

In this study, a classic prognostic factor such as β2-microglobulin was found to be significant at univariate analysis, but it was not retained in the model at multivariate analysis in both PFS and OS prediction. The measurement of β2-microglobulin and serum albumin levels is the basis of the ISS, which is created to standardize the classification of myeloma using more widely available measures of disease.[3,24] However, cutoff levels for these variables are controversial because in advanced disease, the increase in β2-microglobulin levels could be due to both tumor burden or renal failure. Moreover, a recent study suggested that ISS is strongly dependent from age-related comorbidity burden lacking specificity for MM.[34] Moreover, with the introduction of new drugs for treating MM, its prognostic significance should probably be redefined.[5]

In recent years, cytogenetic abnormalities have been reported to predict prognosis in hematologic malignancies, especially in acute leukemias.[31] In myeloma, translocation t(4;14), t(14;16), or deletion of 17p by fluorescence in situ hybridization; deletion of 13 or 13q, t(4;14); or deletion of 17p by conventional cytogenetic were reported to identify high-risk MM patients.[2,35–37] At the time of diagnosis of our patients, cytogenetic tests were not included in the standard workup of MM at our institution; therefore, the available cytogenetic data did not allow an accurate statistical analysis. In MM, however, data from genetic alterations obtained by conventional cytogenetic or fluorescence in situ hybridization are not yet considered adequate enough to suggest their routine use in MM prediction due to technical challenges related to the low proliferation rate of malignant plasma cells and their potential low proportion in bioptic samples.[2,35,36] Moreover, outcome heterogeneity has been observed even in high-risk MM subgroups defined by cytogenetic tests.[35]

However, the main focus of the present study was to compare imaging modalities such as ^{18}F-FDG PET/CT, ^{99m}Tc-MIBI, and MRI of the spine and pelvis in the prediction of prognosis in MM patients, and to the best of our knowledge, it is the first to provide such comparison. The number of focal lesions at ^{18}F-FDG PET/CT or ^{99m}Tc-MIBI scan is an independent predictor of both PFS and OS in myeloma patients therefore providing complementary information on the prognosis of these patients. Moreover, diffuse ^{99m}Tc-MIBI uptake is an independent predictor of disease progression. Conversely, neither focal nor diffuse MRI pattern of distribution independently predicted PFS or OS. ^{18}F-FDG PET/CT and ^{99m}Tc-MIBI resulted to be more accurate in predicting disease outcome than spine and pelvis MRI likely because of their wider whole-body FOV that allows them to detect the consistent number of lesions occurring outside the spine and pelvis in MM patients.

In conclusion, among all imaging and conventional clinical variables tested, ^{18}F-FDG PET/CT and ^{99m}Tc-MIBI provided independent prognostic parameters that can improve prognostic stratification and therapeutic strategies in MM patients. Therefore, it is advisable to perform at least one of these techniques at baseline in every newly diagnosed patient because the presence of ^{18}F-FDG PET/CT or ^{99m}Tc-MIBI focal uptake can be of aid in identifying patients with poor prognosis who may benefit from more aggressive treatments.

REFERENCES

1. Bodet-Milin C, Eugène T, Bailly C, et al. FDG-PET in the evaluation of myeloma in 2012. *Diagn Interv Imaging*. 2013;94:184–189.
2. Munshi NC, Anderson KC, Bergsagel PL, et al. Consensus recommendations for risk stratification in multiple myeloma: report of the International Myeloma Workshop Consensus Panel 2. *Blood*. 2011;117:4696–4700.
3. Bird JM, Owen RG, D'Sa S, et al. Guidelines on the diagnosis and management of multiple myeloma 2011. *Br J Haematol*. 2011;154:32–75.
4. San-Miguel JF, Paiva B, Gutiérrez NC. New tools for diagnosis and monitoring of multiple myeloma. *Am Soc Clin Oncol Educ Book*. 2013;1:313–138.
5. Kyle RA, Rajkumar SV. Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. *Leukemia*. 2009;23:3–9.
6. Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cells mass with presenting clinical features, response to treatment and survival. *Cancer*. 1975;36:842–854.
7. Durie BG, Kyle RA, Belch A, et al. Myeloma management guidelines: a consensus report from the Scientific Advisors of the International Myeloma Foundation. *Hematol J*. 2003;4:379–398.
8. Durie BG. The role of anatomic and functional staging in myeloma: description of Durie/Salmon plus staging system. *Eur J Cancer*. 2006;42:1539–1543.
9. Zamagni E, Cavo M. The role of imaging techniques in the management of multiple myeloma. *Br J Haematol*. 2012;159:499–513.
10. Pace L, Catalano L, Pinto A, et al. Different patterns of technetium-99m sestamibi uptake in multiple myeloma. *Eur J Nucl Med*. 1998;25:714–720.
11. Mele A, Offidani M, Visani G, et al. Technetium-99m sestamibi scintigraphy is sensitive and specific for the staging and the follow-up of patients with multiple myeloma: a multicentre study on 397 scans. *Br J Haematol*. 2007;136:729–735.
12. Catalano L, Pace L, Califano C, et al. Detection of focal myeloma lesions by technetium-99m-sestaMIBI scintigraphy. *Haematologica*. 1999;84:119–124.
13. Mileshkin L, Blum R, Seymour JF, et al. A comparison of fluorine-18 fluorodeoxyglucose PET and technetium-99m sestamibi in assessing patients with multiple myeloma. *Eur J Haematol*. 2004;72:32–37.
14. Andretta C, Catalano L, Pace L, et al. 99mTc-sestamibi scintigraphy in thalidomide-treated refractory or relapsed multiple myeloma patients. *Leuk Lymphoma*. 2003;44:1081–1082.

15. Hung GU, Tsai CC, Tsai SC, et al. Comparison of Tc-99m sestamibi and F-18 FDG-PET in the assessment of multiple myeloma. *Anticancer Res*. 2005;25: 4737–4741.
16. Catalano L, Del Vecchio S, Petruzziello F, et al. Sestamibi and FDG-PET scans to support diagnosis of jaw osteonecrosis. *Ann Hematol*. 2007;86:415–423.
17. Fonti R, Del Vecchio S, Zannetti A, et al. Bone marrow uptake of ^{99m}Tc-MIBI in patients with multiple myeloma. *Eur J Nucl Med*. 2001;28:214–220.
18. Bacovsky J, Myslivecek M, Scudla V, et al. Tc-99m MIBI scintigraphy in multiple myeloma: prognostic value of different Tc-99m MIBI uptake patterns. *Clin Nucl Med*. 2010;35:667–670.
19. Pace L, Catalano L, Del Vecchio S, et al. Washout of (^{99m}Tc) sestamibi in predicting response to chemotherapy in patients with multiple myeloma. *Q J Nucl Med Mol Imaging*. 2005;49:281–285.
20. Fonti R, Del Vecchio S, Zannetti A, et al. Functional imaging of multidrug resistant phenotype by ^{99m}Tc-MIBI scan in patients with multiple myeloma. *Cancer Biother Radiopharm*. 2004;19:165–170.
21. Martin MG, Romero Colas MS, Dourdil Sahun MV, et al. Baseline Tc99-MIBI scanning predicts survival in multiple myeloma and helps to differentiate this disease from monoclonal gammopathy of unknown significance. *Haematologica*. 2005;90:1141–1143.
22. Fonti R, Salvatore B, Quarantelli M, et al. ^{18}F-FDG PET/CT, ^{99m}Tc-MIBI, and MRI in evaluation of patients with multiple myeloma. *J Nucl Med*. 2008;49: 195–200.
23. Moulopoulos LA, Dimopoulos MA. Magnetic resonance imaging of the bone marrow in hematologic malignancies. *Blood*. 1997;90:2127–2147.
24. Koppula B, Kaptuch J, Hanrahan CJ. Imaging of multiple myeloma: usefulness of MRI and PET/CT. *Semin Ultrasound CT MR*. 2013;34:566–577.
25. Regelink JC, Minnema MC, Terpos E, et al. Comparison of modern and conventional imaging techniques in establishing multiple myeloma-related bone disease: a systematic review. *Br J Haematol*. 2013;162:50–61.
26. Walker RC, Brown TL, Jones-Jackson LB, et al. Imaging of multiple myeloma and related plasma cell dyscrasias. *J Nucl Med*. 2012;53:1091–1101.
27. Tan E, Weiss BM, Mena E, et al. Current and future imaging modalities for multiple myeloma and its precursor states. *Leuk Lymphoma*. 2011;52:1630–1640.
28. Bartel TB, Haessler J, Brown TL, et al. F18-fluorodeoxyglucose positron emission tomography in the context of other imaging techniques and prognostic factors in multiple myeloma. *Blood*. 2009;114:2068–2076.
29. Fonti R, Larobina M, Del Vecchio S, et al. Metabolic tumor volume assessed by ^{18}F-FDG PET/CT for the prediction of outcome in patients with multiple myeloma. *J Nucl Med*. 2012;53:1829–1835.
30. Walker R, Barlogie B, Haessler J, et al. Magnetic resonance imaging in multiple myeloma: diagnostic and clinical implications. *J Clin Oncol*. 2007;25:1121–1128.
31. Moulopoulos LA, Gika D, Anagnostopoulos A, et al. Prognostic significance of magnetic resonance imaging of bone marrow in previously untreated patients with multiple myeloma. *Ann Oncol*. 2005;16:1824–1828.
32. Moulopoulos LA, Dimopoulos MA, Kastritis E, et al. Diffuse pattern of bone marrow involvement on magnetic resonance imaging is associated with high risk cytogenetics and poor outcome in newly diagnosed, symptomatic patients with multiple myeloma: a single center experience on 228 patients. *Am J Hematol*. 2012;87:861–864.
33. Nanni C, Zamagni E, Farsad M, et al. Role of ^{18}F-FDG PET/CT in the assessment of bone involvement in newly diagnosed multiple myeloma: preliminary results. *Eur J Nucl Med Mol Imaging*. 2006;33:525–531.
34. Bataille R, Annweiler C, Beauchet O. Multiple myeloma international staging system: "staging" or simply "aging" system? *Clin Lymphoma Myeloma Leuk*. 2013; 13:635–637.
35. Avet-Loiseau H. Role of genetics in prognostication in myeloma. *Best Pract Res Clin Haematol*. 2007;20:625–635.
36. Nahi H, Sutlu T, Jansson M, et al. Clinical impact of chromosomal aberrations in multiple myeolma. *J Intern Med*. 2011;269:137–147.
37. An G, Xu Y, Shi L, et al. t(11;14) multiple myeloma: a subtype associated with distinct immunological features, immunophenotypic characteristics but divergent outcome. *Leuk Res*. 2013;37:1251–1257.

LETTER TO THE EDITOR

Salvage therapy with pegylated liposomial doxorubicin-based regimen in relapsed/refractory multiple myeloma: comments to the article by Romano *et al.*

To the Editor:

Romano A. *et al.* have recently published an interesting study on the efficacy of a salvage regimen with pegylated liposomial doxorubicin, cyclophosphamide, and dexamethasone in relapsed/refractory multiple myeloma patients (rrMM). As the authors clearly demonstrated, this regimen can be really effective and safe also for rrMM patients previously treated with novel agents (median of previous lines: 2, range: 1–6), with clinical response in 12 patients (50%), 29% of which being Very Good Partial Response (VGPR) or better, with 8.7 months Progression Free Survival (PFS) and 21.5 months Overall Survival (OS) (range: 2–44 months), after a median follow-up of 21.5 months (1).

Since 2009, in our Institution, some patients affected by multiple myeloma, relapsed and refractory to most of the available therapeutic options (2–7), have been treated with courses of pegylated liposomial doxorubicin (35 mg/sqm, day 1), cyclophosphamide (800 mg/sqm, day 1), and dexamethasone (20 mg days 1–4), with pegfilgrastim at day +4, every 28 d (Caelyx, Endoxan, Dexamethasone (CED) regimen), until progression of disease.

So far, 31 patients (16 women, 12 men), with median age 63.4 yr (range: 43–84), affected by relapsed and progressive multiple myeloma, whose median number of previous treatments was six (range: 2–11), have been treated with CED schedule (median number of courses: 4.3, range: 2–17). Available results refer to 31 patients completing at least two courses of CED, whereas four patients who received only one cycle were excluded from analysis. Tolerability profile of CED was satisfactory: hematological toxicity was present in all patients, but grade 3 transfusion-dependent anemia or neutropenia was verified in 37% and 46% of cases, without necessity of hospitalization. No severe extrahematologic toxicity was observed: grade 1 gastrointestinal side effect (nausea) in the majority of patients and two grade 3 extrahematological events: acute renal failure in a patient and bradycardia in another patient, both of them not requiring hospitalization. According to International Myeloma Working Group (IMWG) response criteria, after a median follow-up of 6 months of treatment (range: 2–17+), overall response ratio (ORR) was 51% (2 Complete Response (CR), 2 VGPR, 8 Partial Response (PR), 4 Minimal Response (MR)) with 10 disease progressions and five patients in stable disease. Median OS from start of CED was 5.9 months (range: 2–17). These effects appear impressive in patients so far lacking available therapeutic options. Together to Romano's results, our observations underline the efficacy of pegylated liposomial doxorubicin, which seems to give a contribution in a particular severe setting of patients, without significant side effects.

References

1. Romano A, Chiarenza A, Conticello C, Cavalli M, Vetro C, Di Raimondo C, Cunsolo R, Palumbo GA, Di Raimondo F. Salvage therapy with pegylated liposomial doxorubicin, bortezomib, cyclophosphamide, and dexamethasone in relapsed/refractory myeloma patients. *Eur J Haematol* 2014;**93**:207–13.
2. Engelhardt M, Terpos E, Kleber M, *et al.*, European Myeloma Network. European Myeloma Network recommendations on the evaluation and treatment of newly diagnosed patients with multiple myeloma. *Haematologica* 2014;**99**:232–42.
3. Anderson KC, Alsina M, Bensinger W, *et al.* Multiple myeloma, version 1.2013. *J Natl Compr Canc Netw* 2013;**11**:11–7.
4. Barosi G, Merlini G, Billio A, *et al.* SIE, SIES, GITMO evidence-based guidelines on novel agents (thalidomide, bortezomib, and lenalidomide) in the treatment of multiple myeloma. *Ann Hematol* 2012;**91**:875–88.
5. Stewart AK, Palumbo A. Treatment of relapsed multiple myeloma. *N Engl J Med* 2015;**372**:1774–5.
6. Nooka AK, Kastritis E, Dimopoulos MA, Lonial S. Treatment options for relapsed and refractory multiple myeloma. *Blood* 2015;**125**:3085–99.
7. Bladé J, Rosiñol L, Fernández de Larrea C. How I treat relapsed myeloma. *Blood* 2015;**125**:1532–40.

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LETTER TO THE EDITOR

Managing neutropenia by pegfilgrastim in patients affected by relapsed/refractory multiple myeloma treated with bendamustine-bortezomib-dexamethasone

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Dear Editor,

Febrile neutropenia (FN) is a serious side effect of chemotherapy, and even when it does not result in significant morbidity, mortality, and costs, it normally leads to a delay in subsequent chemotherapy treatments [1]. Suboptimal delivery of chemotherapy and reduced relative dose intensity (RDI) adversely affects long-term cancer outcome and survival [2]. FN is a surrogate marker for infections during chemotherapy and is characterized by an absolute neutrophil count (ANC) <1000/mmc and a single body temperature of >38.3 °C or a sustained temperature of ≥38 °C for more than 1 h [1, 3]. Risk of FN is dependent on both patient-specific factors (e.g., type of cancer, disease stage, co-morbid conditions, and age) and the myelotoxicity of the chemotherapy regimen. Once an episode of FN occurs, the risk of FN increases in subsequent chemotherapy cycles [4].

Recombinant granulocyte colony-stimulating factors (G-CSFs) have been developed to stimulate proliferation and differentiation of neutrophils in patients receiving chemotherapy.

The American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) recommend the use of G-CSF as primary prophylaxis (PP) when the overall FN risk is greater than 20 % following myelosuppressive chemotherapy, and secondary prophylaxis (SP) following FN or a dose-limiting neutropenic events [4, 5].

Pegfilgrastim is a pegylated long-acting recombinant form of G-CSF which extends the half-life, requiring less frequent dosing than non-pegylated G-CSF [6]. It is indicated to decrease the incidence of infection, as manifested by FN, in patients with non-myeloid malignancies receiving myelosuppressive chemotherapy associated with a clinically significant incidence of FN [5]. Pegfilgrastim is cleared via a neutrophil-mediated system and requires only a single dose administered subcutaneously once per chemotherapy cycle [6–8].

Multiple myeloma (MM) in advanced phases of disease may be managed by regimens combining agents not frequently employed in early phases of treatment (e.g., anthracyclines, alkylating agents, etc), which have significant myelotoxicity. Bendamustine is a bifunctional alkylating agent that produces both single and double strand breaks in DNA, which has shown good results in association with bortezomib and dexamethasone in heavily pretreated patients [9], but in this schedule myelotoxicity is the main expected side effect [10]. In this context, G-CSFs are often necessary to warrant an effective treatment, counteracting the risks of febrile neutropenia. Their use is bound to frequent evaluation of neutrophil counts which may not be easily performed by patients in home care. Avoiding severe neutropenia by prophylactic long-acting G-CSF, as pegfilgrastim, seems particularly useful in this setting of patients.

The objective of this retrospective study was to evaluate the efficacy and safety of pegfilgrastim in relapsed and refractory MM patients, in treatment with courses of bendamustine-bortezomib-dexamethasone (BVD), in order to determine whether primary prophylaxis with pegfilgrastim is more effective than that with filgrastim [6, 11–13] in terms of incidence of chemotherapy disruptions due to FN, days of hospitalization, and G-CSF-related extra-hematological side effects.

Methods

From December 2012 to February 2016, 47 patients have been considered (25 male and 22 female) with a median age of

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61.3 years (range 37–83) affected by relapsed and refractory MM, treated with several lines of treatments (median 6, r. 2–11), and refractory to the drugs previously received, who were treated with monthly courses of BVD (bendamustine 90 mg/sqm i.v. days 1 and 2; bortezomib 1 mg/sqm s.c. days 1, 4, 8, and 11; and dexamethasone 20 mg per os days 1, 2, 4, 5, 8, 9, 11, and 12, until progression).

All treatments were performed in our outpatient unit. Twenty-four consecutive patients received pegfilgrastim (6 mg) subcutaneously with a single administration on day +4, as primary prophlyaxis, and they were compared to a historical group of twenty-three consecutive patients in which filgrastim (5 μg/kg/day for at least 3 days) had been given, as primary prophylaxis "on demand," if neutrophils count was $<1000 \times 10^9$ cells/L.

All patients performed blood counts twice weekly and received, from day +8 to day +19, considering "day +1" the day in which the chemotherapy protocol starts, prophylactic oral quinolones and anti-fungal drugs.

Results

In filgrastim group, twenty-three consecutive patients, previously treated with several lines of treatments (median 6, r. 3–11) with a median age of 60.7 years (r. 37–78) have been considered. Nadir neutropenia was registered after a median of 9.1 days (r.8–15), with maximum duration of 13 days (median 9.4 days, r. 7–13); median of nadir neutrophil count was 1.15×10^9 cells/L (range $0.3–1.5 \times 10^9$ cells/L). Median number of filgrastim administrations was 4.2 (r. 3–6). Patients have been evaluated after at least three courses of therapy (r. 3–6). Filgrastim was well tolerated in all patients; main side effects were mild fever and bone pain (6/23, 26 %), treated successfully with paracetamol. Three hospitalizations for pneumonia were needed during filgrastim (median days of hospitalization 15, range 8–19); the patients received intravenous antibiotic treatment with resolution of infectious episodes. Four patients (4/23, 17.3 %) disrupted chemotherapy schedules because of neutropenia.

In pegfilgrastim group, twenty-four consecutive patients, previously treated with several lines of treatments (median 6, r. 2–10) with a median age of 62.1 years (r. 43–83) have been considered. Nadir neutropenia, registered at day +11, was 1.484×10^9 cells/L (range $1.04–2.33 \times 10^9$ cells/L). During pegfilgrastim, neutropenia, when present, was shorter than during filgrastim treatment, never longer than 8 days (median 5.9 days, r. 4–8), with a consequent reduction of neutropenia-related infections. Only four patients (16.6 %) needed, after pegfilgrastim, a supplement of three administrations of filgrastim. Patients have been evaluated after at least three courses of therapy (r. 3–6). Apart from the advantage of mono-administration, pegfilgrastim was well tolerated in all patients; main side effects were mild fever and bone pain (3/24, 12.5 %), treated successfully with paracetamol. Moreover, no hospitalization was needed during pegfilgrastim. Only two patients (2/24, 8.3 %) disrupted chemotherapy schedules because of neutropenia.

In Italy, the cost of filgrastim 30-MU vial is 95.18–127.95 euro (depending from producer), while the cost of pegfilgrastim 6 mg is 1.489.50 euro. However, this cost has to be considered together with that of hospitalizations, antibiotic usage, and disruptions of scheduled chemotherapy treatments.

Thus, pegfilgrastim was significantly associated with fewer incidence rate of FN-related chemotherapy disruptions (17.3 % in filgrastim group vs. 8.3 % in pegfilgrastim group, $p = 0.3534$ by $\chi 2$ test), fewer days of hospitalization due to FN (median number 15 days in filgrastim group vs. 0 in the pegfilgrastim group), and fewer G-CSF-related extra-hematological side effects (26 % in filgrastim group vs. 12.5 % in pegfilgrastim group, $p = 0.2987$ by $\chi 2$ test), with consequent improvement of quality of life. However, statistical comparison of the two groups (by $\chi 2$ test) was not properly feasible because of the very small sample size.

Conclusions

In conclusions, in patients affected by relapsed and refractory MM, treated with bendamustine-bortezomib-dexamethasone, primary prophylaxis with pegfilgrastim seems to reduce the incidence of chemotherapy disruptions due to FN, and the days of hospitalization. Moreover, it is better tolerated and may increase the opportunity to maintain the planned schedule of treatment. These results make pegfilgrastim and advantageous option in most cases, both in terms of cost-effectiveness and of quality of life. These preliminary observations need to be validated by controlled clinical trials, involving a larger number of patients.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Crawford J, Dale DC, Lyman GH (2004) Chemotherapy-induced neutropenia: risks, consequences, and new directions for its management. Cancer 100:228–237
2. Pettengell R, Schwenkglenks M, Leonard R et al (2008) Neutropenia occurrence and predictors of reduced chemotherapy delivery: results from the INC-EU prospective observational European neutropenia study. Support Care Cancer 16:1299–1309
3. United States Department of Health & Human Services, National Institutes of Health, National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE). Published: May 28, 2009 (v4.03: June 14, 2010)
4. Smith TJ, Khatcheressian J, Lyman GH et al (2006) 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. J Clin Oncol 24:3187–3205
5. Crawford J, Armitage J, Balducci L, Becker PS, Blayney DW, Cataland SR, Heaney ML, Hudock S, Kloth DD, Kuter DJ, Lyman GH, McMahon B, Rugo HS, Saad AA, Schwartzberg LS, Shayani S, Steensma DP, Talbott M, Vadhan-Raj S, Westervelt P, Westmoreland M, Dwyer M, Ho M (2013) National comprehensive cancer network. Myeloid growth factors. J Natl Compr Cancer Netw 11(10):1266–1290
6. Cerchione C, Catalano L, Pareto AE, Picardi M, Pane F (2015) Pegfilgrastim in primary prophylaxis of febrile neutropenia during chemotherapy of relapsed and refractory multiple myeloma: a real-life experience. Support Care Cancer 23(2):301–302
7. Green MD, Koelbl H, Baselga J et al (2003) A randomized doubleblind multicenter phase III study of fixed-dose single-administration pegfilgrastim versus daily filgrastim in patients receiving myelosuppressive chemotherapy. Ann Oncol 14:29–35
8. Balducci L et al (2007) Elderly cancer patients receiving chemotherapy benefit from first-cycle pegfilgrastim. Oncologist 12:1416–1424
9. Ludwig H, Kasparu H, Leitgeb C, Rauch et al (2014) Bendamustine-bortezomib-dexamethasone is an active and well-tolerated regimen in patients with relapsed or refractory multiple myeloma. Blood 123(7):985–991
10. Link H, Nietsch J, Kerkmann M, Ortner P (2015) Supportive Care Group (ASORS) of the German Cancer Society (DKG). Adherence to granulocyte-colony stimulating factor (G-CSF) guidelines to reduce the incidence of febrile neutropenia after chemotherapy-a representative sample survey in Germany. Support Care Cancer.
11. Ferrara F, Izzo T, Criscuolo C, Ricciardi C, Viola A, Delia R, Carbone A, Celentano M (2011) Comparison of fixed dose pegfilgrastim and daily filgrastim after autologous stem cell transplantation in patients with multiple myeloma autografted on a outpatient basis. Hematol Oncol 29(3):139–143
12. Chan A, Leng XZ, Chiang JY, Tao M, Quek R, Tay K, Lim ST (2011) Comparison of daily filgrastim and pegfilgrastim to prevent febrile neutropenia in Asian lymphoma patients. Asia Pac J Clin Oncol 7(1):75–81
13. Kourlaba G, Dimopoulos MA, Pectasides D, Skarlos DV, Gogas H, Pentheroudakis G, Koutras A, Fountzilas G, Maniadakis N (2015) Comparison of filgrastim and pegfilgrastim to prevent neutropenia and maintain dose intensity of adjuvant chemotherapy in patients with breast cancer. Support Care Cancer 23(7):2045–2051

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ORIGINAL ARTICLE

Pegfilgrastim in primary prophylaxis of febrile neutropenia following frontline bendamustine plus rituximab treatment in patients with indolent non-Hodgkin lymphoma: a single center, real-life experience

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Abstract

Background In this prospective study, the impact of granulocyte colony-stimulating factors (G-2 CSF) administered during induction treatment with bendamustine plus rituximab for indolent non- Hodgkin Llymphoma (NHL) was evaluated by comparing patients who received secondary prophylaxis with filgrastim (control group) versus. patients who received pegfilgrastim as primary prophylaxis (peg-group). The primary endpoint was the incidence rate of febrile neutropenia (FN)- related chemotherapy disruptions (regarding dose-dense and/or dose-intensity of schedule). The Ssecondary endpoint included days of hospitalization due to FN, and G-CSF-related side effects (grade ≥3 WHO toxicity criteria) in each group.

Methods One hundred twenty-two: 122 consecutive patients, with untreated indolent NHL, were referred to our outpatient unit for remission induction immuno-chemotherapy with bendamustine-rituximab. During the first period, 61 patients received secondary prophylaxis with filgrastim, given "on demand" if ANC was <1000/mm3. During the second period, 61 patients received primary prophylaxis with pegfilgrastim in a single administration.

Results Pegfilgrastim was significantly associated with fewer incidence rate of FN-related chemotherapy disruptions (11.4% in the control group vs. 1.6% in the peg-group, p = 0.04) and fewer days of hospitalization due to FN (median number 18 days in the control group vs. 6 in the peg-group, p = 0.04). In terms of G-CSF-related extra-hematological grade III side effects, no significant difference has been found in the two groups (9.8% in the control group vs. 11.5% in the peg-group, p = 0.77). Only one patient stopped the treatment in the peg-group due to intolerance.

Conclusions In patients with indolent NHL, in front-line treatment with bendamustine plus rituximab, primary prophylaxis with pegfilgrastim seems to reduce the incidence of chemotherapy disruptions due to FN, and the days of hospitalization. Moreover, it is well- tolerated and may increase the opportunity to maintain the planned schedule of treatment. These results make pegfilgrastim an advantageous option in most cases both in terms of cost-effectiveness and quality of life. These preliminary observations need to be validated by controlled clinical trials.

Introduction

Bendamustine is a bifunction alkylating agent that produces both single- and double-strand breaks in deoxyribonucleic acid. It is frequently used in association with rituximab as a frontline treatment of indolent (follicular and non-follicular) non-Hodgkin lymphoma (NHL). With this schedule, myelotoxicity, in particular severe neutropenia, is the main expected side effect [1, 2]. Febrile neutropenia (FN) is one of the most important clinical signs of infection during chemotherapy and is characterized by an absolute neutrophil count (ANC) <1000/mm^3 and at least one temperature

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measuring of ≥38 °C [3]. Once an episode of FN occurs, the risk of FN increases in subsequent chemotherapy courses [4–6]. FN may lead to a disruption of chemotherapy program, with delay of cytotoxic agent administration and/or reduction of relative dose intensity, adversely affecting long-term patients' outcome [4–6]. The American Society of Clinical Oncology and the National Comprehensive Cancer Network recommend the use of granulocyte colony-stimulating factors (G-CSF), which have been developed to stimulate the proliferation and differentiation of neutrophils in patients receiving cytotoxic agent treatments [7, 8]. The routine use of G-CSF from the first cycle of myelosuppressive chemotherapy, i.e., primary prophylaxis, is indicated when the overall FN risk is greater than 20% [6, 7]. In the other cases (≤ 20% of risk), it is suggested a secondary prophylaxis, which consists of post-chemotherapy G-CSF administration "on demand" if ANC is <1000/mm^3 [6, 7]. Another important issue is the type of G-CSF to employ [9–11]. Filgrastim is a non-pegylated form of G-CSF, used at the daily dose of 5 μg/kg, until the end of neutropenia, according to the myelosuppressive chemotherapy schedules [7]. Pegfilgrastim is a pegylated long-acting recombinant form of G-CSF which extends the half-life, requiring less frequent administrations than non-pegylated G-CSF [9]. Pegfilgrastim is cleared via a neutrophil-mediated system and requires only a single dose administered once *per* chemotherapy cycle [9–11]. It is indicated to decrease the incidence of infections in patients with non-myeloid malignancies, receiving myelosuppressive chemotherapy [6]. However, data on the optimal G-CSF strategy, i.e., primary vs. secondary prophylaxis and/or non-pegylated vs. pegylated form, are scanty in the setting of patients with NHL undergoing frontline treatment with immuno-chemotherapy schedule [1, 2].

In this prospective study, the impact of G-CSF administered during induction treatment with bendamustine plus rituximab for indolent NHL was evaluated by comparing patients who received secondary prophylaxis with filgrastim (control group) to patients who received pegfilgrastim as primary prophylaxis (peg-group). The primary endpoint was the incidence rate of FN-related chemotherapy disruptions (regarding dose dense and/or dose intensity of schedule). Secondary endpoints included days of hospitalization due to FN and G-CSF-related side effects (grade ≥3 WHO toxicity criteria) in each group.

Patients and methods

Study design

From March 2013 to February 2016, 264 patients with histologically diagnosed and untreated indolent NHL (including grade 1 or 2 follicular lymphoma, lymphoplasmocitic lymphoma, small lymphocytic lymphoma, and marginal zone lymphoma), age ≥18 years, and WHO performance score 0–2 were screened for enrollment. Eligible criteria were features requiring to start immuno-chemotherapy treatment, i.e., Ann Arbor stage III or IV, and impaired hemopoiesis (hemoglobin <10 g/dL, ANC <1500/mm^3 , or platelet count <100 × 10^9/L), presence of B symptoms, large tumor burden (three areas >5 cm, or one area >7.5 cm), bulky disease with impingement on internal organs, progressive disease (defined as a more than 50% increase of tumor mass within 6 months), and/or a hyperviscosity syndrome [12]. Only patients who received bendamustine plus rituximab regimen were included in the study (Fig. 1).

Ninety-nine patients were excluded: 79 because they underwent to other chemo-immunotherapy regimens, while 20 because of severe cardiac disease or previous malignancy, inadequate hepatic, renal, or cardiac function, or infection with HIV or hepatitis B (HbsAg positivity) (Fig. 1).

All patients underwent standard pretreatment screening, including a physical examination, complete blood count, assessment of serum chemistry, serum immune-electrophoresis, measurement of immunoglobulin concentrations, chest radiograph, CT scans of the chest, abdomen, and pelvis, sonography of the abdomen, and bone marrow aspiration and biopsy.

The protocol was approved by the local ethics committee; the study complied with the Declaration of Helsinki and its amendments. It was done in accordance with the Good Clinical Practice guidelines. All patients gave written informed consent.

Strategies of prophylaxis with G-CSF

All patients received frontline immune-chemotherapy courses in our outpatient unit. Treatment included intravenous bendamustine (90 mg/m^2 given over 30–60 min on days 1 and 2 of each cycle) plus rituximab (375 mg/m^2 on day 1 of each cycle), every 4 weeks for up to 6 cycles [1].

Patients were divided into two groups of G-CSF prophylactic strategy. From March 2013 to August 2014 (first period), 61 patients received secondary prophylaxis with filgrastim (5 μg/kg/day *s.c.* for at least 3 days) given on demand if ANC was <1000/mm^3. Since July 2014 (second period), the use of primary prophylaxis with pegfilgrastim (Neulasta®; Amgen) became in our division a standard practice during frontline treatment for NHL with bendamustine plus rituximab. Thus, during the second period, 61 patients received primary prophylaxis with pegfilgrastim (6 mg, injected subcutaneously in a single administration on day 4, from the first course of immuno-chemotherapy).

All neutropenic patients underwent antimicrobial prophylaxis with quinolones and azoles, as elsewhere reported [13].

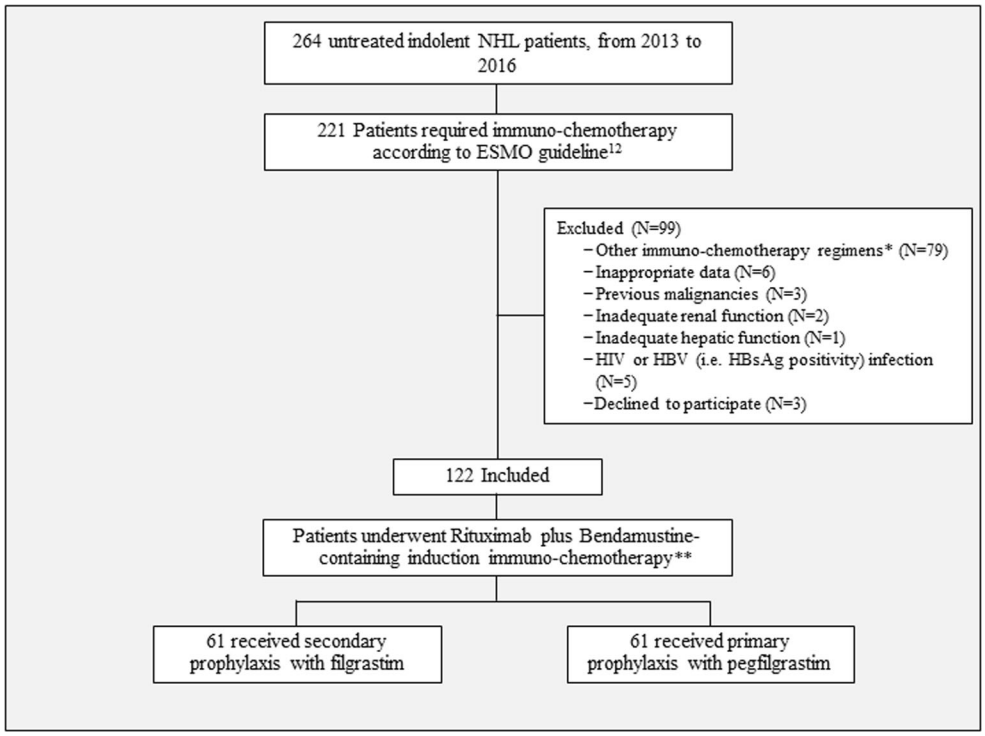

Fig. 1 Flow chart shows patient selection during the study. *Other immuno-chemotherapy regimens included R-CHOP, R-CVP, and R-FC [15]. **Immuno-chemotherapy with rituximab plus bendamustine was conducted according to Rummel et al. study [1]

Study endpoints

We defined as "disrupted chemotherapy" when we delayed treatment cycles for at least 1 week if the leukocyte count was less than 2000/mm^3 before a scheduled cycle ("time-disruption"), or if we noted a leukocyte count less than 1000/mm^3 on two consecutive days between cycles, the dose of bendamustine was decreased to 70 mg/m^2 ("dose-disruption") [1].

During the study period, as part of our Institutional guidelines for post-chemotherapy supportive care, in patients with FN, i.e., an ANC <1000/mm^3 and at least one temperature measuring of ≥38 °C, blood cultures were performed every 24–48 h: the Vitek 2 automated system (bioMérieux, Marcy l'Etoile, France) was used for bloodstream isolate identification and antimicrobial susceptibility testing. Minimum inhibitory concentrations (MICs) were evaluated by using E-test (BioMerieux) strips and classified according to the European Committee on Antimicrobial Susceptibility Testing [14]. If respiratory symptoms or signs appeared, sputum culture and chest radiography or thorax computed tomography (CT) scans were performed. Serum CMV DNA screening was performed in case of neutropenic fever or in patients with fever even in the absence of neutropenia.

We used WHO's toxicity criteria to assess treatment-related toxic effects [3]. Complete blood counts, including differential counts, and physical examination were done twice a week.

Statistical analysis

For the statistical evaluations, the χ^2 test was performed to compare proportions for clinical characteristics and complication rate, and the *t* test was used to compare the quantitative variables of clinical characteristics, disruptions, and hospitalization times between the two groups. *p* values less than 0.05 were considered to indicate a significant difference.

Results

During the study period, among 264 patients with diagnosis of indolent NHL, 122 consecutive patients (63 males and 59 females), older than 18 years (median age, 45.3 years; range, 31–77) with untreated indolent NHL (histological subtypes: follicular, $n = 83$; marginal zone, $n = 32$; small lymphocytic, $n = 4$; and lymphoplasmocytic, $n = 3$), met the inclusion criteria and were included in the final analysis (Table 1). All patients underwent to first-line immuno-chemotherapy with bendamustine plus rituximab.

Findings in the control group

In the 366 cycles of immuno-chemotherapy performed in the control group, the median number of the vials of filgrastim administered was 3 (range, 0–5), started in mean from the second course (range, 1–3). The mean of nadir of ANC was

Table 1 Baseline characteristics of patients in the two study groups

	Control group	Peg-group	*p* value
Total patients	61	61	
Sex			
Male	32 (52.4)	31 (50.8)	0.85
Female	29 (47.6)	30 (49.2)	
Age, years			
Median (range)	45.1 (31–76)	45.4 (33–77)	0.59
Histology [15]			
Follicular	41 (67.2)	42 (68.8)	0.85
Marginal zone	17 (27.9)	15 (24.6)	0.68
Small lymphocytic	2 (3.3)	2 (3.3)	1.00
Lymphoplasmocytic[a]	1 (1.6)	2 (3.3)	0.56
Stage			
II	3 (4.9)	2 (3.3)	0.65
III	12 (19.7)	11 (18.0)	0.81
IV	46 (75.4)	48 (78.7)	0.66
B symptoms	22 (36.1)	19 (31.1)	0.56
Bone marrow involved	40 (65.6)	42 (68.9)	0.69
Extra-nodal involved sites ≥1	46 (75.4)	45 (73.8)	0.84
LDH >240 U/L	22 (36.1)	21 (34.4)	0.85
Prognostic groups according to FLIPI			
Low risk (0–1 risk factor)	5 (12.1)	5 (11.9)	0.96
Intermediate risk (2 risk factors)	15 (36.6)	13 (31.0)	0.59
Poor risk (3–5 risk factors)	21 (51.2)	24 (57.1)	0.58

Unless otherwise indicated, data are number of patients, with percentage in parentheses

FLIPI Follicular Lymphoma International Prognostic Index [16]

[a] Waldenström's macroglobulinemia

1220/mm^3 (range, 300–1700) for a mean duration of 9 days (range, 7–11).

Overall, 7/61 patients (11.5%) disrupted chemotherapy schedules due to FN. Of them, three were time-disruptions and four were dose-disruptions (Table 2).

Five patients (5/61, 8.1%) were hospitalized for pneumonia (median days of hospitalization 18, with a range of 6–22) and received intravenous antibiotic treatment with resolution of infectious episodes (Table 3). Extra-hematological toxicity of grade ≥III (bone pain) was observed in six patients (9.8%), treated successfully with paracetamol. No patient had to stop the immuno-chemotherapy because of filgrastim-related side effects.

Findings in the peg-group

In the peg-group, pegfilgrastim was administered at day 4 for each of the 366 cycles of immuno-chemotherapy performed. The mean of nadir of ANC was 1734/mm^3 (range, 880–2110) for a mean duration of 5 days (range, 3–9).

Overall, only 1/61 patients (1.6%) disrupted chemotherapy schedules due to FN (time-disruption; Table 2).

Three patients (3/61, 4.9%) were hospitalized for pneumonia (median days of hospitalization 6, range 1–21) and received intravenous antibiotic treatment with resolution of infectious episodes. No patient died during pegfilgrastim. Apart from the advantage of mono-administration, pegfilgrastim was well tolerated in all patients. Extra-hematological toxicity of grade III (bone pain) was observed in 7/61 patients (11.5%), managed successfully with paracetamol. Extra-hematological toxicity of grade IV (bone pain) was observed only in one patient (1.6%), who had to stop the treatment.

Peg-group vs. control group

Thus, pegfilgrastim was significantly associated with fewer incidence rate of FN-related chemotherapy disruptions (11.4% in the control group vs. 1.6% in the peg-group, $p = 0.04$) and fewer days of hospitalization due to FN (median number 18 days in the control group

Table 2 Modification of immune-chemotherapy schedule with rituximab plus bendamustine according to Rummel et al. [1] due to neutropenia in the two study groups

	Control group ($N = 61$)	Peg-group ($N = 61$)	p value
Overall chemotherapy disruption	7 (11.5)	1 (1.6)	0.028
Time disruption	3 (4.9)	1 (1.6)	0.31
Median days (range)	16 (6–21)	5[a]	0.04
Dose disruption	4 (6.6)	–	0.04
Percentage	22.3	–	<0.001

Unless otherwise indicated, data are number of patients, with percentage in parentheses

[a] Data are from the only patient in the peg-group obliged to delay chemotherapy due to FN

vs. 6 in the peg-group, $p = 0.04$). In terms of G-CSF-related extra-hematological grade III side effects, no significant difference has been found in the two groups (9.8% in the control group vs. 11.5% in the peg-group, $p = 0.77$). Only one patient was obliged to stop the treatment in the peg-group due to intolerance.

Discussion

Immuno-chemotherapy treatment with rituximab-bendamustine has been approved as a frontline treatment for indolent NHL. It has been demonstrated to be non-inferior to rituximab in combination with cyclophosphamide,

Table 3 Clinical characteristics of neutropenic episodes in the two study groups

Characteristic	Control group ($N = 61$)	Peg-group ($N = 61$)	p value
Neutropenic episodes without infectious symptoms	20 (32.8)	7 (11.5)	0.04
Febrile neutropenia of unknown origin	10 (16.4)	2 (3.3)	0.04
Febrile neutropenia with clinically documented infection	17 (27.8)	5 (8.2)	0.005
Site/source of infection			
Mouth[a]	7 (11.5)	1 (1.6)	0.03
Upper respiratory tract	3 (4.9)	1 (1.6)	0.31
Lower respiratory tract	5 (8.2)	3 (4.9)	0.46
Urinary tract	2	–	
Radiological signs of infection[b]	5 (8.2)	3 (4.9)	0.46
Febrile neutropenia with microbiologically documented infections	7 (11.5)	4 (6.6)	0.34
Bacteremia	2 (3.3)	–	0.15
Gram-positive	2 (3.3)	–	0.15
Enterococcus spp.	1 (1.6)	–	0.31
Staphylococcus spp.	1 (1.6)	–	0.31
Gram-negative	–	–	1.00
Positive sputum culture	2 (3.3)	1 (1.6)	0.56
Gram-positive	1 (1.6)	1 (1.6)	1.00
Staphylococcus spp.	1 (1.6)	–	0.31
Gram-negative	1 (1.6)	–	0.31
Pseudomonas spp.	1 (1.6)	–	0.31
Serum CMV DNA positivity	3 (4.9)	3 (4.9)	1.00
Hospitalization required for FN complications	5 (8.1)	3 (4.9)	0.46
Hospitalization days			
Median (range)	18 (6–22)	6 (1–21)	0.04
ICU recovery	2 (3.3)	–	0.15

Unless otherwise indicated, data are number of patients, with percentage in parentheses

[a] It refers to stomatitis

[b] Chest radiography or CT scans suspected for pneumonia

doxorubicin, vincristine, and prednisone (R-CHOP) in terms of efficacy, but better tolerated. Indolent NHL is characterized by a chronic relapsing-remitting disease course, with patients usually exposed to several successive treatment courses.

In this scenario, a reduction of treatment-related toxicities and an improvement of quality of life should be considered so important as clinical results.

In this prospective study, the impact of G-CSF administered during induction treatment with bendamustine plus rituximab for indolent NHL was evaluated by comparing patients who received secondary prophylaxis with filgrastim (control group) vs. patients who received pegfilgrastim as primary prophylaxis (peg-group).

In this study, we observed a lower rate of febrile neutropenia with clinical signs of infection in the peg-group compared with control group (8.2 vs. 27.8%; $p = 0.005$): in particular, we observed an increased number of stomatitis in the control group compared with the peg-group (11.5 vs. 1.6%; $p = 0.03$).

Conversely, no difference was found between the two groups regarding microbiological documented infection, both for bacterial infection than for those CMV-related. The common prophylaxis to the two study groups with quinolone is likely able to reduce the rate of documented bacterial infections regardless of neutropenia, although the number of patients is probably too low to state this statement with certainty. At the same time, the number of CMV-related infections is equal in the two groups (3 vs. 3), being this type of viral infection non-controllable merely by neutrophils. Indeed, the number of hospitalization required for FN complications was similar in the two study groups (8.1% in the control group vs. 4.9% in the peg-group), being influenced by the CMV-induced pneumonia; while the median hospitalization time was lower in the peg-group, probably due to a more rapid recovery of neutrophil counts.

Moreover, pegfilgrastim demonstrated a reduction of the incidence rate of FN-related chemotherapy disruptions (regarding dose-dense and/or dose-intensity of schedule), of days of hospitalization due to FN, with no differences in G-CSF-related side effects (grade ≥3 WHO toxicity criteria), such as bone and back pain. No clinical significant changes in laboratory parameters or vital signs were observed.

This gives an advantage in pegfilgrastim's group also in terms of quality of life, which should be always considered in this setting of patients.

In conclusion, in patients affected by newly diagnosed indolent NHL, in treatment with bendamustine plus rituximab, primary prophylaxis with pegfilgrastim seems to reduce the incidence of chemotherapy disruptions due to FN, and of the days of hospitalization, with no differences in G-CSF-related side effects. Moreover, it is well-tolerated and may increase the opportunity to maintain the planned schedule of treatment. These results make pegfilgrastim an advantageous option in most cases both in terms of cost-effectiveness and of quality of life. These preliminary observations need to be validated by controlled clinical trials.

Compliance with ethical standards The protocol was approved by the local ethics committee; the study complied with the Declaration of Helsinki and its amendments. It was done in accordance with the Good Clinical Practice guidelines. All patients gave written informed consent.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Rummel MJ, Niederle N, Maschmeyer G et al (2013) Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. Lancet 381(9873):1203–1210. doi:10.1016/S0140-6736(12)61763-2
2. Luminari S, Goldaniga M, Cesaretti M et al (2016) A phase II study of bendamustine in combination with rituximab as initial treatment for patients with indolent non-follicular non-Hodgkin lymphoma. LeukLymphoma Apr 57(4):880–887
3. Common Terminology Criteria for Adverse Events (CTCAE) (2009) Version 4.0, published: May 28, 2009 (v4.03: June 14, 2010)
4. Link H, Nietsch J, Kerkmann M et al (2016) Adherence to granulocyte-colony stimulating factor (G-CSF) guidelines to reduce the incidence of febrile neutropenia after chemotherapy-a representative sample survey in Germany. Support Care Cancer 24(1):367–376
5. Crawford J, Dale DC, Lyman GH (2004) Chemotherapy-induced neutropenia: risks, consequences, and new directions for its management. Cancer 100:228–237
6. Pettengell R, Schwenkglenks M, Leonard R et al (2008) Neutropenia occurrence and predictors of reduced chemotherapy delivery: results from the INC-EU prospective observational European neutropenia study. Support Care Cancer 16:1299–1309
7. Smith TJ, Khatcheressian J, Lyman GH et al (2006) 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. J ClinOncol 24:3187–3205
8. Crawford J, Armitage J, Balducci L, National comprehensive cancer network et al (2013) Myeloid growth factors. J NatlComprCancNetw 11(10):1266–1290
9. Cerchione C, Catalano L, Pareto AE et al (2015) Pegfilgrastim in primary prophylaxis of febrile neutropenia during chemotherapy of relapsed and refractory multiple myeloma: a real-life experience. Support Care Cancer 23(2):301–302
10. Green MD, Koelbl H, Baselga J et al (2003) A randomized double blind multicenter phase III study of fixed-dose single-

administration pegfilgrastim versus daily filgrastim in patients receiving myelosuppressive chemotherapy. Ann Oncol 14:29–35
11. Balducci L, Al-Halawani H, Charu V et al (2007) Elderly cancer patients receiving chemotherapy benefit from first-cycle pegfilgrastim. Oncologist 12:1416–1424
12. Dreyling M, ESMO Guidelines Working Group (2009) Newly diagnosed and relapsed follicular lymphoma: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol 20(Suppl 4):115–118
13. Freifeld GA, Bow EJ, Sepkowitz KA et al (2011) Executive summary: clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Disease Society of America. Clin Infect Dis 52:427–431
14. European Committee on Antimicrobial Susceptibility Testing (2014) Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/EUCAST_breakpoints_v1.3_pdf.pdf. Accessed 3 November 2014
15. Zelenetz AD, Gordon LI, Wierda WG et al (2014) Non-Hodgkin's lymphomas, version 4. J Natl Compr Netw 12(9):1282–1303
16. Solal-Céligny P, Roy P, Colombat P et al (2004) Follicular lymphoma international prognostic index. Blood 104(5):1258–1265

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Case Report

Retreatment with Bendamustine-Bortezomib-Dexamethasone in a Patient with Relapsed/Refractory Multiple Myeloma

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The clinical management of relapsed/refractory multiple myeloma and the correct choice of the most suitable therapy in heavily pretreated and fragile patients are tough clinical issues for clinicians. In advanced phases of disease, the choice of available therapies becomes very poor, and the retreatment with previously adopted and effective therapy, although unpredictable, could be an effective option. In this report, we describe the clinical history of a patient, previously treated with 9 lines of therapy, refractory to bortezomib and IMIDs, for whom the retreatment with bendamustine resulted in a stable disease with good quality of life.

1. Introduction

In advanced multiple myeloma, the choice of the treatment can be difficult, as therapeutic options decrease over time. Both new combinations of previously used drugs and retreatment with a previously adopted and effective therapy can be taken into consideration in patients showing persistent chemosensitivity. In this report, we describe the case of a heavily pretreated patient, refractory to bortezomib and IMIDs, with clinical benefit after retreatment with bendamustine.

2. Case Presentation

In June 2009, this male patient was 67 years old and was diagnosed with IgG λ stage IIIA multiple myeloma (MM). *FISH analysis was performed at diagnosis, and it showed negativity for the most frequent alterations (t(11; 14), t(4; 14), del13q, and del17p)*. First-line therapy was 7 cycles of thalidomide-dexamethasone (TD), followed by radiotherapy on T2. In March 2010 progressive *bone* disease was detected by MRI of the spine showing multiple cervical and dorsal osteolytic lesions. Thus, second line of bortezomib-desametasone (VD), together with zoledronic acid, was performed for 5 cycles, obtaining a partial response. A first ASCT, preceded by thiotepa/melphalan conditioning regimen, was performed in December 2010 leading to a partial response. After a period with stable clinical conditions, in April 2011, disease progression *was documented by the increase of the serum monoclonal component (sMC)*: the patient was treated with 4 courses of lenalidomide-dexamethasone (RD), but the disease progressed. Therefore, a combination of melphalan-lenalidomide-dexamethasone (MRD) was performed for 3 cycles in September 2011, again followed by disease progression, *determined by sMC increase*. At the same time, PET/CT performed for neck pain revealed multiple osteolytic lesions: the most dangerous (C2) was treated with tomotherapy (40 Gy total). Thus, 2 cycles of cyclophosphamide-doxorubicin-dexamethasone (CED) regimen were attempted (1), but the disease was still refractory. Hence, a bendamustine-bortezomib-dexamethasone (BVD) regimen was administered (bendamustine 90 mg/sqm at days 1 and 2, bortezomib 1.3 mg/sqm at days 1, 4, 8, and 11, dexamethasone 20 mg at days 1, 2, 4, 5, 8, 9, 11, and 12, and pegfilgrastim 6 mg at day + 4) (2, 3, and 4) for 6 cycles, resulting in a partial response, followed by a second ASCT, preceded by thiotepa/melphalan conditioning regimen. In February *2014, a further sMC increase suggested disease progression, and* the patient was treated with bortezomib-lenalidomide-dexamethasone (VRD) for 6 cycles with the

Table 1: Patient's history.

Line	Regimen	Cycle (n°)	Responses
1	Thalidomide-dexamethasone + RT	7	Progressive disease
2	Bortezomib-dexamethasone	5	Partial response
3	First auto-BMT (thiotepa-melphalan)	/	Stable disease
4	Lenalidomide-dexamethasone	4	Progressive disease
5	Melphalan-lenalidomide-dexamethasone	3	Progressive disease
6	Doxorubicin-cyclophosphamide-dexamethasone	2	Progressive disease
7	Bendamustine-bortezomib-dexamethasone	6	Partial response
8	Second auto-BMT (thiotepa-melphalan)	/	Stable disease
9	Bortezomib-lenalidomide-dexamethasone	6	Progressive disease
10	Bendamustine-bortezomib-dexamethasone	7	Stable disease
11	Pomalidomide-dexamethasone	4	Progressive disease

result of progressive disease. In November 2014, for disease progression confirmed also by PET/CT scan (Table 1), even considering cardiovascular comorbidities, BVD-retreatment was chosen as tenth line. The patient switched to a stable disease status and clinical conditions were relatively fit for more than one year. The treatment was well tolerated: the only toxicities were grade 2 anemia and grade 3 thrombocytopenia, while severe neutropenia was effectively prevented with pegfilgrastim prophylaxis (6 mg at day + 4 of every courses). No extrahematological side effects were revealed.

Due to *further sMC increase*, in December 2015, 4 courses of pomalidomide-dexamethasone were attempted, in a palliative intent, but the patient died in July 2016.

3. Discussion

After the advent of proteasome inhibitors, international guidelines agree on first-line treatment strategy for ASCT-eligible and noneligible patients [1–3]. However, selecting and managing the correct therapy for a patient with rrMM it is still a tough task for the hematologist, as, after many relapses, available therapeutic options are scanty. A commonly adopted strategy consists in retreating the patient with the same molecules used previously, choosing those which showed the best response or considering new drug combinations, *even if in previous administrations single drugs showed to be ineffective* [4–9].

This strategy seems particularly successful in patients who show persistent chemosensitivity, as in our case, who obtained an overall survival longer than 7 years, which can be considered as an impressive result in a 67-year-old patient affected by MM.

Bendamustine is a well-tolerated agent with a double mechanism of action, alkylating and antimetabolite, with proved effectiveness in treatment of relapsed/refractory [10, 11] and newly diagnosed multiple myeloma [12, 13] and in a relapsing/refractory setting [14–19]. In rrMM it can be used as single agent combined to dexamethasone, but a synergistic effect has been demonstrated when associated with bortezomib.

Bendamustine showed significant efficacy also in a selected setting of patients, such as those who became refractory to bortezomib and IMIDs or multirelapsed after single or double ASCT, demonstrating also an effective opportunity as a bridge to ASCT [10]. To the best of our knowledge, BVD-retreatment for relapsing/refractory MM is still not consolidated, but, as in our case, it could be considered an effective choice in heavily pretreated patients without significant therapeutic options, in a context of a well-tolerated palliative treatment with good quality of life.

Competing Interests

The authors declared that there are no competing interests.

References

[1] M. Engelhardt, E. Terpos, M. Kleber et al., "European myeloma network recommendations on the evaluation and treatment of newly diagnosed patients with multiple myeloma," *Haematologica*, vol. 99, no. 2, pp. 232–242, 2014.

[2] K. C. Anderson, M. Alsina, D. Atanackovic et al., "NCCN guidelines insights: multiple myeloma, version 3. 2016," *Journal of the National Comprehensive Cancer Network*, vol. 14, no. 4, pp. 389–400, 2016.

[3] G. Barosi, G. Merlini, A. Billio et al., "SIE, SIES, GITMO evidence-based guidelines on novel agents (thalidomide, bortezomib, and lenalidomide) in the treatment of multiple myeloma," *Annals of Hematology*, vol. 91, no. 6, pp. 875–888, 2012.

[4] A. K. Stewart and A. Palumbo, "Treatment of relapsed multiple myeloma," *The New England Journal of Medicine*, vol. 372, no. 18, pp. 1774–1775, 2015.

[5] A. K. Nooka, E. Kastritis, M. A. Dimopoulos, and S. Lonial, "Treatment options for relapsed and refractory multiple myeloma," *Blood*, vol. 125, no. 20, pp. 3085–3099, 2015.

[6] J. Bladé, L. Rosiñol, and C. Fernández de Larrea, "How I treat relapsed myeloma," *Blood*, vol. 125, no. 10, pp. 1532–1540, 2015.

[7] P. G. Richardson, W. Xie, S. Jagannath et al., "A phase 2 trial of lenalidomide, bortezomib, and dexamethasone in patients with relapsed and relapsed/refractory myeloma," *Blood*, vol. 123, no. 10, pp. 1461–1469, 2014.

[8] C. Cerchione, M. Lucignano, F. Pane, and L. Catalano, "Salvage therapy with pegylated liposomial doxorubicin-based regimen in relapsed/refractory multiple myeloma: comments to the article by Romano et al," *European Journal of Haematology*, vol. 96, no. 5, p. 544, 2016.

[9] C. Cerchione, L. Catalano, A. E. Pareto, M. Picardi, and F. Pane, "Pegfilgrastim in primary prophylaxis of febrile neutropenia during chemotherapy of relapsed and refractory multiple myeloma: a real-life experience," *Supportive Care in Cancer*, vol. 23, no. 2, pp. 301–302, 2014.

[10] C. Cerchione, L. Catalano, A. E. Pareto et al., "Bendamustine in the management of relapsed and refractory multiple myeloma: a regional real-life experience," *Hematologica*, vol. 100, supplement 1, 2015.

[11] S. Lentzsch, "Bendamustine: the remedy that came in from the cold," *Blood*, vol. 123, no. 7, pp. 948–950, 2014.

[12] M. Gentile, E. Vigna, A. G. Recchia et al., "Bendamustine in multiple myeloma," *European Journal of Haematology*, vol. 95, no. 5, pp. 377–388, 2015.

[13] W. Pönisch, M. Andrea, I. Wagner et al., "Successful treatment of patients with newly diagnosed/untreated multiple myeloma and advanced renal failure using bortezomib in combination with bendamustine and prednisone," *Journal of Cancer Research and Clinical Oncology*, vol. 138, no. 8, pp. 1405–1412, 2012.

[14] M.-V. Mateos, A. Orio, L. Rosiñol et al., "Bendamustine, bortezomib and prednisone for the treatment of patients with newly diagnosed multiple myeloma: results of a prospective phase 2 Spanish/PETHEMA trial," *Haematologica*, vol. 100, no. 8, pp. 1096–1102, 2015.

[15] R. Fenk, M. Michael, F. Zohren et al., "Escalation therapy with bortezomib, dexamethasone and bendamustine for patients with relapsed or refractory multiple myeloma," *Leukemia and Lymphoma*, vol. 48, no. 12, pp. 2345–2351, 2007.

[16] G. Damaj, F. Malard, C. Hulin et al., "Efficacy of bendamustine in relapsed/refractory myeloma patients: results from the French compassionate use program," *Leukemia and Lymphoma*, vol. 53, no. 4, pp. 632–634, 2012.

[17] M. Offidani, L. Corvatta, L. Maracci et al., "Efficacy and tolerability of bendamustine, bortezomib and dexamethasone in patients with relapsed-refractory multiple myeloma: a phase II study," *Blood Cancer Journal*, vol. 3, no. 11, article e162, 2013.

[18] H. Ludwig, H. Kasparu, C. Leitgeb et al., "Bendamustine-bortezomib-dexamethasone is an active and well-tolerated regimen in patients with relapsed or refractory multiple myeloma," *Blood*, vol. 123, no. 7, pp. 985–991, 2014.

[19] P. Rodon, C. Hulin, B. Pegourie et al., "Phase II study of bendamustine, bortezomib and dexamethasone as second-line treatment for elderly patients with multiple myeloma: the Intergroupe Francophone du Myelome 2009-01 trial," *Haematologica*, vol. 100, no. 2, pp. e56–e59, 2015.

Management of iron overload in myelodysplastic syndromes: combined deferasirox and deferoxamine in a patient with liver disease

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Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders of haematopoietic stem cells, characterised by ineffective haematopoiesis leading to peripheral cytopenias and hypercellular bone marrow, with increased propensity to progression to acute myeloid leukaemia. Anaemia is the most common symptom: it may precipitate symptoms in patients with cardiac disorders, thus affecting the patients' outcome.

Approved therapies, such as erythropoiesis-stimulating agents, azacitidine, decitabine and lenalidomide are now available for patients who are ineligible for potentially curative haematopoietic stem cell transplantation. These former options can produce haematological improvement and enhance the quality of life of patients who previously would have received only supportive care.

In this context, supportive red blood cell transfusions represent a life-saving treatment for patients with chronic anaemia, in particular for those who do not respond or have a poor response to available treatments[1]. However, transfusions lead to iron overload, with an increased risk of associated comorbidity and mortality, independently of the underlying haematological disease, in relation to iron toxicity to cardiac, hepatic and endocrine cells. The management of iron overload is problematic because humans lack effective means to excrete excess iron.

Retrospective studies revealed that transfusion-related iron toxicity is associated with reduced survival in MDS patients[2]. This is a particularly relevant problem in low-risk MDS patients, because of these patients' otherwise long-life expectancy. Adequate iron chelation therapy can, however, improve survival and may delay transformation into acute myeloid leukaemia[3-6].

Iron chelation therapy is recommended in MDS to manage iron overload when the patient has, at least, elevated serum ferritin (SF), evidence of iron-related organ dysfunction or is receiving chronic red blood cell (RBC) transfusions. Guidelines from the Italian Society of Haematology recommend iron chelation therapy with deferasirox for the treatment of MDS patients with low/intermediate-1 risk (according to the International Prognostic Scoring Scale, IPSS) after they have received at least 20 units of packed RBC[5].

It is evident from controlled clinical trials, and confirmed by real-life experience[7], that iron overload in many MDS patients is often not adequately managed[4].

Iron chelation therapy should be considered in all patients who require long-term RBC transfusions while it may not be needed in patients with MDS or other acquired refractory anaemias who have an estimated survival of less than 1 year.

Ideally, chelation therapy should be initiated prophylactically, before clinically significant iron accumulation has occurred. Treatment should begin when patients have received between 10 and 20 units of RBC. Patients who have already undergone repeated transfusions without sufficient chelation can also be successfully treated, but they may require more intensive chelating regimens. Iron chelation therapy is recommended by several treatment guidelines for patients who have a low or intermediate-1 IPSS risk and SF >1,000-2,000 ng/mL, depending on transfusion requirements.

Evaluation of the patient before the initiation or adjustment of iron chelation therapy should include a detailed characterisation of the underlying disorder, with thorough documentation of the transfusion and chelation history, determination of body iron load by measurement of hepatic iron and SF, estimation of the rate of transfusional iron loading, and assessment of cardiac iron deposition[8].

Until recently, desferoxamine and deferiprone were the only drugs available for iron chelation therapy and neither was well tolerated by patients.

Deferoxamine was developed more than 40 years ago and, due to its pharmakokinetic properties, in order to be effective, must be administered subcutaneously or intravenously, usually with a portable pump, as a slow infusion over 8-12 hours/day, 5-7 days/week, often resulting in poor compliance. Subcutaneous administration is preferred, except in patients with severe cardiac iron deposition, for whom continuous intravenous deferoxamine is recommended. This regimen is contraindicated in patients with

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thrombocytopenia and the inconvenience often results in low compliance[9-11].

Deferiprone is not approved or recommended for MDS, as it can cause neutropenia and agranulocytosis.

Deferasirox is a once-daily orally administered iron chelator, with established dose-dependent efficacy, approved for the treatment of transfusional iron overload in both adult and paediatric patients with transfusion-dependent anaemia. The initial dose of 10 mg/kg can be increased to 20-30 mg/kg based on the degree of iron load, concentration of SF and extent of iron-related organ damage[12]. The efficacy and safety of deferasirox have been evaluated in patients with β-thalassaemia and a wide range of other disorders, including MDS, sickle cell disease, aplastic anaemia, Diamond-Blackfan anaemia, and other rare anaemias[13]. *In vivo* studies in acute myeloid leukaemia and MDS cell lines showed that deferasirox is a potent nuclear factor-kB inhibitor, which may partly explain the reports regarding its ability to produce haematological improvements[14,15]. In addition to reducing key indicators of total body iron level (SF, liver iron concentration, and toxic labile plasma iron), deferasirox has also been shown to remove cardiac iron and prevent further cardiac iron accumulation. It has an acceptable safety profile: the most commonly reported side effects have been non-progressive changes in serum creatinine levels, gastrointestinal disturbances and skin rashes, with significant increases in alanine transaminase value after 12 months of treatment being possible, in direct correlation with the dose administered[7]. Because of its potential hepatotoxicity, it is usually not recommended for patients with known liver disease.

Nowadays, most patients requiring iron chelation therapy opt for deferasirox because of the convenience of its oral administration, while deferoxamine, which has been proven to reverse iron-induced heart disease and increase long-term survival[16], may be indicated if deferasirox is ineffective, and it may be favoured for severe iron overload, especially with cardiac involvement.

Deferasirox may be better in patients who are unable to tolerate subcutaneous infusions of deferoxamine and it may also be an alternative to deferoxamine after successful clearance of cardiac iron.

To our knowledge, the possibility of iron chelation therapy with a combination of deferasirox and deferoxamine has been reported only in patients with β-thalasssaemia[17-20]. There do not appear to be any data on the use of this combination in MDS patients with liver disease. We describe here the first patient affected by MDS and chronic liver disease in whom combined iron chelation therapy was successfully employed.

Case report

A 62-year old Caucasian man was first seen in our Division for anaemia. He was diagnosed as having MDS-refractory anaemia (low IPSS risk), and hepatitis C virus-correlated liver cirrhosis (Child-Pugh class B) with signs of portal hypertension (portal vein 14.6 mm, splenic vein 14 mm, normal mesenteric vein), and severe splenomegaly (longitudinal diameter 205 mm). Liver function tests at the diagnosis of MDS were: serum albumin 3.2 g/dL, normal coagulation profile, total/direct bilirubin 1.01/0.69; aspartate transaminase 38 U/L (normal values <40 U/L), alanine transaminase 60 U/L (normal values <40 U/L). The patient was initially treated with an erythropoiesis-stimulating agent (30,000 U/week), without success for 6 months, which was then withdrawn, and he continued treatment with only RBC transfusions, requiring two packs/month. Iron chelation therapy was started when he had a SF of 700 ng/mL (normal values 30-400 ng/mL): deferoxamine was given (starting dose 15 mg/kg/day, for 5 days/week, increased up to 25 mg/kg/day, for 5 days/week), in consideration of the patient's pre-existing hepatic disease. However, the patient was unable to take the drug correctly and his transfusion needs increased to two packs of RBC/week, with his SF exceeding 6,000 ng/mL (Figure 1), after 12 months of transfusion treatment. At that time,

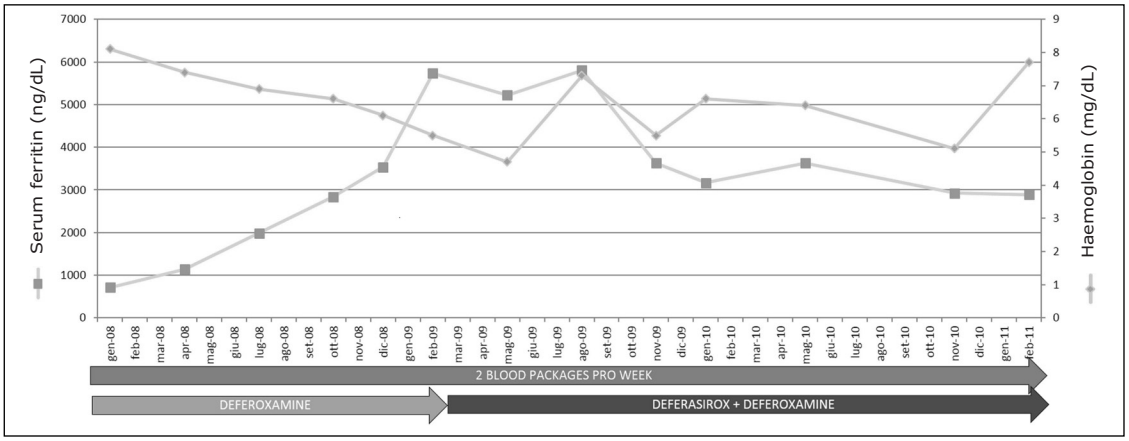

Figure 1 - Time course of serum ferritin (normal values: 30-400 ng/dL) and haemoglobin concentrations.

deferasirox was started (Table I) at the dose of 10 mg/kg/day, after a thorough investigation of the patient's hepatic, renal and cardiac function. After 3 months, neither SF nor other biochemical parameters had changed. The dose of deferasirox was then gradually increased to 30 mg/kg/die after 2 months, without evidence of liver damage. After 5 months of a full dose of deferasirox, the patient's SF concentration was 5,098 ng/mL.

Taking into consideration all risks related to secondary haemochromatosis, after informed consent, combined iron chelation therapy with deferasirox (30 mg/kg/die) and deferoxamine (25 mg/kg/day for 5 days/week) was started. After 3 months the patient's SF had decreased to 3,000 ng/mL. In the meantime, his haemoglobin concentration decreased significantly, so he had to be given two packs of RBC/week. After 2 years of combined therapy, his SF concentration was stable under 3,000 ng/mL and his transfusion requirements gradually decreased (Figure 1). No adverse events were observed and regular monitoring of hepatic (Figure 2), renal and cardiac function did not show any alterations. After 4 years of transfusions and combined iron chelation therapy, the patient died from acute respiratory distress syndrome.

Table I - Characteristics of deferoxamine and deferasirox[8].

Variable	Deferoxamine	Deferasirox
Chelator-iron complex	Hexadentate, 1:1 complex	Tridentate, 2:1 complex
Usual dose	25-50 mg/kg/day	20-40 mg/kg/day
Administration	Subcutaneous or intravenous, 8-10 h/day, 5-7 days/week	Oral, once daily
Plasma half-life	20-30 min	8-16 hr
Route of elimination	Biliary and urinary	Predominantly biliary
Regulatory approval	Approved in USA, Canada, Europe and other countries	Approved in USA, Canada, Europe and other countries
Indication	Transfusional iron overload	Transfusional iron overload
Adverse effects	Irritation at the infusion site, ocular and auditory disturbances, growth retardation and skeletal changes, allergy, respiratory distress syndrome with higher-than-recommended doses	Gastrointestinal disturbances, rash, increase in serum creatinine level; potential foetal renal and hepatic impairment or failure, gastrointestinal haemorrhage

Discussion

Supportive care of MDS patients is based on RBC transfusions, with management of iron overload being an essential, but sometimes overlooked, part of the treatment. In recent years, better understanding of the biological consequences of secondary haemosiderosis in MDS has suggested that iron chelation therapy should be started promptly to prevent serious clinical sequelae in patients with a long life-expectancy. Retrospective analyses indicated that iron overloading has an impact on the outcome of MDS patients and suggested that chelation therapy could improve patients' overall survival.

Until recently, desferoxamine and deferiprone were the only drugs available for the treatment of transfusional iron overload, but deferasirox is changing the clinical scenario of iron chelation therapy.

Deferasirox is a once-daily orally administered iron chelator, with established dose-dependent efficacy, approved for the treatment of transfusional iron overload in both adult and paediatric patients with transfusion-dependent anaemia. The drug has an acceptable safety profile, with the most common side effects reported being non-progressive changes in serum creatinine levels, gastrointestinal disturbances, and skin rash, and dose-related hepato-toxicity.

Deferasirox may allow effective iron chelation therapy in patients intolerant to subcutaneous infusions of deferoxamine.

In our case, the combination of deferasirox and deferoxamine had significant effects on iron overload,

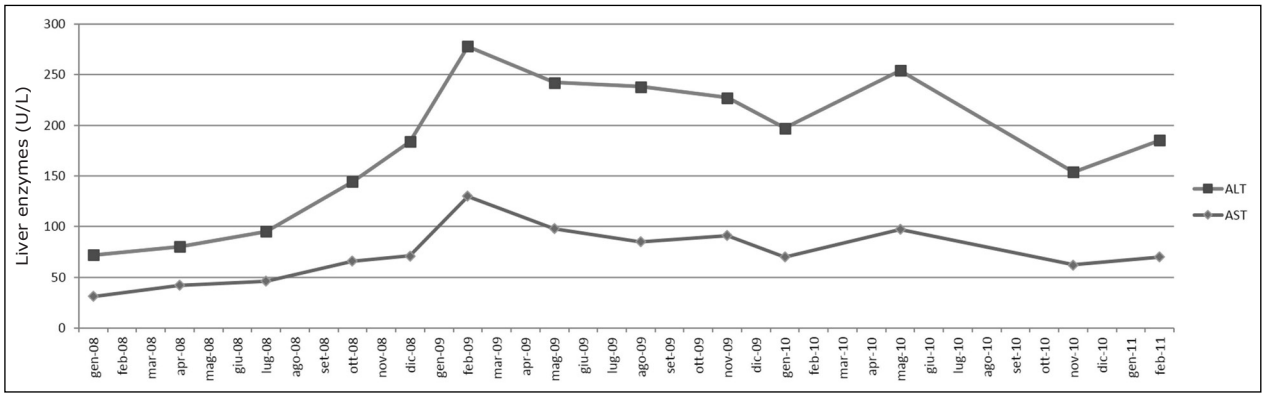

Figure 2 - Time course of liver function parameters: aspartate transaminase (AST, normal values <40 U/L) and alanine transaminase (ALT, normal values <40 U/L).

and proved to be safe in a patient with hepatitis C virus-correlated liver cirrhosis. The patient complied well with the treatment, had a good quality of life, had no side effects and did not require hospitalisation. Moreover, as reported in literature, deferasirox can also improve haematological parameters: our patient had a decrease in transfusional needs during treatment, which could have been related to the deferasirox treatment[2,6,14].

In our opinion, if deferasirox alone is not able to reduce iron overload rapidly, combined treatment with deferoxamine should be considered a safe and useful therapeutic choice, in selected patients, although our preliminary observations need to be validated by controlled clinical trials.

Authorship contributions

CC, FP and LC participated in the conception and design of the study, data analysis and interpretation, drafting the article and revising it critically for important intellectual content, and approved the final version for publication giving final approval for publication. CC also collected the data and is responsible for the overall content as guarantor.

GC, SA, RDP, NP and MP participated in the conception and design of the study, data analysis and interpretation, and approved the final version for publication.

Keywords: deferasirox, deferoxamine, iron overload, iron chelation, myelodysplastic syndromes.

The Authors declare no conflicts of interest.

References

1) Malcovati L, Hellström-Lindberg E, Bowen D, et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood 2013; **122**: 2943-64.
2) Improta S, Villa MR, Volpe A, et al. Transfusion-dependent low-risk myelodysplastic patients receiving deferasirox: long-term follow-up. Oncol Lett 2013; **6**; 1774-8.
3) Neukirchen J, Fox F, Kündgen A, et al. Improved survival in MDS patients receiving iron chelation therapy – a matched pair analysis of 188 patients from the Dusseldorf MDS registry. Leuk Res 2012; **36**; 1067-70.
4) Rose C, Brechignac S, Vassilief D, et al. Does iron chelation therapy improve survival in regularly transfused lower risk MDS patients? A multicenter study by the GFM (Groupe Francophone des Myelodysplasies). Leuk Res 2010; **34**; 864-70.
5) Santini V, Alessandrino PE, Angelucci E, et al. Clinical management of myelodysplastic syndromes: update of SIE, SIES, GITMO practice guidelines. Leuk Res 2010; **34**; 1576-88.
6) Malcovati L. Impact on transfusion dependency and secondary iron overload on the survival of patients with myelodysplastic syndromes. Leuk Res 2007: **31** (Suppl 3): S2-6.
7) Breccia M, Alimena G. Efficacy and safety of deferasirox in myelodysplastic syndromes. Ann Hematol 2013; **92**: 863-70.
8) Brittenham GM. Iron-chelating therapy for transfusional iron overload. N Engl J Med 2011; **364**: 146-56.
9) Nisbet-Brown E, Olivieri NF, Giardina PJ, et al. Effectiveness and safety of ICL670 in iron-loaded patients with thalassemia: a randomised, double-blind, placebo-controlled, dose-escalation trial. Lancet 2003; **361**: 1597-602.
10) Cohen AR. Compassionate use of deferiprone for patients with thalassemia and iron-induced heart disease. ClinicalTrials.gov identifier n. NCT00293098.
11) Borgna-Pignatti C, Cappellini MD, De Stefano P, et al. Cardiac morbidity and mortality in deferoxamine- or deferiprone-treated patients with thalassemia major. Blood 2006; **107**: 3733-7
12) Gattermann N, Finelli C, Porta MD et al. Deferasirox in iron-overload patients with transfusion dependent myelodysplastic syndromes: results from the large 1-year EPIC study. Leuk Res 2101; **34**: 1143-50.
13) Porter J, Galanello R, Saglio G, et al. Relative response of patients with myelodysplastic syndromes and other transfusion-dependent anemias to deferasirox (ICL670): a 1-year prospective study. Eur J Haematol 2008; **80**: 168-76.
14) Gattermann N, Finelli C, Della Porta M, et al. Hematologic responses with deferasirox therapy in transfusion-dependent myelodysplastic syndromes patients. Haematologica 2012; **97**: 1364-71.
15) List AF, Baer MR, Steensma DP, et al. Deferasirox reduces serum ferritin and labile plasma iron in RBC transfusion-dependent patients with myelodysplastic syndrome. J Clin Oncol 2012; **30**: 2134-9.
16) Davis BA, Porter JB. Long-term outcome of continuous 24-hour deferoxamine infusion via indwelling intravenous catheters in high-risk beta-thalassemia. Blood 2000; **95**: 1229-36.
17) Jetsrisuparb A, Komvilaisak P, Wiangnon S, Jetsrisuparb C. Retrospective study on the combination of desferrioxamine and deferasirox for treatment of iron-overloaded thalassemic patients: first evidence of more than 2 years. J Pediatr Hematol Oncol 2010; **32**: 400-3.
18) Lal A, Porter J, Sweeters N, et al. Combined chelation therapy with deferasirox and deferoxamine in thalassemia. Blood Cells Mol Dis 2013; **50**: 99-104.
19) Voskaridou E, Komninaka V, Karavas A, et al. Combination therapy of deferasirox and deferoxamine shows significant improvement in markers of iron overload in a patient with β-thalassemia mayor and severe iron burden. Transfusion 2014; **54**: 646-9.
20) Cassinerio E, Orofino N, Roghi A, et al. Combination of deferasirox and deferoxamine in clinical practice: an alternative scheme of chelation in thalassemia major patients. Blood Cells Mol Dis 2014; **53**: 164-7.

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LETTER TO THE EDITOR

Reply to the letter to the editor "chronic disseminated candidiasis" by Kenneth Rolston

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Dear Editor,

We would like to thank Kenneth Rolston for his comments regarding our recent *Supportive Care in Cancer* article on chronic disseminated candidiasis (CDC) in patients with hematological malignancies on the behalf of SEIFEM (Sorveglianza Epidemiologica Infezioni Fungine in Ematologia) group [1].

We acknowledge the small sample size (N = 20) and the retrospective nature of the study, which is probably not enough capable to lead to significant modifications of the CDC treatment recommendations. However, we would like to underline some aspects.

First, the guidelines of the Infectious Diseases Society of America (IDSA) strongly recommend the first line therapy of CDC with lipid formulation amphotericin B (AmB) 3–5 mg/kg daily [2]. Our data suggest that high-dose (HD) liposomal AmB (5 mg/kg daily) is the better choice for the treatment of CDC. This is likely due to the fungicide action of HD liposomal AmB in the liver and spleen derived from better tissue concentrations (target of liposomal formulation: reticuloendothelial system) than that of triazoles and echinocandins [3]. In addition, the 5 mg/kg daily dosage for liposomal AmB may be useful for less susceptible species, such as *Candida glabrata* and *Candida krusei* [2]. On the other hand, in our series, the majority of patients were receiving triazoles prophylaxis and thus had an increased risk of developing infection with a fluconazole-resistant organism [2]. Moreover, according to the IDSA guidelines, fluconazole (6 mg/kg daily) should be administered only for maintenance therapy [2].

Second, 13/20 (65%) patients received diagnosis of probable CDC according to standard criteria, i.e., an alkaline phosphatase increase, hepatic and/or splenic nodules with typical bull's eye aspect (seen at imaging tools), and blood cultures positive for *Candida* spp. (no polymicrobic sepsis occurred in our series) [4]. Such patients had negative serum galactomannan monitoring and negative thorax radiological assessments; three cases had a serum β-D-glucan assay >80 pg/ml (270, 520, and 370 pg/ml, respectively). Altogether, it is very unlikely that these findings may represent infections due to other organisms, particularly molds. According to the policy of the SEIFEM group, when clinically indicated, we performed liver biopsy using a Menghini-type automatic fine-cutting needle (1.2 mm, 18G) under color ultrasound guidance, as already reported [5, 6]. In fact, the remaining seven patients underwent a mini-invasive procedure that was well tolerated with no discomfort and provided reliable information regarding liver histology, leading to the definitive diagnosis of CDC.

Third, both cases no. 11 and no. 20 died early as a result of CDC (before the definitive microbiological results from blood samples); they were receiving empirical antifungal treatment, respectively, with fluconazole and itraconazole.

Finally, no liposomal AmB-related toxicity of grade ≥3, according to the Common Terminology Criteria for Adverse Events (CTCAE), occurred in our series [7].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Della Pepa R, Picardi M, Sora F, Stamouli M, Busca A, Candoni A, Delia M, Fanci R, Perriello V, Zancanella M, Nosari A, Salutari P, Marchesi F, Pane F, Pagano L, (2016) Successful management of chronic disseminated candidiasis in hematologic patients treated with high-dose liposomal amphotericin B: a retrospective study of the SEIFEM registry. Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer 24(9):3839–3845. doi:10.1007/s00520-016-3208-0
2. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD (2016) Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 62(4):e1–50. doi:10.1093/cid/civ933
3. Vogelsinger H, Weiler S, Djanani A, Kountchev J, Bellmann-Weiler R, Wiedermann CJ, Bellmann R (2006) Amphotericin B tissue distribution in autopsy material after treatment with liposomal amphotericin B and amphotericin B colloidal dispersion. J Antimicrob Chemother 57(6):1153–1160. doi:10.1093/jac/dkl141
4. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE, Herbrecht R, Hope WW, Kibbler CC, Kullberg BJ, Marr KA, Munoz P, Odds FC, Perfect JR, Restrepo A, Ruhnke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaoutis T, Bennett JE, European Organization for R, Treatment of Cancer/Invasive Fungal Infections Cooperative G, National Institute of A, Infectious Diseases Mycoses Study Group Consensus G (2008) Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 46(12):1813–1821. doi:10.1086/588660
5. Pagano L, Mele L, Fianchi L, Melillo L, Martino B, D'Antonio D, Tosti ME, Posteraro B, Sanguinetti M, Trape G, Equitani F, Carotenuto M, Leone G (2002) Chronic disseminated candidiasis in patients with hematologic malignancies. Clinical features and outcome of 29 episodes. Haematologica 87(5):535–541
6. Picardi M, Muretto P, De Rosa G, Selleri C, De Renzo A, Persico M, Rotoli B (2002) Color ultrasound-guided fine needle cutting biopsy for the characterization of diffuse liver damage in critical bone marrow transplanted patients. Haematologica 87(6):652–657
7. Common Terminology Criteria for Adverse Events (CTCAE) (2009) Version 4.0 (May 28, 2009 (v4.03: June 14, 2010))

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ORIGINAL ARTICLE

Randomized comparison of power Doppler ultrasonography-guided core-needle biopsy with open surgical biopsy for the characterization of lymphadenopathies in patients with suspected lymphoma

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Abstract The sensitivity of lymph node core-needle biopsy under imaging guidance requires validation. We employed power Doppler ultrasonography (PDUS) to select the lymph node most suspected of malignancy and to histologically characterize it through the use of large cutting needle. Institutional review board approval and informed consent were obtained for this randomized clinical trial. In a single center between 1 January 2009 and 31 December 2015, patients with lymph node enlargement suspected for lymphoma were randomly assigned (1:1) to biopsy with either standard surgery or PDUS-guided 16-gauge modified Menghini needle. The primary endpoint was the superiority of sensitivity for the diagnosis of malignancy for core-needle cutting biopsy (CNCB). Secondary endpoints were times to biopsy, complications, and costs. A total of 376 patients were randomized into the two arms and received allocated biopsy. However, four patients undergoing CNCB were excluded for inadequate samples; thus, 372 patients were analyzed. Sensitivity for the detection of malignancy was significantly better for PDUS-guided CNCB [98.8%; 95% confidence interval (CI), 95.9–99.9] than standard biopsy (88.7%; 95% CI, 82.9–93; $P < 0.001$). For all secondary endpoints, the comparison was significantly disadvantageous for conventional approach. In particular, estimated cost per biopsy performed with standard surgery was 24-fold higher compared with that performed with CNCB. The presence of satellite enlarged reactive and/or necrotic lymph nodes may impair the success of an open surgical biopsy (OSB). PDUS and CNCB with adequate gauge are diagnostic tools that enable effective, safe, fast, and low-cost routine biopsy for patients with suspected lymphoma, avoiding psychological and physical pain of an unnecessary surgical intervention.

Keywords Lymphoma · Power Doppler ultrasonography · Core-needle cutting biopsy

Introduction

In the case of clinical suspicion of lymphoma, the histological examination of lymphadenopathy is essential for defining a correct diagnosis and for developing a proper treatment plan [1]. An open surgical biopsy (OSB) is still the "gold standard," owing to the large amount of tissue obtained [2]. Preoperative evaluation includes (1) a careful and through physical examination, i.e., palpation of superficial lymph node regions performed by a physician experienced in the management of patients with lymphoma; (2) gray-scale ultrasonography scans (US), i.e., a technology that is readily available in clinical practice and is considered to provide sufficient information for selecting the node to be biopsied [1, 2]; and (3) computed tomography (CT), performed to strengthen the suspicion of lymphoma [2]. However, the possible presence of enlarged reactive or necrotic lymph nodes and/or of

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nonpalpable but histologically significant malignant lymph nodes may impair the success of an OSB. Another limitation is mostly related to patients whose conditions may be too unstable for undergoing general anesthesia and surgical intervention [3]. Thus, a study that has value to decide the primary interventionist diagnostic tool for suspected lymphoma is a clinically important topic. New mini-invasive approaches to this procedure based on imaging-guided methods are now available.

The introduction of the new generation of ultrasonographic and biopsy needle devices, which already have been proven valuable in the management of patients with lymphoma in our cancer center [4–8], provides the opportunity to develop effective combined diagnostic strategy. The modern US instruments merge tissue harmonic compound, which generates an image from multiple imaging lines that strike the target from different angles [9], with power Doppler (PD) which allows the study of the angioarchitecture of lymph node tissue [5, 6, 8, 10]. Neoplastic angiogenesis such as vessel proliferation (endothelial cell migration and proliferation) and abnormal vascularization (tube formation with stenosis, occlusion, and/or dilation and/or arteriovenous shunts) is recognized as being critical for B cell lymphoma pathogenesis [11, 12]. Power Doppler ultrasonography (PDUS) equipment detects fine flow signals, mimicking an angiography of microvascular intranodal network. The result is a high-resolution quality examination that allows better detection of both superficial and deep-seated malignant lymphadenopathies compared with results obtained with gray-scale US [13]. Regarding biopsy needle devices, the latest Menghini needles have ultrathin sharpened cannula with trocar stylet and automatic aspiration with tiny battery-powered vacuum [14]. These characteristics make particularly effective the needle devices with large gauge [15]. Under PDUS guidance, the tip of cutting needle can be careful positioning into the most significant target, obtaining histological suction of the core of nodal lesion [14–16]. Nevertheless, few clear indications for performing such procedure are available. The Lugano classification for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma recommend core-needle biopsy when surgical intervention is not possible and to document relapse [2]. However, the existing guidelines are not evidence based, a uniform program for optimal imaging guidance is lacking, and the characteristics of biopsy needle, i.e., gauge, length, tip configurations, and sampling mechanisms, are still a matter of opinion among experts [1, 14, 16]. Thus, this approach requires validation with randomized studies.

Our trial was intended to test the efficacy of PDUS-guided core-needle cutting biopsy (CNCB) compared with OSB as first-line diagnostic approach for pathologic lymphadenopathies in patients with clinical suspicion of lymphoma. The primary endpoint of the study was the sensitivity for diagnosis of malignancy for each of the two interventionist methods, i.e., percutaneous biopsy by using modified Menghini needle under modern US guidance and standard excisional biopsy. Additional endpoints were times to biopsy, rates of biopsy-related complications, and costs.

Materials and methods

Trial design and participants

Included patients were randomly assigned at 1:1 allocation ratio to receive lymph node biopsy by using one of two methods, OSB (standard group) or PDUS-guided CNCB (core-needle group).

Patients were required to meet the following eligibility criteria: (a) age ≥14 years, (b) lymph node enlargement clinically suspected for lymphoma, and (c) indication to perform nodal biopsy. Patients affected by Epstein-Barr virus, cytomegalovirus, herpes simplex virus, rubella, toxoplasma, or tuberculosis infection, as well as abnormalities of coagulation tests were excluded.

This was a single center study. Eligible patients were registered at the Hematology Division Office of the "Federico II" University of Naples, where the trial was designed and approved by the local Institutional Review Board in the early 2008 (10 January 2008; number of registration, 140/2008).

Interventions

Standard group

In the standard group, all biopsy-related procedures were performed by surgeons experienced in lymph node resection. The patients underwent physical examination and gray-scale US, of whom findings were sufficient to account for the region to be biopsied according to conventional methods [17]. At surgeon's discretion, biopsy was directed to the most superficial and/or largest lymph node. In a day hospital regimen or as inpatients, and under local or general anesthesia (according to the type of intervention scheduled), the lymph nodes were harvested through skin crease incision obtained by free-hand methods. Superficial lymphadenopathy was removed by means of excisional biopsy. Mini-cervicotomy or mediastinotomy were used for removing lymphadenopathy in the anterosuperior mediastinum, and abdominal and pelvic lymphadenopathies were removed by means of laparotomy.

Core-needle group

In the core-needle group, all biopsy-related procedures were performed by two members of the hematology staff (N. Pugliese and *M. Picardi*, with more than 10 years of experience with interventionist PDUS) [4, 5]. The lymph node to undergone CNCB was determined by PDUS assessment as

already reported [8]. In particular, baseline US exploration of all superficial, anterosuperior mediastinum (clavicular, supra-aortic, and prevascular regions), and abdominal and pelvic lymph node areas was carried out. Then, any abnormal [for size (long axis ≥ 2 cm), round shape, hilus absent, and/or hypoechoic parenchyma] lymph node underwent power Doppler examination in accordance with methods already described [5, 6, 8], using a scanner (iU22; Philips Health-care, Bothell, Wash) equipped with tissue harmonic compound technology (SonoCT; Philips), power Doppler sonography, and 5–1 MHz (C5-1 curvilinear; Philips) and 9–3 MHz (L9-3 linear; Philips) broadband probes. The main criterion to select the node to be biopsied was the hypervascularization, i.e., intranodal arterial vessels with high-resistive index value (>0.6) [6, 8]. All CNCB were carried out under US guidance with a puncture adaptor, an aseptic technique (sterile cover of the probe and sterile gel), and cutaneous anesthesia, using a 16-gauge diameter modified Menghini needle 150 mm in length with automatic aspiration (Biomol® HS-Hospital; Rome, Italy).

Reference standard

The reference standard for lymph node involvement was histopathologic examination. It was performed in a single pathology unit by at least three expert hematopathologists (I. Cozzolino, G. Ciancia, G. Pettinato, P. Zeppa, and/or V. Varone, with more than 10 years of experience with hematopathological analysis) [5]. Lymph node samples were routinely fixed in formalin and embedded in paraffin (FFEP). The histologic sections were stained according to standard methods (hematoxylin and eosin, and Giemsa). All cases of lymphoma were diagnosed by a combination of morphologic, immunohistochemical and/or molecular analyses and were classified according to the current WHO criteria [1]. Immunophenotyping was carried out in FFEP slides with antibodies recognizing CD3, CD4, CD8, CD5, CD10, CD15, CD20, CD23, CD30, CD45RB, CD56, CD79a, bcl-2, bcl-6, cyclin D1, PAX-5, Mum-1, Ki-67, ALK-1, and TdT. *Bcl-2*, *Myc*, *Cyclin D1*, and *MALT-1* gene translocations were evaluated by fluorescent in situ hybridization analysis in FFPE slides using commercially available kits, whenever deemed necessary. B or T cell clonality was also investigated by polymerase chain reaction. Epithelial metastatic tumors were identified by monoclonal antibodies to cytokeratin.

Overall, biopsies were categorized as positive for malignancy (samples containing adequate number of cells with morphologic atypia and evidence of monoclonality), negative for malignancy (samples containing adequate number of cells with no evidence of malignancy), or inadequate (specimens too small to confirm or rule out malignancy). Patients classified as having histologic results negative for malignancy underwent strict follow-up by clinicians for the following months, in order to discovery a malignant disease undetected at first biopsy.

In 50 patients of the experimental arm, the biopsy specimens of nodal tissue were studied by the three operators: each one was blinded to the patient's clinical condition and to the histologic results of the other hematopathologists (interobserver reproducibility) [15].

Primary and secondary outcomes

The sensitivity for each arm was defined as the ratio of patients who showed lymph node positive for malignancy at first biopsy compared with the total number of patients with malignancy. In addition, the negative predictive value was defined as the ratio of patients with lymph node negative for malignancies at first biopsy compared to the total number of patients with negative results for malignancy during the follow-up. The likelihood ratio of a negative test was also calculated (1 minus sensitivity divided by specificity).

The waiting time for the performance of biopsy was calculated as the number of days elapsed between indication to lymph node biopsy and the execution of the procedure itself.

After biopsy, patients were strictly monitored in order to look for procedure-related complications. Outpatients were kept under observation for 1 h and were discharged if there were no signs or symptoms suggestive of a significant complication. All patients were encouraged to contact their physicians if they developed symptoms after leaving hospital.

Cost analysis for biopsy procedures was performed by adopting the perspective of the National Healthcare System. Cost calculations for PDUS-guided CNCB were based on the tariffs in the Nomenclature for Outpatient Care, provided by the Italian National Healthcare System (http://www.arsan.campania.it/documents/10157/01088316-4824-4c7e-8671-1418af8f3af7). The costs of OSB were calculated according to the diagnosis-related group tariffs that are currently used to fund in patient health services in Italy (http://www.eumed.it/drg/tariffe_drg.asp).

Sample size

We tested the hypothesis that histological yield obtained with PDUS-guided CNCB resulted in a higher sensitivity than OSB, owing to a more significant lymph node tissue biopsied (i.e., the viable core of malignant lesion was exactly removed). Based on previous studies, we estimated a sensitivity rate at standard biopsy of 78% [5] and at PDUS-guided CNCB of 96.5% [14, 18]; hence, a certain number of patients could be underdiagnosed with OSB approach. To detect more than 10% sensitivity improvement (for the superiority test), 332 patients were needed, when using a two-sided type I error of 5% and 99% statistical power. Assuming a dropout rate of 10%, we set a final sample size of at least 183 patients in each group.

Randomization

Random allocation sequence was carried out by using a computerized system (generated by the study statistician on the basis of the procedure outlined elsewhere) [19]. It was based on a minimization method in which patients were assigned to the two study groups while ensuring equal distribution on the basis of sex, age, presence and type of systemic symptoms (i.e. fever, sweating, and weight loss) and sites of lymph node enlargement at baseline clinical evaluation.

Patients were asked to sign a consent form before randomization, according to the requirements of the Helsinki declaration.

Statistical analysis

For the statistical evaluations, the χ^2 test was performed to compare proportions for clinical and histological characteristics and complication rate, and the *t* test was used to compare the quantitative variables of clinical characteristics, costs, and waiting times to biopsy between the two groups. *P* values less than 0.05 were considered to indicate a significant difference.

Asymptotic 95% confidence intervals for kappa statistic (to assess the level of agreement in diagnostic opinion among all three hematopathologists for the 50 samples of the core of nodal tissue) were computed according to Fleiss et al. [20].

Results

Participants and recruitment

Between 1 January 2009 and 31 December 2015, 376 patients were randomly assigned either to standard group ($N = 187$) or core-needle group ($N = 189$). All randomized patients received allocated biopsy intervention. However, four patients (2.1%) undergoing PDUS-guided CNCB were excluded for inadequate samples (thereafter, these cases underwent an OSB). No other patient was lost to follow-up, nor did any withdraw their consent to participate in the study when a second biopsy was clinically indicated during monitoring. Thus, a total of 372 patients was analyzed for the primary endpoint (standard group, $N = 187$; core-needle group, $N = 185$). Twenty-two patients (5.5%) failed during screening. A common reason for exclusion was contraindications for general anesthesia ($N = 12$). Other reasons were the presence of obesity, potential cause of uninterpretable PDUS scans for deep-seated lymph nodes ($N = 6$), and refused to participate ($N = 4$). A consolidated standard of reporting trials' (CONSORT) diagram summarizes the study in Figure 1.

Patients in both groups were well-balanced with respect to clinical characteristics, in particular symptoms suspected for lymphoma and nodal sites involved at baseline evaluation (Table 1).

Power Doppler ultrasonographic and core-needle features

The average time required for PDUS examination and core-needle biopsy was 40 min (range, 30–50 min). Sites of biopsied lymph nodes were superficial in 140 cases (vs. 160 cases in the standard group) and deep-seated (abdominal or pelvic regions) in 45 cases (vs. 27 cases in the standard group, $P = 0.02$). For each core-needle biopsy, a median of 2 needle passes (range, 1–4) into the nodal tissue was made. Length of core-needle specimens varied from 15 to 70 mm (median, 32 mm). Median-estimated volume of acquired tissue was 185 mm^3 with a range of 92–430 mm^3 (vs. a median volume of 1458 mm^3 with a range of 312–5678 mm^3, in the standard

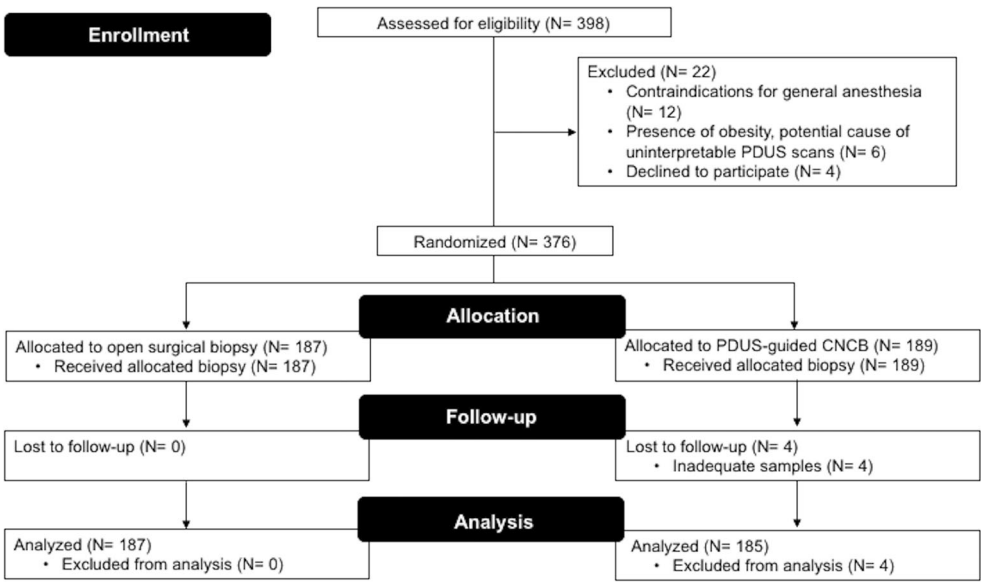

Fig. 1 Flowchart shows patient selection and follow-up during the study (CONSORT). *PDUS* = power Doppler ultrasonography

Table 1 Baseline characteristics of patients in the two study groups

	Standard group	Core-needle group	*P* value
Total patients	187	185	
Sex			
Male	98 (52.4)	86 (46.5)	0.25
Female	89 (47.6)	99 (53.5)	
Age, years			
Median, (range)	46 (18–79)	42 (17–76)	0.61
Symptoms			
Fever	33 (17.6)	31 (16.8)	0.82
Sweat	24 (12.8)	25 (13.5)	0.84
Weight loss	27 (14.4)	26 (14.1)	0.91
Site of clinically suspected lymphadenopathies			
Cervical	93 (49.7)	90 (48.6)	0.83
Axillary/pectoral	41 (21.9)	39 (21.1)	0.84
Antero-superior mediastinum	4 (2.1)	3 (1.6)	0.71
Inguinal	28 (15)	30 (16.2)	0.74
Abdomen-pelvic	21 (11.2)	23 (12.4)	0.72

Note: unless otherwise indicated, data are number of patients, with percentage in parentheses

group). The number of tests (i.e., staining and/or molecular analyses) performed by pathology on core-needle tissue and surgical excisional biopsy was similar in the two study groups.

Interobserver reproducibility of histological assessments of the cores of nodal tissue among the three pathologists had a kappa score of 0.916 (95% CI: 0.756–1.07). Of the 50 samples tested for reproducibility, 49 (98%) were classified identically by the three observers.

Histology

Of the 187 patients undergoing OSB, 149 (80%) cases had lymph nodes positive for malignancy, and 38 (20%) had lymph nodes negative for malignancy (described as benign lymphoid hyperplasia in 37 cases, and sarcoidosis in one case, with steato-fibrotic and/or necrotic changes in 17 of the cases).

Of the 185 patients undergoing PDUS-guided CNCB (all with adequate specimens), 172 (93%) cases had lymph nodes positive for malignancy, and 13 (7%) had lymph nodes negative for malignancy (benign lymphoid hyperplasia in 10 patients, Kikuchi-Fujimoto disease in two patients, and sarcoidosis in one patient; Table 2).

Overall, the 51 patients with lymph nodes negative for malignancy (defined as reactive or inflammatory) were observed for a median follow-up of 10 months (range, 1–24 months). During the follow-up, for 19 of 38 patients in the standard group, the clinicians required a second lymph node biopsy, and a malignancy was finally detected. The second biopsy, which was performed after a median of 5 months (range, 1–9 months) from the first biopsy, demonstrated lymphoma in 16 patients (five diffuse large B cell lymphomas, three grade 1 follicular lymphomas, two small lymphocytic lymphomas, four Hodgkin lymphomas, one mantle cell lymphoma, and one nodal marginal zone lymphoma) and metastatic carcinoma in three patients (Table 3). In contrast, two of the 13 patients who had had diagnosis of a benign lesion at the first biopsy in the core-needle group required a second biopsy (open surgical intervention in both cases) after 6 and 8 months, respectively. Histologic examination showed a malignancy in both cases (one grade 1 follicular lymphoma and one small lymphocytic lymphoma) (Table 3).

The definitive histological findings for each case in the two groups are shown in Tables 2 and 3. Overall, the majority of patients were suffering from lymphomas (B cell non Hodgkin lymphoma, 195 cases; Hodgkin lymphoma, 88 cases; T cell non Hodgkin lymphoma, 12 cases; and metastatic carcinoma, 47 cases).

Accuracy in identifying malignancy

The sensitivity rate of lymph node malignant status was 88.7% [95% confidence interval (CI): 82.9–93] for OSB (149 of 168 patients with lymph node positive for malignancy were identified) with a false negative rate of 10.2% (19 of 168 patients with lymph node positive for malignancy were not identified). By contrast, the sensitivity rate of lymph node malignant status was 98.8% (95% CI: 95.9–99.9) for PDUS-guided CNCB (172 of 174 patients with lymph node positive for malignancy were identified) with a false negative rate of 1.1% (i.e., 2 of 174 patients with lymph node positive for malignancy were not identified). Therefore, the study objective to show superiority of PDUS-guided CNCB versus OSB was achieved, being the sensitivity rate of experimental

Table 2 Histologic diagnosis on lymph node biopsy in the two study groups

	Standard group ($N = 187$)	Core-needle group ($N = 185$)
B cell neoplasms	84 (44.9)	97 (52.4)
Diffuse large B cell lymphoma	32 (17.1)	38 (20.5)
Follicular lymphoma	25 (13.4)	23 (12.4)
CLL/SLL[a]	16 (8.6)	18 (9.7)
Mantle cell lymphoma	7 (3.7)	12 (6.5)
Nodal marginal zone lymphoma	3 (1.6)	5 (2.7)
Primary mediastinal (thymic) large B cell lymphoma	1 (0.5)	1 (0.5)
Hodgkin lymphoma	38 (20.3)	46 (24.9)
Nodular sclerosis	25 (13.4)	30 (16.2)
Mixed cellularity	9 (4.8)	11 (5.9)
Nodular lymphocyte predominant	2 (1.1)	2 (1.1)
Lymphocyte-rich	1 (0.5)	1 (0.5)
Lymphocyte-depleted	1 (0.5)	2 (1.1)
T cell neoplasms	4 (2.1)	8 (4.3)
Anaplastic large cell lymphoma, ALK-positive	2 (1.1)	4 (2.2)
T cell lymphoblastic leukemia/lymphoma	1 (0.5)	2 (1.1)
Peripheral T cell lymphoma	1 (0.5)	1 (0.5)
Anaplastic large cell lymphoma, ALK-negative	–	1 (0.5)
Metastatic carcinoma	23 (12.3)	21 (11.4)
Nonmalignant findings	38 (20.3)	13 (7)
True-negative	19 (10.1)	11 (5.9)
Benign lymphoid hyperplasia	18 (9.6)	8 (4.3)
Sarcoidosis	1 (0.5)	1 (0.5)
Kikuchi-Fujimoto disease	–	2 (1.1)
False-negative	19 (10.1)	2 (1.1)
Benign lymphoid hyperplasia[b]	19 (10.1)	2 (1.1)

Note: unless otherwise indicated, data are number of patients, with percentage in parentheses

ALK anaplastic lymphoma kinase

[a] Chronic lymphocytic leukemia/small lymphocytic lymphoma

[b] With steato-fibrotic and/or necrotic changes in 17 of the cases

approach significantly higher than the standard approach ($P < 0.001$; Table 4).

Noteworthy, the sensitivity rate of lymph nodes positive for lymphoma was 98.7% (95% CI: 95.4–99.8) for PDUS-guided CNCB versus 88.7% (95% CI: 82.3–93.4) for OSB ($P < 0.001$). The negative predictive value was 54.3% (95% CI: 36.6–71.2) for OSB and 84.6% (95% CI: 54.5–98.1) for PDUS-guided CNCB ($P = 0.05$). The negative likelihood ratio was 0.11 (95% CI: 0.07–0.18) for OSB and 0.01 (95% CI: 0.00–0.05) for PDUS-guided CNCB, confirming the value of the PDUS-guided CNCB for detecting lymphoma.

Waiting time to biopsy

The median waiting time for performance of interventionist procedure (from biopsy indication to perform itself) in the core-needle group was 4 days (range, 1–10 days). By contrast, it was 16 days with a range of 5–34 days in the standard group ($P < 0.001$).

Procedure-related complications

Overall, 42 patients, which were in the standard group, underwent biopsy (cervical-clavicular, 17 cases; mediastinum compartments, 4 cases; abdomen-pelvis, 21 cases) under general anesthesia, with an average hospitalization of 2.5 days. All other patients underwent biopsy in a day surgery or outpatient regimen under local anesthesia.

Patients who received standard biopsy had significantly more pain, numbness or paresthesia, larger scars,

Table 3 Findings in the patients who underwent a second lymph node biopsy (all open surgical biopsies) in the two study groups

Patient No.	No. of months between the two biopsies	Biopsy site		Sample volume (mm^3)		Histologic diagnosis	
		First	Second	First	Second	First	Second
1	2	Cervical	Axillary	1597	2154	Benign hyperplasia[b]	Diffuse large B cell lymphoma
2	4	Inguinal	Mesenteric	1460	2092	Benign hyperplasia[b]	Diffuse large B cell lymphoma
3	3	Cervical	Supraclavicular	3200	4230	Benign hyperplasia[b]	Diffuse large B cell lymphoma
4	5	Supraclavicular	Axillary	1539	2129	Benign hyperplasia	Diffuse large B cell lymphoma
5	6	Inguinal	Iliac	5148	2766	Benign hyperplasia	Diffuse large B cell lymphoma
6	1	Cervical	Supraclavicular	2860	1769	Benign hyperplasia[b]	Nodular sclerosis—HL
7	3	Cervical	Cervical	4512	2870	Benign hyperplasia[b]	Nodular sclerosis—HL
8	3	Axillary	Supraclavicular	1955	2350	Benign hyperplasia[b]	Nodular sclerosis—HL
9	4	Inguinal	Cervical	2766	2020	Benign hyperplasia	Nodular sclerosis—HL
10	5	Cervical	Cervical	2030	1980	Benign hyperplasia	Follicular lymphoma Grade I
11	6	Cervical	Axillary	3240	2563	Benign hyperplasia	Follicular lymphoma Grade I
12	7	Inguinal	Inguinal	1780	1201	Benign hyperplasia[b]	Follicular lymphoma Grade I
13	6	Cervical	Supraclavicular	673	1251	Benign hyperplasia[b]	CLL/SLL
14	8	Cervical	Inguinal	1840	2560	Benign hyperplasia[b]	CLL/SLL
15	5	Cervical	Supraclavicular	790	1300	Benign hyperplasia	Mantle cell lymphoma
16	9	Supraclavicular	Axillary	1578	3410	Benign hyperplasia	Nodal marginal zone lymphoma
17	1	Cervical	Supraclavicular	4370	2531	Benign hyperplasia	Metastatic carcinoma
18	2	Inguinal	Inguinal	3594	1589	Benign hyperplasia	Metastatic carcinoma
19	5	Cervical	Supraclavicular	1737	2010	Benign hyperplasia[b]	Metastatic carcinoma
20[a]	6	Supraclavicular	Supraclavicular	230	2130	Benign hyperplasia	Follicular lymphoma Grade I
21[a]	8	Inguinal	Cervical	310	1867	Benign hyperplasia[b]	CLL/SLL

Note: Unless otherwise indicated, data are number of patients, with percentage in parentheses

HL Hodgkin lymphoma, *CLL/SLL* Chronic lymphocytic leukemia/small lymphocytic lymphoma

[a] Patients #20 and #21 had received power Doppler ultrasonography-guided core-needle cutting biopsy as first lymph node biopsy

[b] With intranodal steato-fibrotic and necrotic changes

Table 4 Accuracy of standard biopsy and PDUS-guided CNCB for the diagnosis of malignant lymph nodes

	Standard group (N = 187)	Core-needle group (N = 185)	P value
Sensitivity			
N	149/168	172/174	0.0001
%	88.7	98.8	
95% CI	82.9–93.0	95.9–99.9	
False-negative			
N (%)	19 (10.2)	2 (1.1)	0.0001
Negative predictive value			
N	19/38	11/13	0.014
%	50	84.6	
95% CI	33.4–66.6	54.5–98.1	
Negative likelihood ratio			
value	0.11	0.01	
95% CI	0.07–0.17	0.00–0.05	

CNCB core-needle cutting biopsy, *CI* confidence interval

lymphorrhea, and wound infection than patients who underwent PDUS-guided CNCB (Table 5).

Cost analysis

The total cost of the biopsy program was much lower for the core-needle group than that for the standard group. By using Italian values for direct costs of interventionist procedures, the cost for one OSB was €10,393 for major surgery and €3056 for minor surgery, whereas it was €171 for one PDUS-guided CNCB (including the complete US assessment of superficial and deep-seated lymph node areas). If the cost of additional surgical biopsies in the 19 patients (false negative results) of the standard group and in the two patients (false negative results) of the core-needle group is considered, the total cost of lymph node biopsy with standard approach was approximately 25-fold higher than that with PDUS-guided CNCB ($P < 0.001$; Table 6). C. Salvatore wrote the section devoted to cost analysis and produced Table 6.

Table 5 Biopsy-related complications in the two study groups

	Standard group (N = 187)	Core-needle group (N = 185)	P value
Pain on operated site[a]			
No	46 (24.6)	130 (70.3)	<0.0001
Yes, mild and transient	57 (30.5)	39 (21.1)	0.038
Yes, continuous	84 (44.9)	16 (8.6)	<0.0001
Numbness on operated site			
No	42 (22.5)	134 (72.4)	<0.0001
Yes	145 (77.5)	51 (27.6)	
Swelling on operated site			
No	50 (26.7)	162 (77.6)	0.0008
Yes	137 (73.3)	23 (12.4)	
Esthetic appearance of biopsy scar[b]			
Absent	–	185 (100)	<0.0001
Acceptable	85 (45.5)	–	
Unpleasant	102 (54.5)	–	
Hematoma[c]			
No	177 (94.6)	179 (96.8)	0.31
Yes	10 (5.4)	6 (3.2)	
Lymphorrhoea			
No	178 (85.2)	185 (100)	0.0025
Yes	9 (4.8)	–	
Wound infection			
No	175 (93.6)	185 (100)	0.0005
Yes	12 (6.4)	–	

Note: unless otherwise indicated, data are number of patients, with percentages in parentheses

[a] Postoperative pain was evaluated as absent, mild (not requiring analgesia), or continuous (requiring analgesia)

[b] As judge by the patients themselves 1 month after biopsy

[c] Temporary hemorrhage, spontaneously resolved

Discussion

Routine biopsy of lymphadenopathies by using core-needle under imaging guidance in patients with suspicion of lymphoma is controversial [1, 2]. Usually, such procedures are reserved for lymph nodes that are accessible only with surgical risk or for critical ill patients, and in case of relapse [2]. Most studies on this issue were retrospective, included an imaging support based on traditional radiological tools (such as gray-scale US and CT, which study mostly morphological characteristics, i.e., the dimensional features of lymph node, not distinguishing between viable tumor and inflammation, necrosis and/or fibrosis), and have tested the role of small (≥18-gauge) needle devices with obsolete

Table 6 Cost analysis of biopsy procedures

Examinations and costs	Standard group (N = 187 patients)	Core-needle group (N = 185 patients)
Total no. of biopsy procedures	187	185
Unitary cost for biopsy (€)		
Major surgery[a]	10,393	–
Minor surgery[b]	3056	–
Complete US assessment of superficial and deep-seated nodal areas (€)	–	88
US-guided core-needle cutting biopsy (€)	–	83
Average cost of biopsy procedure per patient (€)	4115	171
Total cost of additional surgical biopsies due to false-negative results (€)[c]	153,445	6112
Total cost of biopsy program (€)	923,016	37,747

[a] Major surgery includes mini-cervicotomy, mediastinotomy and laparotomic bioptic procedure

[b] Minor surgery includes excisional biopsy of superficial lymph nodes

[c] Total cost of additional surgical biopsies for the four patients randomized in the core-needle group, but excluded for inadequate samples, was 41,572 €

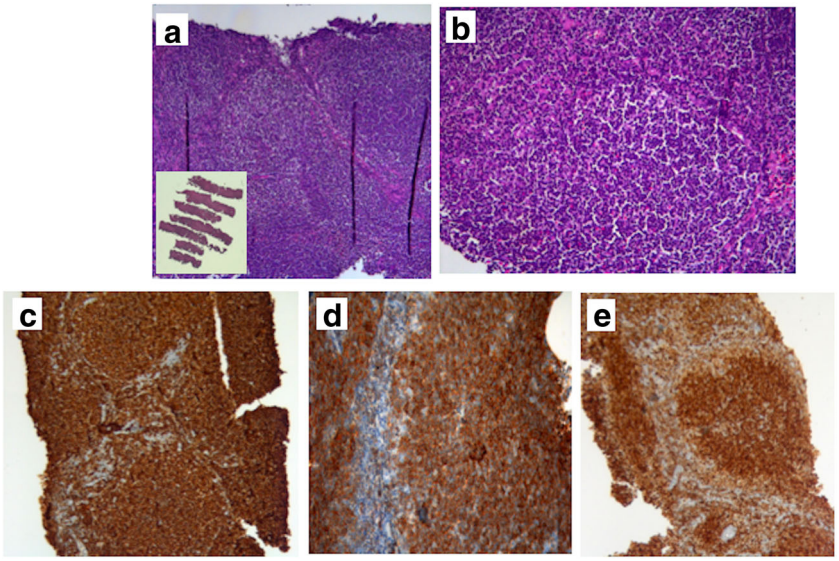

Fig. 2 **a** Inset: low-power image (H&E, ×1) of a core-needle biopsy specimen obtained from a right iliac lymph node: the core-needles reveal large follicular nodules closely packed with a back-to-back arrangement (H&E, ×20). **b** The neoplastic lymphoid follicles are composed of a uniform, small size, cell population (H&E, ×40). **c**, **d**, **e** The immunohistochemical stain strongly highlights CD20 (**c**), CD10 (**d**), and BCL-2 (**e**) (ABC, ×40). These samples are large enough to preserve tissue architecture and to assess the diagnosis of follicular lymphoma

configurations [16]. Thus, it is reasonable to investigate a front line diagnostic combination of new generation imaging equipment and technologically refined cutting needle with large gauge [15].

Our randomized study was an examination of two different interventionist approaches, one surgery-driven (standard arm) and the other one hematology-driven (experimental arm), in patients with lymphadenopathies clinically suspected for lymphoma. Traditionally, whole lymph nodes are resected when it is necessary to determine whether a lymphadenopathy is lymphoma or some other conditions, such as metastases of a nonhematological tumor [1, 2]. In our trial, the entire decision making process for biopsy in the standard arm was left to the surgeon's discretion: the selection of the node to be biopsied was based on physical examination and gray-scale US [17]. In the daily diagnostic service of our surgery unit, as in others [21], power Doppler ultrasonographic technology was limited in its availability for routine clinical practice. In the experimental arm, the selection of the node to be biopsied and biopsy itself were exclusively based on the expertise of hematologists. In fact, the hematological unit kept a modern US equipment available and had it run by experienced operators, who were members of the hematological staff trained in diagnostic PDUS [4, 5]. The goal of the study was to maintain optimal accuracy of the diagnostic work-up of lymphadenopathies, while avoiding psychological and physical pain of an unnecessary surgical intervention.

The primary endpoint in this trial, a greater sensitivity with the experimental approach, was proven being the comparison with standard approach significantly advantageous for PDUS-guided CNCB. The number of cases in which a definite diagnosis of malignancy could not be established at first biopsy

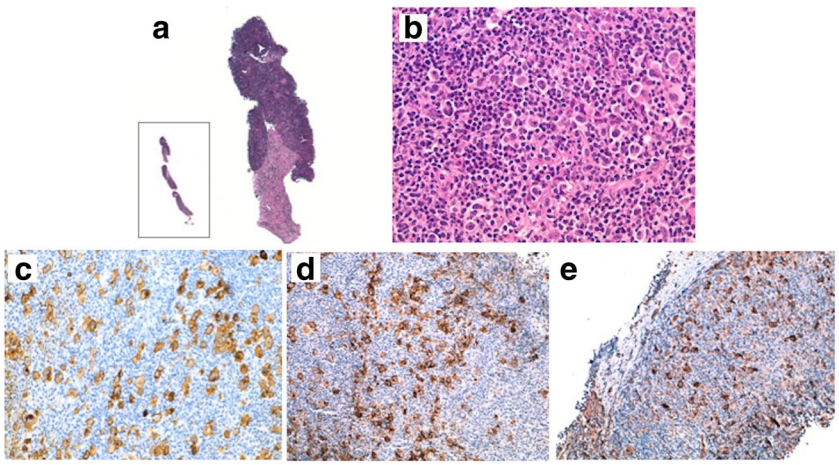

Fig. 3 **a** Inset: low-power image (H&E, ×1) of a core-needle biopsy specimen obtained from a right latero-cervical lymph node: the core-needle appears fragmented due to an obvious fibrosis (H&E, ×5). **b** Higher power views show several Reed-Sternberg cells (H&E, ×40). The Reed-Sternberg cells are CD30 (**c**), CD15 (**d**), and fascin (**e**) positive (ABC, ×40). These samples are large enough to preserve tissue architecture and to assess the diagnosis of nodular sclerosis classical Hodgkin lymphoma

was almost 8 times higher on standard tissue specimens than core-needle material. As a consequence, the clinicians recommended a re-biopsy (OSB) significantly more often in the standard group than experimental group, also considering the four patients with inadequate samples randomized in the core-needle group (19 vs. 6 cases, respectively; $P = 0.006$). Not all lymph nodes may be involved by the main disease entity. There is a risk of removing satellite reactive lymph nodes, thus missing the primary diagnosis of a malignant disease present in another node, which is sometimes deeper seated or even seated in a different anatomic area. An affected lymph node may also undergo necrosis and/or steato-fibrotic changes, which could avert the pathologist from the correct diagnosis. These are all potential sources of inaccuracy in standard excisional biopsy [5]. In our study, PDUS technology accurately selected the most suspected target, imaged all nodal lesion clearly, and simultaneously monitored the entire puncture process (in both superficial and deep-seated regions). The cutting needle had a diameter of 1.6 mm with ultrathinner tip and wall, and powered automatic suction. Although the tissue volume obtained by CNCB was smaller than OSB, the experimental method provided enough tissue for architectural-morphologic pattern assessment, immunohistochemical staining, and/or molecular testing (Figs. 2 and 3) [1].

For all secondary endpoints in this trial, the comparison was significantly disadvantageous for excisional biopsy. Compared with PDUS-guided CNCB, standard approach had significantly more waiting time to allocated interventionist procedure, considerably higher amounts of biopsy-related complications (analgesia required for postoperative pain was about 5-fold higher), and extraordinarily higher costs for the National Healthcare System (performance of one biopsy was 24-fold more expensive with standard approach).

Our study suffers from three major limitations. First, this trial was conducted in one single center. Therefore, studies from other institutions are needed to assess (1) interobserver and interequipment PDUS variability; (2) core-needle specimen quality reproducibility, e.g., tissue harvested, size and preservation; and (3) concordance by pathologists in diagnosing and subtyping lymphoma on core-needle material. Second, a bias error could have been committed due to a more accurate selection of nodal target to be biopsied in the core-needle group leading the study toward a better sensitivity for experimental arm than standard arm. A factor that may have a strong influence to explain such bias is the high specialization (derived from long and extensive experience) [4–8] of hematology team to identify the right lymph node and to biopsy it, as compared to the surgeons. Finally, the rate of failure with PDUS-guided CNCB was 1.6% (6/376 patients randomly allocated to core-needle biopsy procedure). In four patients (those with inadequate samples), stiffened tissue of nodular sclerosis Hodgkin lymphoma (documented at re-biopsy) which was seated in subclavicular area (a particular hindered region) led to the sampling error of CNCB. For the two false negative results (benign lymphoid hyperplasia), the final diagnosis (at second biopsy) was conclusive for small lymphocytic indolent non-Hodgkin lymphoma, suggesting that in some instances, there is a need of a large amount of lymph node tissue for correct histological assessment.

To the best of our knowledge, this study is the first to compare in a randomized fashion the sensitivity of imaging-guided CNCB and OSB in detecting lymphoma. Under optimal study conditions (avoiding patients with obesity), with modern US equipment and an experienced operator, core-needle biopsy is a reliable and cost-effective diagnostic procedure [22]. Histological patterns of lymphoma are recognizable in core material and are useful in diagnosing and subtyping according to the current *WHO classification of tumors of haematopoietic and lymphoid tissues* [1, 15]. A 16-gauge cutting needle is recommended, and at least two passes yielding two tissue cores, with total length of 30–60 mm should be taken. CNCB is less traumatic and well tolerated by patients. It should be recommended as first-line procedure, for both superficial and deep-seated lymph nodes, for patients with a suspected lymphoma, and not merely for patients with poor medical condition when surgical intervention is not possible or to document relapse.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Eligible patients were registered at the Hematology Division Office of the "Federico II" University of Naples, where the trial was designed and approved by the local Institutional Review Board in the early 2008 (10 January 2008; number of registration, 140/2008).

References

1. Campo E, Swerdlow SH, Harris NL et al (2011) The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood 117:5019–5032
2. Cheson BD, Fisher RI, Barrington SF et al (2014) Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol 32:3059–3068
3. Jenkins K, Baker AB (2003) Consent and anaesthetic risk. Anaesthesia 58:962–984
4. Picardi M, Muretto P, De Rosa G et al (2002) Color ultrasound-guided fine needle cutting biopsy for the characterization of diffuse liver damage in critical bone marrow transplanted patients. Haematologica 87:652–657

5. Picardi M, Gennarelli N, Ciancia R et al (2004) Randomized comparison of power Doppler ultrasound-directed excisional biopsy with standard excisional biopsy for the characterization of lymphadenopathies in patients with suspected lymphoma. J Clin Oncol 22:3733–3740
6. Picardi M, Ciancia R, De Renzo A et al (2006) Estimation of bulky lymph nodes by power Doppler ultrasound scanning in patients with Hodgkin's lymphoma: a prospective study. Haematologica 91:960–963
7. Picardi M, Soricelli A, Pane F et al (2009) Contrast-enhanced harmonic compound US of the spleen to increase staging accuracy in patients with Hodgkin lymphoma: a prospective study. Radiology 251:574–582
8. Picardi M, Pugliese N, Cirillo M et al (2014) Advanced-stage Hodgkin lymphoma: US/chest radiography for detection of relapse in patients in first complete remission—a randomized trial of routine surveillance imaging procedures. Radiology 272:262–274
9. Choudhry S, Gorman B, Charboneau JW et al (2000) Comparison of tissue harmonic imaging with conventional US in abdominal disease. Radiographics 20:1127–1135
10. Steinkamp HJ, Wissgott C, Rademaker J, Felix R (2002) Current status of power Doppler and color Doppler sonography in the differential diagnosis of lymph node lesions. Eur Radiol 12:1785–1793
11. Marinaccio C, Nico B, Maiorano E et al (2014) Insights in Hodgkin Lymphoma angiogenesis. Leuk Res 38:857–861
12. Ruan J, Hajjar K, Rafii S, Leonard JP (2009) Angiogenesis and antiangiogenic therapy in non-Hodgkin's lymphoma. Ann Oncol 20:413–424
13. Chan JM, Shin LK, Jeffrey RB (2007) Ultrasonography of abnormal neck lymph nodes. Ultrasound Q 23:47–54
14. Pruneri G, Gigli F, Raviele P et al (2015) Core needle biopsy as a front line diagnostic approach for lymphoma patients. Hematol Oncol 33:247–249
15. Hu Q, Naushad H, Xie Q et al (2013) Needle-core biopsy in the pathologic diagnosis of malignant lymphoma showing high reproducibility among pathologists. Am J Clin Pathol 140:238–247
16. Johl A, Lengfelder E, Hiddemann W, Klapper W (2016) Core needle biopsies and surgical excision biopsies in the diagnosis of lymphoma—experience at the Lymph Node Registry Kiel. Ann Hematol 95:1281–1286
17. Suramme F, Waltieg A, Emering C (2001) Encyclopedie Medico-Chirurgicale. Editions Scientifiques et Mèdicales. Elsevier, Paris
18. de Kerviler E, Benet C, Brière J, de Bazelaire C (2012) Image-guided needle biopsy for diagnosis and molecular biology in lymphomas. Best Pract Res Clin Haematol 25:29–39
19. Begg CB, Iglewicz B (1980) A treatment allocation procedure for sequential clinical trials. Biometrics 36:81–90
20. Fleiss JL, Gurland BJ, Cooper JE (1971) Some contributions to the measurement of psychopathology. Br J Psychiatry 119:647–656
21. Picardi M, Gennarelli N, Ciancia R et al (2004) Improving diagnostic accuracy in patients with suspected lymphoma. J Clin Outcomes Manag 11:691–692
22. Velasco Garrido M, Gerhardus A, Rottingen J-A et al (2010) Developing health technology assessment to address health care system needs. Health Policy 94:196–202

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LETTER TO THE EDITOR

Ruxolitinib rechallenge in combination with hydroxyurea is effective in reverting cachexia and reducing blood transfusion demand and splenomegaly symptoms in a patient with primary myelofibrosis

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Keywords Primary myelofibrosis · Cachexia · Ruxolitinib · Hydroxyurea · Splenomegaly

Dear Editor,

Myelofibrosis (MF) is a myeloproliferative neoplasm (MPN) whose pathogenesis mainly involves JAK/STAT signaling; approximately 65% of patients carry V617F-JAK2 mutation with a gain-of-function mechanism [1, 2]. Hydroxyurea is recommended as the first-line therapy for MF in low and intermediate-1 patients, whereas ruxolitinib, an orally available and selective JAK2-inhibitor, is recommended in International Prognostic Scoring System (IPSS) intermediate-2 and high-risk patients as front-line treatment of symptoms and splenomegaly in non-transplant candidates [3].

We describe the case of a 57-year-old Caucasian man with primary MF (PMF). The patient was 166 cm in height and weighed 60 kg; before diagnosis, he had been in fair physical condition. At diagnosis (November 2012), his main symptoms were early satiety and a sense of fullness in the left upper abdomen that rapidly deteriorated into cachexia. His blood count showed anemia and leukocytosis, and a physical examination revealed splenomegaly (10 cm from costal margin); size measured by ultrasound scan [4] was 22 cm (longitudinal diameter) × 14 cm (transverse diameter) with a spleen volume of 2700 mL. Bone marrow biopsy demonstrated grade 3 fibrosis (MF = 3) and the presence of *JAK2*-V617F mutation. In December 2012, cytoreductive therapy with hydroxyurea (1 and 2 capsules daily on alternate days) was started, obtaining a stable disease for few months. After 3 months, systemic symptoms and splenomegaly worsened. The patient was cachectic, his weight had fallen to 47 kg, and his spleen was of hard consistency and had enlarged, extending to the iliac fossa. He had lack of appetite, he was having difficulty eating, and his quality of life had deteriorated badly. The severity of the patient's condition led to the consideration of other treatment options.

The patient refused allogeneic stem cell transplantation after becoming aware of transplant-related risks and peri-transplant mortality. Therefore, treatment with ruxolitinib was initiated, initially at 10 mg twice daily (bid), and reduced to 5 mg bid in response to grade 3 thrombocytopenia. The patient experienced only partial relief from symptoms and, in September 2014, ruxolitinib was discontinued due to severe leukocytosis and very poor compliance. After hydroxyurea was reintroduced to control leukocytosis, there was a considerable increase in the need for blood transfusions over subsequent months (up to 8 units/month; Fig. 1) and spleen size increased, reaching 27.8 cm longitudinal diameter. Following poor compliance with gastroprotective drugs, the patient required hospitalization in April 2015 for gastric bleeding, and hydroxyurea therapy was stopped due to severe anemia and thrombocytopenia (30,000/mm^3).

Hydroxyurea was reintroduced with palliative intent after 1 month and, in June 2015, low-dose ruxolitinib (5 mg bid) rechallenge was undertaken, in combination with hydroxyurea (1 and 2 capsules daily on alternate days for 5 days/week). The ruxolitinib dose was increased to 10 mg bid after 1 month, and after 2 months (September 2015), the patient

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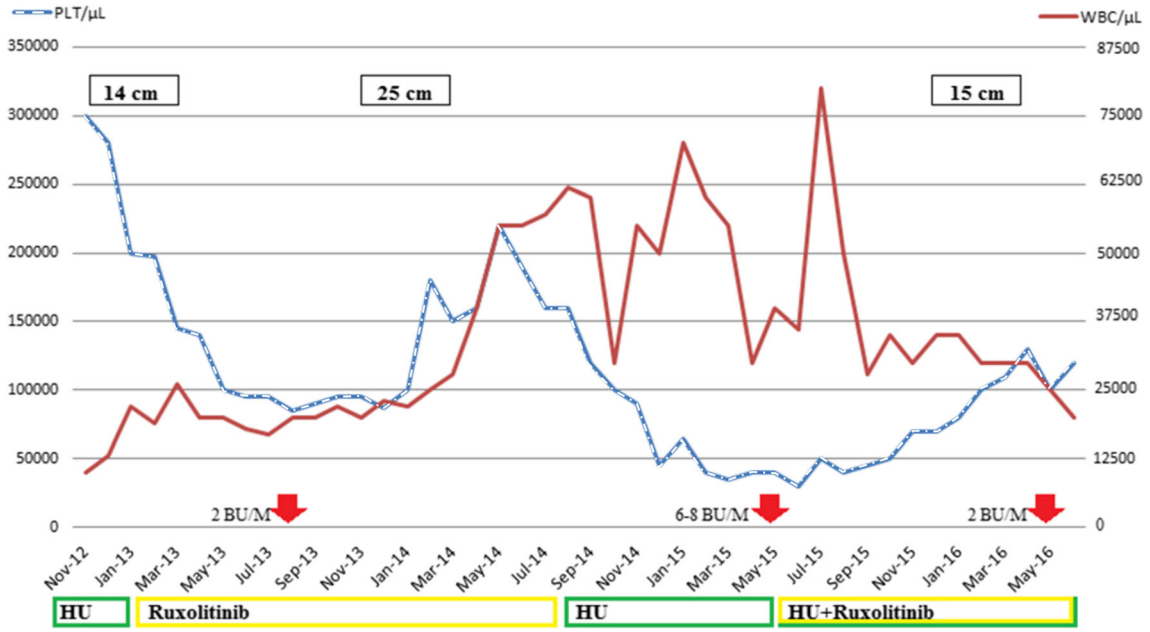

Fig. 1 Patient clinical history showing platelet (PLT) count (*dashed line*), white blood cell (WBC) count (*solid line*), blood units per month (BU/M; *broad arrows*), spleen longitudinal diameter (*black rectangles*), and timeline of hydroxyurea (HU) and ruxolitinib administrations

experienced a dramatic reduction in spleen size with substantial relief from symptomatic splenomegaly (longitudinal diameter 15.6 cm), control of anemia and leukocytosis, and improved nutritional status, with an increase in appetite and an increase in weight to 55 kg. He regained a decent quality of life and was able to resume routine activities, such as shopping.

Resolution of cachexia and substantial improvement in clinical status continued and, as of May 2016, the patient was continuing combination treatment with ruxolitinib (10 mg bid) and hydroxyurea.

Single-agent ruxolitinib is effective in improving splenomegaly, systemic symptoms, and overall survival, compared with placebo and standard treatment, in patients with intermediate-2 or high-risk MF [5, 6]. As with hydroxyurea, significant anemia and thrombocytopenia are the most common side effects, often requiring discontinuation [7]. However, despite the combination of ruxolitinib with a cytoreductive agent, we obtained control of leukocytosis and anemia with concurrent increase in platelet count to stable normal values, without the expected synergic cytotoxic effects. Of interest, the safe and effective use of combination of ruxolitinib plus hydroxyurea in reducing platelet count and splenomegaly in a patient with uncontrolled thrombocytosis on ruxolitinib monotherapy has been described [8]. Furthermore, in addition to its primary anti-myeloproliferative action via JAK2 inhibition, ruxolitinib appears to exert a remarkable improvement in cachexia status [9], as observed in our case.

In conclusion, combined ruxolitinib plus hydroxyurea effectively controlled myeloproliferation without worsening anemia, instead leading to a remarkable decrease in the need for blood transfusions. Our patient's cachectic status was reverted, and overall quality of life dramatically improved.

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Compliance with ethical standards Written informed consent was obtained from the patient for publication.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, Cazzola M, Skoda RC (2005) A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 352(17): 1779–1790. doi:10.1056/NEJMoa051113
2. Tefferi A, Pardanani A (2015) Myeloproliferative neoplasms: a contemporary review. JAMA Oncol 1(1):97–105. doi:10.1001/jamaoncol.2015.89
3. Tefferi A (2014) Primary myelofibrosis: 2014 update on diagnosis, risk-stratification, and management. Am J Hematol 89(9):915–925. doi:10.1002/ajh.23703

4. Picardi M, Martinelli V, Ciancia R, Soscia E, Morante R, Sodano A, Fortunato G, Rotoli B (2002) Measurement of spleen volume by ultrasound scanning in patients with thrombocytosis: a prospective study. Blood 99(11):4228–4230
5. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, Catalano JV, Deininger MW, Miller CB, Silver RT, Talpaz M, Winton EF, Harvey JH Jr, Arcasoy MO, Hexner EO, Lyons RM, Raza A, Vaddi K, Sun W, Peng W, Sandor V, Kantarjian H, investigators C-I (2015) Efficacy, safety, and survival with ruxolitinib in patients with myelofibrosis: results of a median 3-year follow-up of COMFORT-I. Haematologica 100(4):479–488. doi:10.3324/haematol.2014.115840
6. Vannucchi AM, Kantarjian HM, Kiladjian JJ, Gotlib J, Cervantes F, Mesa RA, Sarlis NJ, Peng W, Sandor V, Gopalakrishna P, Hmissi A, Stalbovskaya V, Gupta V, Harrison C, Verstovsek S, Investigators C (2015) A pooled analysis of overall survival in COMFORT-I and COMFORT-II, 2 randomized phase III trials of ruxolitinib for the treatment of myelofibrosis. Haematologica 100(9):1139–1145. doi:10.3324/haematol.2014.119545
7. Al-Ali HK, Griesshammer M, le Coutre P, Waller CF, Liberati AM, Schafhausen P, Tavares R, Giraldo P, Foltz L, Raanani P, Gupta V, Tannir B, Ronco JP, Ghosh J, Martino B, Vannucchi AM (2016) Safety and efficacy of ruxolitinib in an open-label, multicenter, single-arm phase 3b expanded-access study in patients with myelofibrosis: a snapshot of 1144 patients in the JUMP trial. Haematologica 101(9):1065–1073. doi:10.3324/haematol.2016.143677
8. Polverelli N, Catani L, Vianelli N, Baccarani M, Cavo M, Palandri F (2015) Ruxolitinib—but not fedratinib—induced extreme thrombocytosis: the combination therapy with hydroxyurea and ruxolitinib is effective in reducing platelet count and splenomegaly/constitutional symptoms. Ann Hematol 94(9):1585–1587. doi:10.1007/s00277-015-2397-9
9. Mesa RA, Verstovsek S, Gupta V, Mascarenhas JO, Atallah E, Burn T, Sun W, Sandor V, Gotlib J (2015) Effects of ruxolitinib treatment on metabolic and nutritional parameters in patients with myelofibrosis from COMFORT-I. Clin Lymphoma Myeloma Leuk 15(4):214–221 e211. doi:10.1016/j.clml.2014.12.008

Clinical Case Reports

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CASE REPORT

A case of efficacy of bendamustine in heavily pretreated multiple myeloma, refractory to pomalidomide

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Key Clinical Message

In this report, we would like to highlight the efficacy of bendamustine in a heavily pretreated patient, also refractory to pomalidomide. It is conceivable that different therapy combinations in heavily treated Multiple myeloma (MM) have to be explored, without "a priori" exclusion of ancient drugs, even after failure of the ultimate pharmacological options.

Keywords

Bendamustine, heavily pretreated, multiple myeloma, pomalidomide, refractory, relapsed.

Introduction

Multiple myeloma (MM) is a clonal malignant disorder derived from an abnormal plasma cell proliferation in the bone marrow that causes anemia, bones lytic lesions and renal injury. If the neoplasm is limited to a restricted area, as bone marrow or soft tissues, it is defined as plasmacytoma.

The natural history of MM has recurrences of active disease defined as relapse when salvage treatment is needed after an off-therapy period, or refractory disease if nonresponsive during therapy, or progressing within 60 days of last therapy.

In spite of the fact that MM is still an incurable disease, treatment strategies have improved survival in the last decades [1], thanks to the availability of novel agents as proteasome inhibitors (bortezomib, carfilzomib) and immunomodulatory drugs (IMiDs; thalidomide, lenalidomide, and pomalidomide).

In this scenario, the role of many drugs used as salvage therapy after several lines including novel agents in the relapsing/refractory MM is currently undergoing evaluation and, although many new molecules appear promising, their efficacy is still unclear and not always predictable.

Bendamustine is a nitrogen mustard with both purine-analogue and alkylating agent mechanisms; it is widely used in the treatment of chronic lymphocytic leukemia and low-grade lymphomas, but is also approved in Europe as single agent and in several combinations with steroid or novel agents (bortezomib, thalidomide, and lenalidomide) for the treatment of relapsed/refractory MM, especially in heavily pretreated patients [2].

In this report, we describe the efficacy of bendamustine combined with bortezomib and dexamethasone (BVD) in a male patient with a long story of relapsed/refractory MM, previously treated with all available drugs, also refractory to pomalidomide, the most recent available IMiDs [3].

Case Presentation

A 70-year-old man was diagnosed with a scantly secretory IgA kappa MM in April 2008, evolved by IgA MGUS recognized 5 years before. At the time of the evaluation, Durie-Salmon stage was IIIA and ISS was I; PET-CT

showed several areas of intense focal bone involvement. Therapy with thalidomide and dexamethasone (TD) was performed, resulting in a symptomatic stable disease (SD), with persistent positive PET-CT in the same bone regions plus a new localization on sternum. Second-line therapy with bortezomib–dexamethasone (VD) for three cycles until March 2009 was performed, but a CT showed heteroplastic tissue wrapping up D2-D3 vertebra; PET-CT was positive in the same sites. Third-line therapy consisting in lenalidomide/dexamethasone (RD) for five cycles + radiotherapy on D2-D3 was carried out and was followed, in September 2009, by autologous stem cell transplantation after conditioning with high-dose melphalan (200 mg/m^2), and 2 years of interferon (IFN-α2br) maintenance therapy in very good partial response (VGPR). In March 2012, a new relapse in the right shoulder (confirmed by PET-CT) led further radiotherapy, and the patient remained free of disease for more than 3 years without maintenance. In August 2015, a wide and hard-consistency lump appeared in the upper-left quadrant of the abdomen, together with a rising of the Bence-Jones protein urinary concentration (573 mg/L). Ultrasound scan showed a vascularized mass surrounding and adherent to the small bowel and mesentery. Fine-needle aspiration cytology (FNAC) was performed on the mass, showing clonal plasma cells and allowing diagnosis of extramedullary plasmocytoma. Consequently, salvage therapy with CED (cyclophosphamide, doxorubicin and dexamethasone) [4] plus radiotherapy on the mass was performed in September 2015, but it was interrupted after the first course due to marrow toxicity (grade III thrombocytopenia). Volume and solidity of the mass were reduced, but Bence-Jonesprotein increased (progressive disease, PD). Hence, in January 2016, the patient was switched to pomalidomide and dexamethasone, but, after the first course, the disease still progressed.

In February 2016, a seventh-line salvage treatment was performed employing bendamustine, bortezomib, dexamethasone (BVD: bendamustine 90 mg/sqm IV days 1 and 2, bortezomib 1.3 mg/mq s.c. days 1, 4, 8, 11, dexamethasone 20 mg oral solution/IV days 1, 2, 4, 5, 8, 9, 11, 12 and pegfilgrastim 6 mg s.c. day 4, every 28 days) [5] with the unexpected result of a VGPR, achieved after only one course of treatment, and reduction of more than 90% of urinary K chain concentration and dramatic shrinkage of the abdominal tumor mass. Unfortunately, the patient died before the second course due to progressive cachexia.

Discussion

Although multiple myeloma is defined as a noncurable disease, nowadays its prognosis is significantly better thanks to a wide spectrum of active drugs. Hence, we often have to face long-survivor patients receiving several lines of therapy, raising questions on how to combine different treatments and how to manage sequential effects.

Nowadays, available treatments for relapsed/refractory MM are as follows: rechallenge with previously used agents (bortezomib- or IMID-based treatments), with or without autologous bone marrow transplantation, new-generation IMIDs (pomalidomide), bendamustine-based treatments, or other alkylating agents. Another option, waiting for new drugs, such as new-generation proteosome inhibitors (carfilzomib) and monoclonal antibodies (daratumumab and elotuzumab), are combinations of previously used agents, which often demonstrate a synergistic effect also in refractory and heavily pretreated patients.

In this report, we would like to highlight the efficacy of bendamustine in a heavily pretreated patient, also refractory to pomalidomide, the newest available IMiDs. To our knowledge, this sequence of treatment has never been reported before. Moreover, it is relevant the clinical setting where these drugs were administered, as MM finally progressed to massive extramedullary plasmacytoma (intra-abdominal mass), suggesting that changing in the disease site, hence of disease biology, could have also affected the response to the therapies, particularly active in the lymphoma context [6]. It is conceivable that different therapy combinations in heavily treated MM have to be explored, without "a priori" exclusion of ancient drugs, even after failure of the ultimate pharmacological options.

Authorship

CC: participated in study conception and design, data analysis and interpretation, article drafting and revising it critically for important intellectual content, and gave final approval for publication. He also collected the data. He is responsible for the overall content as guarantor. IM and DS: collaborated in clinical follow-up of the patient; MDP, DN, MP: participated in study conception and design, data analysis and interpretation and gave final approval for publication; FP and LC: participated in study conception and design, data analysis and interpretation, article drafting and revising it critically for important intellectual content and gave final approval for publication. All authors read and approved the final manuscript.

Conflict of Interest

None declared.

References

1. Bladè, J., L. Rosiñol, and C. Fernández de Larrea. 2015. How I treat relapsed myeloma. Blood 125: 1532–1540.
2. Ludwig, H., H. Kasparu, C. Leitgeb, E. Rauch, W. Linkesch, and N. Zojer, et al. 2014. Bendamustine-bortezomib-dexamethasone is an active and well-tolerated regimen in patients with relapsed or refractory multiple myeloma. Blood 123:985–991.
3. Richardson, P. G., D. S. Siegel, R. Vij, C. C. Hofmeister, R. Baz, S. Jagannath, et al. 2014. Pomalidomide alone or in combination with low-dose dexamethasone in relapsed and refractory multiple myeloma: a randomized phase 2 study. Blood 123:3208–3209.
4. Cerchione, C., M. Lucignano, F. Pane, and L. Catalano. 2016. Salvage therapy with pegylated liposomial doxorubicin-based regimen in relapsed/refractory multiple myeloma: comments to the article by Romano et al.. Eur. J. Haematol. 96:544.
5. Cerchione, C., L. Catalano, I. Peluso, D. Nappi, M. Di Perna, and D. Salvatore, et al. 2016. Managing neutropenia by pegfilgrastim in patients affected by relapsed/refractory multiple myeloma treated with bendamustine-bortezomib-dexamethasone. Support. Care Cancer 24:4835–4837.
6. Bladé, J., C. Fernández de Larrea, L. Rosiñol, M. T. Cibeira, R. Jiménez, and R. Powles. 2011. Soft-tissue plasmacytomas in multiple myeloma: incidence, mechanisms of extramedullary spread, and treatment approach. J. Clin. Oncol. 29:3805–3812.

