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Seminars in Oncology

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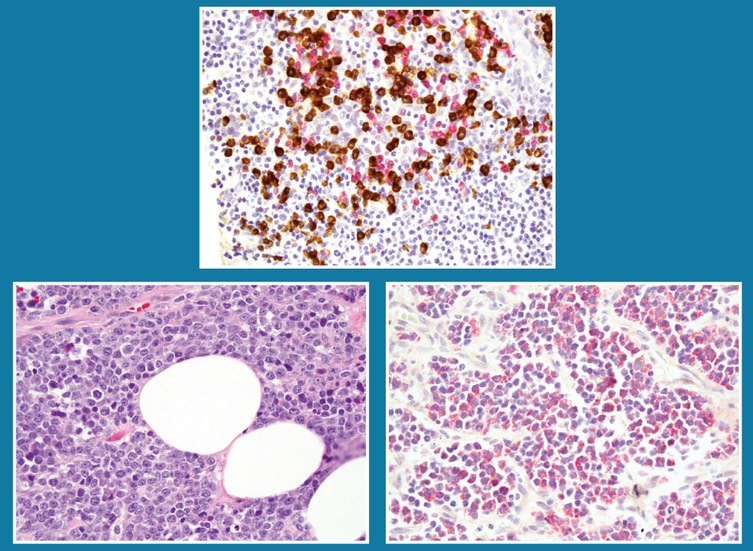

Integrating Novel Therapies Into Treatment of Multiple Myeloma

Andrzej J. Jakubowiak, MD, PhD
Guest Editor

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CONTENTS

On the Cover: Reactive plasma cell proliferations typically are polyclonal. The reactive population contains a mix of lambda and kappa light chain expressing cells. The top figure shows such a collection with kappa (brown) and lambda (red) expressing constituents. In this example, light chain expression is detected by chromogenic in situ hybridization (Bond III, Leica Biosystems). The bottom left figure represents marrow sampling from a 70-year-old woman who presented with signs and symptoms of a vertebral fracture. The marrow space was filled by a homogenous population of cells with abundant cytoplasm and large nuclei. Nuclear chromatin is clumped and nucleoli are prominent. The cells were $CD138^{+}$. A diagnosis of multiple myeloma/plasmacytoma was made. The bottom right figure shows a restricted pattern of lambda light chain expression (red chromogen). Cytogenetic studies were ordered for risk stratification.

Seminars in Oncology

FUTURE ISSUES

Cancer Survivorship
Kevin C. Oeffinger, MD, *Guest Editor*

Lung Cancer
David Carbone, MD, *Guest Editor*

Tumor Microenvironment
Ubaldo Martinez-Outschoorn, MD, and Michael Lisanti, MD, PhD, *Guest Editors*

PREVIOUS ISSUES

Vol 40, No 4 June 2013	**Renal Cancer** August 2013 Brian I. Rini, MD, FACP, and Tom Powles, MRCP, MD, *Guest Editors*
Vol 40, No 3 June 2013	**Prostate Cancer** Adam P. Dicker, MD, PhD, Leonard G. Gomella, MD, FACS, and Wm. Kevin Kelly, DO, *Guest Editors*
Vol 40, No 2 April 2013	**Cardio-oncology: The Relationships Between the Heart and Cancer** Marc L. Schwartz, MD, *Guest Editor*
Vol 40, No 1 February 2013	**The Evolving Landscape of Neuroendocrine Tumors** Emily K. Bergsland, MD, *Guest Editor*
Vol 39, No 6 December 2012	**Haploidentical Bone Marrow Transplantation** Neal Flomenberg, MD, *Guest Editor*

Please visit *www.seminoncol.org*
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Sarcoidosis Mimicking Metastatic Bone Disease in Head and Neck Cancer

Cecilia Arana Yi, Peter McCue, Marc Rosen, Mitchell Machtay, Rita Axelrod, and Gloria J. Morris

Seminars in Oncology

At times we encounter clinical problems for which there are no directly applicable evidence-based solutions, but we are compelled by circumstances to act. When doing so we rely on related evidence, general principles of best medical practice, and our experience. Each "Current Clinical Practice" feature article in *Seminars in Oncology* describes such a challenging presentation and offers treatment approaches from selected specialists. We invite readers' comments and questions, which, with your approval, will be published in subsequent issues of the Journal. It is hoped that sharing our views and experiences will better inform our management decisions when we next encounter similar challenging patients. Please send your comments on the articles, your challenging cases, and your treatment successes to me at dr.gjmorris@gmail.com. I look forward to a lively discussion.

Gloria J. Morris, MD, PhD
Current Clinical Practice
Feature Editor

According to national cancer statistics for 2010,[1] head and neck cancers are estimated to account for about 3% of new cancer cases in the United States, with more than 49,000 new cases diagnosed and nearly resultant 12,000 deaths.[1–3] Squamous cell carcinoma and its variants are the most common histologic types of head and neck cancers, specifically those of the oral cavity, and pharyngeal and laryngeal cancers, with alcohol and tobacco abuse being common etiologic risk factors in their development.[1] More recently, human papilloma virus (HPV) infection has emerged as a risk factor for the development of squamous cell cancers of the oropharynx, including tonsils and base of tongue.[1,4]

Carcinoma of the ethmoid sinus appears to have a different etiology. Adenocarcinoma is the most common malignancy of the ethmoid sinus and may be associated with occupational dust exposure.[5,6] The percentage of distant metastasis from primary ethmoid sinus cancer varies greatly from 6%–30% and occurs predominantly in the bone and meninges.[7]

Positron emission tomography (PET)/computed tomography (CT) scanning is a valuable tool for the diagnosis and monitoring of cancer and cancer metastasis. Nevertheless, its specificity is hampered by non-oncologic medical conditions such as sarcoidosis. Here, we present a case of a locally advanced ethmoid sinus carcinoma with F-18 fluoro-2-deoxyglucose (FDG)-PET/CT scans that were suspicious for skeletal metastases in follow-up but which biopsy proved to be granulomatous disease.

CASE PRESENTATION

A 42-year-old white woman presented with stage III T3N0M0 clear cell adenocarcinoma of the ethmoid sinus. Her initial FDG-PET/CT revealed increased metabolic activity in the right ethmoid air cells and extensive mediastinal and hilar lymphadenopathy. Biopsies of the right ethmoid mass revealed clear cell adenocarcinoma; mediastinal node biopsies showed noncaseating granulomatous lymphadenitis consistent with sarcoidosis. She then underwent a craniofacial resection, followed by adjuvant chemoradiotherapy (clinical trial) with cisplatin and bortezomib and achieved complete remission after treatment.

Two years later, PET/CT demonstrated multiple osseous metastases located in the right and left iliac bone and left lower anterior rib, with a standardized uptake value (SUV) of 8.7, and stable mediastinal lymphadenopathy (Figure 1). She had headaches, occasional sinus infections, and intermittent bilateral hip pain. A CT-guided left iliac bone biopsy revealed granulomatous inflammation without acid-fast organisms or fungi (Figure 2). Four years after therapy completion, she presented with severe hip pain and fatigue. The PET/CT scan revealed again mediastinal and retroperitoneal hypermetabolic lymphadenopathy (maximum SUV of 5.55) and extensive osseous metabolic uptake (maximum SUV of 19.47)

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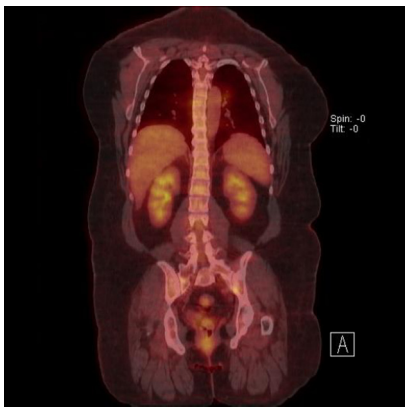

Figure 1. PET/CT scan showed FDG uptake in the right and left iliac bone.

(Figure 3) within the right hemivertebral body of C7, C3, and T12, within the bilateral sacral ala, left ilium, ribs, lower sternum, and left acromial process, which was concerning for early bone metastases and no evidence of local sinus recurrence. A biopsy of the right sacral ala demonstrated again noncaseating granulomatous inflammation. Staining was negative for cytokeratin (Figure 4). After this evaluation, she was started on systemic oral corticosteroid therapy with notable improvement in her bone pain and fatigue. A repeated PET/CT scan performed 6 months after corticosteroid treatment showed complete resolution of hypermetabolic mediastinal and retroperitoneal lymphadenopathy, as well as near-complete resolution of hypermetabolic osseous metastases (Figure 5). The patient has continued to do well clinically, with no evidence of recurrent cancer to date.

We have posed the following clinical questions: (1) What is the likelihood of metastatic disease from a pathologic point of view given these PET/CT findings? (2) Did this patient have definitive surgery from an ear, nose, and throat (ENT) standpoint? (3) What modalities would be recommended for further surveillance that would distinguish metastases, given the similar appearance of sarcoid in the bones? (4) How would one approach further treatment in the event of recurrence, given she is on chronic steroids?

PATHOLOGIST'S EXPERT OPINION

The staging and monitoring of oncologic patients often result in positive imaging results. When faced with the radiographic finding of a skeletal hotspot, the most logical clinical course is to rule out metastatic disease. Sometimes the addition of a plain film will clarify the situation, but more often than not the patient comes to biopsy. The pathology decision tree starts with spread of the primary tumor. However, an undiagnosed primary process is always a consideration. This category of diseases includes both neoplastic and non-neoplastic lesions. Incidental primary bone tumors can include osteomas, nonossifying fibromas, and angiomas. Various infections may result in positive scans and run the gamut of bacteria, mycobacteria, mycoses, and parasites. Additionally, inflammatory foci can be seen in states such as sarcoidosis, healing fracture

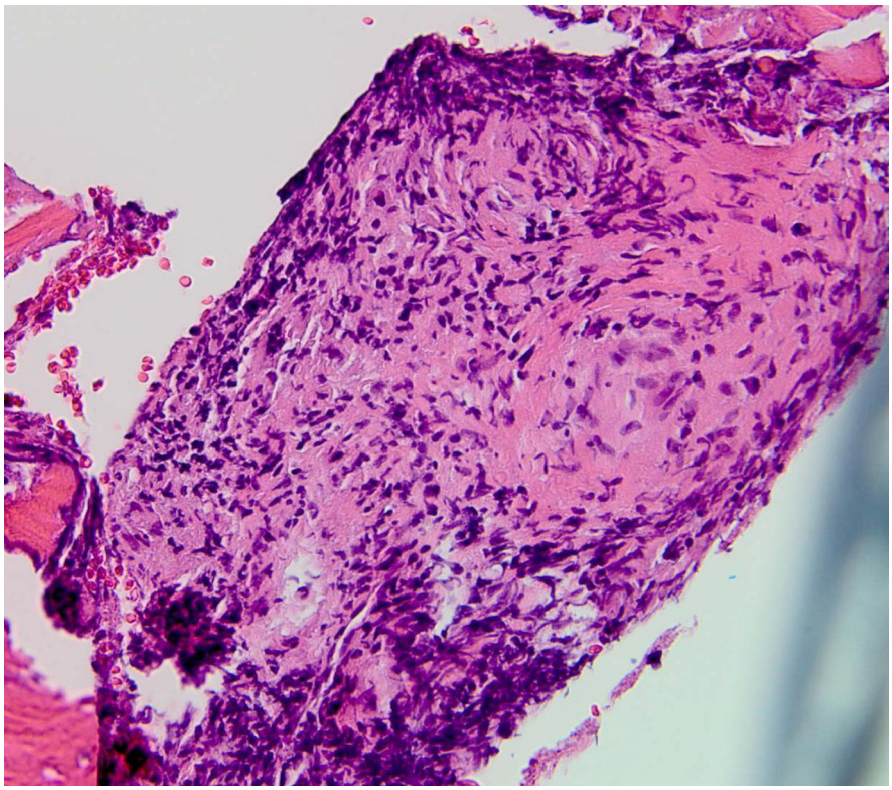

Figure 2. High-power photomicrograph of biopsy specimen from the left iliac bone shows non-caseating granuloma. Hematoxylin and eosin stain (H&E) x100.

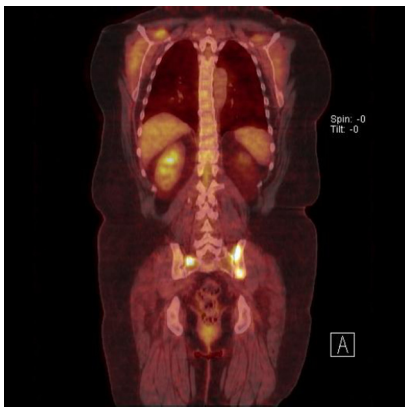

Figure 3. PET/CT scan shows intense FDG-uptake mediastinal and retroperitoneal hypermetabolic lymphadenopathy (not shown) and extensive osseous metabolic uptake (maximum SUV of 19.47) within the bilateral sacral ala.

sites, and various metabolic disturbances.

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OTOLARYNGOLOGIST'S EXPERT OPINION

This is an interesting case for many reasons. The lesion was first identified by an outside otolaryngologist in 2005. He performed a standard endoscopic procedure for nasal obstruction and polyposis. Despite the fact that there was skull base erosion on preoperative imaging, a diagnosis of carcinoma was never established. The patient had recurrence of her symptoms in 2006 and was referred to Jefferson for further evaluation. Our imaging at that point was very suspicious for a sinonasal neoplasm. Biopsy at that point revealed clear cell adenocarcinoma and a metastatic workup with PET scan revealed the thyroid and mediastinal findings. Definitive surgery of the primary tumor was delayed so that the thyroid and mediastinal lesions could be evaluated. The lesions were consistent with thyroiditis and noncaseating granulomas. Definitive surgery followed by chemoradiation was completed in November 2006. Repeat biopsy of the primary site in 2007 revealed no evidence of local recurrence. There continued to be no evidence of local recurrence, but follow-up PET scan in 2007 was consistent with bony metastasis. Extensive workup since then has continued to show no evidence of local or distant metastasis. The PET scan did show uptake at the primary site initially and it certainly was reasonable to assume metastatic disease. I think this case does not diminish the usefulness of PET scanning and emphasizes the need to confirm PET findings with definitive biopsy rather than to assume the patient has metastatic disease.

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RADIATION ONCOLOGIST'S EXPERT OPINION

This is an unusually dramatic case of a not so unusual scenario —the difficulty and imperfection in using FDG-PET scan for staging/restaging. The only type of PET scan approved and reimbursed for oncology is FDG-PET. High FDG uptake on PET indicates glucose hypermetabolism, which is very typical of malignancy. However,

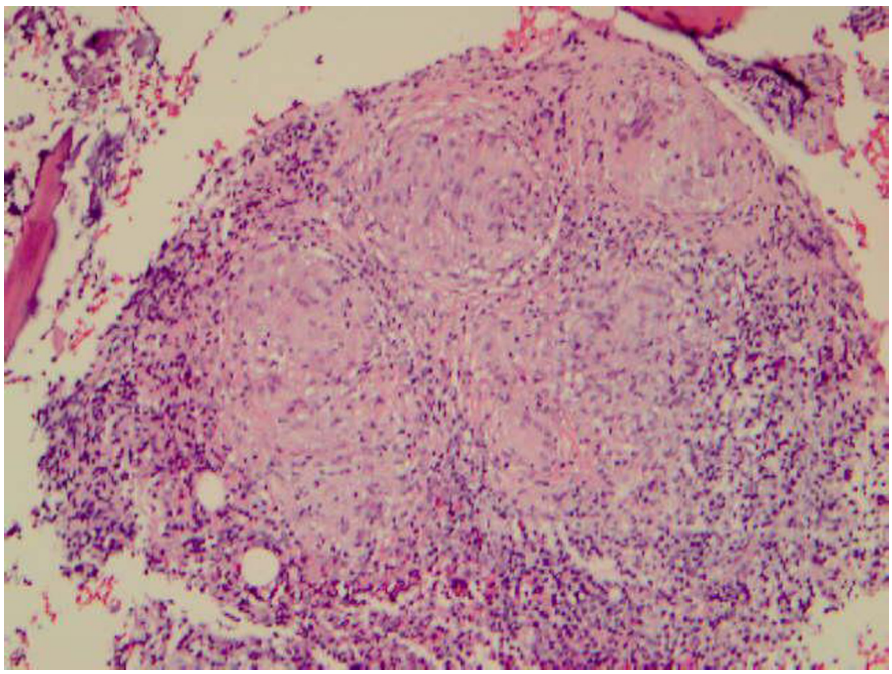

Figure 4. High-power photomicrograph of biopsy specimen from the right sacral ala shows noncaseating granuloma. H&E 100

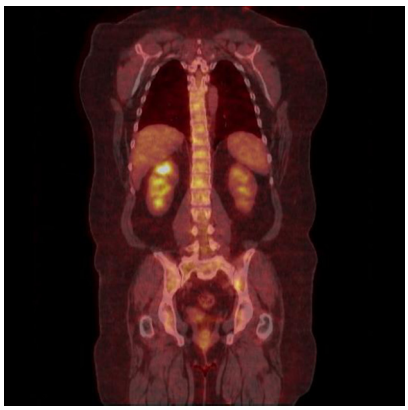

Figure 5. PET/CT scan after corticosteroid treatment shows near complete resolution of bone lesions.

elevated FDG uptake is also very common in inflammatory states, including autoimmune diseases (like sarcoidosis), infection, and post-radiation effects. This case illustrates the importance of skepticism in the interpretation of FDG-PET; simply assuming the patient has incurable metastatic disease could be catastrophic. When in doubt, biopsy is necessary, particularly if it can be done without much morbidity and in a compliant patient who is interested in and amenable to biopsy.

Given that we are almost certainly not going to stop using FDG-PET scanning for staging and restaging, is there anything else that can be done noninvasively? There are several areas of encouraging research aimed at improving the specificity of PET scans. First, it has been suggested that dual/multiple time point image acquisition can assist in differentiating tumor from inflammation. On serial imaging, over a number of hours, inflammatory lesions tend to demonstrate progressively lower SUV, while tumors tend to retain their high SUV (or may display an even higher SUV than on the first reading). Second, using other tracers (instead of or in addition to FDG) may be promising. F18-labeled 3'-deoxy-3'fluorothymidine (FLT), which measures DNA synthesis and thus indirectly proliferation, may be more specific than FDG-PET. Unfortunately, FLT is not approved by the US Food and Drug Administration at this time, and thus it is only available for use under investigational new drug (IND)-approved clinical research.

Again, the stakes are high in diagnostic imaging of oncology. No one wants to misdiagnose metastatic disease, especially in a young "curative-intent" patient. A healthy dose of skepticism should be a mandatory coproduct in each FDG syringe.

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DISCUSSION

The utility of FDG-PET/CT in head and neck carcinoma is well documented.[8,9] FDG-PET/CT is helpful in assessing response to chemoradiotherapy and in evaluating local and distant metastases. It allows for the visualization not only of malignant tissues but also of inflammatory lesions.[10] The sensitivity in the detection of head and neck cancer varies between 47% and 100%, while the specificity ranges from 86%–100%.[11]

Sarcoidosis is a multisystem inflammatory disorder that affects the lungs and skin most often; the incidence of osseous involvement is 5%.[12] Pulmonary and skeletal sarcoidosis have been described to mimic metastatic disease in people with and without preexisting malignant conditions.[13–16]

A link between sarcoidosis and cancer has been reported in previous studies,[17,18] and its presence seems to be associated with worse prognosis, likely due to immune deregulation.[19] Sarcoidosis also has been reported at diagnosis, during treatment, and in the surveillance of cancer patients.[20,21]

Sarcoid-like reaction in mediastinal lymph nodes also has been reported in malignant tumors, such as Hodgkin lymphoma, and breast and lung cancers.[13,22] Chemotherapy also can cause fluctuations in serum levels of cytokines that play an important role in granuloma formation. Although the incidence of sarcoidosis associated with malignancy is rare, there are published data to correlate its presence with worsening survival.[19] To our knowledge, there are no previous reports of adenocarcinoma of the ethmoid sinus associated with sarcoidosis.[23]

As discussed before, the accumulation of FDG in inflammatory sites could lead to false-positive findings, especially in patients with a known history of cancer.[24] Therefore, sarcoidosis and other granulomatous conditions might be included in the differential diagnosis, and a biopsy of the suspicious metastatic site should be required for confirmatory diagnosis.

In our case, the abnormal PET/CT was concerning for the presence of skeletal involvement in locally advanced clear cell adenocarcinoma of the ethmoid sinus. However, her good performance status, the chronicity of the symptoms, and intermittent presentation of the radiological findings warranted a confirmatory bone biopsy that was finally consistent with sarcoidosis.

CONCLUSIONS

PET/CT is a valuable tool in the staging of head and neck cancer and other malignancies, but our case illustrates once again that false positives do occur. Sarcoidosis can and does mimic metastatic disease on PET/CT scan. Clinical judgment is needed to determine the role of confirmatory biopsy, particularly in cases with a preexisting diagnosis of sarcoidosis or accompanying bilateral hilar

adenopathy that would warrant further investigation.

When or if recurrent, treatment is with single-agent or combination systemic chemotherapy, as well as utilization of palliative adjunctive measures including radiotherapy to areas of symptomatic disease. Multiple agents with systemic activity have been reported in the literature[25–32] and additional options include targeted therapy, as well as participation in clinical trials. The choice of agents would not be impeded by the diagnosis of sarcoidosis in this patient. Fortunately, she was spared of the use systemic chemotherapy after judicious bone biopsy ruled out widespread metastases.

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REFERENCES

1. Clinical practice guidelines in oncology. Head and Neck Cancers National Comprehensive Cancer Network Version 1.2012. http://www.nccn.org/professionsals/physician_gls/pdf/head-and-neck.pdf.
2. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010;60:277–300.
3. American Cancer Society. Cancer facts & figures 2010. Atlanta, GA: American Cancer Society; 2010.
4. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst. 2000;92:709–20.
5. Robin PE, Powell DJ, Stansbie JM. Carcinoma of the nasal cavity and paranasal sinuses: incidence and presentation of different histological types. Clin Otolaryngol Allied Sci. 1979;4(6):431–56.
6. Choussy O, Ferron C, Vedrine PO, et al. Adenocarcinoma of athmoid: a GETTEC retrospective multicenter study of 418 cases. Laryngoscope. 2008;118(3):437–43.
7. de Gabory L, Maunoury A, Maurice-Tison S, et al. Long-term single-center results of management of ethmoid adenocarcinoma: 95 patients over 28 years. Ann Surg Oncol. 2010;17(4):1127–34.
8. Limmer LA, Branstetter BF, Nayak JV, et al. Current use of 18F-fluorodeoxyglucose positron emission tomography and combined positron emission tomography and computed tomography in squamous cell carcinoma of the head and neck. Laryngoscope. 2005;115(11):2029–34.
9. Pfister DG, Ang K, Brockstein B, et al. NCCN practice guidelines for head and neck cancers. Oncology (Williston Park). 2000;14(11A):163–94.
10. Xu GZ, Zhu XD, Li MY. Accuracy of whole-body PET and PET-CT in initial M staging of head and neck cancer: a meta-analysis. Head Neck. 2011;33(1):87–94.
11. Zanatian AM, Sutton DK, Couch ME, et al. Use, accuracy, and implications for patient management of [18F]-2-fluorodeoxyglucose-positron emission/computerized tomography for head and neck tumors. Laryngoscope. 2005;115(7):1186–90.
12. Teirstein AS, Machac J, Almeida O, et al. Results of 188 whole-body fluorodeoxyglucose positron emission tomography scans in 137 patients with sarcoidosis. Chest. 2007;132(6):1949–53.
13. Takanami K, Kaneta T, Yamada T, et al. FDG PET for esophageal cancer complicated by sarcoidosis mimicking mediastinal and hilar lymph node metastases: two case reports. Clin Nucl Med. 2008;33(4):258–61.
14. Talmi D, Smith S, Mulligan ME. Central skeletal sarcoidosis mimicking metastatic disease. Skeletal Radiol. 2008;37(8):757–61.
15. Ludwig V, Fordice S, Lamar R, et al. Unsuspected skeletal sarcoidosis mimicking metastatic disease on FDG positron emission tomography and bone scintigraphy. Clin Nucl Med. 2003;28(3):176–9.
16. Sartoris DJ, Resnick D, Resnik C, et al. Musculoskeletal manifestations of sarcoidosis. Semin Roentgenol. 1985;20(4):376–86.
17. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet. 2003;361(9363):1111–8.
18. Ji J, Shu X, Li X, et al. Cancer risk in hospitalized sarcoidosis patients: a follow-up study in Sweden. Ann Oncol. 2009;20(6):1121–6.
19. Boffetta P, Rabkin CS, Gridley G. A cohort study of cancer among sarcoidosis patients. Int J Cancer. 2009;124(11):2697–700.
20. Shu X, Li X, Sundquist K, et al. Survival in cancer patients with previous hospitalization for sarcoidosis: a Swedish population-based cohort study during 1964-2006. Ann Oncol. 2011;22(6):1427–34.
21. Umezu H, Chida M, Inoue T, et al. Sarcoidosis development during induction chemotherapy for lung cancer mimicked progressive disease. Gen Thorac Cardiovasc Surg. 2010;58(8):434–7.
22. Hunsaker AR, Munden RF, Pugatch RD, Mentzer SJ. Sarcoid-like reaction in patients with malignancy. Radiology. 1996;200(1):255–61.
23. Kamiyoshihara M, Hirai T, Kawashima O, et al. Sarcoid reactions in primary pulmonary carcinoma: report of seven cases. Oncol Rep. 1998;5(1):177–80.
24. Rosenbaum SJ, Lind T, Antoch, Bockisch A. False-positive FDG PET uptake—the role of PET/CT. Eur Radiol. 2006;16(5):1054–65.
25. Vermorken JB, Trigo J, Hitt R, et al. Open-label uncontrolled, multicenter phase II study to evaluate the efficacy and toxicity of cetuximab as a single agent in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck who failed to respond to platinum-based therapy. J Clin Oncol. 2007;25:2171–7.
26. Colevas AD. Chemotherapy options for patients with metastatic or recurrent squamous

cell carcinoma of the head and neck. J Clin Oncol. 2006;24: 2644–52.
27. Forastiere AA, Shank D, Neuberg D, et al. Final report of a phase II evaluation of paclitaxel in patients with advanced squamous cell carcinoma of the head and neck: an Eastern Cooperative Oncology Group trial (PA390). Cancer. 1998;82:2270–4.
28. Gibson MK, Li Y, Murphy B, et al. Randomized phase III evaluation of cisplatin plus fluorouracil versus cisplatin plus paclitaxel in advanced head and neck cancer (E1395): an intergroup trial of the Eastern Cooperative Oncology Group. J Clin Oncol. 2005;23: 3562–7.
29. Forastiere AA, Metch B, Schuler DE, et al. Randomized comparison of cisplatin plus fluorouracil plus fluorouracil versus methotrexate in advanced squamous cell carcinoma of the head and neck: a Southwest Oncology Group study. J Clin Oncol. 1992;10:1245–51.
30. Vermorken JB, Mesla R, Reivera F, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med. 2008; 359:1116–27.
31. Samlowski WE, Moon J, Kuebler JP, et al. Evaluation of the combination of docetaxel/carboplatin in patients with metastatic or recurrent squamous cell carcinoma of the head and neck (SCCHN): a Southwest Oncology Group phase II study. Cancer Invest. 2007;25:182–8.
32. Bertness B, Goldwasser MA, Flood W, et al. Phase III randomized trial of cisplatin plus placebo compared with cisplatin plus cetuximab in metastatic/recurrent head and neck cancer: an Eastern Cooperative Oncology Group study. J Clin Oncol. 2005;23:8646–54.

Introduction: Recent Advances in the Understanding and Management of Multiple Myeloma ☆

When I entered the myeloma field in 2001, the disease was mostly managed with conventional chemotherapy with the addition of high-dose chemotherapy in younger, transplant-eligible patients. Life expectancy was in 3- to 4-year range, not very different than two to three decades earlier. The introduction of thalidomide in late 1990s, the first of the novel agents for myeloma, coincided with an increased awareness of the disease among the general public, in part thanks to the efforts of a few champions with this diagnosis. The increased awareness led to expanded research funding generated by existing and new organizations and newly formed consortia committed to accelerating the development of new drugs and new treatments.

Indeed, in a little more than just 10 years, the landscape of multiple myeloma is dramatically different. We have witnessed the development of new therapies at an unprecedented speed, which has led to the approval of a number of new drugs and combinations, starting with bortezomib, followed by lenalidomide, then bortezomib with liposomal doxorubicin, and most recently carfilzomib and pomalidomide. The development of new drugs and treatments helped trigger an increase in broader research efforts leading to significant advances in our understanding of the biology of the disease. In particular, we have increased our insight into the genetic and molecular foundations of myeloma, including characterization of its heterogeneity. Importantly, we have learned about the value of generating a strong preclinical rationale before moving to clinical evaluations, establishing the framework of "bench-to-bedside" development of new drugs and treatments. This was well illustrated in the development and ultimate approval of the combination of bortezomib and pegylated liposomal doxorubicin for treatment of relapsed myeloma. Most importantly, the increasing number of therapies and improved understanding of how to treat myeloma, led to a dramatic improvement in not only our ability to control the disease and limit organ damage but to produce meaningfully and dramatically prolonged patient survival, which by some recent projections has at least tripled compared to where we were just over a decade ago. These improvements are so promising that some of us, recently more and more, dare to set a goal of "functional cure" for new therapies.

In this issue of *Seminars in Oncology* a group of established leaders in multiple myeloma research, together with their colleagues, review the progress in their specific areas of expertise and interest. This issue is divided into sections, with the first one or two articles of each section focused on a review of recent progress in an area, and the last providing additional discussion or commentary regarding the most challenging or controversial issues covered by the other experts. We start with a review of the current understanding of the biology of the disease and its implication for the development of treatment strategies. This section is followed by the review of our understanding of the heterogeneity of myeloma and its implications for the development of biology-based personalized therapy. Next we summarize the important developments in the treatment of newly diagnosed patients with myeloma and discuss "hot topics" such as the importance of complete response and depth of response, the role of transplant, and the projections of the evolution of initial treatment strategies, for both "transplant" and "nontransplant" patients. We then review the progress in the treatment of relapsed and relapsed refractory myeloma with a special attention to recently approved agents and the most promising agents in development for treatment of the disease. We conclude with a commentary on how the most recently developed and currently evaluated agents are predicted to contribute to further progress in the treatment of myeloma and how we see their integration into existing treatment paradigms.

Despite the advances of the last decade, challenges remain. The majority of patients are still

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expected to relapse and in the course of their disease the established treatments become less and less effective with shorter and shorter duration of response. In addition, a relatively small (10%–15%) yet significant proportion of patients with poor risk factors, which is becoming better defined, are expected to have much shorter life expectancy than standard-risk patients, and unfortunately, their median overall survival is not very different from a decade ago. We hope that this issue of *Seminars in Oncology* not only provides a concise summary of the state of the field but will help to generate new ideas, providing additional stimulus to further innovations that lead to further prolongation of life for all myeloma patients, including patients with high-risk disease, and hopefully to finding a cure.

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Decoding the Pathophysiology and the Genetics of Multiple Myeloma to Identify New Therapeutic Targets

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In recent years, significant progress has been achieved in the characterization of the transcriptional profiles, gene mutations and structural chromosomal lesions in myeloma cells. These studies have identified many candidate therapeutic targets, which are recurrently deregulated in myeloma cells. However, these targets do not appear, at least individually, to represent universal driver(s) of this disease. Furthermore, evaluation of these recurrent lesions does not suggest that they converge to a single molecular pathway. Detailed integration of molecular and functional data for these candidate targets and pathways will hopefully dissect which of them play more critical roles for each of the different individual molecular defined subtypes of this disease. This review focuses on how recent updates in our understanding of myeloma pathogenesis and molecular characterization may impact ongoing and future efforts to develop new therapeutics for this disease.
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In multiple myeloma (MM), the complex pathophysiology and heterogeneous genetic lesions of the tumor cells, compounded by the genomic instability and molecular evolution of the disease in a given patient over time, pose major challenges to the efforts to improve the treatment outcome for this presently incurable hematologic malignancy. Despite significant advances in our understanding of the biology of MM, and the development of new therapeutics with notable clinical activity, MM patients eventually relapse to all available therapeutic options currently available. Translating promising investigational therapies from preclinical evaluation to successful clinical trials and individualizing the use of these agents for patients with specific molecularly defined subtypes of MM remains a formidable task, since conventional preclinical models may not always faithfully represent the tumor–microenvironment interactions operative in patients. In this review, we will focus on how recent updates in our understanding of MM pathogenesis and genetics may lead to a shift in the direction for development of new therapeutic regimens.

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MOLECULAR PROFILING AND GENOME SEQUENCING STUDIES: IMPLICATIONS FOR THE IDENTIFICATION OF NOVEL THERAPEUTIC TARGETS FOR MM

During the last decade, focused (eg, fluorescence in situ hybridization [FISH]-based cytogenetics) and open-ended molecular profiling studies (including evaluation of copy number changes and gene expression profiles) were performed on primary patient-derived MM samples or MM cell lines.[1–15] These studies stimulated development of molecular marker-driven classification systems, including (1) characterization of hyperdiploid versus non-hyperdiploid MM[1–3,7,16]; (2) TC (translocation and cyclin D classification) system; and (3) gene expression profiling (GEP)-based classification systems, such as those developed by the University of Arkansas (UAMS)[14] or the HOVON (Hemato-Oncologie voor Volwassenen Nederland; Haemato-Oncology Foundation for Adults in the in the Netherlands).[15] These molecular classification systems "collapse" the

heterogeneous MM patient population into smaller and relatively more homogeneous subgroups, in order to provide better insight into the biology and candidate therapeutic targets for each subtype. The biological and clinical implications of these studies on the heterogeneity of MM are described in more detail in other articles in this issue.

Over the last 2–3 years, there has been increased interest in next-generation sequencing (NGS) of MM cells. The first comprehensive open-ended NGS study of this kind involved massively parallel sequencing of 38 genomes from patient-derived MM cells and their comparison to matched normal DNAs.[17] This study determined that essentially all mutations previously known to be highly recurrent in MM, including *NRAS/KRAS* and *TP53* mutations, were consistently detected with NGS technologies. As anticipated, the high-resolution and open-ended nature of these sequencing studies also identified mutations not previously recognized in MM: some of these had been previously detected in other cancers (eg, activating mutations of the kinase *BRAF*), while others are not highly recurrent, if at all present, in other neoplasms (eg, mutations of *FAM46C* or *DIS3*) studied so far. Interestingly, some of these recurrent but infrequent mutations converge to discrete molecular pathways and biological functions. For example, infrequent mutations also were observed in several transcription factors critical for plasma cell biology/myelomagenesis, such as XBP1 (the critical regulator of the differentiation towards the plasma cell lineage and a regulator of unfolded protein response genes); IRF4 (another transcription factor with a central role in myelomagenesis[18]); and PRDM1 (BLIMP1), which is transcriptionally regulated by IRF4.[17] A high proportion of patients harbor mutations in different genes regulating RNA processing and protein homeostasis (eg, *FAM46C* and *DIS3*) and the unfolded protein response, or diverse components of nuclear factor kappa B (NF-κB) signaling or its regulation (consistent with the previously known role of NF-κB signaling in MM biology[4]).

Several genes for histone-modifying enzymes also have been found to be recurrently mutated in MM cells, eg, *MLL, MLL2, MLL3, UTX, MMSET* (*WHSC1*) and *WHSC1L1*.[17–19] Of note, the transcription factor HOXA9, which is regulated, at least in part, by histone methyltransferase-related genomic events, is highly expressed in a subset of MM patients, while depletion of HOXA9 was reported to significantly decrease MM cell proliferation.[17] These results raise the possibility that multiple distinct mutations in genes for histone-modifying enzymes may converge to concordant functional outcome(s), through downstream mediators, such as HOXA9 or other transcriptional networks regulating MM cell proliferation, survival, and drug resistance. The nature of these networks may be highly variable among samples from different MM subtypes. For example, in MM patients with t(4;14) translocation, there is overexpression of MMSET, a histone methyltransferase and transcriptional repressor,[20,21] which induces an increase in lysine 36 methylation of histone H3 and a decrease in lysine 27 methylation across the genome.[22,23] The histone methyltransferase activity of MMSET was found to be essential for growth stimulation by MMSET, and MMSET is assumed to be a major epigenetic regulator in t(4;14) MM.[20,24] To further underline the complexity of epigenetic regulation in MM, a recent report showed that histone demethyltransferases also may have a role in MM pathogenesis.[25]

Congruent with a role of epigenetic changes in the pathogenesis of MM, methylation profiling studies indicate a pattern of global DNA hypomethylation and gene-specific hypermethylation during the multistep transformation from normal plasma cells to monoclonal gammopathy of undetermined significance (MGUS), and to MM (normal plasma cells > MGUS > MM). Interestingly, global re-methylation of the genome appears to occur again during the transformation of MM to plasma cell leukemia (PCL), and involves genes implicated in cell–cell signaling and cell adhesion,[26] which may conceivably contribute to tumor cell independence from the bone marrow (BM) microenvironment during transformation to PCL.

The recurrent nature of new mutations on cascades with pathophysiological relevance for MM[17] reinforces the notion that a low frequency of mutation(s) in any individual gene should not necessarily be interpreted as lack of clinical or therapeutic relevance of this gene. However, further follow-up validation studies are needed to probe the biological significance of mutations in certain pathways (eg, coagulation cascade genes), which are typically not expressed at substantial levels in MM cells.

Studies of Longitudinal Genetic Changes in MM Samples

Recent whole-genome sequencing (WGS) studies of sequential samples from a MM patient with t(4;14) molecular subtype identified changes in the mutational landscape in MM cells of that patient from diagnosis to later relapses, and ultimately to PCL.[19] Single-nucleotide variants (SNVs) were detected in several genes that were previously identified as mutated in both the Multiple Myeloma Research Consortium (MMRC) cohort[17] and the Catalogue of Somatic Mutations in Cancer (COSMIC) database,[27] including: *AFF1, C12orf42, CSMD3, LRRC4C, PCDH7, PTPRD, PPFIBP1, RB1,* and *ZKSCAN3*. The frequency of some SNVs within the tumor cell population fluctuated considerably between samples collected at different time points in the course of the

disease.[19,28] These findings imply that tumor heterogeneity can already be present at diagnosis and that the dominance of certain tumor clone(s) over others can shift over time, which may be influenced by the specific classes of agents used during the course. In addition to described lesions,[19] another similar pilot study from three high-risk myeloma patients' cells[29] detected mutation in Cereblon (*CRBN*, which was recently proposed as a main mediator of the anti-MM activity of lenalidomide and pomalidomide[30,31]), *PSMG2* (a gene encoding for a proteasome assembly protein), and *NR3C1* (which encodes for the glucocorticoid receptor), raising the possibility that these mutations contribute, at least in part, to resistance of these patients to immunomodulatory drugs (IMIDs), proteasome inhibitors, or dexamethasone, respectively. Larger studies will be needed to characterize the longitudinal changes in the genome of MM cells in patients. One such study by the MMRC (CoMMpass project) will seek to correlate the molecular and clinical data of 1000 newly diagnosed symptomatic MM patients over a period of 8 years.[32]

Roles of MicroRNAs in the Regulation of Gene Expression in MM

The discovery of microRNAs (miRNAs) created a new area of study on post-transcriptional regulation of gene expression. A large number of miRNAs are reported to be dysregulated in MM[33–36] and to influence different aspects of its pathogenesis, including cell-cycle progression, p53, and MYC.[37] Notably, recurrent mutations in *DIS3*, *FAM46C*, and *SF3B1*, as identified in NGS studies of MM patients, also may have a potential role in RNA processing,[17] and could potentially cooperate with other molecular lesions, to alter the levels of specific miRNAs. miRNA molecules that have been extensively studied in MM include miR-29b,[38,39] miR-34a,[40,41] and the mir-17-92 cluster.[42] MiR-29b is downregulated in approximately 60% of MM patients and MM cell lines and is suppressed when MM cells are co-cultured with bone marrow stromal cells (BMSCs).[38] Furthermore, miR-29b exhibited anti-MM effects in vitro and in vivo by targeting diverse oncogenic pathways (including suppression of CDK6 and MCL-1) and by leading to an aberrant methylation pattern of MM cells.[38,39] Besides, miR-29b impairs osteoclast differentiation and supresses osteoclast activation triggered by MM cells.[43] MiR-34a has a tumor-suppressor activity and is transcriptionally regulated by p53. *TP53* loss/mutation or silencing through promoter methylation decreased miR-34a expression in MM cells, while restoration of miR-34a expression could suppress the levels of Bcl2, CDK4/6, and YY1, as well as sensitize MM cells to bortezomib.[40] Synthetic miR-34a can downregulate BCL2, CDK6, and NOTCH1 at both the mRNA and protein levels,[41] as well as achieve in vivo activity against *TP53*-mutated MM xenografts without major systemic toxicity. Several other miRNAs affecting the *TP53* pathway have been described, including miR-32, miR-192, miR-194, and miR-215.[33,44]

Interestingly, recent studies have identified a miRNA-based risk-stratification system, which can significantly improve the predictive power of International Staging System (ISS)/FISH risk stratification in patients from the UK Myeloma IX clinical trial, in a manner independent of gene expression-derived prognostic signatures, including those determined by studies of the UAMS or Intergroupe Français du Myélome (IFM), as well as the Myeloma IX study itself.[42] In this miRNA-based risk stratification, the mir-17-92 cluster was prominently involved and correlated with the activity of Myc and E2F3, underscoring the importance of the Myc/E2F/miR-17-92 negative feedback loop in MM pathogenesis.

KEY DYSREGULATED PATHWAYS IN MM AS THE FOCUS FOR DEVELOPMENT OF NEW THERAPEUTIC APPROACHES

The genetic and epigenetic dysregulation observed in MM cells involves a large number of molecular pathways that could conceivably represent targets for therapeutic interventions.

Activation of NF-κB signaling is common in MM, due to loss-of-function mutations in negative regulators of this pathway,[4,7] and/or of stroma-induced NF-κB signaling in MM cells.[45,46] Bortezomib suppresses the transcriptional activity of NF-κB, and this event may contribute to the anti-MM activity of this compound. However, NF-κB–independent functions of the proteasome may account for a significant part of the activity of bortezomib and other members of this drug class.

The Ras/Raf/MEK/ERK pathway is also frequently dysregulated in MM.[2] Specifically, inhibition of MEK/MAPK exhibits preclinical anti-MM activity,[47] which is enhanced by concomitant inhibition of PI3K/Akt in 75% of primary MM samples tested with this combination. Resistance to this combination was exclusively observed in RAS wild-type cases,[47] suggesting that RAS mutations may identify patients in whom combined inhibition of these pathways should be explored in future studies. MEK regulates MAF transcription in MM cells,[48] suggesting that inhibition of Ras/MEK/ERK may be particularly warranted in MM patients with c-maf overexpression.

Dysregulation of the PI3K/AKT/mTOR pathway is also important in MM pathophysiology[2]: even though genes involved in this cascade are not frequently

mutated in MM,[17] phospho-AKT is detected in 50% of cases[49] due to activation of this pathway by cytokines/growth factors or adhesion molecule-mediated interactions with BSMCs.[50] It is also plausible that MM cells harbor genetic lesions, which facilitate the constitutive activation of PI3K/AKT. For example, the mTOR-interacting protein DEPTOR (DEP domain containing mTOR-interacting protein), suppresses S6K1 but relieves feedback inhibition from mTORC1 to PI3K signaling, thereby activating Akt. DEPTOR levels are low in most human cancers, but DEPTOR is overexpressed in MM cells with cyclin D1/D3 or c-MAF/MAFB translocations.[51] Interestingly, high levels of DEPTOR correlate with response to thalidomide in MM patients.[52] Other studies indicate that SCF (Fbxo9) and CK2 regulate Akt activity in the context of growth factor deprivation and promote survival of MM cells.[53] Given the role of the PI3K/AKT/mTOR in MM, several efforts have been undertaken to inhibit its function, in some cases with multi-targeted agents, eg, the PI3K/mTOR inhibitors BEZ235[54] and BTG-226,[55] other mTOR-targeting agents,[56] and Akt inhibitors.[57]

Developmental pathways are frequently dysregulated in human cancers and the progress of this research in other cancers led to similar interest in their role in MM.

The Notch family of receptors and ligands are dysregulated early in MM progression.[58] Interactions of MM cells with BMSCs has been reported to activate Notch-signaling, both in tumor cells and BMSC, and then to induce secretion of interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), and insulin-like growth factor-1 (IGF-1).[59] Notch receptors are expressed by MM cells and Notch ligand Dll1 is expressed on BMSCs. Dll1 is involved in tumor migration[58] and also has been reported to contribute to bortezomib resistance,[60] while blockade of the Notch pathway could reverse this effect and increase MM cell sensitivity to bortezomib. Notch signaling also has been reported to induce CXCR4/SDF-1 expression[61] and, conversely, Notch inhibition suppresses MM cell migration, proliferation, and resistance to apoptosis by reducing CXCR4 and SDF-1 levels.[61]

The Hedgehog (Hh) pathway has been reported to regulate the function of MM cells with stem cell–like features,[62,63] conceivably recapitulating the physiological role of this pathway in regulating normal stem cells during embryogenesis. After early development, the Hh pathway is typically silenced in most normal tissues, except during tissue homeostasis and repair, implying that therapeutic interventions against this pathway can be achieved without catastrophic toxicities against most healthy tissues. This notion has been supported by the safety profile of ismodegib, a Hh inhibitor approved for treatment of advanced basal cell carcinoma (BCC).[64] Hh genes are overexpressed in MGUS and MM patients, compared to healthy donors and aberrant Hh signaling has been detected in MM, through activation of both canonical (Hh receptor Smoothened [Smo]-dependent) and noncanonical (Smo-independent) pathways.[62] BMSCs are a source of sonic hedgehog (Shh) ligand, which suggests a paracrine role for stroma-derived Hh signals.

The Wnt pathway has been studied extensively in MM. The Wnt inhibitor DKK1, a suppressor of osteoblast maturation and function, is considered to be an important contributor to the impaired bone remodeling in MM. Anti-DKK1 neutralizing antibodies have been tested as a strategy to suppress MM-associated bone loss.[65] More recently, the Wnt pathway has been studied as a regulator of MM cell proliferation and biological aggressiveness. For example, DKK1 exhibits a biphasic pattern of expression during the course of MM, with overexpression during the earlier stages (during which DKK1 contributes to bone lytic lesions) and low or nearly undetectable DKK1 levels in more advanced MM (and MM cell lines).[65,66] This pattern may be due to a negative feedback loop of Wnt/β-catenin signaling in MM cells. DKK1 may act as tumor-suppressor, since low levels of DKK 1 correlate with enhanced Wnt pathway activation in MM cells[66] and transcriptional silencing of DKK1 by CpG-island methylation of DKK1 promoter is associated with increased Wnt pathway activation in advanced MM: in contrast, demethylation of the DKK1 promoter restores DKK1 expression, resulting in inhibition of β-catenin/T-cell factor (TCF)-mediated gene transcription in MM lines.[66] In addition, early preclinical data suggest that Wnt antagonists inhibit MM cell proliferation.[67] Further studies will be needed to evaluate the impact of inhibition of DKK1 in advanced MM.

Targeting Transcriptional Regulators With Oncogenic Properties in MM: CCND, MAF/MAFB, MMSET/FGFR3

As in many other neoplasms, MM is a disease of dysregulated transcriptional programs. Interestingly, several early oncogenic events in MM involve transcription factors such as D-type cyclins (CCND) or c-maf.[1,14,68,69] Moreover, an increasing volume of data points to c-myc[1,6,70–72] and IRF4[18,30,31,46,73] as important regulators of MM biology.

Many transcription factors typically lack structural motifs amenable to selective binding of currently available classes of chemical entities, which may explain, at least in part, the challenges to direct inhibition of MM-associated transcription factors.[74,75] An approach to bypass these challenges involves targeting of other, upstream or downstream, components of the molecular pathways

involving such transcription factors. For example, cyclin-dependent kinases (CDKs) can be targeted instead of D-type cyclins themselves. Specifically, the broad-spectrum CDK inhibitor flavopiridol exhibits preclinical anti-MM activity and has been evaluated in clinical trials for other hematologic malignancies. RNAi knockdown of CDK5 emerged among the top "hits" in an in vitro screen for bortezomib sensitizers, and the CDK5 inhibitor, dinaciclib (SCH727965), is in phase I/II clinical trial.[76] CDK9, a key regulator of transcriptional elongation, is targeted by SNS-032 and AT7519 (inhibitors of CDK9 and other CDKs), which are also being evaluated in MM.[65,77] Although no selective pharmacologic inhibitors exist for c-maf, which is overexpressed in approximately 30% of MM cases,[48] MEK inhibitors can inhibit c-maf expression in cells from both the MMSET (Multiple Myeloma SET Domain) or MAF subtypes of MM, suggesting a role for MEK inhibitors to indirectly target c-maf function.

MYC contributes to progression of normal plasma cells to MM, and stochastic activation of the MYC transgene in MGUS-prone mice leads to development of MM.[78] Secondary Myc rearrangements are observed in 16% of newly diagnosed patients, with increasing prevalence during disease progression (and detection in >90% of MM cell lines), indicating a role for MYC both early and late in myelomagenesis. Recently, major progress in therapeutic targeting of c-myc was achieved with the development of JQ1, the prototypic bromodomain inhibitor. The BET bromodomain (BRD) protein BRD4 contains recognition domains for chromatin-dependent signal transduction to RNA polymerase and regulates c-Myc expression and function. BRD4 has a role in marking select M/G_1 phase genes in mitotic chromatin as transcriptional memory and regulates postmitotic transcription via direct interaction with the positive transcription elongation factor complex b (P-TEFb). Because c-Myc regulates promoter-proximal pause release of Pol II, also through the recruitment of P-TEFb, it was hypothesized that targeting BET bromodomains could result in inhibition of c-Myc–dependent transcription. Consistent with this hypothesis, BET inhibition using the small molecule JQ1 resulted in downregulation of MYC transcription and subsequent genome-wide suppression of Myc-dependent target genes.[72] JQ1 induces a potent in vitro antiproliferative effect via cell cycle arrest and cellular senescence, but not cell death, and the cellular processes recover after treatment withdrawal. JQ1 also effectively reduces tumor growth rate in murine models, and produces complete remissions in the genetically engineered c-myc–driven Vk*myc model.[72] However, JQ1 treatment was not able to decrease tumor burden (compared to pretreatment baseline) in cell line xenograft models (of either subcutaneous or diffuse orthotopic bone lesions).[72] Furthermore, those Vk*myc mice which exhibited decreases in MM burden (as measured by human monoclonal M protein levels in the peripheral blood) with JQ1 treatment, the tumor burden increased again after treatment was stopped. Collectively, these data indicate that Myc is a prominent target and that inhibition of Myc via BRD4 inhibitor shows efficacy in preclinical models, as validated by multiple follow-up studies.[79] However, the ability of MM cells to survive despite treatment remains a limitation, and ongoing efforts are addressing strategies to develop and optimize BET bromodomain inhibitor-based combination regimens.

Until the development of proteasome inhibitors, MM patients with t(4;14) (15% of MM cases) typically exhibited suboptimal response to conventional and high-dose chemotherapy.[80] The overexpression of FGFR3 in MM cells of these patients suggested that kinase inhibitors or monoclonal antibodies against this receptor could achieve major clinical responses.[3,81] FGFR3 tyrosine kinase inhibitors are typically active preclinically only against MM cells with activating FGFR3 mutations, while anti-FGFR3 monoclonal antibodies exhibit activity against both wild-type and mutant FGFR3-expressing cells.[82–84] Several FGFR3-targeting agents have been tested clinically, but so far there are no definitive reports of major clinical responses from these studies. This may be due to the fact that t(4;14) dysregulates not only FGFR3, but also MMSET expression. In fact, more recent preclinical data suggest that MMSET may actually represent a more significant driver of the biology of t(4;14) MM than FGFR3 itself.[20,21,24] To date, selective MMSET inhibitors have not yet been developed although progress in structural biology studies of MMSET protein are poised to facilitate the structure-function–based drug design of such agents.[2]

BRAF V600E mutations are detected with variable frequency (2%–4%) in several cohorts[17] of MM cases. The small molecule inhibitor, vemurafenib, already approved for use in *BRAF*-mutant melanoma, could potentially be used to treat *BRAF*-mutant MM patients. Interestingly, *BRAF* V600E mutation patients have been reported in small case series to have relatively short progression-free survival after first-line of treatment and high prevalence of extramedullary disease,[85] although further confirmation is warranted

Loss of *p53* function is a relatively rare (~10%) molecular event in newly diagnosed MM patients, but *TP53* mutations/deletions are associated with adverse clinical outcome in MM.[1,6,9,86] There are no available clinically applicable approaches to restore p53 function in cells with deletion of both alleles. In terms of mutant p53, there are efforts to develop small-molecule inhibitors,[87] for example, MIRA-1, which

are capable of restoring wild-type conformation and function to mutant p53, and which have in vitro and in vivo anti-MM activity regardless of p53 mutation.[88] APR-246 (PRIMA-1MET) is capable of restoring transcriptional activity of unfolded wild-type or mutant p53, through inhibition of MDM2, and has already reached phase I clinical trial.[89]

ARRY-520, a novel kinesin spindle protein (KSP) inhibitor, was reported to have preclinical anti-MM activity through degradation of MCL-1.[90] This agent has shown promising early clinical results as a single agent and as part of combined therapy.[91,92]

XPO1/CRM1 was identified in RNAi screening studies to be essential for MM cell proliferation.[93] *XPO1* codes for the protein exportin 1, a nuclear transport protein that exports tumor-suppressor proteins from the nucleus. *XPO1* expression is increased with disease progression.[94] Oral selective inhibitors of nuclear export (SINE) compounds,[95,96] such as KPT-276 and KPT-330 (currently in phase 1 clinical trials), exhibit anti-MM activity in vitro and in vivo, as well as impair osteoclastogenesis and bone resorption via RANKL inhibition, without impacting osteoblasts.

Immune-Based Therapies

Immune dysregulation has been proposed to play a critical role in MM progression,[97] hence the importance of developing potent and selective anti-MM immunotherapeutic strategies. In MM, the number of dendritic cells (DCs) appears to be increased, but their immunophenotype is consistent with immature DCs with inefficient antigen presentation/processing properties.[58] This has led to ongoing studies to test DCs/MM fusion cell vaccination in combination with inhibition of PD-1, which promotes T-cell tolerance, after autologous stem cell transplantation.[98]

Another development is the use of monoclonal antibodies against specific surface molecules in MM cells, namely, CD38 (daratumumab)[99] and CS1 (elotuzumab),[100–103] which both show promising results in phase I/II clinical trials. For example, recent report (2013 European Hematology Association meeting) of results from a phase I/II trial of single-agent daratumumab in patients with relapsed or refractory MM indicated a response rate of approximately 30% for the entire study population and 67% for patients treated with 4 mg/kg and upwards. These results are promising because these responses to monotherapy with a monoclonal antibody have not been previously observed in patients with disease refractory to proteasome inhibition and IMIDs. Another area of active research relates to myeloid-derived suppressor cells (MDSCs), which represent a heterogeneous group of immature myeloid cells ($CD11b^{+}CD14^{-}HLA\text{-}DR^{-/low}CD33^{+}CD15^{+}$) that are capable of suppressing immune responses.[104–107] MDSCs were recently evaluated in MM patients and were found to be increased in the peripheral blood and BM of MM patients.[105] MM cells induce MDSC development from healthy donor peripheral blood mononuclear cells, whereas interaction with MDSCs supports MM proliferation and suppresses T-cell–mediated immune responses. Inhibition of the tumor-promoting and immune-suppressive functions of MDSCs in MM represents an interesting potential immune-based therapeutic strategy.

IMPLICATIONS OF THE TUMOR–MICROENVIRONMENT INTERACTION ON THE FUTURE OF MM TREATMENT

MM is a prototypical disease for the study of interactions between tumor cells and their microenvironment. A major focus in many studies has been the role of BMSCs and specifically how adhesion of MM cells to BMSCs can trigger paracrine, autocrine, and/or juxtacrine (cell adhesion–mediated) activation of proliferative and anti-apoptotic signaling cascades in MM cells.[46,108–113] Major progress has been recently achieved in the mechanistic understanding of these events, and on characterizing the extensive impact of these events on MM cell response to diverse therapeutics.

The development of the compartment-specific bioluminescence imaging (CS-BLI) platform has allowed high-throughput scalable quantification of the response of large numbers of MM cell lines to a very large number of established and investigational therapeutics both in the presence and in the absence of different types and sources of BMSCs.[46] CS-BLI–based studies documented that MM cells acquire resistance in the presence of BMSCs not only to glucocorticoids, anthracyclines, and alkylating agents but also to a broader range of agents, including diverse investigational therapeutics, from various chemical classes.[46] In the context of interaction with BMSCs, MM cells actually may become more sensitive to certain classes of therapeutics,[46] a phenomenon termed "microenvironment-dependent synthetic lethality." A notable example of this principle involves multitargeted kinase inhibitors, which inhibit JAK kinases,[46] which may be explained by the role of JAK kinases as downstream effectors of signaling events induced by the IL-6/IL-6 receptor interaction or other gp130 receptor systems on the surface of MM cells. Microenvironment-dependent synthetic lethality may have profound implications for the future of drug development in MM and other neoplasms: it suggests that a substantial number of investigational agents with anti-tumor activity exhibited in the context of tumor–microenvironment interactions may have been missed in the past by

conventional preclinical development assays, which are based on culture of tumor cells in isolation and therefore did not routinely incorporate the element of interaction between tumor cells and BMSCs (or other accessory cells).

Significant advances have been achieved in our mechanistic understanding of the molecular cascades triggered in MM cells by their interaction with BMSCs. Transcriptional profiling analysis of MM cell lines interacting with immortalized BMSCs indicate that MM cells exhibit increased transcriptional output of diverse oncogenic pathways, including Ras, PI3K/Akt, and NF-κB, as well as transcriptional signatures for MYC, IRF4, and other transcriptional regulatory programs that are important for MM cells in particular, or malignant cells more broadly.[46] The pleiotropic nature of these molecular events implies that it may be difficult to abrogate the molecular consequences of tumor stroma interactions through suppression of single growth factor(s), cytokine(s), respective receptors, or downstream signaling cascades.[46] Consistent with these observations, neutralization of IL-6 activity in MM–BMSC co-cultures decreased but did not completely abrogate the ability of BMSCs to induce resistance of MM cells to conventional agents, such as doxorubicin.[46]

MM cells in the local microenvironment interact not only with BMSCs but also with other accessory cells (eg, osteoclasts[114–117]), as well as with diverse growth factors and cytokines. Tumor cell–BMSC adhesion has been reported to trigger excessive osteoclast activation and increased MM cell proliferation and survival, through both direct cell–cell contact-mediated interactions, as well as the release of soluble factors, such as IL-6, BAFF, and APRIL.[118] These observations combined with recent reports of potential survival advantage from bisphosphonate treatment in MM patients[119] suggest that inhibition of osteoclast function in MM may have a favorable impact on survival of patients through effects that are independent of the direct impact of osteoclasts on bone resorption and the clinical sequelae of decreased skeletal events.

DCs and macrophages recently have attracted considerable interest in terms of their possible role as accessory cells supportive of MM cells in the BM milieu. Plasmacytoid dendritic cells (pDCs) in the BM of MM patients have been proposed to mediate, at least in part, the immune deficiency in this disease, as well as to promote MM cell proliferation, survival, and drug resistance.[120] Targeting toll-like receptors with CpG oligodeoxynucleotides both restores pDC immune function and abrogates pDC-induced MM cell growth. Targeting pDC–MM interactions may thus serve as a therapeutic strategy for MM. Myeloid dendritic cells (mDCs) in the BM also interact with MM cells via CD28–CD80/CD86(B7) mechanism(s).[121,122] CD28, a co-stimulatory receptor on T cells, is overexpressed in MM cells during disease progression and is associated with adverse survival. Engagement of CD28 on MM cells by its ligand CD80/CD86 on mDCs directly stimulates the increase in MM cells of prosurvival signaling via PI3K/Akt/FoxO3a/Bim, as well as NF-κB, leading to protection against cytotoxic agents and growth factor withdrawal-induced apoptosis. The interaction with MM cells induces in mDCs a B7-mediated upregulation of IL-6 (involved in cross-talk with the Notch pathway) and the immunosuppressive enzyme IDO. This MM–mDC interaction and its protective effect on MM cells can be suppressed by lenalidomide or anti-CD28 antibody. Interestingly, the MM–DCs interaction was recently reported to contribute to genomic instability in MM: cell-to-cell contact between MM and DCs rapidly induces the genomic mutator activation-induced cytidine deaminase (AID) and AID-dependent DNA double-strand breaks (DSBs) in MM cells.[123] AID-mediated genomic damage led to the malignant progression of plasma cells in vivo and this induction can be inhibited by blockade of RANKL interactions. Macrophages also have been reported to provide MM cells with proliferative and anti-apoptotic signals. PSGL-1 (P-selectin glycoprotein ligand-1)/selectins and ICAM-1/CD18 play an important role in macrophage-mediated MM drug resistance.[124] Interaction of macrophages and MM cells activates Src and Erk1/2 kinases, as well as c-myc pathways, as well as suppresses caspase activation induced by chemotherapy. $CD14^{+}$ monocytic cells freshly explanted from MM BM exhibited a predominantly pro-inflammatory transcriptomic profile when compared to normal monocytes. Constitutive activation of MAP3K8 kinase-dependent cascades appears to regulate the inflammatory activity of monocytes/macrophages, which acquire a pro-inflammatory transcriptional profile in the MM microenvironment.[125] MAP3K8 is required for tumor necrosis factor alpha (TNFα)-mediated ERK activation, while inhibition of MAP3K8 results in apoptosis of MM cells despite their contact with primary stromal cells.

In addition to the known effects of proteasome inhibitors or IMIDs, new classes of therapeutics are also capable of targeting the local BM/bone microenvironment and the support that it provides to the MM cells. For example, targeting the hypoxic niche is under evaluation using drugs that inhibit molecular targets in the hypoxia signaling pathway (eg, HIF-1α inhibitors), as well as selective hypoxia-activated prodrugs (eg, TH-302).[126]

KEY CONCEPTS

The recent advances in MM research have identified novel therapeutic targets that may translate into improvements in treatment outcome. However, none of the molecular lesions recurrently present in MM cells are universal driver(s) of this disease. Furthermore, evaluation of recurrent lesions does not suggest convergence of these molecular defects to one single pathway. Instead, multiple molecular cascades seem to be concomitantly dysregulated in this disease, even in its early stages. However, there is room for optimism that these advances in our molecular understanding of the disease will translate into therapeutic benefit. The last decade has seen major progress in development of new chemical entities that selectively engage molecular targets with potential therapeutic value for different cancers, including myeloma. Moreover, even though the molecular complexity and heterogeneity of MM cells is daunting, there has been increasing emphasis on identification of molecular targets that play central role(s) in the biology of this disease which thereby represent targets for therapeutic interventions. Importantly, preclinical and clinical translation of novel agents with multi-targeted features (eg, proteasome inhibitors and immunomodulatory drugs), as well as use of preclinical models to inform the design of combination regimens incorporating new targeted therapies along with these agents, has great potential to further improve patient outcome.

CONCLUSION

Our understanding of the molecular pathophysiology of MM has significantly expanded over the last decade. Recurrent mutations in MM oncogenes have been found, but many of these are either currently not targetable with existing therapies or too infrequent to enable rapid clinical development of specific therapeutic agents. However, progress in the study of several different pathways involved in MM pathogenesis has already provided valuable insights into new, and potentially beneficial strategies to treat MM.

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REFERENCES

1. Bergsagel PL, Kuehl WM. Molecular pathogenesis and a consequent classification of multiple myeloma. J Clin Oncol. 2005;23:6333–8.
2. Morgan GJ, Kaiser MF. How to use new biology to guide therapy in multiple myeloma. Hematology/Education Program of the American Society of Hematology American Society of Hematology Education Program. 2012;2012:342–9.
3. Kuehl WM, Bergsagel PL. Molecular pathogenesis of multiple myeloma and its premalignant precursor. J Clin Invest. 2012;122:3456–63.
4. Keats JJ, Fonseca R, Chesi M, et al. Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma. Cancer Cell. 2007;12:131–44.
5. Billecke L, Penas EM, May AM, et al. Similar incidences of TP53 deletions in extramedullary organ infiltrations, soft tissue and osteolyses of patients with multiple myeloma. Anticancer Res. 2012;32:2031–4.
6. Tiedemann RE, Gonzalez-Paz N, Kyle RA, et al. Genetic aberrations and survival in plasma cell leukemia. Leukemia. 2008;22:1044–52.
7. Bergsagel PL, Davies F, Avet-Loiseau H. Epidemiology, etiology, and molecular pathogenesis of multiple myeloma. In: Richardson PG, Anderson KC, editors. Multiple myeloma. 2nd ed. Chicago: Remedica; 2011:1–18.
8. Nair B, van Rhee F, Shaughnessy JD, Jr., et al. Superior results of Total Therapy 3 (2003-33) in gene expression profiling-defined low-risk multiple myeloma confirmed in subsequent trial 2006-66 with VRD maintenance. Blood. 2010;115:4168–73.
9. Walker BA, Leone PE, Chiecchio L, et al. A compendium of myeloma-associated chromosomal copy number abnormalities and their prognostic value. Blood. 2010;116:e56–65.
10. Bergsagel PL, Mateos MV, Gutierrez NC, Rajkumar SV, San Miguel JF. Improving overall survival and overcoming adverse prognosis in the treatment of cytogenetically high-risk multiple myeloma. Blood. 2012;121:884–92.
11. Fonseca R, Blood E, Rue M, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. Blood. 2003;101:4569–75.
12. Boyd KD, Ross FM, Chiecchio L, et al. A novel prognostic model in myeloma based on co-segregating adverse FISH lesions and the ISS: analysis of patients treated in the MRC Myeloma IX trial. Leukemia. 2012;26:349–55.
13. Avet-Loiseau H, Malard F, Campion L, et al. Translocation t(14;16) and multiple myeloma: is it really an independent prognostic factor? Blood. 2011;117:2009–11.
14. Zhan F, Huang Y, Colla S, et al. The molecular classification of multiple myeloma. Blood. 2006;108:2020–8.

15. Broyl A, Hose D, Lokhorst H, et al. Gene expression profiling for molecular classification of multiple myeloma in newly diagnosed patients. Blood. 2010;116:2543–53.
16. Kumar S, Fonseca R, Ketterling RP, et al. Trisomies in multiple myeloma: impact on survival in patients with high-risk cytogenetics. Blood. 2012;119:2100–5.
17. Chapman MA, Lawrence MS, Keats JJ, et al. Initial genome sequencing and analysis of multiple myeloma. Nature. 2011;471:467–72.
18. Shaffer AL, Emre NC, Lamy L, et al. IRF4 addiction in multiple myeloma. Nature. 2008;454:226–31.
19. Egan JB, Shi CX, Tembe W, et al. Whole-genome sequencing of multiple myeloma from diagnosis to plasma cell leukemia reveals genomic initiating events, evolution, and clonal tides. Blood. 2012;120:1060–6.
20. Martinez-Garcia E, Popovic R, Min DJ, et al. The MMSET histone methyl transferase switches global histone methylation and alters gene expression in t(4;14) multiple myeloma cells. Blood. 2011;117:211–20.
21. Walker BA, Wardell CP, Melchor L, et al. Intraclonal heterogeneity and distinct molecular mechanisms characterize the development of t(4;14) and t(11;14) myeloma. Blood. 2012;120:1077–86.
22. Brito JL, Walker B, Jenner M, et al. MMSET deregulation affects cell cycle progression and adhesion regulons in t(4;14) myeloma plasma cells. Haematologica. 2009;94:78–86.
23. Pei H, Zhang L, Luo K, et al. MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. Nature. 2011;470:124–8.
24. Popovic R, Martinez E, Zhang Q, et al. MMSET dysregulates gene expression in myeloma through global and focal changes in H3K36 and H3K27 methylation. ASH Annual Meeting Abstracts. 2012;120:523.
25. LoBello J, Gale M, Watanabe A, Hostetter G, Fonseca R, Salhia B. The role of the histone demethyltransferase gene JMJD1C and H3K9 methylation in multiple myeloma. ASH Annual Meeting Abstracts. 2012;120: 3527.
26. Walker BA, Wardell CP, Chiecchio L, et al. Aberrant global methylation patterns affect the molecular pathogenesis and prognosis of multiple myeloma. Blood. 2011;117:553–62.
27. Forbes SA, Bindal N, Bamford S, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res. 2011;39:D945–50.
28. Keats JJ, Chesi M, Egan JB, et al. Clonal competition with alternating dominance in multiple myeloma. Blood. 2012;120:1067–76.
29. Egan J, Kortuem KM, Shi C-X, et al. The myeloma genome in drug refractory extra-medullary disease identifies mutations in proteasome, cereblon and glucocorticoid pathways. ASH Annual Meeting Abstracts. 2012;120:3968.
30. Zhu YX, Braggio E, Shi CX, et al. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. Blood. 2011; 118:4771–9.
31. Lopez-Girona A, Mendy D, Ito T, et al. Cereblon is a direct protein target for immunomodulatory and antiproliferative activities of lenalidomide and pomalidomide. Leukemia. 2012;26:2326–35.
32. Needle MN, Harrison B, Hoban C, et al. The Multiple Myeloma Research Foundation (MMRF) CoMMpassSM study: a longitudinal study in newly-diagnosed multiple myeloma patients to assess genomic profiles, immunophenotypes and clinical outcomes. ASH Annual Meeting Abstracts. 2012;120:3980.
33. Pichiorri F, Suh SS, Ladetto M, et al. MicroRNAs regulate critical genes associated with multiple myeloma pathogenesis. Proc Natl Acad Sci U S A. 2008;105:12885–90.
34. Zhou Y, Chen L, Barlogie B, et al. High-risk myeloma is associated with global elevation of miRNAs and overexpression of EIF2C2/AGO2. Proc Natl Acad Sci U S A. 2010;107:7904–9.
35. Benetatos L, Vartholomatos G. Deregulated microRNAs in multiple myeloma. Cancer. 2012;118:878–87.
36. Tagliaferri P, Rossi M, Di Martino MT, et al. Promises and challenges of microRNA-based treatment of multiple myeloma. Curr Cancer Drug Targets. 2012; 12:838–46.
37. Chang TC, Yu D, Lee YS, et al. Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet. 2008;40:43–50.
38. Amodio N, Martino MTD, Foresta U, et al. MiR-29b exerts anti-multiple myeloma activity by targeting key oncogenic pathways and modulating DNA methylation profile. ASH Annual Meeting Abstracts. 2012;120:2941.
39. Amodio N, Leotta M, Bellizzi D, et al. DNA-demethylating and anti-tumor activity of synthetic miR-29b mimics in multiple myeloma. Oncotarget. 2012;3:1246–58.
40. Stebner E, Neri P, Johnson J, et al. Mir-34a sensitizes multiple myeloma (MM) cells to the proteasome inhibitor bortezomib. ASH Annual Meeting Abstracts. 2011;118:138.
41. Di Martino MT, Leone E, Amodio N, et al. Synthetic miR-34a mimics as a novel therapeutic agent for multiple myeloma: in vitro and in vivo evidence. Clin Cancer Res. 2012;18:6260–70.
42. Wu P, Agnelli L, Walker BA, et al. Improved risk stratification in myeloma using microrna-based classifier. ASH Annual Meeting Abstracts. 2012;120:932.
43. Rossi M, Pitari MR, Amodio N, et al. miR-29b negatively regulates human osteoclastic cell differentiation and function: implications for the treatment of multiple myeloma-related bone disease. J Cell Physiol. 2013;228:1506–15.
44. Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer. 2012;13:83–96.
45. Chauhan D, Uchiyama H, Akbarali Y, et al. Multiple myeloma cell adhesion-induced interleukin-6 expression in bone marrow stromal cells involves activation of NF-kappa B. Blood. 1996;87:1104–12.
46. McMillin DW, Delmore J, Weisberg E, et al. Tumor cell-specific bioluminescence platform to identify stroma-induced changes to anticancer drug activity. Nature Med. 2010;16:483–9.

47. Steinbrunn T, Stuhmer T, Sayehli C, Chatterjee M, Einsele H, Bargou RC. Combined targeting of MEK/MAPK and PI3K/Akt signalling in multiple myeloma. Br J Haematol. 2012;159:430–40.
48. Annunziata CM, Hernandez L, Davis RE, et al. A mechanistic rationale for MEK inhibitor therapy in myeloma based on blockade of MAF oncogene expression. Blood. 2011;117:2396–404.
49. Zollinger A, Stuhmer T, Chatterjee M, et al. Combined functional and molecular analysis of tumor cell signaling defines 2 distinct myeloma subgroups: Akt-dependent and Akt-independent multiple myeloma. Blood. 2008;112:3403–11.
50. Hideshima T, Bergsagel PL, Kuehl WM, Anderson KC. Advances in biology of multiple myeloma: clinical applications. Blood. 2004;104:607–18.
51. Peterson TR, Laplante M, Thoreen CC, et al. DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. Cell. 2009;137:873–86.
52. Boyd KD, Walker BA, Wardell CP, et al. High expression levels of the mammalian target of rapamycin inhibitor DEPTOR are predictive of response to thalidomide in myeloma. Leuk Lymphoma. 2010;51:2126–9.
53. Fernandez-Saiz V, Targosz BS, Lemeer S, et al. SCF (Fbxo9) and CK2 direct the cellular response to growth factor withdrawal via Tel2/Tti1 degradation and promote survival in multiple myeloma. Nature Cell Biol. 2013;15:72–81.
54. McMillin DW, Ooi M, Delmore J, et al. Antimyeloma activity of the orally bioavailable dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235. Cancer Res. 2009;69:5835–42.
55. Baumann P, Schneider L, Mandl-Weber S, Oduncu F, Schmidmaier R. Simultaneous targeting of PI3K and mTOR with NVP-BGT226 is highly effective in multiple myeloma. Anticancer Drugs. 2012;23:131–8.
56. Maiso P, Liu Y, Morgan B, et al. Defining the role of TORC1/2 in multiple myeloma. Blood. 2011;118:6860–70.
57. Mimura N, Ohguchi H, Cirstea D, et al. TAS-117, a novel selective Akt inhibitor demonstrates significant growth inhibition in multiple myeloma cells in vitro and in vivo. ASH Annual Meeting Abstracts. 2012;120:942.
58. Colombo M, Mirandola L, Platonova N, et al. Notch-directed microenvironment reprogramming in myeloma: a single path to multiple outcomes. Leukemia. 2013;27:1009–18.
59. Manier S, Sacco A, Leleu X, Ghobrial IM, Roccaro AM. Bone marrow microenvironment in multiple myeloma progression. J Biomed Biotechnol. 2012;2012:157496.
60. Xu D, Hu J, De Bruyne E, et al. Dll1/Notch activation contributes to bortezomib resistance by upregulating CYP1A1 in multiple myeloma. Biochem Biophys Res Commun. 2012;428:518–24.
61. Mirandola L, Apicella L, Colombo M, et al. Anti-Notch treatment prevents multiple myeloma cells localization to the bone marrow via the chemokine system CXCR4/SDF-1. Leukemia. 2013;27:1558–66.
62. Blotta S, Jakubikova J, Calimeri T, et al. Canonical and noncanonical Hedgehog pathway in the pathogenesis of multiple myeloma. Blood. 2012;120:5002–13.
63. Peacock CD, Wang Q, Gesell GS, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. Proc Natl Acad Sci U S A. 2007;104:4048–53.
64. Axelson M, Liu K, Jiang X, et al. US Food and drug administration approval: vismodegib for recurrent, locally advanced, or metastatic basal cell carcinoma. Clin Cancer Res. 2013;19:2289–93.
65. Pozzi S, Fulciniti M, Yan H, et al. In vivo and in vitro effects of a novel anti-Dkk1 neutralizing antibody in multiple myeloma. Bone. 2013;53:487–96.
66. Kocemba KA, Groen RW, van Andel H, et al. Transcriptional silencing of the Wnt-antagonist DKK1 by promoter methylation is associated with enhanced Wnt signaling in advanced multiple myeloma. PloS One. 2012;7:e30359.
67. Narayanan BA, Doudican NA, Park J, et al. Antagonistic effect of small-molecule inhibitors of Wnt/beta-catenin in multiple myeloma. Anticancer Res. 2012;32:4697–707.
68. Hurt EM, Wiestner A, Rosenwald A, et al. Overexpression of c-maf is a frequent oncogenic event in multiple myeloma that promotes proliferation and pathological interactions with bone marrow stroma. Cancer Cell. 2004;5:191–9.
69. Bergsagel PL, Kuehl WM, Zhan F, Sawyer J, Barlogie B, Shaughnessy J, Jr. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. Blood. 2005;106:296–303.
70. 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;109:3489–95.
71. Chesi M, Robbiani DF, Sebag M, et al. AID-dependent activation of a MYC transgene induces multiple myeloma in a conditional mouse model of post-germinal center malignancies. Cancer Cell. 2008;13:167–80.
72. Delmore JE, Issa GC, Lemieux ME, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell. 2011;146:904–17.
73. Schuster SR, Kortuem KM, Zhu YX, et al. Cereblon expression predicts response, progression free and overall survival after pomalidomide and dexamethasone therapy in multiple myeloma. ASH Annual Meeting Abstracts. 2012;120:194.
74. Tiedemann RE, Mao X, Shi CX, et al. Identification of kinetin riboside as a repressor of CCND1 and CCND2 with preclinical antimyeloma activity. J Clin Invest. 2008;118:1750–64.
75. Tchakarska G, Le Lan-Leguen A, Roth L, Sola B. The targeting of the sole cyclin D1 is not adequate for mantle cell lymphoma and myeloma therapies. Haematologica. 2009;94:1781–2.
76. Kumar SK, LaPlant BR, Chng WJ, et al. Phase 1/2 trial of a novel CDK inhibitor dinaciclib (SCH727965) in patients with relapsed multiple myeloma demonstrates encouraging single agent activity. ASH Annual Meeting Abstracts. 2012;120:76.

77. Tong WG, Chen R, Plunkett W, et al. Phase I and pharmacologic study of SNS-032, a potent and selective Cdk2, 7, and 9 inhibitor, in patients with advanced chronic lymphocytic leukemia and multiple myeloma. J Clin Oncol. 2010;28:3015–22.
78. Bergsagel PL, Affer M, Glebov OK, et al. ASH Annual Meeting Abstracts. Promiscuous cryptic rearrangements of the MYC locus cis-dysregulate MYC expression and are present in the majority of patients with hyperdiploid myeloma. ASH Annual Meeting Abstracts. 2012;120:724.
79. Mertz JA, Conery AR, Bryant BM, et al. Targeting MYC dependence in cancer by inhibiting BET bromodomains. Proc Natl Acad Sci U S A. 2011;108:16669–74.
80. Chang H, Sloan S, Li D, et al. The t(4;14) is associated with poor prognosis in myeloma patients undergoing autologous stem cell transplant. Br J Haematol. 2004;125:64–8.
81. Quintero-Rivera F, El-Sabbagh Badr R, Rao PN. FGFR3 amplification in the absence of IGH@-FGFR3 fusion t(4;14) in myeloma. Cancer Genet Cytogenet. 2009;195:92–3.
82. Qing J, Du X, Chen Y, et al. Antibody-based targeting of FGFR3 in bladder carcinoma and t(4;14)-positive multiple myeloma in mice. J Clin Invest. 2009;119:1216–29.
83. Trudel S, Li ZH, Wei E, et al. CHIR-258, a novel, multitargeted tyrosine kinase inhibitor for the potential treatment of t(4;14) multiple myeloma. Blood. 2005;105:2941–8.
84. Chesi M, Bergsagel PL. Many multiple myelomas: making more of the molecular mayhem. Hematology/Education Program of the American Society of Hematology American Society of Hematology Education Program. 2011;2011:344–53.
85. Andrulis M, Lehners N, Capper D, et al. Targeting the BRAF V600E mutation in multiple myeloma. Cancer Disc. 2013.
86. Sheth N, Yeung J, Chang H. p53 nuclear accumulation is associated with extramedullary progression of multiple myeloma. Leuk Res. 2009;33:1357–60.
87. Ooi MG, Hayden PJ, Kotoula V, et al. Interactions of the Hdm2/p53 and proteasome pathways may enhance the antitumor activity of bortezomib. Clin Cancer Res. 2009;15:7153–60.
88. Saha MN, Jiang H, Yang Y, Reece D, Chang H. Small molecule MIRA-1 induces p53-independent apoptosis in multiple myeloma cells through activation of the p38 MAPK signaling pathway. ASH Annual Meeting Abstracts. 2012;120:2937.
89. Lehmann S, Bykov VJ, Ali D, et al. Targeting p53 in vivo: a first-in-human study with p53-targeting compound APR-246 in refractory hematologic malignancies and prostate cancer. J Clin Oncol. 2012; 30:3633–9.
90. Tunquist BJ, Woessner RD, Walker DH. Mcl-1 stability determines mitotic cell fate of human multiple myeloma tumor cells treated with the kinesin spindle protein inhibitor ARRY-520. Mol Cancer Therapeut. 2010;9:2046–56.
91. Shah JJ, Zonder JA, Cohen A, et al. The novel KSP inhibitor ARRY-520 is active both with and without low-dose dexamethasone in patients with multiple myeloma refractory to bortezomib and lenalidomide: results from a phase 2 study. ASH Annual Meeting Abstracts. 2012;120:449.
92. Shah JJ, Weber DM, Thomas SK, et al. Phase 1 study of the novel kinesin spindle protein inhibitor ARRY-520 + carfilzomib in patients with relapsed and/or refractory multiple myeloma. ASH Annual Meeting Abstracts. 2012;120:4082.
93. Tiedemann RE, Zhu YX, Schmidt J, et al. Identification of molecular vulnerabilities in human multiple myeloma cells by RNA interference lethality screening of the druggable genome. Cancer Res. 2012; 72:757–68.
94. Schmidt J, Braggio E, Chesi M, et al. Genome wide studies in multiple myeloma identify XPO1/CRM-1 as a critical target validated using the selective inhibitor of nuclear export (SINE) KPT-276. ASH Annual Meeting Abstracts. 2012;120:573.
95. Schmidt J, Braggio E, Kortuem KM, et al. Genome-wide studies in multiple myeloma identify XPO1/CRM1 as a critical target validated using the selective nuclear export inhibitor KPT-276. Leukemia. Epub 2013 Jun 11, 10.1038/leu.2013. Available from: www.nature.com.
96. Tai Y-T, Landesman Y, Acharya C, et al. CRM1 blockade by novel inhibitors of nuclear export (SINEs) inhibits multiple myeloma cell growth, osteoclastogenesis, and myeloma-induced osteolysis. ASH Annual Meeting Abstracts. 2012;120:326.
97. Borrello I. Can we change the disease biology of multiple myeloma? Leuk Res. 2012;36Suppl 1:S3–12.
98. Rosenblatt J, Avivi I, Vasir B, et al. Blockade of PD-1 in combination with dendritic cell/myeloma fusion cell vaccination following autologous stem cell transplantation. ASH Annual Meeting Abstracts. 2012; 120:578.
99. Plesner T, Lokhorst H, Gimsing P, Nahi H, Lisby S, Richardson PG. Daratumumab, a CD38 monoclonal antibody in patients with multiple myeloma—data from a dose-escalation phase I/II study. ASH Annual Meeting Abstracts. 2012;120:73.
100. Jakubowiak AJ, Benson DM, Bensinger W, et al. Phase I trial of anti-CS1 monoclonal antibody elotuzumab in combination with bortezomib in the treatment of relapsed/refractory multiple myeloma. J Clin Oncol. 2012;30:1960–5.
101. Lonial S, Vij R, Harousseau JL, et al. Elotuzumab in combination with lenalidomide and low-dose dexamethasone in relapsed or refractory multiple myeloma. J Clin Oncol. 2012;30:1953–9.
102. Richardson PG, Jagannath S, Moreau P, et al. A phase 2 study of elotuzumab (Elo) in combination with lenalidomide and low-dose dexamethasone (Ld) in patients (pts) with relapsed/refractory multiple myeloma (R/R MM): updated results. ASH Annual Meeting Abstracts. 2012;120:202.
103. Zonder JA, Mohrbacher AF, Singhal S, et al. A phase 1, multicenter, open-label, dose escalation study of elotuzumab in patients with advanced multiple myeloma. Blood. 2012;120:552–9.
104. Brimnes MK, Vangsted AJ, Knudsen LM, et al. Increased level of both CD4+FOXP3+ regulatory T

cells and CD14+HLA-DR(-)/low myeloid-derived suppressor cells and decreased level of dendritic cells in patients with multiple myeloma. Scand J Immunol. 2010;72:540–7.
105. Gorgun GT, Whitehill G, Anderson JL, et al. Tumor-promoting immune-suppressive myeloid-derived suppressor cells in the multiple myeloma microenvironment in humans. Blood. 2013;121:2975–87.
106. Ramachandran IR, Martner A, Pisklakova A, et al. Myeloid-derived suppressor cells regulate growth of multiple myeloma by inhibiting T cells in bone marrow. J Immunol. 2013;190:3815–23.
107. Van Valckenborgh E, Schouppe E, Movahedi K, et al. Multiple myeloma induces the immunosuppressive capacity of distinct myeloid-derived suppressor cell subpopulations in the bone marrow. Leukemia. 2012;26:2424–8.
108. Negri JM, McMillin DW, Delmore J, et al. In vitro anti-myeloma activity of the Aurora kinase inhibitor VE-465. Br J Haematol. 2009;147:672–6.
109. McMillin DW, Delmore J, Negri J, et al. Microenvironmental influence on pre-clinical activity of polo-like kinase inhibition in multiple myeloma: implications for clinical translation. PloS One. 2011;6:e20226.
110. Cao S, McMillin DW, Tamayo G, Delmore J, Mitsiades CS, Clardy J. Inhibition of tumor cells interacting with stromal cells by xanthones isolated from a Costa Rican Penicillium sp. J Natural Prod. 2012;75:793–7.
111. McMillin DW, Delmore J, Negri JM, et al. Compartment-specific bioluminescence imaging platform for the high-throughput evaluation of antitumor immune function. Blood. 2012;119:e131–8.
112. McMillin DW, Mitsiades CS. High-throughput approaches to discover novel immunomodulatory agents for cancer. Oncoimmunology. 2012;1:1406–8.
113. McMillin DW, Negri JM, Mitsiades CS. The role of tumour-stromal interactions in modifying drug response: challenges and opportunities. Nature Rev Drug Disc. 2013;12:217–28.
114. Kaiser MF, Heider U, Mieth M, et al. Induction of CXCL1 in osteoblasts by myeloma cells promotes migration of osteoclast precursors. ASH Annual Meeting Abstracts. 2012;120:441.
115. Fulciniti M, Tassone P, Hideshima T, et al. Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. Blood. 2009;114:371–9.
116. Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy JD, Jr. Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. Blood. 2007;109:2106–11.
117. Edwards CM, Edwards JR, Lwin ST, et al. Increasing Wnt signaling in the bone marrow microenvironment inhibits the development of myeloma bone disease and reduces tumor burden in bone in vivo. Blood. 2008;111:2833–42.
118. Gorgun GT, Whitehill G, Anderson JL, et al. Tumor promoting immune suppressive myeloid derived suppressor cells in multiple myeloma microenvironment. Blood. 2013;121:2975–87.
119. 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.
120. Chauhan D, Singh AV, Brahmandam M, et al. Functional interaction of plasmacytoid dendritic cells with multiple myeloma cells: a therapeutic target. Cancer Cell. 2009;16:309–23.
121. Nair JR, Murray M, Koorella C, Carlson LM, Boise LH, Lee KP. Targeting the cellular and molecular components of CD28 mediated survival signaling in multiple myeloma. ASH Annual Meeting Abstracts. 2012;120:722.
122. Murray ME, Nair JR, Lee KP. CD28-mediated pro-survival signaling in multiple myeloma. ASH Annual Meeting Abstracts. 2012;120:941.
123. Koduru S, Wong E, Strowig T, et al. Dendritic cell-mediated activation-induced cytidine deaminase (AID)-dependent induction of genomic instability in human myeloma. Blood. 2012;119:2302–9.
124. Zheng Y, Yang J, Qian J, et al. PSGL-1/selectin and ICAM-1/CD18 interactions are involved in macrophage-induced drug resistance in myeloma. Leukemia. 2013;27:702–10.
125. Hebron E, Hope C, Kim J, et al. MAP3K8 kinase regulates myeloma growth by cell-autonomous and non-autonomous mechanisms involving myeloma-associated monocytes/macrophages. Br J Haematol. 2013;160:779–84.
126. Hu J, Handisides DR, Van Valckenborgh E, et al. Targeting the multiple myeloma hypoxic niche with TH-302, a hypoxia-activated prodrug. Blood. 2010;116:1524–1527.

Clinical Translation in Multiple Myeloma: From Bench to Bedside

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The outlook for patients with multiple myeloma (MM) has improved significantly with the development of new and more effective therapies, particularly the immunomodulatory agents and proteasome inhibitors. Preclinical and correlative science investigations have played a critical role in these advances, providing important insights regarding mechanisms of neoplasia, inhibition of tumor growth, and drug resistance. This review highlights the evolution of drug development in MM, the manner in which preclinical models have contributed to the process of drug discovery, and important insights gained during the current era of MM drug development.
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The outlook for patients with multiple myeloma (MM) has improved dramatically with the development of new, more effective therapeutic agents, particularly the immunomodulatory drugs and proteasome inhibitors.[1,2] This progress has been catalyzed by advances in the understanding of MM biology and the specific cellular and extracellular processes that drive proliferation of the malignant plasma cell clone. The development of increasingly sophisticated preclinical models that approximate unique characteristics of the MM tumor cell microenvironment has enhanced translation of discovery from the bench to bedside through identification of promising therapeutic targets and compounds likely to hit them. This article reviews the evolution of drug development in MM, the manner in which preclinical models have contributed to the process of drug discovery, and important insights gained during this most recent era of drug development in MM.

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EARLY STAGES OF CHEMOTHERAPEUTIC INTERVENTION IN MM

Systematic study of MM therapy began in the 1960s. By this time, MM was known to be a bone marrow malignancy involving plasma cells characterized by production of clonal immunoglobulin.[3,4] Study of nitrogen mustard in lymphoma had demonstrated the significant anti-tumor potential of alkylating agents in hematologic neoplasia, and investigations of this drug class in MM soon followed. Several groups reported on the single-agent activity of both melphalan[5–7] and cyclophosphamide[8,9] in MM. Prednisone[10,11] and pulse-dose dexamethasone[12] also were shown to have anti-MM activity in this time period.

Combination chemotherapy with an alkylating agent and corticosteroid in MM was subsequently introduced by Alexanian and colleagues, who demonstrated the superiority of melphalan and prednisone (MP) over single-agent melphalan.[13] The emergence of MP as a standard in MM treatment was an important milestone in the field, as it confirmed the ability of combination chemotherapy to induce significant, sustained responses, albeit in no more than 50% of patients treated with the regimen.

Efforts to improve on MP with the addition of other chemotherapeutic drug classes such as taxanes and anthracyclines resulted in more intensive combination regimens such as vincristine, carmustine, melphalan, cyclophosphamide, and prednisone (VBMCP)[14] and vincristine, doxorubicin, and dexamethasone (VAD).[15] While these combinations produced higher response rates than MP, they did not increase the duration of remission or overall survival (OS).[16,17]

A NEW PARADIGM IN MM DRUG DEVELOPMENT

The paradigm for treatment of MM changed in the 1990s following a series of key observations in the laboratory. On the one hand, MM was shown to be associated with increased levels of circulating angiogenic cytokines such as vascular endothelial growth factor (VEGF) and with increased bone marrow vascularization.[18,19] Meanwhile, thalidomide was shown to possesses anti-angiogenic properties[20] and anti-tumor effect in an in vivo animal model.[21] On the basis of observations from critical preclinical investigations, thalidomide was evaluated in relapsed and refractory MM, wherein it demonstrated significant activity in heavily pretreated patients.[22]

Thus, a drug with a high level of single-agent activity in MM was identified. It was subsequently shown that thalidomide exerts pleiotropic anti-tumor effects in MM, including enhancement of T-cell and NK cell activity, disruption of adhesion between MM cells and surrounding stromal cells, and induction of caspase-8–mediated apoptosis.[23] The agent proved to be a suitable therapeutic partner in combination regimens; in studies involving patients with newly diagnosed MM, thalidomide plus MP (MPT) outperformed MP alone,[24,25] while thalidomide plus dexamethasone (TD) proved to be superior to dexamethasone alone.[26]

The introduction of thalidomide proved to be a prologue to additional steps in MM drug development that have advanced the field still further. Since 2000, nine new treatments have received US Food and Drug Administration approval for MM, an unprecedented rate of drug development in MM, and indeed in the broader arena of medical oncology. A cornerstone of this progress has been the utilization of sophisticated in vitro and in vivo preclinical models that recapitulate the intra- or extramedullary tumor cell environment, and so can potentially meaningfully inform clinical studies.

PRECLINICAL MODELS IN MM

Preclinical studies consist of both in vitro and in vivo animal studies. The objectives of in vitro studies are to define the impact of target genes and/or their products (proteins) in MM cells by using molecular biological approaches (knockdown or overexpression) or small molecule inhibitors (SMIs), as well as antibodies (Abs). Primary endpoints of the studies are not only proliferation, but also drug resistance, adhesion, and migration of MM cells. Cell proliferation studies can be assessed by inhibition of multiple targets using selective SMIs. Since the bone marrow (BM) microenvironment plays a crucial role in MM pathogenesis, the effect of SMIs or knockdown/overexpression of targets also should be evaluated in the presence of cellular components of the BM microenvironment (ie, BM stromal cells, osteoclasts, dendritic cells).[27] The results from cell line studies also should be confirmed in primary tumor cells from MM patients to define clinical relevance.

For validation of SMIs or Abs in vivo, several different models have been used. First, the plasmacytoma model in which MM cells are subcutaneously injected into severe combined immunodeficiency (SCID) mice;[28] second, the diffuse model in which MM cells are injected intravenously and cells predominantly localize in mouse bone marrow[29]; third, the SCID-hu model in which a fetal human bone chip is implanted subcutaneously into SCID mice, followed by direct injection of human MM cells into the chip;[30] and fourth, the Vk*MYC transgenic model that spontaneously develops a high rate of monoclonal gammopathy, evolving to many features of MM.[31,32] In these mouse models, not only tumor growth inhibition but also inhibition of neoangiogenesis or alteration of bone remodeling can be examined.[33]

Since the majority of the preclinical studies are conducted using MM cell lines, there are several limitations. For example, cell lines proliferate with a 24- to 48-hour doubling time, whereas primary MM cells do not spontaneously proliferate. In this context, evaluating SMIs modulating cell cycle and/or mitosis in primary MM cells is challenging and in vitro results may not predict drug efficacy in clinical trials.

BORTEZOMIB—A CASE STUDY

The development of the proteasome inhibitor bortezomib highlights the manner in which preclinical models have enhanced translation of a compound with promising yet unproven activity in MM from the laboratory to the clinic. Reports that proteasome inhibition induces apoptosis in chronic lymphocytic leukemia (CLL) cells brought initial attention to the potential anti-tumor effect of this drug class.[34,35] The effect of proteasome inhibition in MM was evaluated in a series of investigations using MM cell lines and patient-derived MM cells grown on a bone marrow stromal cell (BMSC) monolayer. These studies identified critical mechanisms through which bortezomib thwarts MM tumor growth, including inhibition of nuclear factor kappa light chain–enhancer of activated B cells (NF-kB), induction of caspase-8/-9–mediated apoptosis, cleavage of DNA repair enzymes, and disruption

of IL-6–induced activation of ERK, STAT3, and AKT pathways.[36,37]

Initial clinical evidence of bortezomib's anti-MM effect came from a phase I study of the drug in 27 patients with refractory hematologic malignancies.[38] The clearest signal of activity emerged from nine patients with plasma cell dyscrasias, all of whom experienced a decrease in paraprotein concentration, including one patient with a complete response (CR). After two phase II clinical trials of bortezomib monotherapy provided further evidence of activity in MM,[39,40] a phase III study comparing bortezomib to high-dose dexamethasone confirmed the drug's efficacy in relapsed and refractory disease based on improvement in overall response rate, progression-free survival (PFS), and OS.[41]

Further advances in the development of bortezomib involved combination strategies that were again based on highly informative preclinical data. Several preclinical studies demonstrated synergy between bortezomib and conventional agents such as doxorubicin and melphalan[42,43] and with the immunodulatory agents thalidomide and lenalidomide.[44] Rigorous efforts evaluating such combinations in clinical trials have culminated in multiple phase III studies confirming the effectiveness of bortezomib-containing regimens in both relapsed and refractory as well as newly diagnosed MM. These combinations include bortezomib-dexamethasone,[45] bortezomib plus liposomal doxorubicin,[46] bortezomib-dexamethasone plus MP,[47] bortezomib plus TD,[48,49] and bortezomib-doxorubicin-dexamethasone.[50] In addition, there are compelling data from phase I/II clinical trials regarding combinations of lenalidomide-bortezomib-dexamethasone,[51,52] as well as cyclophosphamide-bortezomib-dexamethasone.[53,54]

INSIGHTS DERIVED AND FUTURE STEPS

As the rapid translation of laboratory discovery in the development of bortezomib illustrates, preclinical models play a pivotal role the arena of MM therapeutics. They provide valuable insights regarding the mechanisms of tumor growth, inhibition of tumor growth, and drug resistance. They also provide an experimental platform through which to systematically screen and identify compounds and drug combinations with greatest potential for efficacy in the clinic.

There are, of course, important limitations to these models. Perhaps most importantly, MM is biologically heterogeneous, and as such tumor models cannot fully account for behavior of the disease in a human host. Moreover, even with sophisticated animal models, drug metabolism and pharmacokinetic characteristics can only be approximated prior to human study. Likewise, drug toxicities associated with a given agent can only be fully characterized in the context of clinical trials.

In spite of these limitations, preclinical investigation will undoubtedly remain at the forefront of drug discovery in MM. The experimental models will be further refined as knowledge of MM genetics and tumor biology deepens and as such they will have even greater capacity to inform drug discovery by providing insight on agents that are most likely to prove effective in the clinic. The impact of such progress is plain to see, as patients with MM experience better outcomes as a result of more effective therapies that are informed by rigorously conducted preclinical study.[55–57]

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REFERENCES

1. Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008;111(5):2516–20.
2. Venner CP, Connors JM, Sutherland HJ, et al. Novel agents improve survival of transplant patients with multiple myeloma including those with high-risk disease defined by early relapse (<12 months). Leuk Lymphoma. 2011;52(1):34–41.
3. Wright JH. A case of multiple myeloma. J Boston Soc Med Sci. 1900;4(8):195–204.
4. Longsworth LG, Shedlovsky T, Macinnes DA. Electrophoretic patterns of normal and pathological human blood serum and plasma. J Exp Med. 1939;70(4):399–413.
5. Blokhin N, Larionov L, Perevodchikova N, Chebotareva L, Merkulova N. Clinical experiences with sarcolysin in neoplastic diseases. Ann N Y Acad Sci. 1958;68(3):1128–32.
6. Bergsagel DE, Sprague CC, Austin C, Griffith KM. Evaluation of new chemotherapeutic agents in the treatment of multiple myeloma. IV. L-Phenylalanine mustard (NSC-8806). Cancer Chemother Rep. 1962;21:87–99.
7. Hoogstraten B, Sheehe PR, Cuttner J, et al. Melphalan in multiple myeloma. Blood. 1967;30(1):74–83.
8. Korst DR, Clifford GO, Fowler WM, Louis J, Will J, Wilson HE. Multiple myeloma. II. Analysis of cyclophosphamide therapy in 165 patients. JAMA. 1964;189:758–62.
9. Tourtellotte CR, Call MK. Prolonged remission of myeloma with cyclophosphamide. Arch Intern Med. 1964;113:758–63.
10. Salmon SE, Shadduck RK, Shilling A. Intermittent high-dose prednisone (NSC-10023) therapy for multiple myeloma. Cancer Chemother Rep. 1967;51:179–87.

11. Maas RE. A comparison of the effect of prednisone and a placebo in the treartment of multiple myeloma. Cancer Chemother Rep. 1962;16:257–9.
12. Alexanian R, Dimopoulos MA, Delasalle K, Barlogie B. Primary dexamethasone treatment of multiple myeloma. Blood. 1992;80:887–90.
13. Alexanian R, Haut A, Khan AU, et al. Treatment for multiple myeloma. Combination chemotherapy with different melphalan dose regimens. JAMA. 1969;208 (9):1680–5.
14. Lee BJ, Sahakian G, Clarkson BD, Krakhoff IH. Proceedings: combination chemotherapy of multiple myeloma with alkeran, cytoxan, vincristine, prednisone, and BCNU. Cancer. 1974;1974(33):533–8.
15. Samson D, Gaminara E, Newland A, et al. Infusion of vincristine and doxorubicin with oral dexamethasone as first-line therapy for multiple myeloma. Lancet. 1989;ii:882–5.
16. Myeloma Trialists' Collaborative Group. Combination chemotherapy versus melphalan plus prednisone as treatment for multiple myeloma: an overview of 6,633 patients from 27 randomized trials. J Clin Oncol. 1998;16:3832–42.
17. Blade J, San Miguel J, Fontanillas M, et al. Increased conventional chemotherapy does not improve survival in multiple myeloma: long-term results of two PETHEMA trials including 914 patients. Hematol J. 2001;2:272–8.
18. Vacca A, Ribatti D, Roncali L, et al. Bone marrow angiogenesis and progression in multiple myeloma. Br J Haematol. 1994;87:503–8.
19. Vacca A, Di Loreto M, Ribatti D, et al. Bone marrow of patients with active multiple myeloma: angiogenesis and plasma cell adhesion molecules LFA-1, VLA-4, LAM-1, and CD44. Am J Hematol. 1995;50(1):9–14.
20. D'Amato RJ, Loughman MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci U S A. 1994;91:4082–5.
21. Verheul HM, Panigrahy D, Yuan J, D'Amato RJ. Combination oral antiangiogenic therapy with thalidomide and sulindac inhibits tumour growth in rabbits. Br J Cancer. 1999;79(1):114–8.
22. Singhal S, Mehta J, Desikan R, et al. Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med. 1999;341(21):1565–71.
23. Hideshima T, Chauhan D, Shima Y, et al. Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. Blood. 2000;96(9):2943–50.
24. Palumbo A, Bringhen S, Caravita T, et al. Oral melphalan and prednisone chemotherapy plus thalidomide compared with melphalan and prednisone alone in elderly patients with multiple myeloma: randomised controlled trial. Lancet. 2006;367:825–31.
25. Facon T, Mary JY, Hulin C, et al. Melphalan and prednisone plus thalidomide versus melphalan and prednisone alone or reduced-intensity autologous stem cell transplantation in elderly patients with multiple myeloma (IFM 99-06): a randomised trial. Lancet. 2007;370:1209–18.
26. Rajkumar SV, Blood E, Vesole D, Fonseca R, Greipp PR. Phase III clinical trial of thalidomide plus dexamethasone compared with dexamethasone alone in newly diagnosed multiple myeloma: a clinical trial coordinated by the Eastern Cooperative Oncology Group. J Clin Oncol. 2006;24:431–6.
27. Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. Nat Rev Cancer. 2007;7(8):585–98.
28. Chauhan D, Tian Z, Nicholson B, et al. A small molecule inhibitor of ubiquitin-specific protease-7 induces apoptosis in multiple myeloma cells and overcomes bortezomib resistance. Cancer Cell. 2012; 22(3):345–58.
29. Mitsiades CS, Mitsiades NS, McMullan CJ, et al. Antimyeloma activity of heat shock protein-90 inhibition. Blood. 2006;107(3):1092–100.
30. Tassone P, Neri P, Carrasco DR, et al. A clinically relevant SCID-hu in vivo model of human multiple myeloma. Blood. 2005;106(2):713–6.
31. Chesi M, Matthews GM, Garbitt VM, et al. Drug response in a genetically engineered mouse model of multiple myeloma is predictive of clinical efficacy. Blood. 2012;120(2):376–85.
32. Delmore JE, Issa GC, Lemieux ME, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell. 2011;146(6):904–17.
33. Tai YT, Landesman Y, Acharya C, et al. CRM1 inhibition induces tumor cell cytotoxicity and impairs osteoclastogenesis in multiple myeloma: molecular mechanisms and therapeutic implications. Leukemia. 2013 Apr 16 [Epub ahead of print].
34. Delic J, Masdehors P, Omura S, et al. The proteasome inhibitor lactacystin induces apoptosis and sensitizes chemo- and radioresistant human chronic lymphocytic leukaemia lymphocytes to TNF-alpha-initiated apoptosis. Br J Cancer. 1998;77(7):1103–7.
35. Chandra J, Niemer I, Gilbreath J, et al. Proteasome inhibitors induce apoptosis in glucocorticoid-resistant chronic lymphocytic leukemic lymphocytes. Blood. 1998;92(11):4220–9.
36. Hideshima T, Mitsiades C, Akiyama M, et al. Molecular mechanisms mediating antimyeloma activity of proteasome inhibitor PS-341. Blood. 2003;101:1530–4.
37. Hideshima T, Richardson P, Chauhan D, et al. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res. 2001;61(7): 3071–3076.
38. Orlowski RZ, Stinchcombe TE, Mitchell BS, et al. Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. J Clin Oncol. 2002;20(22):4420–7.
39. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med. 2003;348(26):2609–17.
40. Jagannath S, Barlogie B, Berenson J, et al. A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br J Haematol. 2004;127(2): 165–172.
41. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005;352(24): 2487–98.

42. Ha PK, Benoit NE, Yochem R, et al. A transcriptional progression model for head and neck cancer. Clin Cancer Res. 2003;9(8):3058–64.
43. Mitsiades N, Mitsiades CS, Richardson PG, et al. The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. Blood. 2003; 101(6):2377–80.
44. Mitsiades N, Mitsiades CS, Poulaki V, et al. Molecular sequelae of proteasome inhibition in human multiple myeloma cells. Proc Natl Acad Sci U S A. 2002;99(22): 14374–9.
45. 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(30):4621–9.
46. Orlowski RZ, 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(25):3892–901.
47. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008;359(9): 906–17.
48. 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(9758):2075–85.
49. 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(8):1589–96.
50. 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(24):2946–55.
51. Richardson PG, Weller E, Jagannath S, et al. Multicenter, phase I, dose-escalation trial of lenalidomide plus bortezomib for relapsed and relapsed/ refractory multiple myeloma. J Clin Oncol. 2009;27 (34):5713–9.
52. Richardson PG, Weller E, Lonial S, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood. 2010;116(5):679–86.
53. Reece DE, Rodriguez GP, Chen C, et al. Phase I-II trial of bortezomib plus oral cyclophosphamide and prednisone in relapsed and refractory multiple myeloma. J Clin Oncol. 2008;26(29):4777–83.
54. Kumar SK, Flinn I, Noga SJ, et al. Bortezomib, dexamethasone, cyclophosphamide and lenalidomide combination for newly diagnosed multiple myeloma: phase 1 results from the multicenter EVOLUTION study. Leukemia. 2010;24(7):1350–6.
55. Richardson PG, Hideshima T, Mitsiades C, Anderson KC. The emerging role of novel therapies for the treatment of relapsed myeloma. J Natl Compr Cancer Network. 2007;5(2):149–62.
56. Laubach JP, Mahindra A, Mitsiades CS, et al. The use of novel agents in the treatment of relapsed and refractory multiple myeloma. Leukemia. 2009;23(12):2222–32.
57. Lonial S, Mitsiades CS, Richardson PG. Treatment options for relapsed and refractory multiple myeloma. Clin Cancer Res. 2011;17(6):1264–77.

Myeloma: Classification and Risk Assessment

Rafael Fonseca and Jorge Monge

Multiple myeloma (MM) is a heterogeneous disease for which several new treatments are available. Much has been learned about its biology over the past 15 years. We now understand that there are various subtypes of the disease, each one associated with different outcomes and clinical pathological features. While a detailed classification of the disease into at least seven or eight major subtypes is possible, a practical clinical approach classifies the disease into high-risk and not-high-risk MM. This classification has allowed for tailored approaches to therapy and treatment planning. Furthermore, the discussion of outcomes with patients should include risk stratification, as the prospects for survival are quite different depending on whether the patient has high-risk MM or not. The tools for measuring risk subcategory are widely available and now routinely employed in the clinic. The continued search for genetic abnormalities that underlie the biology of MM may allow for even better precision therapy in the future.
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Multiple myeloma (MM) is a neoplasm that arises from the malignant proliferation of plasma cells.[1] MM always arises as a progression from the monoclonal gammopathy of undetermined significance (MGUS).[2–4] The last 15 years have witnessed heretofore unprecedented understanding of the biology of the disease. It is now clear that at least some of the genetic abnormalities that ultimately give rise to MM arise as a consequence of errors during the normal process of plasma cell development.[5] Specifically, and much like other B-cell lymphomas, the presence of chromosome translocation involving the immunoglobulin heavy chain (IgH) genes accounts for approximately half of MM cases.[6–8] The other half of MM is hyperdiploid MM, for which, despite some progress, we still lack full understanding of the genetic drivers.[6,7,9,10]

CLINICAL STAGES OF THE PLASMA CELL NEOPLASMS

There are various stages in the development of plasma cells disorders. The earliest stage that can be recognized in the clinic is MGUS.[11] However, it is quite likely that earlier stages of plasma cell proliferation exist (mini-MGUS or pre-MGUS) but that the level of proliferation is minimal and therefore not easily identifiable in the clinic. To detect MGUS, patients must have a significant concentration of a monoclonal protein such that it is identifiable (or measurable) in a protein electrophoresis or patients must have an abnormality in the serum-free light chain assay. The same genetic abnormalities that are present in MM are typically present in patients with MGUS.[12–16] Patients with MGUS, by definition, will have none of the complications associated with MM.[11] These patients also have a lower prevalence of the genetic aberrations believed to be indicators of clonal progression, such as deletions of chromosome 17.[12–16] An intermediate stage between MGUS and active MM is known as smoldering myeloma (SMM).[17] Patients with SMM also have a lower prevalence of genetic aberrations believed to be associated with disease progression. One interesting feature of patients with early-stage plasma cell tumors is that they appear to have a lower frequency of genomic instability as opposed to patients with MM (unpublished observations).

The progression of MGUS and SMM to MM is believed to result from the acquisition of secondary genetic changes, although other factors such as microenvironment changes as well as deregulation of the immune system may contribute in supporting, or allowing, this progression.[18,19] The specific changes that result in this progression are not fully identified, although some, such as mutations of *RAS*, are observed in a significantly higher proportion of MM cases than MGUS.[20–22]

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Conflicts of interest: R.F. has received a patent for the prognostication of MM based on genetic categorization of the disease. R.F. has received consulting fees from Medtronic, Otsuka, Celgene, Genzyme, BMS, Lilly, Onyx, Binding Site, Millennium and AMGEN. R.F. also has sponsored research from Cylene and Onyx. J.M. has no conflicts to disclose.

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MM has some racial association being twice as common in patients of African descent.[23–25] Very few studies have attempted to understand the biology of the disease in this population.[26–28] One study reported that African-American patients with MM have a lower frequency IgH translocation.[26] Another study reported that the prevalence of MGUS is higher in patients of African origin.[24] It is quite likely than that these differences are secondary to genetic susceptibility factors, although the precise mechanisms is not known.[26,27]

FROM CYTOGENETICS TO MOLECULAR CLASSIFICATION OF RISK ASSESSMENT

Biologically, MM can be broadly categorized into two genetic categories; hyperdiploid MM and non-hyperdiploid MM.[6–8] The latter is characterized by a high prevalence of chromosomal translocations.[6–8]

Hyperdiploid Myeloma

Hyperdiploid MM is observed by fluorescence in situ hybridization (FISH) in about 50% of the cases,[6–8] and is generally believed to be associated with a better outcome. These patient's clonal cells harbor multiple trisomies of the odd-numbered chromosomes, with the exception of chromosome 13.[6,7] Hyperdiploidy is observed even in the earliest stages of the disease, such as in MGUS,[15,29] and is therefore considered to be an initiating pathogenetic event, although the mechanisms for this remain unclear.[9,10,30] Patients with hyperdiploidy detected by FISH are found to have a better prognosis and longer survival than their counterparts.[31] This prognostic benevolence is lost in cases where hyperdiploidy is also associated with other genetic markers of progression or aggressiveness such as −17p13.[10] The prognostic impact of hyperdiploidy by karyotype analysis is unknown, since in and of itself, abnormal metaphases are an indicator of poor prognosis.[32] The classic patient with hyperdiploid MM is an elderly male, who has IgG-kappa MM and many bone lytic lesions.

Non-hyperdiploid MM

Non-hyperdiploid MM is characterized by a relative high prevalence of IgH chromosome translocations (>85%).[7,8] Most of these patients, with the exception of most of those with a t(11;14)(q13;q32) translocation, have an aggressive disease course, characterized by rapid relapse and shorter survival.[33–40] These patients also have a higher frequency of progression of genetic events such as chromosome 13 and 14 deletion, chromosome 17 abnormalities, chromosome 1q amplification, and 1p deletion.[33–39] High-risk MM is more common in patients with non-hyperdiploid MM.[33–39]

The three major subtypes of chromosome abnormalities include the t(11;14)(q13;q32), t(4;14)(p16;q32), and t(14;16)(q32;q23).

Translocation t(11;14)(q13;q32)

The t(11;14)(q13;q32) chromosomal translocation is one of the most common genetic abnormalities in MM.[35,41–43] This translocation results in increased expression of the cyclin D1 gene (*CCND1*). This chromosomal translocation has been observed in MGUS as well as in MM.[16,44] Patients with this abnormality can be subdivided into two categories: one with a rather indolent course and one associated with more aggressive features.[45] Gene expression profiling has been able to categorize individual cases into these two categories.[45] This chromosome aberration is also quite common in patients with primary plasma cell leukemia and light chain amyloidosis.[46–48] It is notable that many of these patients have a very stable genome (with fewer copy number aberrations and interstitial breakpoints), and therefore this likely explains the overall better outcome for many of them.

High-Risk Translocations

The two major translocations associated with aggressive disease are the t(4;14)(p16;q32) (observed in 15% of cases) and the t(14;16)(q32;q23) (observed in 5% of cases).[33,35] The t(4;14)(p16;q32) is associated with dysregulation of the expression of *FGFR3* and *MMSET*.[49] The t(14;16)(q32;q23) is associated with increased expression of C-maf.[50] These two translocations have been associated in most studies with a more aggressive clinical course, although their impact as prognostic factors has been diminished (although not fully eliminated) by the introduction of proteasome inhibitors.[51] Some studies have questioned the usefulness of the t(14;16)(q32;q23) as a predictor,[52] but the consensus is that it is still important as a prognostic factor.[53]

Progression Genetic Events

Chromosome 17 Deletions

Chromosome 17 deletions have been identified as crucial in the identification of patients with high-risk MM.[33,54,55] Most of them involve the short arm of chromosome 17, and almost always include the TP53.[56,57] Interestingly, mutations of TP53 have not been routinely detected in these patients, different from what is observed in chronic lymphocytic leukemia, and in fact only a small minority of them have this mutation.[58] These patients have more

aggressive variants of MM with a short time to relapse, extramedullary disease, and CNS involvement.[33,59] All primary genetic subtypes of the disease can acquire chromosome 17 deletions as part of disease progression. Patients with this abnormality have a worse prognosis, with 17p13 deletions being the single most powerful genetic marker for risk stratification.

Other Genetic Factors of Importance

Other genetic factors associated with outcome have been identified as well. Notable among these are chromosome 1 abnormalities.[60,61] Chromosome 1p deletions often coexist with chromosome 1q duplications or amplification. Both of these abnormalities have been associated with more aggressive disease. Deletions of chromosome 14 are more common in patients who have hypodiploid MM, and therefore also have been associated with a more aggressive clinical course.[56,62] The importance of MYC abnormalities as a prognostic risk factor has not been fully tested,[63,64] although one large clinical study showed no effect.[65] Hypodiploidy, usually detected by karyotype only, is also an important negative prognostic risk factor.[66–71]

High-Risk Classification Via Gene Expression Profiling

Several studies have addressed the role of gene expression profiling as a prognostic marker for MM.[10,39,45,72–75] The group from the University of Arkansas Medical Sciences has conducted the most comprehensive studies.[39,45] They have shown that utilization of RNA-based microarrays can accurately identify 15% of patients who have very aggressive disease.[29,52] This team also has used gene expression profiling for the identification of other important clinical outcomes such as responsiveness to bortezomib.[73,76–78] The test is now commercially available and can be performed by reference laboratories. The clinical implications for these results have been validated in multiple data sets. Other research teams have identified various other gene expression profiling signatures that can aid in prognostication.[10,39,45,72–75] Most of these signatures are not overlapping, and it is not clear whether any of them can perform any better in routine clinical care than the 70-gene signature developed by the University of Arkansas. Other models have explored gene expression profiling to detect high-risk MM cases, including novel genetic signatures,[75,79] array-based comparative hybridization,[80] and a centrosome-based index[74] (Table 1).

Next-Generation Sequencing, Clonal Evolution, and Tides

A thorough analysis of MM cases using next-generation sequencing (NGS) confirmed previously known genetic mutations, and discovery of new altered pathways.[62] Interestingly, these new recurrent mutations would not not normally have been sought by rational hypothesis testing, and included seemingly unrelated genes such as those involved in the coagulation cascade. Similar efforts have reported in the search of tyrosine kinase mutations.[81] Unfortunately, and despite the technological sophistication, as of yet these studies have failed to yield major new insights into the biology of the disease and further analysis is underway. However, they have paved the way for the thorough understanding of the subclonal nature of MM.

Combining high-throughput genomic tools such as NGS with single-cell analysis such as FISH, we and others have shown the existence of multiple clones within a single patient with MM.[82–84] While these clones are all related, and originate from a common ancestor, multiple branches of evolution arise. This branching diversity creates a complex adaptive system that can favor clonal selection and drug resistance.[82–84] Understanding the potential clinical implications of subclones in MM as a prognostic marker for the disease would seem to be an important avenue of research. It seems logical that patients who have a greater number of clones will have greater adaptability, and therefore potentially should be classified as high risk exclusively on the presence of this phenotype/genotype.

Genomic Instability

One question that has not been solved is why certain genetic markers are associated with more aggressive disease? Recent data suggest that the genetic markers that identify high-risk MM also correlate with patients who have greater degrees of genomic instability.[85] In fact, Chung et al have shown that genomic instability, a characteristic measured by the surrogate marker of genomic complexity, is a major prognostic factor for MM.[85] Data from our group have shown that patients with greater degrees of genomic instability will tend to have a shorter survival and more aggressive phenotypes.[85] Perhaps it is genomic instability that should be considered the most important prognostic factor for disease. Genomic instability is likely the most important determinant for clonal aggressiveness by allowing the genomic evolution of clones capable of one-drug resistance, making this permissive environment more significant than the specifics of the genetic aberrations observed.

Table 1. Gene Expression Profiling as a Prognostic Marker in Multiple Myeloma

GEP Signature	Outcome Measured	*P* Value	Outcome Measured	*P* Value
UAMS 70-gene signature[39]	EFS (HR of high *v* low risk)		OS (HR of high *v* low risk)	
Training cohort (n = 351)	4.51	<.001	5.16	<.001
Test cohort (n = 181)	3.41	.002	4.75	<.001
H-MM signature[10]	PFS (median in days)		Response to bortezomib	
Cluster 3 *v* others	253 *v* 127	.13	70% *v* 29%	.02
	Median survival (mo)			
Cluster 3 *v* cluster 1	122 *v* 27	.04		
Centrosome index (CI)[72]	OS (hazard ratio)		OS in patients with PI >2 (median in mo)	
High *v* low CI	1.95	.04	30.6 *v* 45.6	.04
	PFS (median in mo)		OS (median in mo)	
Enrolled in bortezomib trials (high *v* low CI)	2.8 *v* 4.9	.02	11.5 *v*. 20.9	.0002
IFM 15-gene signature[73]	Mean 3-yr survival		OS (hazard ratio)	
High *v* low risk	47.4% *v* 90.5%	NR	6.8	.001
Treatment response by GEP-defined risk[77]				
GEP high-risk	EFS (hazard ratio)		OS (hazard ratio)	
2003-33 trial	2.57	<.001	2.43	.001
2006-66 trial	2.77	.019	3.00	.016
EMC 92 -gene signature[79]	% of patients identified as high risk		OS (hazard ratio)	
UAMS-TT2 data set	19.4		3.4	<.001
UAMS-TT3 data set	16.2		5.23	<.001
MRC-IX data set	20.2		2.38	<.001

Abbreviations: GEP, gene expression profiling; UAMS, University of Arkansas for Medical Sciences; H-MM, Hyperdiploid Multiple Myeloma; EMC, Erasmus University Medical Center; IFM, Intergroupe Francophone du Myelome; OS, overall survival; PFS, progression-free survival.

Predictive Biomarkers, Cereblon

It is logical to expect that a number of genetic mutations will ultimately confer resistance to certain drugs used for the treatment of MM. The first such important discovery was the identification of cereblon (*CRBN*) as a key target of immunomodulatory agents.[86,87] While it is unclear whether all activity of immunomodulatory agents is mediated via *CRBN*, the lack of expression of this gene has a high degree of correlation with drug resistance to immunomodulatory agents. *CRBN* was recently described as the genetic factor for teratogenicity of immunomodulatory agents, and subsequently tested as a biomarker in MM. While more studies are needed, the marker predicts with high accuracy response and survival parameters among patients treated with immunomodulatory agents.[86,87]

PROGNOSIS AND TREATMENT OUTCOME: A MOVING TARGET

Conceptual Discussion

Many variables contribute to the development of prognostic systems for patients with MM, including disease biology itself, use of specific drugs, and sequencing of agents. The complexity is such that one might want to consider various prognostic systems for MM that include stage of the disease, medications being used, previous treatment administered, and other host factors and comorbidities. This high complexity to establish prognosis is somewhat specific to MM, and unlike that of most solid tumors since these patients will commonly be treated with all active medications at one point of their disease course or another (it is not unusual to see a patient was receiving six-line therapy for the disease). Currently available prognostic markers mostly estimate overall survival but will be limited in their ability to precisely determine which is the best sequence for use of the agents and the duration of responses to specific treatment strategies. The situation is further complicated as most MM patients are now treated with combinatorial strategies, adding complexity to the decision-making process.

Combinatorial strategies, discussed elsewhere in this issue of *Seminars*, have resulted in superior outcomes with regard to response, and early indicators suggest that deep responses (eg, complete responses) will translate to improvements in overall survival.[38,88] Another complexity is that many of these novel agents have improved the survival and outcomes for patients, irrespective of risk status.[89,90]

However, one of the most notable findings in risk prognostication research in MM is that high-risk patients fare better when they are treated with proteasome inhibitors.[51,90–93] This observation led to the first set of risk-stratified treatment recommendations suggesting that earlier introduction of proteasome inhibitors during induction is indicated for high-risk patients.[94] However, nowadays it is nearly ubiquitous that most MM patients, irrespective of risk, will be treated with proteasome inhibitors as first-line therapy. Thus, the contribution of risk stratification as a tool for drug selection for induction is limited. We recommend the utilization of risk stratification for patient counseling and post stem cell transplantation (SCT) therapy. It also has been noted that obtaining a complete response is of crucial importance for patients who have high-risk MM.[95]

One valuable use for risk stratification is patient counseling. With the advent of so many new drugs, some have called MM a "chronic disease." A patient diagnosed with high-risk MM is not one with a "chronic disease" and has a higher risk of mortality in the 2–3 years post diagnosis.[38,77] While some of the aforementioned studies suggest abrogation of traditional genetic classifiers by the introduction of proteasome inhibitors, long-term follow-up, and sufficiently powered studies, suggest that high-risk MM patients still fare worse.[89,92,93] Although the introduction of proteasome inhibitors has nevertheless improved outcomes for some high-risk MM patients,[38,90] many still have a shorter duration of response and survival.[88,91] The discussion in the scientific literature will sometimes prematurely claim that a novel combination "overcomes high-risk MM," but longer duration of follow-up usually confirms early reports as pyrrhic victories if only used during induction.[92,93] However, recent clinical data suggest that the use of proteasome inhibitors as consolidation therapy is beneficial for patients with t(4;14)(p16;q32).[38,90] It is important to recognize that at the time of diagnosis, patients with high-risk MM already have clinicopathologic features of more aggressive disease (eg, greater tumor burden, more hypercalcemia, etc) and also have a higher propensity for faster relapse.[33,36,39] Furthermore, many of them present with greater degrees of clonal evolution. Unfortunately, high-risk MM remains a challenge and a much greater threat to MM patients than standard disease.

Melphalan and Autologous Stem Cell Transplantation Following Doublets

Multiple studies have shown that genetic factors associated with high-risk disease predict outcomes in patients treated with conventional forms of chemotherapy (eg, melphalan-based therapy) and also with simple inductions (eg, vincristine, doxorubicin, and dexamethasone [VAD] or bortezomib and dexamethasone [VD]) followed by SCT.[36,37,96] These studies showed a much shorter progression-free survival and

overall survival among patients with high-risk MM treated with these agents. Recently Avet-Loiseau and colleagues showed that a short (4 months) induction with bortezomib in combination with dexamethasone improves the outcomes of MM patients with t(4;14)(p16;q32) but not for those with −17p13.[51] Older studies have shown that induction with thalidomide and dexamethasone (TD) or VAD followed by a single autologous SCT in patients with t(4;14)(p16;q32) would result in nearly ubiquitous relapse in the 12 months post SCT.[26,27,59] Simple inductions (doublets) and autologous SCT seem thus incapable of abrogating the effects of high-risk genetic markers.

Modern Induction (triplets)

The use of combinatorial strategies has resulted in a much greater proportion of patients achieving deep responses,[95] and as mentioned previously, early indicators suggest a beneficial effect on long-term outcomes including overall survival in some.[88,93] While clinical trials are addressing the role of carfilzomib as part of induction regimens,[97] most current clinical practice is based on the combination of bortezomib and dexamethasone with either cyclophosphamide (CyBORD)[98] or lenalidomide (RVD).[99] Similar combinations are being pursued with great success now replacing bortezomib with carfilzomib. One such study showed a dramatic rate of deep responses among newly diagnosed MM patients treated with carfilzomib, Lenalidomide, and dexamethasone.[97] All of these regimens are highly active and achieve deep responses in the majority of cases. Many authors still favor the use of combinations including cyclophosphamide due to lower cost, immediate availability, good tolerability, and no need to adjust doses in patients with renal dysfunction. However, both strategies (ie, adding either cyclophosphamide or lenalidomide to proteasome inhibitors and dexamethasone) are appropriate and similar. Details regarding the various options for induction are discussed elsewhere in this issue of *Seminars*.

There is one hypothetical situation where lenalidomide may be preferable over cyclophosphamide in these combinations. In patients with high-risk MM, the clonal cells are capable of greater genomic instability, and thus introduction of alkylating agents when the tumor burden is high might be deleterious by allowing clonal evolution favoring the creation of drug-resistance clones. While the rationale for this is largely hypothetical, and the fact that lenalidomide also may be genotoxic (based on the reported second primary malignancies), favoring lenalidomide in the induction of high-risk MM seems reasonable. This hypothesis remains to be proven but may be a reason to choose non–alkylator-based regimens in newly diagnosed high-risk MM.

RISK STRATIFICATION FOR CONSOLIDATION AND MAINTENANCE

Overview

Performing an autologous SCT remains one of the mainstays for treatment of eligible MM patients. Given the aforementioned discussion it seems that risk stratification will have a greater influence on patient counseling and selection of post SCT therapy. But also given the possible genotoxic effects of high-dose melphalan in tumor cells, it seems reasonable to seek maximal tumor bulk reduction in cases of high-risk MM.

An interesting conceptual development for MM treatment, based on the results of several large clinical trials, is that the duration of therapy seems to be important for optimal outcomes. This approach has been explored in the setting of maintenance post SCT,[100,101] consolidation after SCT,[38,82,83,90] and maintenance after induction in non-SCT candidates.[92,102]

While the terms "consolidation" and "maintenance" are used to describe the temporal association of various treatments, the reality is that many of them represent semantic variations for (merely) continuation of therapy. Whether there is a biologic difference between consolidation, intensification, or maintenance is unclear, and mostly reflects physicians' perception of treatment toxicity (eg, maintenance is "less toxic"). It has become increasingly clear that longer duration of therapy may be important.[103,104] A potential rationale for this might be that MM cells have a very low proliferative rate so that it is only because of prolonged exposure that opportunities to expose mitotic cells to anti-MM treatments exist.

Maintenance and Risk Stratification

Two large clinical trials have evaluated lenalidomide as maintenance after SCT, one of which showed improvements in overall survival.[100,101] The other study (Intergroupe Francophone du Myelome [IFM]) has not shown improvement in overall survival, but did find a very significant increase in progression-free survival.[100] This last study, despite randomization, had a greater proportion of high-risk MM patients among those receiving lenalidomide maintenance, which no doubt could have had a major impact on the study results (10% *v* 20%). In another maintenance study, the use of thalidomide appeared to be deleterious to patients with high-risk MM.[105] It is possible that incomplete therapeutic interventions (eg, low-dose "maintenance") in the context of high-risk MM engenders subclone selection, and therefore disease aggressiveness ensues. Overall maintenance as a strategy seems to be a laudable goal, but its

Table 2. Maintenance Therapies After Stem Cell Transplantation

Regimen	Length of Treatment	CR or VGPR (%)	PFS (mo)	3-yr OS (%)	EFS (mo)	Second Primary Cancers	Adverse Events
Lenalidomide *v* placebo[100]	Until relapse	84 *v* 76 ($P = .009$)	41 *v* 23 (HR 0.5, $P < .001$)	80 *v* 84 (HR 1.25, $P = .29$)	40 *v* 23 ($P < .001$)	3.1 *v* 1.2 per 100 patient-years ($P = .002$)	Thromboembolic events: 6% *v* 2% ($P = .01$)
Lenalidomide *v* placebo[101]	Until progression	NR	46 *v* 27 ($P < .001$)	88 *v* 80 (HR 0.62, $P = .053$)	43 *v* 27 ($P < .001$)	7.8% *v* 2.6% (NR)	Thromboembolic events: 1% *v* 0% (NR)
Thalidomide *v* no maintenance[105]	Until progression	NR	Overall: 23 *v* 15 ($P < .001$) Adverse iFISH: 9 *v* 12 ($P = .48$)	Overall: no difference (HR 0.91, 95% CI 0.72–1.17; $P = .40$) Adverse iFISH: worse OS ($P = .009$)	NR	NR	Any serious adverse reaction: 9.1% *v* 2.6% ($P = .0001$)
Thalidomide/ bortezomib *v* thalidomide *v* alfa2-IFN[94]	Up to 3 years, discontinued at disease progression	NR	78% *v* 63% *v* 49% at 2 years ($P = .01$)	No difference	NR	NR	NR

Abbreviations: CR, complete response; VGPR, very good partial response; PFS, progression-free survival; OS, overall survival; EFS, event-free survival; NR, no reported; HR, hazards ratio; CI, confidence interval; iFISH, interphase fluorescence in situ hybridization.

application in the various risk categories needs to be explored further. It is possible that selection of patients based on post SCT disease burden (none to minimal to measurable) and risk category could be used to determine who is more likely to derive benefit from maintenance (Table 2).

Post SCT Consolidation and Risk Stratification

More recent studies have addressed the role of combinatorial strategies with new agents in the post SCT setting. These studies have shown an improvement in depth of responses and various measurements of survival. Some have shown that addition of bortezomib in the setting of post SCT consolidation is beneficial for patients with high-risk disease, mainly those with t(4;14)(p16;q32).[89] One large study showed that bortezomib administration improved outcome for all cytogenetic subtypes, in particular patients with 17p13.[89] This is in contradiction to the IFM study but could very well be explained by the longer duration of therapy in the German study.[51] In another study, Cavo and colleagues showed that two cycles of consolidation after SCT with the combination of bortezomib, thalidomide, and dexamethasone improved the quality and duration of responses.[90] More importantly, they showed that for patients with the t(4;14)(p16;q32) the administration of bortezomib eliminated the prognostic significance of the marker.[90] Given the low number of cases no specific analysis could be done for 17p13. The group from the University of Arkansas has shown the benefit of adding bortezomib in the context of their Total Therapy 3 protocol[38](Table 3).

Minimal Residual Disease and Risk Stratification

A growing body of literature has shown that more precise estimates of residual disease after SCT, and similar strategies will be important to estimate outcome, and presumably later to determine the need for additional or continued therapy.[106–112] This literature is exemplified by both studies that use molecular methods and flow cytometry.[106–112] The use of flow cytometry is favored given that is appears to be as sensitive and more widely available, although it is still technically challenging.[106–110] Integrating these new assays into large clinical trials that also determine risk stratification will be key to incorporate both variables into the decision process regarding need of additional treatment.

CONSENSUS RECOMMENDATION FOR PROGNOSTIC TESTING AND CLASSIFICATION

International recommendations for testing state that patients should have some measurement of genetic variability as an integral part of proper disease management.[53] A practical and simple approach is to identify high-risk genetic subtypes using one of two methods: FISH or gene expression profiling (Table 4). If FISH is to be used, the probes should include, at a minimum, testing for the t(4;14)(p16;q32), t(14;16)(q32;q23), and −17p13.[53] This testing can be done on samples submitted to a central laboratory. The testing should be done only on samples where the plasma cells have been selected for scoring, either by magnetic beads purification or by costaining with antibodies that detect the MM cells (eg, cytoplasmic immunoglobulin-FISH).

Table 3. Post Stem Cell Transplantation Consolidation Therapies by Chromosomal Abnormalities

Regimen	Chromosomal Abnormality	PFS	*P* Value	3-yr OS (%)	*P* Value
Bortezomib *v* thalidomide[89]					
	del(17p13)	26.2 *v* 12 mo	.024	69 *v* 17	.028
	t(4;14)	25.3 *v* 21.7 mo	.12	66 *v* 44	.37
	+1q21	28.2 *v* 23.6 mo	.22	77 *v* 62	.10
	del(13q14)	27.4 *v* 25.2 mo	.27	81 *v* 61	.072
VTD *v* TD[113]					
	All patients	HR 0.63 (95% CI, 0.45–0.88)	.0061	86 *v* 84	.3
	del(13q)	HR 0.49 (95% CI, 0.31–0.79)	.0039	NR	
	t(4;14)	HR 0.51 (95% CI, 0.29–0.88)	.0174	NR	

Table 4. Recommendations for Testing and Risk Classification

High-Risk Markers	Standard Risk
FISH	
t(4;14)(p16;q32)	Hyperdiploidy
t(14;16)(q32;q23)	t(11;14)(q13;q32)
del17p13	
GEP	
UAMS 70-gene or 17-gene signature	
Other signatures	

Not doing this will greatly diminish the sensitivity of the assay, and will result in unacceptable high rates of false-negative results, particularly for deletions. Oftentimes the samples submitted for FISH analysis (third pull from an aspirate) will contain a much lower percentage of plasma cells than the first aspirate (hemodiluted) and the presence of deletions will be within the range of normal for a given probe, even if all cells have an abnormality.

An alternative is to test samples by gene expression profiling. It is important to follow the laboratory recommendations for sample collection and submission, but if done properly these samples can be submitted to a centralized commercial reference laboratory.

Testing can be repeated, but the major genetic subtypes of the disease will not change over time. Change is likely to be observed only for the high-risk genetic markers (eg, -17), signatures (high-risk GEP), as well as the acquisition of secondary genetic factors associated with progression (1p/1q aberrations). In conclusion it is important to consider risk stratification as an integral part of the management of MM patients, information that is useful for treatment planning and also for patient counseling. Few markers are able to fully discriminate outcome, and likely never will. Development of new biomarkers, predictive of resistance (such as *CRBN* deletions), and perhaps of therapeutic intervention, is needed to further advance the field of MM therapeutics.

REFERENCES

1. Rajkumar SV, Kyle RA. Multiple myeloma: diagnosis and treatment. Mayo Clin Proc. 2005;80:1371–82.
2. Rajkumar SV, Lacy MQ, Kyle RA. Monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. Blood Rev. 2007;21:255–65.
3. Landgren O, Kyle RA, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. Blood. 2009;113:5412–7.
4. Weiss BM, Abadie J, Verma P, Howard RS, Kuehl WM. A monoclonal gammopathy precedes multiple myeloma in most patients. Blood. 2009;113:5418–22.
5. Bergsagel PL, Kuehl WM, Zhan F, Sawyer J, Barlogie B, Shaughnessy J, Jr. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. Blood. 2005;106:296–303.
6. Debes-Marun C, Dewald G, Bryant S, et al. Chromosome abnormalities clustering and its implications for pathogenesis and prognosis in myeloma. Leukemia. 2003;17:427–36.
7. Smadja NV, Leroux D, Soulier J, et al. Further cytogenetic characterization of multiple myeloma confirms that 14q32 translocations are a very rare event in hyperdiploid cases. Genes Chromosomes Cancer. 2003;38:234–9.
8. Fonseca R, Debes-Marun CS, Picken EB, et al. The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma. Blood. 2003;102:2562–7.
9. Rio-Machin A, Ferreira BI, Henry T, et al. Downregulation of specific miRNAs in hyperdiploid multiple myeloma mimics the oncogenic effect of IgH translocations occurring in the non-hyperdiploid subtype. Leukemia. 2013;27:925–31.
10. Chng WJ, Kumar S, Vanwier S, et al. Molecular dissection of hyperdiploid multiple myeloma by gene expression profiling. Cancer Res. 2007;67:2982–9.
11. Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Melton LJ, 3rd. Long-term follow-up of 241 patients with monoclonal gammopathy of undetermined significance: the original Mayo Clinic series 25 years later. Mayo Clin Proc. 2004;79:859–66.
12. Ackermann J, Meidlinger P, Zojer N, et al. Absence of p53 deletions in bone marrow plasma cells of patients with monoclonal gammopathy of undedetermined significance. Br J Haematol. 1998;103:1161–3.
13. Kaufmann H, Ackermann J, Baldia C, et al. Both IGH translocations and chromosome 13q deletions are early events in monoclonal gammopathy of undetermined significance and do not evolve during transition to multiple myeloma. Leukemia. 2004;18:1879–82.
14. Konigsberg R, Ackermann J, Kaufmann H, et al. Deletions of chromosome 13q in monoclonal gammopathy of undetermined significance. Leukemia. 2000;14:1975–9.
15. Chng WJ, Van Wier SA, Ahmann GJ, et al. A validated FISH trisomy index demonstrates the hyperdiploid and nonhyperdiploid dichotomy in MGUS. Blood. 2005;106:2156–61.
16. Fonseca R, Bailey RJ, Ahmann GJ, et al. Genomic abnormalities in monoclonal gammopathy of undetermined significance. Blood. 2002;100:1417–24.
17. Kyle RA, Remstein ED, Therneau TM, et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. N Engl J Med. 2007;356:2582–90.

18. Dhodapkar KM, Barbuto S, Matthews P, et al. Dendritic cells mediate the induction of polyfunctional human IL17-producing cells (Th17-1 cells) enriched in the bone marrow of patients with myeloma. Blood. 2008;112:2878–85.
19. Dhodapkar MV. Myeloid neighborhood in myeloma: cancer's underbelly? Am J Hematol. 2009;84:395–6.
20. Rasmussen T, Kuehl M, Lodahl M, Johnsen HE, Dahl IM. Possible roles for activating RAS mutations in the MGUS to MM transition and in the intramedullary to extramedullary transition some plasma cell tumors. Blood. 2005;105:317–23.
21. Chng WJ, Gonzalez-Paz N, Price-Troska T, et al. Clinical and biological significance of RAS mutations in multiple myeloma. Leukemia. 2008;22:2280–4.
22. Billadeau D, Jelinek DF, Shah N, LeBien TW, Van Ness B. Introduction of an activated N-ras oncogene alters the growth characteristics of the interleukin 6-dependent myeloma cell line ANBL6. Cancer Res. 1995;55:3640–6.
23. Landgren O, Gridley G, Turesson I, et al. Risk of monoclonal gammopathy of undetermined significance (MGUS) and subsequent multiple myeloma among African American and white veterans in the United States. Blood. 2006;107:904–6.
24. Landgren O, Katzmann JA, Hsing AW, et al. Prevalence of monoclonal gammopathy of undetermined significance among men in Ghana. Mayo Clin Proc. 2007;82:1468–73.
25. Landgren O, Weiss BM. Patterns of monoclonal gammopathy of undetermined significance and multiple myeloma in various ethnic/racial groups: support for genetic factors in pathogenesis. Leukemia. 2009;23:1691–7.
26. Baker A, Braggio E, Jacobus S, et al. Uncovering the biology of multiple myeloma among African Americans: a comprehensive genomics approach. Blood. 2013;121:3147–52.
27. Greenberg AJ, Vachon CM, Rajkumar SV. Disparities in the prevalence, pathogenesis and progression of monoclonal gammopathy of undetermined significance and multiple myeloma between blacks and whites. Leukemia. 2012;26:609–14.
28. Weiss BM, Minter A, Abadie J, et al. Patterns of monoclonal immunoglobulins and serum free light chains are significantly different in black compared to white monoclonal gammopathy of undetermined significance (MGUS) patients. Am J Hematol. 2011; 86:475–8.
29. Brousseau M, Leleu X, Gerard J, et al. Hyperdiploidy is a common finding in monoclonal gammopathy of undetermined significance and monosomy 13 is restricted to these hyperdiploid patients. Clin Cancer Res. 2007;13:6026–31.
30. Agnelli L, Fabris S, Bicciato S, et al. Upregulation of translational machinery and distinct genetic subgroups characterise hyperdiploidy in multiple myeloma. Br J Haematol. 2007;136:565–73.
31. Trendle MC, Greipp PR, Leong T, et al. Prognostic significance of the S-phase fraction of light chain restricted cytoplasmic immunoglobulin (cIg) positive plasma cells in patients with newly diagnosed multiple myeloma (meeting abstract). Proc Annu Meet Am Soc Clin Oncol. 1996:15.
32. Dewald GW, Kyle RA, Hicks GA, Greipp PR. The clinical significance of cytogenetic studies in 100 patients with multiple myeloma, plasma cell leukemia, or amyloidosis. Blood. 1985;66:380–90.
33. Fonseca R, Blood E, Rue M, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. Blood. 2003;101:4569–75.
34. Keats JJ, Reiman T, Maxwell CA, et al. In multiple myeloma, t(4;14)(p16;q32) is an adverse prognostic factor irrespective of FGFR3 expression. Blood. 2003;101:1520–9.
35. Avet-Loiseau H, Facon T, Grosbois B, et al. Oncogenesis of multiple myeloma: 14q32 and 13q chromosomal abnormalities are not randomly distributed, but correlate with natural history, immunological features, and clinical presentation. Blood. 2002;99: 2185–91.
36. 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;109:3489–95.
37. Chang H, Sloan S, Li D, et al. The t(4;14) is associated with poor prognosis in myeloma patients undergoing autologous stem cell transplant. Br J Haematol. 2004;125:64–8.
38. Pineda-Roman M, Zangari M, van Rhee F, et al. VTD combination therapy with bortezomib-thalidomide-dexamethasone is highly effective in advanced and refractory multiple myeloma. Leukemia. 2008;22: 1419–27.
39. 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: 2276–84.
40. Avet-Loiseau H, Durie BG, Cavo M, et al. Combining fluorescent in situ hybridization data with ISS staging improves risk assessment in myeloma: an International Myeloma Working Group collaborative project. Leukemia. 2013;27:711–7.
41. Fonseca R, Harrington D, Oken MM, et al. Prognostic significance of 13q- deletions and the t(11;14)(q13; q32) in myeloma (MM); an interphase FISH Study of 351 patients entered into the Eastern Cooperative Oncology Group E9487 clinical trial. Blood. 2000;96:547a.
42. Nishida K, Taniwaki M, Misawa S, Abe T. Nonrandom rearrangement of chromosome 14 at band q32.33 in human lymphoid malignancies with mature B-cell phenotype. Cancer Res. 1989;49: 1275–81.
43. Chesi M, Bergsagel PL, Brents LA, Smith CM, Gerhard DS, Kuehl WM. Dysregulation of cyclin D1 by translocation into an IgH gamma switch region in two multiple myeloma cell lines. Blood. 1996;88: 674–81.
44. Avet-Loiseau H, Facon T, Daviet A, et al. 14q32 translocations and monosomy 13 observed in monoclonal gammopathy of undetermined significance delineate a multistep process for the oncogenesis

of multiple myeloma. Intergroupe Francophone du Myelome. Cancer Res. 1999;59:4546–50.
45. Zhan F, Huang Y, Colla S, et al. The molecular classification of multiple myeloma. Blood. 2006;108: 2020–8.
46. Tiedemann RE, Gonzalez-Paz N, Kyle RA, et al. Genetic aberrations and survival in plasma cell leukemia. Leukemia. 2008;22:1044–52.
47. Hayman SR, Bailey RJ, Jalal SM, et al. Translocations involving heavy-chain locus are possible early genetic events in patients with primary systemic amyloidosis. Blood. 2001;98:2266–8.
48. Harrison C, Mazullo H, Cheung K, et al. Chromosomal abnormalities in systemic amyloidosis. In: Proceedings of the VIII International Myeloma Workshop. 2001; Banff, Alberta, Canada; 2001:P18.
49. Chesi M, Nardini E, Brents LA, et al. Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. Nature Genet. 1997;16:260–4.
50. Chesi M, Bergsagel PL, Shonukan OO, et al. Frequent dysregulation of the c-maf proto-oncogene at 16q23 by translocation to an Ig locus in multiple myeloma. Blood. 1998;91:4457–63.
51. 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: 4630–4.
52. Avet-Loiseau H, Malard F, Campion L, et al. Translocation t(14;16) and multiple myeloma: is it really an independent prognostic factor? Blood. 2011;117: 2009–11.
53. Fonseca R, Bergsagel PL, Drach J, et al. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. Leukemia. 2009;23:2210–21.
54. 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; 112:4235–46.
55. 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;92:802–9.
56. Keats JJ, Fonseca R, Chesi M, et al. Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma. Cancer Cell. 2007;12: 131–44.
57. Carrasco DR, Tonon G, Huang Y, et al. High-resolution genomic profiles define distinct clinicopathogenetic subgroups of multiple myeloma patients. Cancer Cell. 2006;9:313–25.
58. Chng WJ, Price-Troska T, Gonzalez-Paz N, et al. Clinical significance of TP53 mutation in myeloma. Leukemia. 2007;21:582–4.
59. Chang H, Qi C, Yi QL, Reece D, Stewart AK. p53 gene deletion detected by fluorescence in situ hybridization is an adverse prognostic factor for patients with multiple myeloma following autologous stem cell transplantation. Blood. 2005;105:358–60.
60. 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;108:1724–32.
61. Fonseca R, Van Wier SA, Chng WJ, et al. Prognostic value of chromosome 1q21 gain by fluorescent in situ hybridization and increase CKS1B expression in myeloma. Leukemia. 2006;20:2034–40.
62. Chapman MA, Lawrence MS, Keats JJ, et al. Initial genome sequencing and analysis of multiple myeloma. Nature. 2011;471:467–72.
63. Chng WJ, Huang GF, Chung TH, et al. Clinical and biological implications of MYC activation: a common difference between MGUS and newly diagnosed multiple myeloma. Leukemia. 2011;25:1026–35.
64. Gabrea A, Martelli ML, Qi Y, et al. Secondary genomic rearrangements involving immunoglobulin or MYC loci show similar prevalences in hyperdiploid and nonhyperdiploid myeloma tumors. Genes Chromosomes Cancer. 2008;47:573–90.
65. Avet-Loiseau H, Gerson F, Magrangeas F, Minvielle S, Harousseau JL, Bataille R. Rearrangements of the c-myc oncogene are present in 15% of primary human multiple myeloma tumors. Blood. 2001;98:3082–6.
66. Smadja NV, Bastard C, Brigaudeau C, Leroux D, Fruchart C. Hypodiploidy is a major prognostic factor in multiple myeloma. Blood. 2001;98: 2229–38.
67. Barlogie B, Alexanian R, Dixon D, Smith L, Smallwood L, Delasalle K. Prognostic implications of tumor cell DNA and RNA content in multiple myeloma. Blood. 1985;66:338–41.
68. Calasanz MJ, Cigudosa JC, Odero MD, et al. Hypodiploidy and 22q11 rearrangements at diagnosis are associated with poor prognosis in patients with multiple myeloma. Br J Haematol. 1997;98: 418–25.
69. Fassas AB, Spencer T, Sawyer J, et al. Both hypodiploidy and deletion of chromosome 13 independently confer poor prognosis in multiple myeloma. Br J Haematol. 2002;118:1041–7.
70. Morgan RJ, Jr., Gonchoroff NJ, Katzmann JA, Witzig TE, Kyle RA, Greipp PR. Detection of hypodiploidy using multi-parameter flow cytometric analysis: a prognostic indicator in multiple myeloma. Am J Hematol. 1989;30:195–200.
71. Van Wier S, Braggio E, Baker A, et al. Hypodiploid multiple myeloma is characterized by more aggressive molecular markers than non-hyperdiploid multiple myeloma. Haematologica. 2013 Epub ahead of print.
72. Chng WJ, Braggio E, Mulligan G, et al. The centrosome index is a powerful prognostic marker in myeloma and identifies a cohort of patients that might benefit from aurora kinase inhibition. Blood. 2008;111:1603–9.

73. 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;26:4798–805.
74. Anguiano A, Tuchman SA, Acharya C, et al. Gene expression profiles of tumor biology provide a novel approach to prognosis and may guide the selection of therapeutic targets in multiple myeloma. J Clin Oncol. 2009;27:4197–203.
75. Broyl A, Hose D, Lokhorst H, et al. Gene expression profiling for molecular classification of multiple myeloma in newly diagnosed patients. Blood. 2010;116:2543–53.
76. Mulligan G, Mitsiades C, Bryant B, et al. Gene expression profiling and correlation with outcome in clinical trials of the proteasome inhibitor bortezomib. Blood. 2007;109:3177–88.
77. Nair B, van Rhee F, Shaughnessy JD, Jr., et al. Superior results of Total Therapy 3 (2003-33) in gene expression profiling-defined low-risk multiple myeloma confirmed in subsequent trial. 2006-66 with VRD maintenance. Blood. 2010;115: 4168–73.
78. Shi J, Tricot GJ, Garg TK, et al. Bortezomib down-regulates the cell-surface expression of HLA class I and enhances natural killer cell-mediated lysis of myeloma. Blood. 2008;111:1309–17.
79. Kuiper R, Broyl A, de Knegt Y, et al. A gene expression signature for high-risk multiple myeloma. Leukemia. 2012;26:2406–13.
80. Avet-Loiseau H, Li C, Magrangeas F, et al. Prognostic significance of copy-number alterations in multiple myeloma. J Clin Oncol. 2009;27:4585–90.
81. Hucthagowder V, Meyer R, Mullins C, et al. Resequencing analysis of the candidate tyrosine kinase and RAS pathway gene families in multiple myeloma. Cancer Genet. 2012;205:474–8.
82. Keats JJ, Chesi M, Egan JB, et al. Clonal competition with alternating dominance in multiple myeloma. Blood. 2012;120:1067–76.
83. Walker BA, Wardell CP, Melchor L, et al. Intraclonal heterogeneity and distinct molecular mechanisms characterize the development of t(4;14) and t(11;14) myeloma. Blood. 2012;120:1077–86.
84. Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. Nat Rev Cancer. 2012;12:335–48.
85. Chung T-H, Mulligan G, Fonseca R, Chng W-J. A novel measure of chromosome instability can account for prognostic difference in multiple myeloma. PLoS One. 2013;8:e66361.
86. Broyl A, Kuiper R, van Duin M, et al. High cereblon expression is associated with better survival in patients with newly diagnosed multiple myeloma treated with thalidomide maintenance. Blood. 2013;121:624–7.
87. Egan JB, Kortuem KM, Kurdoglu A, et al. Extramedullary myeloma whole genome sequencing reveals novel mutations in Cereblon, proteasome subunit G2 and the glucocorticoid receptor in multi drug resistant disease. Br J Haematol. 2013;161:748–51.
88. 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: 2946–55.
89. Neben K, Lokhorst HM, Jauch A, et al. Administration of bortezomib before and after autologous stem cell transplantation improves outcome in multiple myeloma patients with deletion 17p. Blood. 2012;119:940–8.
90. Cavo M, Pantani L, Petrucci MT, et al. Bortezomib-thalidomide-dexamethasone is superior to thalidomide-dexamethasone as consolidation therapy after autologous hematopoietic stem cell transplantation in patients with newly diagnosed multiple myeloma. Blood. 2012;120:9–19.
91. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008;359:906–17.
92. Mateos MV, Oriol A, Martinez-Lopez J, et al. Maintenance therapy with bortezomib plus thalidomide or bortezomib plus prednisone in elderly multiple myeloma patients included in the GEM2005MAS65 trial. Blood. 2012;120:2581–8.
93. 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;120:1589–96.
94. Stewart AK, Bergsagel PL, Greipp PR, et al. A practical guide to defining high-risk myeloma for clinical trials, patient counseling and choice of therapy. Leukemia. 2007;21:529–34.
95. Haessler J, Shaughnessy JD, Jr., Zhan F, et al. Benefit of complete response in multiple myeloma limited to high-risk subgroup identified by gene expression profiling. Clin Cancer Res. 2007;13:7073–9.
96. 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;106:2837–40.
97. 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;120:1801–9.
98. 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;23:1337–41.
99. Richardson PG, Weller E, Lonial S, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood. 2010;116:679–86.
100. Attal M, Lauwers-Cances V, Marit G, et al. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366: 1782–91.

101. McCarthy PL, Owzar K, Hofmeister CC, et al. Lenalidomide after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366:1770–81.
102. Palumbo A, Sezer O, Kyle R, et al. International Myeloma Working Group guidelines for the management of multiple myeloma patients ineligible for standard high-dose chemotherapy with autologous stem cell transplantation. Leukemia. 2009;23: 1716–30.
103. Dimopoulos MA, Delforge M, Hajek R, et al. Lenalidomide, melphalan, and prednisone, followed by lenalidomide maintenance, improves health-related quality of life in newly diagnosed multiple myeloma patients aged 65 years or older: results of a randomized phase III trial. Haematologica. 2013;98:784–8.
104. Falco P, Cavallo F, Larocca A, et al. Lenalidomide-prednisone induction followed by lenalidomide-melphalan-prednisone consolidation and lenalidomide-prednisone maintenance in newly diagnosed elderly unfit myeloma patients. Leukemia. 2013;27:695–701.
105. Morgan GJ, Gregory WM, Davies FE, et al. The role of maintenance thalidomide therapy in multiple myeloma: MRC Myeloma IX results and meta-analysis. Blood. 2012;119:7–15.
106. 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;112:4017–23.
107. Paiva B, Vidriales MB, Perez JJ, et al. Multiparameter flow cytometry quantification of bone marrow plasma cells at diagnosis provides more prognostic information than morphological assessment in myeloma patients. Haematologica. 2009;94:1599–602.
108. Paiva B, Vidriales MB, Mateo G, et al. The persistence of immunophenotypically normal residual bone marrow plasma cells at diagnosis identifies a good prognostic subgroup of symptomatic multiple myeloma patients. Blood. 2009;114:4369–72.
109. Paiva B, Gutierrez NC, Rosinol L, et al. High-risk cytogenetics and persistent minimal residual disease by multiparameter flow cytometry predict unsustained complete response after autologous stem cell transplantation in multiple myeloma. Blood. 2012;119:687–91.
110. Paiva B, Vidriales MB, Rosinol L, et al. A multiparameter flow cytometry immunophenotypic algorithm for the identification of newly-diagnosed symptomatic myeloma with an MGUS-like signature and long-term disease control. Leukemia. 2013 [Epub ahead of print].
111. Ladetto M, Pagliano G, Ferrero S, et al. Correlation between clinical outcome and disease kinetics by quantitative PCR in myeloma patients following post-transplant consolidation with bortezomib, thalidomide and dexamethasone. Blood (ASH Annual Meeting Abstracts). 2009;114:960.
112. Ladetto M, Pagliano G, Ferrero S, et al. Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma. J Clin Oncol. 2010;28:2077–84.
113. 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;376:2075–85.

Individualized Therapy in Multiple Myeloma: Are We There?

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Multiple myeloma (MM), a heterogeneous plasma cell dyscrasia with a variety of clinical presentations and outcomes, is undergoing a treatment renaissance. While new drug classes have been discovered, a subset of high-risk MM remains relatively refractory to treatment. Current risk stratifications models, such as Durie-Salmon and the International Staging System, estimate disease burden and prognosis. Cytogenetics and gene expression profiles can help further identify more aggressive disease. Additionally, molecular and immunophenotypic assessment of minimal residual disease (MRD) and different imaging studies can identify patients at higher risk for relapse. It is now an opportune time to develop algorithms to combine all of the currently available clinical and genomic information to begin to inform specific therapeutic intervention in individual patients or at least smaller subgroups with similarly behaving disease. Semin Oncol 40:567-576 © 2013 Published by Elsevier Inc.

Multiple myeloma (MM) has significant heterogeneity in its disease course. Almost one quarter of patients die within 2 year but 10% can live almost 15 years. With development of new classes of agents, the 5-year overall survival (OS) has improved from 31.1% in 1999 to 42.2% today.[1] However, the 1-year survival has improved only modestly, increasing from 74.2% to 76.5%. This lack of improvement can be explained best by the intrinsic behavior of the myeloma clone, as well as comorbidities affecting the host. The incidence of MM increases with age and the median age of diagnosis in the United States is 69 years.[1] Thus, patients can have significant comorbidities that require reduction in the dosage of therapeutic agents or simply do not tolerate side effects. In addition, frail patients are not appropriate for high-dose therapy, such as autologous stem cell transplantation (ASCT). Lastly, some patients elect less intensive therapies for social or personal reasons.

The myeloma clone also may have intrinsic properties that can make it more resistant to treatment. Cytogenetics and gene expression profiling (GEP) are improving our recognition and understanding of these properties. Recent evaluation for minimal residual disease (MRD) after aggressive treatment is playing an increasing prognostic role in the management of MM. Current trends are showing survival benefits of multiple drug cocktails, ASCTs, and maintenance chemotherapy, all at the risk of increasing toxicity. The ultimate goal in management of MM is to prolong OS while minimizing toxicity from the disease and its treatment. In this article, we will discuss current clinical, biochemical, and genomic correlates of disease behavior, as well as the emerging role of novel markers of response to identify not only patient subgroups but also individual patient characteristics to select specific therapy to provide further improvement in long-term outcome and to establish a pathway for the future individualized therapy.

EXISTING RISK STRATIFICATION MODELS: STRENGTHS AND WEAKNESSES

The Durie-Salmon Staging System[2] (DSS) and the International Staging System[3] (ISS) are the two most commonly used risk stratification models. The DSS system stratifies the tumor mass of myeloma by

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assessing serum hemoglobin, calcium, creatinine, paraprotein level, urinary light chain excretion, and the number of lytic lesions on skeletal survey. Evaluation of lytic lesions is highly subjective, resulting in the interoperator variability. Moreover, other variables, specifically serum creatinine and hemoglobin, can be affected by underlying non-myeloma comorbidities, rather than myeloma tumor burden. Furthermore, positron emission tomography/computed tomography (PET/CT) and magnetic resonance imaging (MRI) are now being used more frequently and could provide a better estimate of the tumor burden than a skeletal survey. Lastly, DSS is less predictive of outcome since novel agents have better efficacy in reducing tumor burden.[3,4] Thus, it is not as reproducible as ISS and not used as frequently.

The ISS, designed from a statistical analysis of survival data, found two independent variables that correlated with survival: serum β_2-microglobulin and albumin. The ease of use and uniform distribution of stages has allowed wide use of the ISS.[5,6] However, ISS has number of shortcomings as well. For example, with ASCT, as well as with use of novel agents, the predictive value between ISS stage I and II diminishes.[6,7] Second, β_2-microglobulin besides higher tumor burden can be elevated with diminished renal function conveying higher risk. Prior to routine use of newer drugs, renal failure conveyed poor survival outcome. With the use of novel agents and ability to achieve higher depth of responses, improvement in renal function is frequently observed. For example, bortezomib, used alone or in combination in patients starting hemodialysis, improved hematologic response rates to 75% and long-term dialysis was spared in 15%.[8] A recent series with newly diagnosed MM showed an improvement in renal function in bortezomib-based, thalidomide-based, and lenalidomide-based therapy in 77%, 55%, and 43% of patients with renal insufficiency.[9] Thus, it remains to be seen if ISS stage III remains prognostic if renal function is improved.

The variables used in ISS and DSS system are clinical correlates of the disease, rather than markers of the biology of myeloma. Neither system assesses parameters such as proliferation or genetics. Thus, newer stratification to individualized therapy focuses on cytogenetics and GEP. Cytogenetic analysis is widely available, but only approximately 30% of clones have abnormal karyotypes.[10,11] Compared to other hematologic malignancies, cytogenetic analysis in MM is complicated by low proliferative activity and difficulty obtaining plasma cells on a bone marrow aspirate. Use of fluorescence in situ hybridization (FISH) thus has become widely acceptable as it can detect chromosomal changes in interphase cells and can be informative in almost all patients with MM.[12,13] More recent techniques such as high-density comparative genomic hybridization (aCGH) and single-nucleotide polymorphism (SNP) arrays have confirmed genomic aberrations in all myeloma patients.[14,15]

Deletion of 17p (del(17p)), affecting only 11% of MM,[16] is the most clinically relevant cytogenetic abnormality. The gene coding for *p53*, a key tumor-suppressor gene that regulates cell cycle arrest after DNA damage, is located on the short arm of chromosome 17. This abnormality is associated with a particularly poor prognosis and a multivariate analysis showed a hazard ratio (HR) of 3.29 and 3.93 for event-free survival (EFS) and OS, respectively. Since chemotherapy commonly cause DNA damage, the absence of *p53* could explain chemotherapy resistance and the shorter progression-free survival (PFS). *p53* mutations tend to accumulate with relapse[17] and are present in 20%–50% of primary plasma cell leukemia.[18] A recent report has suggested that this risk of poor outcome is especially significant if 60% of cells demonstrate del(17p) abnormality. Patients with this abnormality may require individualization of therapy as discussed below.

Two translocations involving the immunoglobulin heavy chain gene rearrangement (IGH) on chromosome 14 have poor prognostic significance in MM: t(4;14) and t(14;16). Early studies with standard-dose chemotherapy showed an almost 50% reduction in median survival in the presence of t(4;14).[16,19] Even with aggressive treatment with ASCT, t(4;14) was associated with poor outcomes.[20] However, bortezomib-based therapy and Total Therapy 3 (TT3) seem to at least partially overcome its negative prognostic effects.[21,22] The recent Intergroupe Francophone du Myélome (IFM) analysis suggested that t(4;14) more strongly impacts outcomes in patients with high, rather than low, β_2-microglobulin, suggesting other additional factors play a significant role.[16] The t(14;16) and t(14;20) are relatively rare cytogenetic abnormalities that are linked to poor outcomes, although this has not been explored clinically in great depth.

Deletion of chromosome 13 (del(13)) is a common chromosomal abnormality in MM. This likely occurs early in the disease course, since del(13) is seen both in monoclonal gammopathy of unknown significance (MGUS) and MM. Early studies have shown decreased hematologic response rates (RR), EFS, and OS compared to normal cytogenetics.[23] Cytogenetic analysis, compared to FISH, detected fewer patients (14% *v* 51%, respectively) but was associated with higher relapse rates at 3 years (61% *v* 38%, $P = .02$) and death at 3 years (43% *v* 35%, $P = .1$).[24] The largest series of more than 1,000 patients found that while del(13) did negatively impact survival, it lost its prognostic value in the absence of del(17p) and/or

t(4;14).[16] Therefore, the International Myeloma Working Group (IMWG) recommends routine FISH analysis for 17p13, t(4;14), and t(14;16), especially in design of clinical trials, but not for del13.

CGH, SNP, and GEP have now allowed identification of prognostically important groups based on perturbation of specific genes. Two groups have developed a 15-gene model[25] and 70-gene models[26] based on GEP of CD138-purified MM cells and differentiated patients into high- and low-risk groups to accurately predict survival. The high-risk group accounted for 13%–25% of patients and accurately predicted poor survival despite treatment. These assays were developed in patients who were treated with two ASCTs. Subsequent recent studies have continued to demonstrate prognostic value of GEP-based signatures in predicting outcome even with use of novel agent–based therapies and thereby in selection of therapeutic interventions. It is interesting to note that despite their prognostic relevance there is not a single common gene between the 70-gene and 15-gene signatures, suggesting both the redundancy in the biological system and also need for further improvement in definitions of prognostic groups. The 15-gene GEP assay focuses on genes affecting proliferation and chromosomal abnormalities, whereas the 70-gene GEP has almost one third either over- or under-expressed genes on chromosome 1. A potential shortcoming is that these GEP identify only the high risk MM and do not further stratify low or intermediate risk clones. A third recent 92 gene-based GEP signature described by the HOVON group is able to predict reduced overall survival number of other datasets with a HR of 3.40 for the Total Therapy 2 (TT2) study, 5.23 for the TT3 study, 2.38 for the Medical Research Council (MRC)-IX study and 3.01 for the Assessment of Proteosome Inhibition for Extending Remission (APEX) study ($P < .0001$ in all studies). Each of these signatures identifies limited number of patients at high risk where one could investigate more intensive intervention, including more aggressive maintenance therapy. As shown in Table 1, two other high-throughput genomic methods have been applied to identify risk. A genome-wide analysis using high-density SNP arrays found three independent lesions—amp(1q23.3), amp(5q31.3), and del(12p13.31)—that were predictive of outcome following a multivariate analysis.[15] After adjusting for established prognostic factors, amp(5q31.3) was found to be a favorable marker and del(12p13.31) and amp (1q23.3) unfavorable ones. Along with β_2-microglobulin, these markers successfully stratified the 5-year survival and this model was independently validated.[15]

EMERGING ROLE OF NOVEL MARKERS OF RESPONSE

Although pretherapeutic factors influence the algorithm to individualize the therapy, with the use of combination novel agent–based regimens, where responses can be observed in more than 90% of the patients and complete responses in over 30%–40% patients, post-therapy response status is also becoming an important marker to prognosticate and to inform further individualized therapeutic decisions. For example, patients not achieving at least a partial remission after three-drug combination regimen require more specific modification in their therapy to obtain greater cytoreduction, and also more intense consolidation and maintenance therapy to sustain the response.[27] Lower expression of Cereblon, a ubiquitin ligase, is reported to suggest

Table 1. Genomic Studies to Identify Patients With High-Risk MM

Study	Type	No. of Genes/ Loci	% High Risk	Survival: Low *v* High Risk
Carrasco et al, 2006[14]	aCGH	-	-	EFS improved, OS - NS
Shaughnessy et al, 2007[26]	GEP	70/17	13%	2-year OS: 91% *v* 54%
Deceaux et al, 2008[25]	GEP	15	25%	3-year OS: 91% *v* 47%
Avet-Loiseau et al, 2009[15]	SNP	3 loci	30%	5-year OS 78% *v* median of 33.7 mo
Dickens et al, 2010[64]	GEP	97/6	25%	48 *v* 32 mo
Walker et al, 2010[65]	SNP/ FISH	12	NR	Significant impact on survival
Shaughnessy et al, 2011[66]	GEP	80	9-11%	2-year OS 92% s 60%
Kuiper et al, 2012[67]	GEP	92	22%	Median 39.8 mo *v* NR

Abbreviations: MM, multiple myeloma; aCGH, high-density comparative genomic hybridization; GEP, gene expression profiling; SNP, single-nucleotide polymorphism; FISH, fluorescence in situ hybridization; EFS, event-free survival; NR, not reached; OS, overall survival; NS, not significant.

resistance to therapy with immunomodulatory drugs.[28,29] However, larger studies looking at Cereblon protein expression may be necessary to confirm this finding and to apply it to clinical practice. Resistance to bortezomib has been evaluated using number of techniques, including high-throughput RNAi screen.[30] The genes identified include the proteasome subunits PSMA5, PSMB2, PSMB3, and PSMB7, as well as Aurora kinase A, CDK5, and modulators of aggresome pathway. With the emerging data that depth of response has a clear relation with survival outcome, this algorithm will be now extended to see whether a maximal response has been obtained and whether further individualized therapy is needed to attain MRD. This also has led to redefinition of responses and development of methods to estimate true MRD.

Methods are being evaluated to improve our ability to detect MRD. These methods include incorporation of serum free light chain (FLC) in the complete remission (CR) definition, immunophenotypic methods (iCR), and molecular methods (mCR). In 2006, the IMWG incorporated serum FLC as part of its response criteria in MM.[31] sCR, defined as normalization of serum FLC and absence of residual clonal cell in the bone marrow by immunohistochemistry or immunofluorescence, can predict an eventual response in 85%, correlates with survival outcomes, and has a sensitivity of 1 clonal cell in 10^2–10^3 cells.[32] iCR can have sensitivity of 1 clonal cell in 10^4 cells with seven-color flow cytometry and correlates with improved time to progression (TTP), PFS, and OS.[33] mCR, evaluated using polymerase chain reaction (PCR) with allele-specific oligonucleotides (ASOs) of the IgH region, has sensitivity of detecting 1 clonal cell in $10^{5–6}$ cells.[34] MCR can be achieved in both allogeneic stem cell transplantation and ASCT in 27%–69%[35–38] and 15%–21% of patients, respectively.[39,40] While PFS is prolonged in mCR, the data are still conflicting for OS.[39,41] iCR and mCR remain investigational techniques due to technical proficiency required to perform these tests, but will likely have major role in the future in determining post-transplant individualized therapy.[42]

PET and MRI imaging have been explored as markers of response. Two studies have shown that a high standardized uptake value (SUV) prior to treatment correlated with shorter PFS.[43,44] The Arkansas Group reported their experience using PET/CT during TT3.[45] After induction chemotherapy, a PET/CT CR, defined as no focal lesions or extramedullary disease, correlated with improved OS (HR 0.33, $P = .001$) and EFS (HR 0.47, $P = .013$), when compared to less than CR. A retrospective series evaluated the role of PET/CT in hematologic non-CR after treatment with ASCT or allogeneic stem cell transplantation.[46] The sensitivity for detecting a lesion in a hematologic very good partial response (VGPR) was only 34.1%, but was as high as 63.6% in stable disease, both of which are of lower sensitivity than in pretreatment PET/CTs. Twenty percent of detected lesions were extramedullary and likely would have been missed by a skeletal survey. The maximum SUV did not correlate with any prognostic markers. Thus, PET imaging may have a prognostic role after induction chemotherapy and can improve detection of focal or extramedullary lesions in patients with a less than VGPR. Similarly, survival benefits of resolution of MRI-identified lesions have been described.[47] An important question now is, do we further intervene and individualize therapy based on residual disease based on imaging?

SYNTHESIZING FACTORS TO INDIVIDUALIZE THERAPY

In selecting the most appropriate treatment for a patient, a number of clinical, biochemical, and genomic correlates are considered (Table 2). Clinical factors, including age and the frailty of the patient, may preclude specific therapy such as high-dose therapy. The goal of treatment of elderly patients remains maximal response and this can be typically achieved not just with a melphalan-prednisone–based regimen but also with novel agent combinations.[48] Typically, dose adjustments and alternate schedules or routes of administration can be considered. Comorbidities, such as cardiomyopathy and neuropathy, limit the dose, respectively, of corticosteroids and bortezomib. Disease-related variables, such as renal failure, bone disease, and anemia, help determine initial cytoreductive therapies, as well as supportive care treatment. Routinely used biochemical variables, including β_2-microglobulin and lactate dehydrogenase, are markers of tumor volume or growth and provide prognostic information in newly diagnosed MM. As discussed above, cytogenetics, including FISH, remain relevant for prognosis and treatment. del(13q) and t(4;14) have decreasing roles with novel agents, but del(17p) remains a marker of poor prognosis and warrants aggressive treatment with three-drug combinations, ASCT, and consideration of allogeneic stem cell transplant early on in the treatment.[49]

As of yet no clear algorithm based on peer-reviewed evidence is available. Treatment algorithms published so far are largely based on expert opinion and analysis of a vast amount of data. For example, Mayo Clinic's Stratification of Myeloma and Risk-Adapted Therapy (mSMART) provides guidelines for patient stratification into high-, intermediate-, and standard-risk groups based on cytogenetics, FISH, and GEP[50]; however, selection of therapy for

Table 2. Factors To Be Considered for Individualization

Factor	Identified Group With Therapeutic Impact	Impact
Clinical		
Age	Older age	Modify dose and schedule
Comorbidities Frailty	Frail patient	Precludes high-dose therapy
Cardiac function	Presence of cardiac dysfunction including cardiomyopathy	Caution with corticosteroids
Neurology	Neuropathy	Caution with bortezomib
Myeloma-related renal failure	Renal failure: creatinine $>$ 2.0 mg/dL	Prefer bortezomib-based regimen
Predominant bone disease	Presence and extent of bone disease	Early use of bisphosphonates
Immunoparesis	Suppressed uninvolved immunoglobulin/frequent infections	Consider prophylactic antibiotics or intravenous immunoglobulin
Plasma cell leukemia	Aggressive high-risk disease	3-drug induction regimen, ASCT and 2-drug maintenance
Biochemical		
ISS/ β_2-microglobulin	High-risk stage III	3-drug induction regimen, ASCT and 2-drug maintenance
LDH	High LDH with aggressive high-risk disease	3-drug induction regimen, ASCT and 2-drug maintenance
MRD	MRD negative patient	Improved outcome/survival
Genomics		
Cytogenetics/FISH		
del(17p)	Aggressive high-risk disease	3-drug induction regimen, ASCT and 2-drug maintenance
del(13q)	Loses prognostic value in absence of del(17p) and/or t(4;14)	No specific intervention recommended
t(4;14)	High-risk disease	Bortezomib-based 3-drug regimen
t(14;16), t(14;20)	Aggressive high-risk disease	Bortezomib-based 3-drug regimen
GEP	High risk group using 70-, 15-, or 92-gene signature	3-drug induction regimen, ASCT and 2-drug maintenance
SNP profile	Can identify a high-risk group but data not independently validated	Not available commercially and no specific intervention recommended based on SNP profile
aCGH profile	Limited data and not validated	Not available commercially and no specific intervention recommended based on aCGH profile
Genome/exome sequencing	limited data; high potential for future identification of targets to individualize therapy	Not available commercially and no specific intervention recommended based on mutational profile

Abbreviations: ASCT, autologous stem cell transplantation; ISS, International Staging System; LDH, lactate dehydrogenase; MRD, minimal residual disease; FISH, fluorescence in situ hybridization; GEP, gene expressin profiling; SNP, single-nucleotide polymorphism; aCGH, high-density comparative genomic hybridization; GEP, gene expression profiling.

these groups is not yet based on patients' specific molecular or genomic characteristics. The National Comprehensive Cancer Network (NCCN) guidelines are intentionally broad and include most available treatment regimens; Palumbo et al published recommended dose reductions based on age and comorbidities, which supplements shortcomings of other guidelines.[51]

The individualization of therapy does not end with initial induction and consolidation but has to be incorporated into therapy of relapsed patients as well. Besides the prognostic factors described above, which determine the intervention as well as outcome, upon relapse additional variables are incorporated into treatment decision. The depth and duration of the initial response provides valuable

information. For example, in patients with a long remission post-ASCT re-transplantation can be considered. In patients refractory to treatment or those with an aggressive relapse, an appropriate alternate therapy to fit a patient's responsiveness or resistance to previous therapy will need to be considered to individualize therapy. Moreover, those patients who have initially indolent disease by genomic analysis could acquire new genomic change that may portend poor outcome, requiring a change in the therapeutic approach

HOW TO MOVE FORWARD IN INDIVIDUALIZING THERAPY?

The current molecular methods identify a subset of patients, 15%–20%, with high-risk disease and shorter (<2 year) survival. A number of methods are available to identify this risk group. Patients should be evaluated for these risk features at the time of diagnosis. Those who are not categorized as high risk should have their risk status rechecked at the time of relapse. The patients classified as high-risk have poor-risk despite aggressive intervention, including high-dose theapy and ASCT. However, that does not mean that such intervention should not be utilized. Although the benefit from ASCT in these high-risk patients is not same as in standard-risk patients, it still provides improvement in both PFS and OS. In all trials except TT3 and possibly bortezomib-based therapy surrounding ASCT,[52,53] del(17p) correlates with the worst prognosis. Even with aggressive treatment with two ASCT, the median survival in patients with del(17p) was only 22 months.[16] GEP shows promise in determining which patients may benefit from aggressive consolidation therapy. Patients with high-risk disease based on 70- and 92-gene GEP had shorter survivals.[25,26] In the 70-gene model developed by the Arkansas group, patients in the high-risk group have 2-year OS and EFS of 50% and 54%, respectively.[26] In the 15-gene model developed by the IFM group, patients with high-risk disease have shorter survival compared to those with low-risk disease (HR 6.06, $P < .001$).[25] Importantly, it appears that achieving MRD-negative status in higher risk MM can provide additional information about relapse risk.[54] Patients with mCR or iCR are likely to have a durable remission, whereas those with positive MRD and poor cytogenetics likely will have short PFS, suggesting the need for further intervention.[55] In one study in patients with poor-risk cytogenetics and positive MRD at 100 days, the median PFS was 6 months and OS was 21 months.[56] Lastly, patients who relapse early from hematologic CR, defined as relapse within 1 year of ASCT, have poor outcomes with a median OS of 39 months.[56]

In summary, there is an agreement that high-risk disease should be treated aggressively with multiagent induction chemotherapy, high-dose melphalan/ASCT, further consolidation, and then maintenance chemotherapy, although evidence from randomized trials is lacking. High-risk patients with del(17p) seem to be most sensitive to a bortezomib-based treatments and ASCT (Table 3). In the HOVON-65/GMMG-HD4 trial,[53] of the 37 patients with del(17p), those randomized to the bortezomib-based arm had longer median PFS (26.2 *v* 12 months, $P = .024$) and 3-year survival (69% *v* 17%, $P = .028$). In the bortezomib arm, del(17p) was not an independent predictor of PFS and OS by multivariate analysis, suggesting an aggressive bortezomib- and stem cell transplant–based therapy may compensate, at least in part, for del(17p) MM. Lenalidomide and

Table 3. Role of Novel Agents in Individualizing Therapy

Novel Agents/ Combinations	Clinical Impact
Bortezomib-based	Improves OS in t(4;14); in 3-drug combination, may improve OS in del (17p)
Lenalidomide-based	Improves outcome del(13q) and t(4;14); in combination with bortezomib, may improve outcome in del(17p)
Pomalidomide-based and carfilzomib-based	Genomic subgroups remain to be evaluated
Alkylating agent-based therapy[18]	Significant efficacy in high proliferative disease.
ASCT	High-risk cytogenetics predict unsustained CR (HR 17.3)
Allogeneic SCT	Auto-allo tandem SCT may positively impact outcome in del(13), del(17p) and t(4;14)

Abbreviations: OS, overall survival; ASCT, autologous stem cell transplantation; SCT stem cell transplantation; CR, complete response; HR, hazards ratio.

Table 4. Common Genomic Abnormalities and Specific Agents to Individualize Therapy

Cytogenetic Abnormality	Target	Potential Drug/Drug Classes
t(4;14)	*FGFR3*	FGFR inhibitor: Dovitinib, AB1010, MGFR18775
	MMSET	Histone methyltransferase inhibitors; MEK inhibitor: selumetinib
t(11;14)	*Cyclin D1*	CDK inhibitor: seleciclib; dinaciclib HDAC inhibitors
t(14;16)	*MAF*	MEK inhibitor: selumetinib
Mutations	*B-RAF*	BRAF inhibitor: vemurafenib
	RAS	Farnesyl transferase inhibitor: tipifarnib MEK inhibitor: selumetinib p38/MAPK inhibitor: SCIO-469

thalidomide do not reduce the deleterious effect in del (17p) MM, and thus should be used in combination.[57,58] The success of this study and TT3 in del (17p) stresses the importance and success of frequent, aggressive, multi-agent therapy, incorporating ASCT.

Plasma cell leukemia (PCL), defined as the presence of >2 x 10^9/L peripheral blood plasma cells or plasmacytosis of $>20\%$ of circulating leukocytes, has the worst prognosis of plasma cell dyscrasias. Primary PCL (pPCL), which can develop independent of multiple myeloma, has a 6-month median survival but this can be increased to 1.8 years with aggressive treatment using a combination of agents.[59] Although both are plasma cell dyscrasias, pPCL has a different molecular profile from MM.[60] Unfavorable prognostic factors including high-risk cytogenetics and GEP, as well as elevated β_2-microglobulin, are almost always observed.[18] Proteosome inhibitors and immunomodulatory agnets have been studied as initial induction therapies for PCL. Lenalidomide (in combination with dexamethasone) is the only novel agent to be studied prospectively in pPCL. It resulted in a VGPR in 34.7% and an overall response rate of 60.8%.[61] In this study, all seven transplanted patients are still in remission. Several retrospective studies have shown the benefit of bortezomib-based combination chemotherapy regimen. The largest trial reported an overall response rate of 79% and $>$VGPR of 38%.[62]

FUTURE DIRECTION OF PERSONALIZED THERAPY

In the past as well as the current era, personalized therapy for MM routinely occurs when chemotherapy is tailored to a patient's comorbidities. For example, the dose of chemotherapy is reduced in the frail, bortezomib is used with caution in patients with underlying neuropathies, and lenalidomide is reduced in renal failure.[51] Prognostic implications of cytogenetics have been known for a while, but poor-risk cytogenetics may start affecting treatment decisions. Bortezomib seems to overcome the increased risk associated with t(4;14). TT3 and the HOVON-65/GMMG-HD4 regimen may prolong survival in patients with del(17p). As seen in Table 4, a number of cytogenetic changes have been correlated with expression of specific genes that can now be targeted for specific effects. For example, patients with t(4:14) clearly have upregulation of the *FGFR3* and *MMSET* genes. New classes of drugs that specifically inhibit function of these genes are under investigation. Demonstration of the efficacy of such therapies will usher in the era where drug selection can be made truly on the basis of expressed gene products. However, due to heterogeneity in tumor clones, it may still be necessary to continue to include those agents that have broader activity. Further advancements in genomic technology are also required to detect precise abnormalities that change transcriptome and its eventual biological effects. Moreover, bone marrow stromal cell interactions protect the myeloma clone and may require more complex studies for effective individualization. Genomics could be used to assess the interaction between these two and provide targets for treatment.[63] Future directions should focus on integrative oncogenetic profiles and more sensitive assessment of agents with specific targeting of gene effect in the context of cellular milieu as well as patient's clinical status.

REFERENCES

1. SEER Cancer Statistics Review. 2012. Accessed 10/31/2012, http://seer.cancer.gov/csr/1975_2009_pops09/.
2. Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. Cancer. 1975;36:842–54.
3. Greipp PR, San Miguel J, Durie BG, et al. International staging system for multiple myeloma. J Clin Oncol. 2005;23:3412–20.

4. Barlogie B, Jagannath S, Desikan KR, et al. Total therapy with tandem transplants for newly diagnosed multiple myeloma. Blood. 1999;93:55–65.
5. Hungria VT, Maiolino A, Martinez G, et al. Confirmation of the utility of the International Staging System and identification of a unique pattern of disease in Brazilian patients with multiple myeloma. Haematologica. 2008;93:791–2.
6. Hari PN, Zhang MJ, Roy V, et al. Is the International Staging System superior to the Durie-Salmon staging system? A comparison in multiple myeloma patients undergoing autologous transplant. Leukemia. 2009;23: 1528–34.
7. Kyle RA, Rajkumar SV. Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. Leukemia. 2009;23:3–9.
8. Chanan-Khan AA, Kaufman JL, Mehta J, et al. Activity and safety of bortezomib in multiple myeloma patients with advanced renal failure: a multicenter retrospective study. Blood. 2007;109:2604–6.
9. Dimopoulos MA, Roussou M, Gkotzamanidou M, et al. The role of novel agents on the reversibility of renal impairment in newly diagnosed symptomatic patients with multiple myeloma. Leukemia. 2013;27:423–9.
10. Sawyer JR, Waldron JA, Jagannath S, Barlogie B. Cytogenetic findings in 200 patients with multiple myeloma. Cancer Genet Cytogenet. 1995;82:41–9.
11. Calasanz MJ, Cigudosa JC, Odero MD, et al. Cytogenetic analysis of 280 patients with multiple myeloma and related disorders: primary breakpoints and clinical correlations. Genes Chromosomes Cancer. 1997;18:84–93.
12. Flactif M, Zandecki M, Lai JL, et al. Interphase fluorescence in situ hybridization (FISH) as a powerful tool for the detection of aneuploidy in multiple myeloma. Leukemia. 1995;9:2109–14.
13. Drach J, Schuster J, Nowotny H, et al. Multiple myeloma: high incidence of chromosomal aneuploidy as detected by interphase fluorescence in situ hybridization. Cancer Res. 1995;55:3854–9.
14. Carrasco DR, Tonon G, Huang Y, et al. High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. Cancer Cell. 2006;9:313–25.
15. Avet-Loiseau H, Li C, Magrangeas F, et al. Prognostic significance of copy-number alterations in multiple myeloma. J Clin Oncol. 2009;27:4585–90.
16. 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;109:3489–95.
17. Hawkins JM, Moorman AV, Hoffbrand AV, et al. Association of 17p loss with late-stage or refractory disease in hematologic malignancy. Cancer Genet Cytogenet. 1994;77:134–43.
18. van de Donk NW, Lokhorst HM, Anderson KC, Richardson PG. How I treat plasma cell leukemia. Blood. 2012;120:2376–89.
19. Fonseca R, Blood E, Rue M, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. Blood. 2003;101:4569–75.
20. Chang H, Sloan S, Li D, et al. The t(4;14) is associated with poor prognosis in myeloma patients undergoing autologous stem cell transplant. Br J Haematol. 2004;125:64–8.
21. 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;28:4630–4.
22. Barlogie B, Anaissie E, van Rhee F, et al. Incorporating bortezomib into upfront treatment for multiple myeloma: early results of total therapy 3. Br J Haematol. 2007;138:176–85.
23. Tricot G, Barlogie B, Jagannath S, et al. Poor prognosis in multiple myeloma is associated only with partial or complete deletions of chromosome 13 or abnormalities involving 11q and not with other karyotype abnormalities. Blood. 1995;86:4250–6.
24. Shaughnessy J, Jr., Tian E, Sawyer J, et al. Prognostic impact of cytogenetic and interphase fluorescence in situ hybridization-defined chromosome 13 deletion in multiple myeloma: early results of total therapy II. Br J Haematol. 2003;120:44–52.
25. 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;26:4798–805.
26. 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;109:2276–84.
27. Cavo M, Pantani L, Petrucci MT, et al. Bortezomib-thalidomide-dexamethasone is superior to thalidomide-dexamethasone as consolidation therapy after autologous hematopoietic stem cell transplantation in patients with newly diagnosed multiple myeloma. Blood. 2012;120:9–19.
28. Zhu YX, Braggio E, Shi CX, et al. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. Blood. 2011;118:4771–9.
29. Broyl A, Kuiper R, van Duin M, et al. High cereblon expression is associated with better survival in patients with newly diagnosed multiple myeloma treated with thalidomide maintenance. Blood. 2013;121:624–7.
30. Zhu YX, Tiedemann R, Shi CX, et al. RNAi screen of the druggable genome identifies modulators of proteasome inhibitor sensitivity in myeloma including CDK5. Blood. 2011;117:3847–57.
31. Durie BG, Harousseau JL, Miguel JS, et al. International uniform response criteria for multiple myeloma. Leukemia. 2006;20:1467–73.
32. Dispenzieri A, Zhang L, Katzmann JA, et al. Appraisal of immunoglobulin free light chain as a marker of response. Blood. 2008;111:4908–15.
33. Rawstron AC, Orfao A, Beksac M, et al. Report of the European Myeloma Network on multiparametric flow cytometry in multiple myeloma and related disorders. Haematologica. 2008;93:431–8.
34. Hart AJ, Jagasia MH, Kim AS, Mosse CA, Savani BN, Kassim A. Minimal residual disease in myeloma: are we there yet? Biol Blood Marrow Transplant. 2012;12:1790–9.

35. Martinelli G, Terragna C, Zamagni E, et al. Molecular remission after allogeneic or autologous transplantation of hematopoietic stem cells for multiple myeloma. J Clin Oncol. 2000;18:2273–81.
36. Corradini P, Cavo M, Lokhorst H, et al. Molecular remission after myeloablative allogeneic stem cell transplantation predicts a better relapse-free survival in patients with multiple myeloma. Blood. 2003;102:1927–9.
37. Raab MS, Cremer FW, Breitkreutz IN, et al. Molecular monitoring of tumour load kinetics predicts disease progression after non-myeloablative allogeneic stem cell transplantation in multiple myeloma. Ann Oncol. 2005;16:611–7.
38. Cavo M, Terragna C, Martinelli G, et al. Molecular monitoring of minimal residual disease in patients in long-term complete remission after allogeneic stem cell transplantation for multiple myeloma. Blood. 2000;96:355–7.
39. Korthals M, Sehnke N, Kronenwett R, et al. The level of minimal residual disease in the bone marrow of patients with multiple myeloma before high-dose therapy and autologous blood stem cell transplantation is an independent predictive parameter. Biol Blood Marrow Transplant. 2012;18:423–31.
40. Ladetto M, Pagliano G, Ferrero S, et al. Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma. J Clin Oncol. 2010;28:2077–84.
41. Ladetto M, Ferrero S, Drandi D, et al. Long-term results of the GIMEMA VRD Consolidation Trial in autografted multple myeloma patients (VEL-03-096): impact of minimal residual disease detection by real time quantitative PCR on late recurrence and overall survival. In: 53rd ASH Annual Meeting Exposition. San Diego, CA. Blood. 2011.
42. Avet-Loiseau H. Ultra high-risk myeloma. Hematology Am Soc Hematol Educ Program. 2010;2010:489–93.
43. Dimitrakopoulou-Strauss A, Hoffmann M, Bergner R, Uppenkamp M, Haberkorn U, Strauss LG. Prediction of progression-free survival in patients with multiple myeloma following anthracycline-based chemotherapy based on dynamic FDG-PET. Clin Nucl Med. 2009;34:576–84.
44. 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;118:5989–95.
45. 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;114:2068–76.
46. Derlin T, Weber C, Habermann CR, et al. 18F-FDG PET/CT for detection and localization of residual or recurrent disease in patients with multiple myeloma after stem cell transplantation. Eur J Nucl Med Mol Imaging. 2012;39:493–500.
47. Schmidt GP, Reiser MF, Baur-Melnyk A. Whole-body MRI for the staging and follow-up of patients with metastasis. Eur J Radiol. 2009;70:393–400.
48. Chanan-Khan AA, Lonial S, Weber D, et al. Lenalidomide in combination with dexamethasone improves survival and time-to-progression in patients >/=65 years old with relapsed or refractory multiple myeloma. Int J Hematol. 2012;96:254–62.
49. Kroger N, Badbaran A, Zabelina T, et al. Impact of high-risk cytogenetics and achievement of molecular remission on long-term freedom from disease after autologous-allogeneic tandem transplantation in patients with multiple myeloma. Biol Blood Marrow Transplant. 2013;19:398–404.
50. Mikhael JR, Dingli D, Roy V, et al. Management of newly diagnosed symptomatic multiple myeloma: updated Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) consensus guidelines. 2013. Mayo Clin Proc. 2013;88:360–76.
51. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med. 2011;364:1046–60.
52. Shaughnessy JD, Zhou Y, Haessler J, et al. TP53 deletion is not an adverse feature in multiple myeloma treated with total therapy 3. Br J Haematol. 2009;147:347–51.
53. Neben K, Lokhorst HM, Jauch A, et al. Administration of bortezomib before and after autologous stem cell transplantation improves outcome in multiple myeloma patients with deletion 17p. Blood. 2012;119:940–8.
54. 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;112:4017–23.
55. Paiva B, Martinez-Lopez J, Vidriales MB, et al. Comparison of immunofixation, serum free light chain, and immunophenotyping for response evaluation and prognostication in multiple myeloma. J Clin Oncol. 2011;29:1627–33.
56. Paiva B, Gutierrez NC, Rosinol L, et al. High-risk cytogenetics and persistent minimal residual disease by multiparameter flow cytometry predict unsustained complete response after autologous stem cell transplantation in multiple myeloma. Blood. 2012;119:687–91.
57. van de Donk NW, Gorgun G, Groen RW, et al. Lenalidomide for the treatment of relapsed and refractory multiple myeloma. Cancer Manag Res. 2012;4:253–68.
58. Boyd KD, Ross FM, Tapper WJ, et al. The clinical impact and molecular biology of del(17p) in multiple myeloma treated with conventional or thalidomide-based therapy. Genes Chromosomes Cancer. 2011;50:765–74.
59. Usmani SZ, Nair B, Qu P, et al. Primary plasma cell leukemia: clinical and laboratory presentation, gene-expression profiling and clinical outcome with Total Therapy protocols. Leukemia. 2012;26:2398–405.
60. Usmani SZ, Nair B, Qu P, et al. Primary plasma cell leukemia: clinical and laboratory presentation, gene-expression profiling and clinical outcome with Total Therapy protocols. Leukemia. 2012;26:2398–405.
61. Musto P, D'Auria F, Petrucci MT, et al. Final results of a phase II study evaluating lenalidomide in combination with low dose dexamethasone as first line therapy for primary plasma cell leukemia [abstract]. Blood (ASH Annual Meeting Abstract). 2011;118:2925.
62. D'Arena G, Valentini CG, Pietrantuono G, et al. Frontline chemotherapy with bortezomib-containing combinations improves response rate and survival in primary plasma cell

leukemia: a retrospective study from GIMEMA Multiple Myeloma Working Party. Ann Oncol. 2012;23:1499–502.
63. Munshi NC, Avet-Loiseau H. Genomics in multiple myeloma. Clin Cancer Res. 2011;17:1234–42.
64. Dickens NJ, Walker BA, Leone PE, et al. Homozygous deletion mapping in myeloma samples identifies genes and an expression signature relevant to pathogenesis and outcome. Clin Cancer Res. 2010;16:1856–64.
65. Walker BA, Leone PE, Chiecchio L, et al. A compendium of myeloma-associated chromosomal copy number abnormalities and their prognostic value. Blood. 2010;116:e56–65.
66. Shaughnessy JD, Jr., Qu P, Usmani S, et al. Pharmacogenomics of bortezomib test-dosing identifies hyperexpression of proteasome genes, especially PSMD4, as novel high-risk feature in myeloma treated with Total Therapy 3. Blood. 2011;118:3512–24.
67. Kuiper R, Broyl A, de Knegt Y, et al. A gene expression signature for high-risk multiple myeloma. Leukemia. 2012;26:2406–13.

Initial Treatment of Nontransplant Patients With Multiple MyelomaC. Cerrato and A. Palumbo

Chiara Cerrato and Antonio Palumbo

During the last two decades, many steps forward have been made in the treatment of multiple myeloma (MM) thanks to the introduction of the novel agents thalidomide, lenalidomide, and bortezomib. Despite this, MM remains an incurable disease. Elderly patients (≥65 years) represent the majority of subjects. Differently from younger (<65 years) and fit patients, elderly patients are usually not eligible for transplantation. Gentler approaches with novel agents plus conventional chemotherapy with melphalan-prednisone are commonly adopted in this setting. Data show that a sequential approach including induction followed by consolidation/maintenance therapy is an optimal strategy to improve patient outcome. In addition, second-generation novel agents are currently under investigation and may represent valuable alternative treatment options in the future.
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Multiple myeloma (MM) is an incurable plasma cell disorder that accounts for approximately 10% of all hematologic cancers and 1% of all cancers; the median age at diagnosis is 70 years.[1]

MM usually evolves from an asymptomatic stage defined as monoclonal gammopathy of undetermined significance (MGUS).[2,3] Treatment should be started in symptomatic myeloma only, which is characterized by the presence of organ damage (CRAB features): hypercalcemia, renal failure, anemia, and bone disease.[4,5]

The choice of the treatment should be based on scientific evidence and patients' characteristics such as age or comorbidities.

The tests recommended for the diagnosis of myeloma are routine and include complete blood cell count, chemistry panel, serum protein electrophoresis on acetate strip to estimate the M band by densitometer, immunofixation, a technique for the identification of proteins after separation by either conventional electrophoresis or isoelectric focusing, quantification of immunoglobulin, 24-hour urine collection for proteinuria, urinalysis using electrophoresis or immunofixation, and, finally, measurement of both urine M-component and albumin levels.[6] Additional tests to evaluate bone marrow plasma cell infiltration include aspirate plus trephine biopsy with testing for cytogenetics, fluorescent in situ hybridization (FISH), and immunophenotyping. Bone survey, including spine, pelvis, skull, humeri, and femurs, is requested. β_2-microglobulin, C-reactive protein, and lactate dehydrogenase also may be assessed, while free–light chain (FLC) ratio in serum may be evaluated when conventional M-component quantification is negative or equivocal.[7] The final diagnosis of myeloma requires 10% or more clonal plasma cells infiltration on bone marrow examination and CRAB features.[8]

Prognostic factors play a crucial role in MM. The International Staging System (ISS) defines three risk groups; stage I with median survival of 62 months (serum β_2-microglobulin <3.5 mg/L and serum albumin ≥35 g/L), stage II with median survival of 44 months (serum β_2-microglobulin >3.5 mg/L and serum albumin <35 g/L or serum β_2-microglobulin 3.5–5.5 mg/L), and stage III with median survival of 29 months (serum β_2-microglobulin ≥5.5 mg/L).[9] Serum FLC ratio incorporated into the ISS can improve the risk stratification.[10,11] To detect chromosomal abnormalities cytogenetics and FISH can be used. The presence of deletion 17p13 or t(4;14) or t(14;16) or chromosome 1 abnormalities is associated with a poor prognosis.[12,13]

In Europe, patients younger than 65 years of age are commonly considered eligible for autologous stem

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cell transplantation (ASCT), whereas patients older than 65 years or with comorbidities are usually not considered ASCT candidates, as they are more susceptible to side effects that may cause treatment interruption. Therefore, gentler approaches seem to be more appropriate. However, since biologic age can be different from chronologic age, this age cutoff can no longer be considered the only criterion, and it is necessary to evaluate comorbidities to determine whether a patient is a good candidate for transplantation.[14] In addition, elderly patients may be classified as very fit, with an excellent performance status (Karnofsky score >80%)[15] and no comorbidities (Charlson score 0)[16]; fit, with a good performance status (Karnofsky score 60%–80%) and very limited comorbidities (Charlson ≤2); or unfit, with a poor performance status (Karnofsky score <60%) and relevant comorbidities (Charlson >2). Based on this classification, different therapeutic approaches may be adopted.

EVIDENCE-BASED SELECTION OF INITIAL TREATMENT

For more than 40 years, the combination of oral melphalan and prednisone (MP) was the conventional therapy for elderly patients.[17] The introduction of novel agents such as the first in-class proteasome inhibitor bortezomib and the immunomodulatory drugs (IMIDs) thalidomide and lenalidomide has improved the efficacy of MM treatment.[18–20] The use of novel agents in fact increased response rates, as recently defined,[21] including the complete response (CR) rate, which was shown to be associated with improved survival.[22]

Here follows a description of the main novel agent–based regimens that can be adopted for the treatment of elderly patients with MM.

Thalidomide-Based Therapies

Five randomized studies have compared the combination of MP plus thalidomide (MPT) with the conventional combination MP.[23–27] The three-drug combination is now considered one of the standards of care for elderly patients as outcome was superior in the MPT group; the partial response (PR) rates were 42%–76% versus 28%–48% with MPT and MP, respectively, and progression-free survival (PFS) times were 14–28 versus 10–19 months. An overall survival (OS) advantage was observed only in the French studies (Table 1).[23,24]

The main adverse event (AE) associated with MPT was grade 3–4 neutropenia (16%–48%), and it was mainly related to melphalan (Table 2). In the five MPT trials, the rate of thrombocytopenia was 3%–14%, peripheral neuropathy 6%–23%, and venous thromboembolism (VTE) 3%–12%. When administering MPT, antithrombotic prophylaxis is recommended. MPT is now considered a standard of care in this setting; it is commonly used for the treatment of fit patients and also unfit ones, with appropriate dose reductions (Table 3).

Thalidomide was also assessed in association with an alternative alkylating agent, cyclophosphamide, and a corticosteroid, dexamethasone. The Medical Research Council (MRC) compared the combination of thalidomide-cyclophosphamide-dexamethasone attenuated (CTDa) with the standard MP; the overall response rate was significantly higher with CTDa than MP (63.8% *v* 32.6%), primarily because of increases in the rate of CRs (13.1% *v* 2.4%) and very good partial responses (VGPRs; 16.9% *v* 1.7%). PFS and OS were similar between groups.

CTDa was associated with higher rates of thromboembolic events, constipation, infection, and neuropathy than MP.[28] Overall, more patients died in the MP group (62%) compared with the CTDa group (57%) mainly due to disease progression and myeloma-related infections. Based on the higher response rates, CTDa can be considered an alternative to standard MP. CTDa was particularly advantageous in terms of PFS and OS for patients with favorable interphase FISH.[28]

Bortezomib Based-Therapies

A randomized trial of 682 patients comparing bortezomib plus MP (VMP) with the former standard MP demonstrated a statistically significant improvement in PR rate (71% *v* 35%) and in all other efficacy endpoints, such as CR rate (30% *v* 4%), median time to progression (TTP; 24 months *v* 17 months), and 3-year OS (69% *v* 54%).

The incidence of nonhematologic AEs was higher with VMP than with MP. The rate of grades 3–4 peripheral sensory neuropathy was 14% with VMP versus 0% with MP. Gastrointestinal complications were more frequent in the VMP group (19% *v* 5%). Treatment-related deaths were similar in both groups (2%). The frequency of herpes zoster infection was higher in the VMP group; therefore, acyclovir prophylaxis is always recommended.[29,30]

Today, VMP is considered a new standard of care for elderly patients together with MPT. A randomized trial compared VMP combination with the regimen bortezomib-thalidomide-prednisone (VTP). There were no significant differences in 2-year TTP, PFS, or OS, but the incidence of cardiac toxicity was higher in patients treated with VTP (8.5% *v* 0%). In the VTP arm, a significantly higher rate of patients discontinued treatment (17% *v* 8%, $P = .03$). In the VMP arm, patients had a higher rate of hematologic AEs, particularly neutropenia (37% *v* 21%) and thrombocytopenia (22% *v* 12%). VMP was considerably better tolerated, with no difference in survival.

Table 1. Efficacy of Novel Agent–Containing Induction Regimens

Combination	No. of Patients	Schedule	At Least PR	CR	PFS/EFS/TTP	OS	References
MPT	125	M: 0.25 mg/kg, d 1–4 P: 2 mg/kg, d 1–4 T: 400 mg/d for twelve 6-week cycles	76%	13%	50% at 28 mo	50% at 52 mo	23
MPT	113	M: 0.25 mg/kg, d 1–4 P: 2 mg/kg, d 1–4 T: 100 mg/d for twelve 6-week cycles	62%	7%	50% at 24 mo	50% at 44 mo	24
MPT	182	M: 0.25 mg/kg, d 1–4 P: 100 mg/d, d 1–4 For 6-week cycles until plateau T: 400 mg/d until plateau, reduced to 200 mg/d until progression	57%	13%	50% at 15 mo	50% at 29 mo	25
MPT	165	M: 0.25 mg/kg P: 1 mg/kg, d 1–5 T: 200 mg/d for 8 4-week cycles, followed by T: 50 mg/d until relapse	66%	23%[†]	67% at 24 mo	29% at 24 mo	26
MPT	167	M: 4 mg/m^2, d 1–7 P: 40 mg/m^2 d 1–7 for six 4-week cycles T: 100 mg/d until relapse	76%	15%	50% at 22 mo	50% at 45 mo	27
CTD	426		64%	13%	50% at 13 mo	50% at 33,2 mo	28
VMP	344	M: 9 mg/m^2, d 1–4 P: 60 mg/m^2, d 1–4 V: 1.3 mg/m^2, d 1,4,8,11,22,25,29,32 for first four 6-week cycles; d 1, 8, 15, 22 for subsequent five 6-week cycles	71%	30%	50% at 22 mo	41% at 36 mo	29–30
VMP	130	M: 9 mg/m^2, d 1–4 P: 60 mg/m^2, d 1–4 V: 1.3 mg/m^2 twice weekly (d 1, 4, 8, 11, 22, 25, 29, and 32) for one 6-week cycle, followed by once weekly (d 1, 8, 15, and 22) for five 5-week cycles	89%	20%	50% at 34 mo	74% at 36 mo	31
VMPT–VT	254	M: 9 mg/m^2, d 1–4 P: 60 mg/m^2, d 1–4 V: 1.3 mg/m^2, d 1, 8, 15, 22 T: 50 mg, d 1–42 for nine 5-week cycles *Maintenance* V: 1.3 mg/m^2 every 15 d T: 50 mg/d	89%	38%	56% at 36 mo	89% at 36 mo	32
Rd	222	R: 25 mg, d 1–21 d: 40 mg, d 1, 8, 15, 22 for a 4-week cycle	68%	4%	50% at 25 mo	87% at 24 mo	36
MPR	152	M: 0.18 mg/kg, d 1–4 P: 2 mg/kg, d 1–4 R: 10 mg, d 1–21 for nine 4-week cycles Maintenance: R: 10 mg, d 1–21	77%	16%	55% at 24 mo	92% at 12 mo	39

[†] CR plus VGPR.

[*]Disease-free survival.

Abbreviations: PR, partial response; CR, complete response; PFS, progression-free survival; EFS, event-free survival; TTP, time to progression; OS, overall survival; TD, thalidomide-dexamethasone; MPT, melphalan-prednisone-thalidomide; VMP, bortezomib-melphalan-prednisone; VTP, bortezomib-thalidomide-prednisone; MPR, melphalan-prednisone-lenalidomide; VMPT-VT, bortezomib-melphalan-prednisone-thalidomide followed by bortezomib-thalidomide maintenance; Rd, lenalidomide plus low-dose dexamethasone; CTD, cyclophosphamide-thalidomide-dexamethasone; NA, not available; mo, months.

Table 2. Safety (grades 3–4 adverse events) of Novel Agent–Containing Induction Regimens

Regimen	N	Neutropenia	Thrombocytopenia	Peripheral neuropathy	VTE	References
MPT	129	16%	3%	8%	9%	23
MPT	125	48%	14%	6%	12%	24
MPT	113	23%	NA	20%	6%	25
MPT	165	NA	NA	23%	3%	26
MPT	182	25%	8%	6%	8%	27
CTD	427	NA	NA	7%	16%	28
VMP	344	40%	38%	13%	1%	29,30
VMP	130	39%	27%	7%	1%	31
VMPT	254	38%	22%	12%	5%	32
Rd	220	20%	5%	2%	12%	36
MPR	152	70%	37%	0%	3%	39

Abbreviations: TD, thalidomide-dexamethasone; MPT, melphalan-prednisone-thalidomide; VMP, bortezomib-melphalan-prednisone; VTP, bortezomib-thalidomide-prednisone; MPR, melphalan-prednisone-lenalidomide; VMPT-VT, bortezomib-melphalan-prednisone-thalidomidefollowed by bortezomib-thalidomidemaintenance; Rd, lenalidomide plus low-dose dexamethasone; CTD, cyclophosphamide-thalidomide-dexamethasone; NA, notavailable.

Therefore, it confirmed its role as a standard of care in MM patients ineligible for the ASCT.[31]

A phase III trial compared a four-drug combination including MP plus bortezomib and thalidomide (VMPT) followed by maintenance with bortezomib-thalidomide (VT) with the new standard VMP.[32] In the VMPT-VT arm, VGPR and CR rates were higher (VGPR: 55% *v* 45%; CR: 39% *v* 21%). After a median follow-up of 17.8 months, patients included in the VMPT arm showed a significantly longer 2-year PFS (70% *v* 58%). After a median follow-up of 47.2 months, an OS advantage with VMPT-VT over VMP was detected: median OS was not reached in the VMPT-VT arm and was 58.2 months in the VMP arm.[33] Neutropenia and cardiac complications were higher in the VMPT-VT arm than in the VMP arm, and mainly consisted of neutropenia (38% *v* 28%) and cardiac toxicity (10% *v* 5%). In order to reduce the overall incidence of grade 3–4 peripheral neuropathy, bortezomib administration was reduced in both arms from a twice-weekly schedule to a once-weekly infusion (1.3 mg/m^2 on days 1, 8, 15, 22). Of note, the once-weekly schedule did not appear to have a negativel impact on efficacy in this patient population.[34]

Both VMP and VMPT-VT are suitable options for fit patients. In unfit patients, VMP with appropriate dose modifications is also a feasible and attractive option (Table 3).

Lenalidomide-Based Therapies

The combination of lenalidomide with high-dose dexamethasone (RD) has been compared to high-dose dexamethasone alone in a phase III randomized trial.[35] Outcome was improved with RD, in particular the 1-year PFS (77% *v* 55%). Incidence of treatment discontinuation due to grades 3–4 AEs such as neutropenia and non-neutropenic infections was higher in the RD arm.

Another trial compared RD with the combination of lenalidomide with low-dose dexamethasone (Rd).[36] RD induced higher overall response rate compared with Rd (79% *v* 68%, respectively,

Table 3. Treatment Strategies for Elderly Myeloma Patients Based on Patients' Status

Patient Status	Suggested Approach
Very fit Karnofsky performance status ≥80%	Reduced-intensity autologous transplantation
Charlson index = 0	VMP/VMPT-VT
Fit Karnofsky performance status 60%–80%	MPR-R/Rd
Charlson index ≤2	MPT (full-dose regimens)
Unfit Karnofsky performance status <60%	MPT/VMP
Charlson index >2	Rd (reduced-dose regimens)

Abbreviations: VMP, bortezomib-melphalan-prednisone; VCD, bortezomib-ciclophosphamide-dexamethasone; VRD, bortezomib-lenalidomide-dexamethasone; VMPT-VT, bortezomib-melphalan-prednisone-thalidomide/bortezomib-thalidomide; MPR-R, melphalan-prednisone-lenalidomide with lenalidomide maintenance; Rd, lanalidomide–low-dose dexamethasone; MPT, melphalan-prednisone-thalidomide; VD, bortezomib-dexamethasone.

$P = .008$). Nevertheless, TTP and PFS were not improved in the high-dose group, and the 1-year OS was 96% with Rd compared to 87% with RD ($P = .0002$), related to higher incidence of early deaths and of AEs in patients treated with RD, with a negative impact on survival. Considering its efficacy and good toxicity profile, Rd can be considered a good option for patients not eligible for ASCT.

A recent study evaluated a sequential approach with lenalidomide plus MP (MPR) followed by lenalidomide maintenance (MPR-R) as compared to MPR and MP with no maintenance. MPR-R prolonged the median PFS by 17 months in comparison to MPR and MP (31 *v* 14 *v* 13 months; $P < .001$). The 3-year OS was similar among the three treatment arms (70% *v* 62% *v* 66%). AEs were mainly hematologic: grade 4 neutropenia was reported in 35% of MPR-R patients and 32% of MPR patients. Concerns about the increased risk of second primary malignancies (SPMs) with lenalidomide have been recently raised. The 3-year rate of SPM was 7% with both MPR-R and MPR, and 3% with MP. However, the benefit associated with MPR-R seem to outweigh the increased risk of SPMs.[37]

Both Rd and MPR-R are effective and safe in fit elderly MM patients. In addition, because of the good tolerability and the survival benefit, the two-drug regimen Rd seems an appropriate option also for unfit MM patients (Table 3).

AUTOLOGOUS STEM CELL TRANSPLANTATION

Patients over 65 years of age are commonly considered ineligible for standard melphalan (MEL200; 200 mg/m^2) followed by ASCT. Yet, for very fit elderly patients, the option of reduced intensity transplantation (MEL100; 100 mg/m^2) can be considered.

MP has been compared with intermediate-dose melphalan and reduced-intensity ASCT in two different studies.[23,38] In patients aged 65–70 years, the response rate was better in the ASCT arm, but there was no difference in PFS or OS. Reduced intensity ASCT led to better event-free survival (EFS) as well.

The second trial included patients aged 65–75 years, and compared reduced-intensity ASCT with the standard MP and also with the combination MPT. Patients treated with MPT had a longer PFS and OS compared with those treated with MP or MEL100, but no differences between MP and MEL100 were found: at a median follow-up of 51.5 months, the median OS was 33.2 months in the MP arm, 51.6 months in the MPT arm, and 38.3 months for patients treated with MEL100.

Based on available data on elderly patients, reduced-intensity transplantation is suggested in patients with excellent clinical conditions, namely, fit elderly patients with no comorbidities and between 65 and 70 years of age (Table 3).

ROLE OF EXTENDED TREATMENT AND MAINTENANCE IN TRANSPLANT INELIGIBLE PATIENTS

The most important goal of maintenance therapy is to maintain outcome after induction, to prolong the duration of response, and to prolong survival.[39] No specific guidelines are available at the moment; we have only few data about the efficacy of maintenance regimen in elderly patients. The first maintenance therapy consisted of continuing chemotherapy after successful induction with the combination MP.[40,41]

Thalidomide-Based Strategies

The advantage of using thalidomide is the oral administration. Thalidomide maintenance has been evaluated in four studies where patients had been treated with MPT induction.

In the first trial, the dose of thalidomide was 100 mg/day. The median PFS was significantly longer in the arm with thalidomide maintenance than in the control arm (25 months *v* 15 months, respectively). Median OS was 47.6 months with maintenance versus 45 months with no maintenance.[27,42]

In the second trial, the dose of thalidomide was reduced to 50 mg/d. In the MPT arm followed by thalidomide maintenance, an advantage in terms of OS was detected (40 months *v* 31 months).[26]

In the Nordic study, thalidomide was given at the dose of 200 mg/d; there was no advantage in terms of PFS (15 months *v* 14 months) and OS (29 months *v* 32 months).[25]

In all of these studies, the administration of thalidomide was associated with development of peripheral neuropathy.

Thalidomide maintenance has been assessed in two others studies that demonstrated an improvement of PFS but no OS advantage.[43] Another trial compared two different maintenance regimens. The first consisted of thalidomide associated with interferon; the second included interferon alone.[43] All of the patients had received an induction regimen with either thalidomide-dexamethasone (TD) or MP. A PFS improvement was observed with thalidomide-interferon maintenance (27.2 *v* 13.2 months). Also in this case, grades 3–4 neuropathy occurred in 7% versus 0%.

The last trial that tested the use of thalidomide as a maintenance regimen was the MRC Myeloma IX trial where patients could receive either MP or CTDa and then were randomized to receive thalidomide maintenance or no maintenance. The PFS improvement was higher in patients assigned to

maintenance, in particular in those who had already received thalidomide as induction therapy.[44]

The best dosage for thalidomide in maintenance therapy should range between 50 and 100 mg/d. The prolonged administration of this drug can affect a patient's quality of life.[45]

The trials described showed an advantage in PFS, but the follow-up is still too short to detect a survival advantage with thalidomide maintenance. In the context of elderly MM, more trials are including maintenance as part of the therapeutic approach. Lenalidomide and bortezomib are more often used compared with thalidomide as maintenance. Maintenance therapy is usually administered for 2 years or until relapse. However, clinical trials to assess the optimal duration of maintenance are needed.

Lenalidomide-Based Strategies

Lenalidomide seems to be a potentially better maintenance approach in elderly patients.

Lenalidomide maintenance after MPR (MPR-R) has been evaluated recently in a phase III trial, in comparison with MPR or MP inductions only.[37] Lenalidomide maintenance significantly improved the median PFS compared with MPR alone (26 months *v* 7 months). A longer follow-up is needed to assess the impact of the maintenance regimen on OS. Lenalidomide was associated with an increased incidence of SPMs compared with MP (3%), but no differences between MPR-R and MPR were seen (7% in both arms).

A phase II study on patients aged 65–75 years evaluated lenalidomide plus prednisone as consolidation followed by lenalidomide alone as maintenance (RP-R), after bortezomib-doxorubicin-dexamethasone (PAD) induction and reduced-intensity transplantation with melphalan 100 mg/m^2.[46] This sequential approach resulted in a 2-year PFS of 69% and a 2-year OS of 86%. RP-R significantly improved response achieved after induction, with the CR rate increasing from 12% to 40%. Neutropenia remained the major toxicity, with a grade 3–4 event occurring in 16% of patients. The available data suggest that lenalidomide is a good maintenance approach in this setting and it may be preferred to thalidomide because of the lack of neurologic side effects.

Bortezomib-Based Strategies

The Spanish group evaluated the efficacy of bortezomib associated with either thalidomide (VT) or prednisone (VP) as maintenance regimen after induction with VMP or bortezomib-thalidomide- prednisone.[47] After a median follow-up of 38 months from start of maintenance, the CR rate increased from 24% at the end of induction (mean value obtained after VMP and bortezomib-thalidomide-prednisone inductions) to 42%, with a slightly higher rate with VT compared with VP (46% *v* 39%). Median PFS was also longer with VT than VP (39 *v* 32 months), although this advantage was not statistically significant. Similarly, OS was only slightly longer with VT than with VP (5-year OS: 69% *v* 50%).[47]

The most important AE associated with the use of bortezomib is peripheral neuropathy, which occurred in 9% of VT patients and in 3% of VP patients.

Another trial evaluated VT maintenance after the four-drug induction regimen VMPT in comparison with standard VMP followed by no maintenance.[32] The maintenance regimen consisted of 1.3 mg/m^2 of bortezomib every 14 days, thalidomide at 50 mg/day for 2 years or until relapse. At a median duration of maintenance of 23.8 months, the 4-year PFS was 65% in the VMPT-VT arm and 49% in the VMP group, with 33% reduced risk of death for patients receiving VT maintenance. VT maintenance was also well tolerated; hematological toxicity occurred in 5% of patients, peripheral neuropathy in 7% of patients.[33]

Bortezomib as a single agent at the dose of 1.6 mg/m^2 was also evaluated in patients treated with bortezomib-dexamethasone (VD), bortezomib-thalidomide-dexamethasone (VTD), or VMP as induction therapy.[48] The most frequent AE was grade 3–4 peripheral neuropathy, which occurred in 5% of patients. Dose reductions are a good option to reduce neurologic toxicity and to allow patients to stay longer on treatment. Based on the available trials, bortezomib maintenance seems beneficial and well tolerated in elderly patients, with a neurologic toxicity lower than thalidomide. Its benefits are particularly evident when a reduced-schedule is used, and it is a valuable maintenance option when combined with thalidomide.

CONCLUSION

The introduction of new drugs that can be differently combined with conventional chemotherapy or low-dose dexamethasone has changed substantially the treatment paradigm for patients with MM, and a wider variety of treatment options is now available for elderly patients.

Physicians now have the opportunity to choose the best treatment regimen according to patient characteristics; compliance also has to be considered, especially for elderly patients.

The goal of therapy in elderly patients is to achieve and maintain maximal response while limiting treatment-related toxicities as much as possible. Therefore, an optimal treatment should always balance efficacy outcome with its toxicity profile. Randomized phase III trials found that MPT, MPV, and MPR-R were more effective than the standard of care MP. VMPT combination followed by VT maintenance was more effective than VMP and

can be another option to treat patients older than 65 years. Before choosing treatment, a careful geriatric assessment of the patients is needed. MPT, VMP, MPR-R, and VMPT-VT full-dose regimens should be recommended in elderly patients with good clinical conditions. If patients have a high risk of thromboembolism, VMP should be preferred. For patients with renal failure, VMP and MPT should be considered. In those with pre-existing neuropathy, MPR is well tolerated and thus proved to be a feasible option. In patients ≥75 years of age or frail with comorbidities, MPT or VMP with lower doses or two-drug combinations such as Rd are suggested.

Management of side effects during the induction regimen is a crucial aspect. Supportive care and dose modifications can be considered to manage myelosuppression.

Consolidation and maintenance therapies have shown promising results, prolonging remission duration and giving PFS advantages. Thalidomide, Lenalidomide, and bortezomib as single agents are well tolerated and can be administrated after induction in a sequential approach.

In patients older than 65, thalidomide maintenance is a good option after MPT, but neuropathy is a major concern. Lenalidomide maintenance proved to be a valid strategy after MPR induction. Bortezomib is commonly used as maintenance, yet dose reductions are needed to reduce the risk of peripheral neuropathy. Also, when choosing the consolidation/maintenance approach, the benefits and risks associated with each approach have to be carefully considered.

Second-generation novel agents, such as carfilzomib, pomalidomide, elotuzumab, and bendamustine, are currently being evaluated. Future trials may validate their role, and they may be a good alternative option to improve treatment outcome also in the elderly setting.

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REFERENCES

1. Kyle RA, Rajkumar SV. Multiple myeloma. Blood. 2008;111(6):2962–72.
2. Kyle RA, Buadi F, Rajkumar SV. Management of monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM). Oncology (Williston Park). 2011;25(7):578–86.
3. Rajkumar SV, Dispenzieri A, Kyle RA. Monoclonal gammopathy of undetermined significance, Waldenström macroglobulinemia, AL amyloidosis, and related plasma cell disorders: diagnosis and treatment. Mayo Clin Proc. 2006;81(5):693–703.
4. Kyle RA, Rajkumar SV. Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. Leukemia. 2009;23(1):3–9.
5. Kyle RA, Remstein ED, Therneau TM, et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. N Engl J Med. 2007;356(25):2582–90.
6. Csako G. Immunofixation electrophoresis for identification of proteins and specific antibodies. Methods Mol Biol. 2012;869:147–71.
7. 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(2):215–24.
8. Rajkumar SV. Multiple myeloma: 2013 update on diagnosis, risk-stratification, and management. Am J Hematol. 2013;88(3):225–35.
9. Kyle RA, Durie BGM, Rajkumar SV, et al. Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management. Leukemia. 2010;24(6):1121–7.
10. Jakob C, Sterz J, Liebisch P, et al. Incorporation of the bone marker carboxy-terminal telopeptide of type-1 collagen improves prognostic information of the International Staging System in newly diagnosed symptomatic multiple myeloma. Leukemia. 2008;22(9):1767–72.
11. Snozek CLH, Katzmann JA, Kyle RA, et al. Prognostic value of the serum free light chain ratio in newly diagnosed myeloma: proposed incorporation into the international staging system. Leukemia. 2008;22 (10):1933–7.
12. Fonseca R, Barlogie B, Bataille R, et al. Genetics and cytogenetics of multiple myeloma: a workshop report. Cancer Res. 2004;64(4):1546–58.
13. Dewald GW, Therneau T, Larson D, et al. Relationship of patient survival and chromosome anomalies detected in metaphase and/or interphase cells at diagnosis of myeloma. Blood. 2005;106(10):3553–8.
14. Ferlay J, Shin H-R, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127(12):2893–917.
15. Karnofsky D, Burchenal J. The clinical evaluation of chemotherapeutic agents in cancer. In MacLeod CM, editor. Evaluation of chemotherapeutic agents. New York: Columbia University Press; 1949:196.
16. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40(5):373–83.
17. Myeloma Trialists' Collaborative Group. Combination chemotherapy versus melphalan plus prednisone as treatment for multiple myeloma: an overview of 6,633 patients from 27 randomized trials. Myeloma Trialists' Collaborative Group. J Clin Oncol. 1998;16 (12):3832–42.
18. Singhal S, Mehta J, Desikan R, et al. Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med. 1999;341(21):1565–71.
19. Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008;111(5):2516–20.
20. Brenner H, Gondos A, Pulte D. Recent major improvement in long-term survival of younger patients with multiple myeloma. Blood. 2008;111(5):2521–6.

21. Durie BGM, Harousseau J-L, Miguel JS, et al. International uniform response criteria for multiple myeloma. Leukemia. 2006;20(9):1467–73.
22. Gay F, Larocca A, Wijermans P, et al. Complete response correlates with long-term progression-free and overall survival in elderly myeloma treated with novel agents: analysis of 1175 patients. Blood. 2011;117(11):3025–31.
23. Facon T, Mary JY, Hulin C, et al. Melphalan and prednisone plus thalidomide versus melphalan and prednisone alone or reduced-intensity autologous stem cell transplantation in elderly patients with multiple myeloma (IFM 99-06): a randomised trial. Lancet. 2007;370(9594):1209–18.
24. Hulin C, Facon T, Rodon P, et al. Efficacy of melphalan and prednisone plus thalidomide in patients older than 75 years with newly diagnosed multiple myeloma: IFM 01/01 trial. J Clin Oncol. 2009;27(22):3664–70.
25. Waage A, Gimsing P, Fayers P, et al. Melphalan and prednisone plus thalidomide or placebo in elderly patients with multiple myeloma. Blood. 2010;116(9):1405–12.
26. Wijermans P, Schaafsma M, Termorshuizen F, et al. Phase III study of the value of thalidomide added to melphalan plus prednisone in elderly patients with newly diagnosed multiple myeloma: the HOVON 49 study. J Clin Oncol. 2010;28(19):3160–6.
27. Palumbo A, Bringhen S, Liberati AM, et al. Oral melphalan, prednisone, and thalidomide in elderly patients with multiple myeloma: updated results of a randomized controlled trial. Blood. 2008;112(8):3107–14.
28. Morgan GJ, Davies FE, Gregory WM, et al. Cyclophosphamide, thalidomide, and dexamethasone as induction therapy for newly diagnosed multiple myeloma patients destined for autologous stem-cell transplantation: MRC Myeloma IX randomized trial results. Haematologica. 2012;97(3):442–50.
29. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008;359(9):906–17.
30. Mateos M-V, Richardson PG, Schlag R, et al. Bortezomib plus melphalan and prednisone compared with melphalan and prednisone in previously untreated multiple myeloma: updated follow-up and impact of subsequent therapy in the phase III VISTA trial. J Clin Oncol. 2010;28(13):2259–66.
31. Mateos M-V, Oriol A, Martínez-López J, et al. Bortezomib, melphalan, and prednisone versus bortezomib, thalidomide, and prednisone as induction therapy followed by maintenance treatment with bortezomib and thalidomide versus bortezomib and prednisone in elderly patients with untreated multiple myeloma: a randomised trial. Lancet Oncol. 2010;11(10):934–41.
32. Palumbo A, Bringhen S, Rossi D, et al. Bortezomib-melphalan-prednisone-thalidomide followed by maintenance with bortezomib-thalidomide compared with bortezomib-melphalan-prednisone for initial treatment of multiple myeloma: a randomized controlled trial. J Clin Oncol. 2010;28(34):5101–9.
33. Palumbo A, Bringhen S, Rossi D, Cavalli M, Ria R, Gentilini S. Overall survival benefit for bortezomib-melphalan-prednisone-thalidomide followed by maintenance with bortezomib-thalidomide (VMPT-VT) versus bortezomib-melphalan-prednisone (VMP) in newly diagnosed multiple myeloma patients. Blood (ASH Annual Meeting Abstracts); 2012;120:200.
34. Bringhen S, Larocca A, Rossi D, et al. Efficacy and safety of once-weekly bortezomib in multiple myeloma patients. Blood. 2010;116(23):4745–53.
35. Zonder JA, Crowley J, Hussein MA, et al. Lenalidomide and high-dose dexamethasone compared with dexamethasone as initial therapy for multiple myeloma: a randomized Southwest Oncology Group trial (S0232). Blood. 2010;116(26):5838–41.
36. 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(1):29–37.
37. Palumbo A, Hajek R, Delforge M, et al. Continuous lenalidomide treatment for newly diagnosed multiple myeloma. N Engl J Med. 2012;366(19):1759–69.
38. Palumbo A, Bringhen S, Petrucci MT, et al. Intermediate-dose melphalan improves survival of myeloma patients aged 50 to 70: results of a randomized controlled trial. Blood. 2004;104(10):3052–7.
39. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med. 2011;364(11):1046–60.
40. Remission maintenance therapy for multiple myeloma. Arch Intern Med. 1975 ;135(1):147-52.
41. Belch A, Shelley W, Bergsagel D, et al. A randomized trial of maintenance versus no maintenance melphalan and prednisone in responding multiple myeloma patients. Br J Cancer. 1988;57(1):94–9.
42. Palumbo A, Bringhen S, Caravita T, et al. Oral melphalan and prednisone chemotherapy plus thalidomide compared with melphalan and prednisone alone in elderly patients with multiple myeloma: randomised controlled trial. Lancet. 2006;367(9513):825–31.
43. Ludwig H, Adam Z, Tóthová E, et al. Thalidomide maintenance treatment increases progression-free but not overall survival in elderly patients with myeloma. Haematologica. 2010;95(9):1548–54.
44. Morgan GJ, Gregory WM, Davies FE, et al. The role of maintenance thalidomide therapy in multiple myeloma: MRC Myeloma IX results and meta-analysis. Blood. 2012;119(1):7–15.
45. Attal M, Harousseau J-L, Leyvraz S, et al. Maintenance therapy with thalidomide improves survival in patients with multiple myeloma. Blood. 2006;108(10):3289–94.
46. Palumbo A, Gay F, Falco P, et al. Bortezomib as induction before autologous transplantation, followed by lenalidomide as consolidation-maintenance in untreated multiple myeloma patients. J Clin Oncol. 2010;28(5):800–7.
47. Mateos M-V, Oriol A, Martínez-López J, et al. Maintenance therapy with bortezomib plus thalidomide or bortezomib plus prednisone in elderly multiple myeloma patients included in the GEM2005MAS65 trial. Blood. 2012;120(13):2581–8.
48. Niesvizky R, Flinn IW, Rifkin RM. Phase 3b UPFRONT study: safety and efficacy of weekly bortezomib maintenance therapy after bortezomib-based induction regimens in elderly, newly diagnosed multiple myeloma patients. Blood (ASH Annual Meeting Abstracts). 2010;116:3026.

Initial Treatment of Transplant Candidates With Multiple Myeloma

Philippe Moreau and Cyrille Touzeau

Over the last decade, thalidomide, bortezomib, and lenalidomide have been introduced into the armamentarium of myeloma therapies. These novel agents have improved the rate of complete remission both before and after autologous stem cell transplantation (ASCT) without substantially increasing toxicity, which has important implications as the achievement of high-quality responses is a significant prognostic factor for outcome. This review will focus on the most recent results of novel agent-based induction therapies, as well as on interesting developments in the transplant phase that are aimed at improving the results of conditioning regimens.
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When considering the impact of high-dose therapy (HDT) and autologous stem cell transplantation (ASCT) in treatment of multiple myeloma, we have to distinguish two distinct time periods, the first one before and the second one after the introduction of novel agents. The former period, which corresponds to the 1990s, provided the proof-of-concept regarding the benefit of early ASCT and resulted in the procedure becoming the standard of care. The incorporation of the novel agents into the transplant procedure has led to an improvement in response rates, progression-free survival (PFS), and overall survival (OS) but also, paradoxically, to the questioning of the role of ASCT as part of frontline treatment.[1] This comes at the very time when important advances in the understanding of the biology of the disease are leading some physicians to believe that a risk-adapted strategy should be routinely used, with biological parameters guiding treatment decisions in daily practice. These points will be discussed in detail in other articles of this issue of *Seminars in Oncology*.

Over the last decade, thalidomide, bortezomib, and lenalidomide have been introduced into the therapeutic armamentarium. These novel agents have improved the rate of complete response (CR) both before and after ASCT without substantially increasing toxicity, which has important implications as the achievement of high-quality responses is a significant prognostic factor for outcome.[1] In addition, novel agent-based consolidation therapy following ASCT has resulted in the achievement of molecular CRs[2,3] and recent data also show that maintenance strategies following HDT may dramatically increase PFS.[4,5] This review will focus on the most recent results of novel agent-based induction therapies, as well as on interesting developments in the transplant phase that are aimed at improving the results of conditioning regimens. The role of consolidation and maintenance are discussed elsewhere in this issue by Cavo et al.

INCORPORATING NOVEL AGENTS INTO THE INITIAL TREATMENT STRATEGY: EVIDENCED-BASED SELECTION OF PRETRANSPLANT INDUCTION

Until 2000, the combination of vincristine, doxorubicin, and dexamethasone (VAD) was the induction regimen most widely used prior to ASCT and was considered the standard of care.[1] The primary objective of incorporating novel agents in this setting is to increase the CR rate not only prior to but also after ASCT. A further objective of incorporating novel agents during induction is to reduce the proportion of patients requiring a second ASCT because of a suboptimal response (less than very good partial response [VGPR]) to the first ASCT step.[6]

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Thalidomide was the first novel agent to be compared to VAD either in combination with dexamethasone (TD),[7] or with doxorubicin plus dexamethasone (TAD).[8,9] Overall, the benefit of TD or TAD compared to VAD remained modest. Other thalidomide combinations also have been evaluated. The CTD regimen (cyclophosphamide, thalidomide, and dexamethasone) was investigated in a large randomized study in the United Kingdom, and results showed high CR rates both before and after ASCT.[10]

The second novel agent to become available, bortezomib, was investigated in combination with dexamethasone in a large trial by the Intergroupe Francophone du Myélome (IFM; IFM 2005-01) and prospectively compared to VAD.[11] Post-induction CR or near-CR (nCR), at least VGPR, and overall response rates were significantly higher with bortezomib plus dexamethasone versus VAD. The superior response rates in the bortezomib plus dexamethasone induction arms of the trial translated into better response rates after HDT. This improvement also had an impact on the overall outcome: the median PFS was 36 versus 30 months with bortezomib plus dexamethasone versus VAD, respectively. Survival was not superior in the bortezomib plus dexamethasone arms of the study, possibly due to effective salvage regimens at the time of relapse. The level of response achieved with bortezomib plus dexamethasone post-induction in the IFM 2005-01 trial is now considered the goal of current therapies, and, as a result, bortezomib plus dexamethasone has become the backbone of induction therapy prior to ASCT to which other more complex regimens are being compared.

The addition of a third agent to the bortezomib plus dexamethasone regimen, such as thalidomide (VTD),[12] doxorubicin (VDD or PAD),[13,14] lenalidomide (VRD),[15] or cyclophosphamide (VCD),[16] has been tested in several small phase II studies and the outcomes appear even better, with response rates of around 90% and CR rates of up to 24%. In all of these studies, the frequent, rapid, and deep responses consistently translated into improved outcomes.

Three prospective studies have already shown that VTD is superior to TD or bortezomib plus dexamethasone.[17–19] The Italian myeloma study group prospectively compared VTD to TD in 474 patients with newly diagnosed MM prior to tandem ASCT. They found that VTD resulted in higher CR and at least VGPR rates as compared to TD, which translated into a better PFS after HDT.[17] However, the addition of bortezomib to the TD regimen was associated with a significant increase in the incidence of grade 3–4 adverse events (AEs) (56% *v* 33%), including peripheral neuropathy (PN) (10% *v* 2%). The Spanish myeloma study group also compared VTD to TD and to another, more complex chemotherapy regimen that included bortezomib prior to ASCT in 386 patients. They confirmed that VTD was able to achieve the highest pre- and post-ASCT CR rates.[18] In the IFM 2007-02 trial, four cycles of the "standard" bortezomib plus dexamethasone induction regimen were prospectively compared to four cycles of VTD with lower doses of bortezomib (1 mg/m^2 instead of 1.3 mg/m^2) and thalidomide (100 mg/d instead of 200 mg/d, which was the dose in the Italian and Spanish trials) in order to reduce the rate of neuropathy.[19] Again, VTD was found to result in superior CR plus VGPR rates both before and after ASCT. The reduction in the doses of both bortezomib and thalidomide was associated with a reduction in the incidence of neurotoxicity, with grade ≥2 PN occurring in 14% of the patients in the VTD arm. The IFM 2007-02 study, therefore, confirmed the superiority of a three-drug combination over a two-drug combination as induction therapy prior to ASCT. The results of a phase III randomized prospective trial comparing VAD versus PAD as induction prior to HDT have also been reported.[20] This study corroborated the superiority of the bortezomib-based three-drug induction regimen over VAD; OS was also superior in the bortezomib arm of the trial.

As yet, no data are available that could allow us to draw conclusions regarding the superiority of one triplet combination, such as VTD, VRD, VCD, or PAD, over the other from phase III randomized trials. However, the IFM group will initiate a trial to compare VTD versus VCD in a randomized fashion in the near future.

Four-drug combinations also have been evaluated, such as VRD plus pegylated liposomal doxorubicin,[21] VRD plus cyclophosphamide (VDCR),[22] or VTD plus cyclophosphamide (VTDC),[23] and compared to three-drug regimens in at least two different randomized phase II trials. The phase II EVOLUTION trial, which was not specifically designed to compare induction regimens prior to ASCT, evaluated VDC, VDR, and VDCR in 140 previously untreated MM.[22] Patients received V (bortezomib) 1.3 mg/m^2 (days 1, 4, 8, 11) and D (dexamethasone) 40 mg (days 1, 8, 15), with either C (cyclophosphamide) 500 mg/m^2 (days 1, 8) and R (lenalidomide) 15 mg (days 1–14; VDCR) or R 25 mg (days 1–14; VDR), C 500 mg/m^2 (days 1, 8; VDC) or C 500 mg/m^2 (days 1, 8, 15; VDC-modified) in 3-week cycles (maximum, eight cycles). As patients were allowed to go off-study for an ASCT after four cycles, responses were first evaluated at four cycles. After four cycles, 80%, 73%, 63%, and 82% of patients in the VDCR, VDR, VDC, and VDC-modified arms had a confirmed response. The responses seen with VDCR appeared to be similar but not superior to those seen with the VDR or VDC-modified arms.

However, the incidence of toxicities with VDCR appeared to be higher than in the other arms, especially hematologic toxicity. The authors therefore concluded that no substantial advantage was derived from VDCR over the three-drug combinations, and considered that the VDR and VCD-modified regimens should be preferred in clinical practice and for further comparative testing. Another recent phase II study prospectively investigated bortezomib 1.3 mg/m^2, thalidomide 100 mg, and dexamethasone 40 mg, with (n = 49 patients) or without (n = 49 patients) cyclophosphamide 400 mg/m^2 for four 21-day cycles (VTDC *v* VTD), followed by ASCT.[23] The primary end point was the combined CR/nCR rate following induction therapy. Fifty-one percent of patients receiving VTD achieved a CR or nCR compared to 44% of patients on the VTDC arm, with confirmed CRs in 29% and 31%, and overall response rates of 100% and 96%, respectively; a VGPR or better was observed in 69% in both arms. Post-ASCT, combined CR/nCR rates were 85% (VTD) and 77% (VTDC). Three-year overall survival was 80% (both arms). Grade 3–4 AEs and serious AEs were observed in 47% and 22% (VTD) and 57% and 41% (VTDC) of patients, respectively. Importantly, the primary health-related quality-of-life score (European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C30 [EORTC QLQ-C30] Global Health score) steadily increased with VTD during induction and reached a clinically relevant difference versus baseline post-transplantation. The conclusions of this important trial were the following: both VTD and VTDC regimens are highly active induction regimens; however, VTDC was associated with increased toxicity and the suggestion of transient decreases in quality of life as assessed by the Global Health score, without an increase in activity.

Overall, current data are not supporting the use of four-drug combinations as part of induction therapy. Thus, based on response rates, depth of response, and PFS as surrogate markers for outcome, three-drug combinations, mainly VTD, VRD, VCD, or PAD are currently the standard of care prior to ASCT.[1,12–20,21–23]

Promising novel agents are under evaluation. The second-in-class proteasome inhibitor, carfilzomib, has recently been combined with lenalidomide and dexamethasone (CRD) as part of frontline therapy in untreated MM.[24] This combination produces unprecedented CR rates and could be one of the most attractive induction regimens prior to ASCT. A prospective comparison of CRD versus VRD is planned in cooperative groups. In the near future, oral proteasome inhibitors, such as MLN9708 or oprozomib, will be combined with dexamethasone, lenalidomide, or the third-in-class immunomodulatory drug, pomalidomide, which potentially has greater activity. For example, MLN9708 is currently tested in combination with lenalidomide and dexamethasone as part of frontline therapy in a phase I/II study.[25] These oral combinations may turn out to be the more effective options, and they could certainly be the more convenient regimens for both patients and physicians.[26]

The high efficacy of the novel agents has led some groups to test these agents upfront without incorporating an ASCT step and interesting results have been reported. Lenalidomide plus low-dose dexamethasone (Len/dex) as part of frontline therapy without ASCT yielded similar survival rates at 2 years as compared with Len/dex followed by ASCT in a non-randomized trial conducted by the Eastern Cooperative Oncology Group (ECOG).[27] Furthermore, in a non-randomized phase II trial of lenalidomide-bortezomib-dexamethasone in the upfront setting, in which the choice of proceeding to HDT or not was based on physician or patient preference, no difference in outcome was seen for the two approaches.[15] Finally, a recent phase I/II trial of carfilzomib in combination with lenalidomide and low-dose dexamethasone as frontline treatment showed that this triplet combination without ASCT was able to induce an impressive rate of stringent CR, and a 24-month PFS rate of 92%.[24] Based on these results, many colleagues have begun to consider the use of such novel agent-based therapies without the upfront application of ASCT as an alternative to early transplantation and the role of ASCT itself has become a matter of debate: should it be used upfront or as a salvage treatment at the time of progression in patients initially treated with novel agents? This unresolved issue will be discussed elsewhere within this issue of *Seminars*.

TRANSPLANT PHASE: RECENT PROGRESS AND FUTURE DIRECTIONS

While many studies have been performed in the induction, consolidation, and maintenance settings, few trials have been dedicated to conditioning regimens prior to ASCT. Nevertheless, several attempts have recently been made to improve this step of the HDT procedure. The current standard conditioning regimen is melphalan 200 mg/m² (Mel200) administered intravenously (IV). This regimen was first introduced by the London group in the early 1990s in a series of 53 patients.[28] At that time, total body irradiation (TBI) was still the most frequently used preparative regimen for ASCT, based on the experience of the Arkansas group.[29] In the prospective, randomized IFM 90 trial, which demonstrated for the first time the superiority of HDT over conventional therapy in a series of 200 patients, the conditioning regimen prior to ASCT consisted of

8 Gy TBI plus melphalan 140 mg/m² (Mel140/TBI).[30] Other studies undertook comparisons of conditioning regimens, but most of these were based on data from international[31] or national[32] registries and not on prospective randomized trials. The IFM group was the first to initiate such a trial comparing Mel200 to melphalan 140 mg/m² plus 8 Gy TBI (Mel140/TBI).[33] A total of 282 patients with newly diagnosed MM were prospectively randomized; 140 were treated with Mel140/TBI (arm A) and 142 with Mel200 (arm B). Disease response to four cycles of VAD before randomization and ASCT were identical in the two arms. In arm B, hematologic recovery was significantly faster regarding both neutropenia and thrombocytopenia. In addition, transfusion requirements were significantly lower, and the median duration of hospitalization was significantly shorter in arm B. The incidence of severe mucositis was significantly increased in arm A, and five toxic deaths (3.6%) were observed in this arm, as compared to none in arm B. Following HDT, the CR, VGPR, and partial response (PR) rates were comparable in the two arms. Furthermore, the median duration of event-free survival (EFS) was similar (21 *v* 20.5 months), but the 45-month survival rate was 65.8% in arm B versus 45.5% in arm A ($P = .05$). This difference was attributed in part to the use of better salvage regimens after relapse in patients treated with Mel200. The results from the trial suggested that Mel200 was at least as effective as Mel140/TBI but that it was a less toxic conditioning regimen. Melphalan 200 mg/m² therefore became the preferred preparative regimen.

A variety of strategies have been explored with the aim of improving on the results of Mel200, including dose escalation, and the addition of other chemotherapeutic agents, radionuclides, or novel agents, such as bortezomib.

Higher doses of melphalan prior to ASCT have been tested since the non-hematologic toxicity of the agent is low. In a phase I dose-escalation study Phillips et al investigated incremental increases of 20 mg/m^2 from a melphalan starting dose of 220 mg/m^2 and demonstrated that the maximum tolerated dose of melphalan was 280 mg/m^2 when used in combination with the cryoprotective agent amifostine.[34] Only 18 of the 58 patients examined in this series presented with MM, which was mainly of advanced nature prior to ASCT, and it was therefore not possible to draw any conclusion regarding the clinical outcome or to define the antitumor efficacy of this regimen. Another small phase II study showed encouraging results with melphalan 220 mg/m² followed by ASCT in relapsed/refractory patients.[35] The regimen was generally tolerable, the most frequent adverse event being severe mucositis. In order to further increase the response rate without increasing the toxicity of HDT, a combination of anti-IL6 monoclonal antibody and dexamethasone was subsequently added to melphalan 220 mg/m² in 16 patients with advanced MM.[36] A strong inhibition of interleukin-6 activity was observed in all patients and was correlated with the high CR rate achieved with this combination therapy. Nevertheless, in the absence of a randomized trial comparing melphalan at 200 mg/m² versus 220 mg/m² or more, the impact of the higher dose is unknown.

The addition of another cytotoxic agent has not yet resulted in convincing improvements as the apparent increase in anti-myeloma activity occurred at the expense of increased toxicity. In the Spanish PETHEMA/GEM2000 trial, the first 225 patients who were enrolled in the study received the combination of oral busulfan 12 mg/kg plus melphalan 140 mg/m^2 (BuMel), but because of a high frequency of veno-occlusive disease (VOD), the protocol was amended[37] and the next 542 patients received Mel200.[38] The investigators subsequently compared the outcome of the two cohorts of patients, and found that the transplant-related mortality was significantly increased in the BuMel group due to VOD. Although the median PFS was significantly longer with BuMel, survival was similar in both cohorts. Since then, busulfan IV has become available and this formulation may reduce toxicity and result in greater efficacy. The Spanish group recently compared IV busulfan (BU) 9.6 mg/kg and MEL140 versus MEL200 as a conditioning regimen before ASCT for newly diagnosed patients with MM.[39] Fifty-one patients received IV BU plus MEL140 while 102 patients were treated with MEL200 in a 1:2 matched control analysis. No differences in the overall and CR rates were observed after ASCT between the groups. After a median follow-up of 63 and 50 months in the control and BU plus MEL groups, the PFS was 24 and 33 months, respectively ($P = .10$). The most frequent toxicities included mucositis and febrile neutropenia in both groups. No case of VOD was observed. Transplant-related mortality was 4% and 2% in BU plus MEL and control groups, respectively. The authors concluded that IV BU plus MEL may be considered an effective and well-tolerated alternative to a MEL-only approach as a conditioning regimen for patients with MM who are candidates for ASCT.

Another strategy that has been explored in an attempt to enhance the activity of HDT prior to ASCT is the addition of a radionuclide to high-dose melphalan. Giralt et al reported the results of a combination of Holmium 166 (^{166}Ho-DOTMP), a radiotherapeutic that localizes specifically to the skeleton and can deliver high doses of radiation to the bone and bone marrow, with either high-dose melphalan alone (Mel140 or Mel200) or Mel140/TBI.[40] In a phase I/II dose-escalation study of

high-dose ^{166}Ho-DOTMP plus melphalan, 83 patients received ^{166}Ho-DOTMP to deliver a nominal radiation dose of 20, 30, or 40 Gy to the bone marrow. The CR rates achieved after the procedure were encouraging (23% in primary refractory patients, and 40% in first remission consolidation cases), but long-term follow-up of the patients revealed two significant late toxicities: grade 2–3 hemorrhagic cystitis described in 23 patients, and renal toxicity of grade 3 or higher observed in 14 cases with eight patients developing a severe form of sustained renal impairment associated with microangiopathic hemolytic anemia, thrombocytopenia, uncontrolled hypertension, and elevated lactate dehydrogenase. This delayed toxicity is an important drawback of this conditioning regimen precluding its further use.

Another β emitter, 153 samarium, conjugated with the diphosphonate compound EDTMP in the radiopharmaceutical ^{153}Sm-EDTMP, which avidly concentrates in bone, also has been investigated in MM patients in combination with high-dose melphalan. In a phase I study, a total of 12 patients received escalating doses of ^{153}Sm-EDTMP (n = 3 per group; 6, 12, 19.8, and 30 mCi/kg) and a fixed dose of Mel200 followed by ASCT.[41] No dose-limiting toxicity was seen, and to better standardize the marrow compartment radiation dose, the study was modified such that an additional six patients were treated at the targeted absorbed radiation dose to the red marrow of 40 Gy, based on a trace-labeled infusion 1 week prior to the therapy. No delayed toxicity was observed, and the overall response rate was 94%, including seven VGPRs and five CRs. Subsequently, a total of 46 patients (29 in first response and 17 with relapsed or refractory disease) were enrolled in the phase II study investigating the same combination calculated to deliver 40 Gy to the bone marrow prior to Mel200 and ASCT.[42] The adverse events attributable to the radioisotope were mild and manageable. Post-transplant, 33% of patients had achieved a CR and another 26% had achieved a VGPR. Study patients were compared to 102 patients who were simultaneously treated off-study with single agent high-dose melphalan conditioning, and no difference regarding response rates, PFS or OS among patients treated with or without ^{153}Sm-EDTMP was seen. The authors concluded that this regimen warrants further study in the phase III setting.

Synergistic effects between bortezomib and melphalan have been reported both in vitro[43] and in vivo.[44] Combining bortezomib and high-dose melphalan is consequently a logical and attractive approach to improve the efficacy of the conditioning regimen. Furthermore, the combination is expected to be well tolerated because of the absence of overlapping toxicities. These observations formed the basis for a phase II trial conducted by the IFM, which was aimed at evaluating CR and VGPR rates, as well as toxicity of the combination of bortezomib and melphalan administered as a conditioning regimen. Fifty-four newly diagnosed patients received bortezomib (1 mg/m^2 x 4) and Mel200 (Bor-HDM),[45] and overall, 70% of patients achieved at least a VGPR, including 17 patients with a CR (32%) after ASCT. No toxic deaths were observed and bortezomib did not increase the hematologic toxicity. Only one case of grade 3–4 peripheral neuropathy was reported. A matched control analysis was conducted comparing this cohort to patients from the IFM 2005-01 trial, who were treated with Mel200 single-agent prior to HDT.[11] Patients were matched for response to induction therapy and type of induction, and the CR rate was found to be higher in the group receiving Bor-HDM conditioning (35% *v* 11%; $P = .001$), regardless of induction therapy. The results suggest that Bor-HDM is a well tolerated and promising conditioning regimen. These findings were recently confirmed in the relapse setting in a small series of heavily pretreated patients,[46] and in a phase I/II trial performed in the US involving 39 patients.[47] In this latter trial, only patients who did not achieve a VGPR following one or more induction regimens were enrolled and were randomized to receive a single escalating dose of bortezomib (1.0, 1.3, or 1.6 mg/m^2) either 24 hours before or 24 hours after Mel200. No severe adverse effects were reported, and the overall response rate was 87%, with 51% achieving a VGPR or better.

Overall, Mel200 is still considered the standard of care, but this regimen could be modified in the future based on the results of ongoing or planned phase III trials. The Spanish group has initiated a randomized study comparing Mel200 to IV busulfan + melphalan and the IFM group will in the near future commence a prospective trial designed to compare Mel200 to Bortezomib plus high-dose melphalan.

CONCLUSIONS

Recent studies examining novel agents as induction treatment prior to ASCT have shown that three-drug combinations are the standard of care. Nevertheless, no randomized trial has as yet prospectively compared one combination versus the others. Regarding the transplant phase, the available data confirm that Mel200 should continue to be considered the gold standard conditioning regimen for patients undergoing ASCT for MM. Nevertheless, avenues aimed at further improving response rates have been opened, and the most exciting areas of research involve the combinations of Mel200 with novel agents, such as bortezomib, or with other chemotherapeutic agents,

such as IV busulfan. These combinations warrant testing in future randomized trials.

REFERENCES

1. Moreau P, Avet-Loiseau H, Harousseau JL, Attal M. Current trends in autologous stem-cell transplantation for myeloma in the era of novel therapies. J Clin Oncol. 2011;29:1898–906.
2. Ladetto M, Pagliano G, Ferrero S, et al. Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma. J Clin Oncol. 2010;28:2077–84.
3. Cavo M, Pantani L, Petrucci MT, et al. Bortezomib-thalidomide-dexamethasone is superior to thalidomide-dexamethasone as consolidation therapy following hematopoietic stem cell transplantation in patients with newly diagnosed multiple myeloma. Blood. 2012;120: 9–19.
4. McCarthy PL, Owzar K, Hofmeister CC, et al. Lenalidomide after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366:1770–81.
5. Attal M, Lauwers-Cances V, Marit G, et al. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366:1782–91.
6. Attal M, Harousseau JL, Facon T, et al. Single versus double autologous stem-cell transplantation for multiple myeloma. N Engl J Med. 2003;349:2495–502.
7. Macro M, Divine M, Uzunhan Y, et al. Dexamethasone + thalidomide (Dex/Thal) compared to VAD as pretransplant treatment in newly diagnosed multiple myeloma (MM): a randomized trial. Blood. 2006;108: abstract 57.
8. Lokhorst HM, Schmidt-Wolf I, Sonneveld P, et al. Thalidomide in induction treatment increases the very good partial remission rate before and after high-dose therapy in previously untreated multiple myeloma. Haematologica. 2008;93:124–7.
9. Lokhorst HM, van der Holt B, Zweegman S, et al. A randomized phase 3 study on the effect of thalidomide combined with adriamycin, dexamethasone, and high-dose melphalan, followed by thalidomide maintenance in patients with multiple myeloma. Blood. 2010;115: 1113–20.
10. Morgan GJ, Davies FE, Owen RG, et al. Thalidomide combinations improve response rates; results from the MRC IX study. Blood. 2007;110: abstract 3593.
11. 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;28:4621–9.
12. Wang M, Giralt S, Delasalle K, Handy B, Alexanian R. Bortezomib in combination with thalidomide-dexamethasone for previously untreated multiple myeloma. Hematology. 2007;12:235–9.
13. Jakubowiak AJ, Kendall T, Al-Zoubi A, et al. Phase II trial of combination therapy with bortezomib, pegylated liposomal doxorubicin, and dexamethasone in patients with newly diagnosed myeloma. J Clin Oncol. 2009;27:5015–22.
14. Popat R, Oakervee HE, Hallam S, et al. Bortezomib, doxorubicin and dexamethasone (PAD) front-line treatment of multiple myeloma: updated results after long-term follow-up. Br J Haematol. 2008;141:512–6.
15. Richardson PG, Weller E, Lonial S, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood. 2010;116:679–86.
16. 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: 1337–41.
17. 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 randomized phase 3 study. Lancet. 2010;376:2075–85.
18. Rosiñol 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;120:1589–96.
19. Moreau P, Avet-Loiseau H, Facon T, et al. Bortezomib plus dexamethasone versus reduced-dose bortezomib, thalidomide plus dexamethasone as induction treatmentbeforeautologous stem cell transplantation in newlydiagnosed multiple myeloma. Blood. 2011;118: 5752–8.
20. Sonneveld P, Schmidt-Wolf I, van der Holt B, et al. Bortezomib induction and maintenance treatment in patients withnewlydiagnosed multiple myeloma: results of the randomized phase III HOVON-65/ GMMG-HD4 trial. J Clin Oncol. 2012;30:2946–55.
21. Jakubowiak AJ, Griffith KA, Reece DE, et al. Lenalidomide, bortezomib, pegylated liposomal doxorubicin, and dexamethasone in newly diagnosed multiple myeloma: a phase 1 / 2 multiple myeloma research consortium trial. Blood. 2011;118:535–43.
22. 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;119:4375–82.
23. Ludwig H, Viterbo L, Greil R, et al. Randomized phase II study of bortezomib, thalidomide, and dexamethasone with or without cyclophosphamide as induction therapy in previously untreated multiple myeloma. J Clin Oncol. 2013;31:247–55.
24. 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: 1801–9.
25. Kumar SK, Berdeja JG, Niesvizky R, et al. Phase 1/2 study of weekly MLN9708, an investigational oral proteasome inhibitor, in combination with lenalidomide and dexamethasone in patients with previously

untreated multiple myeloma. Blood. 2012;120: abstract 332.
26. Moreau P, Richardson PG, Cavo M, et al. Proteasome inhibitors in multiple myeloma : 10 years later. Blood. 2012;120:947–59.
27. Siegel DS, Jacobus S, Rajkumar SV, et al. Outcome with lenalidomide plus dexamethasone followed by early autologous stem cell transplantation in the ECOG E4A03 randomized clinical trial. Blood. 2010;116: abstract 38.
28. Cunningham D, Paz-Ares L, Milan S, et al. High-dose melphalan and autologous bone marrow transplantation as consolidation in previously untreated myeloma. J Clin Oncol. 1994;12:759–63.
29. Barlogie B, Alexanian R, Dicke KA, et al. High dose chemoradiotherapy and autologous bone marrow transplantation for resistant multiple myeloma. Blood. 1987;70:869–72.
30. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. N Engl J Med. 1996;335:91–7.
31. Björskstrand B. European Group for Blood and Marrow Transplantation Registry studies in multiple myeloma. Semin Hematol. 2001;38:219–25.
32. Lahuerta JJ, Martinez-Lopez J, Grande C, et al. Conditioning regimens in autologous stem cell transplantation for multiple myeloma: a comparative study of efficacy and toxicity from the Spanish registry for transplantation in multiple myeloma. Br J Haematol. 2000;109:138–47.
33. Moreau P, Facon T, Attal M, et al. Comparison of 200 mg/m^2 melphalan and 8Gy total body irradiation plus 140 mg/m^2 melphalan as conditioning regimen for peripheral blood stem cell transplantation in patients with newly diagnosed multiple myeloma :final analysis of the Intergroupe Francophone du Myelome 9502 trial. Blood. 2002;99:731–5.
34. Philips GL, Meisenberg BR, Reece DE, et al. Activity of single-agent melphalan 220 to 300mg/m^2 with amifostine cytoprotection and autologous hematopoietic stem cell support in non-Hodgkin and Hodgkin lymphoma. Biol Bone Marrow Transplant. 2004;10: 473–83.
35. Moreau P, Milpied N, Mahé B, et al. Melphalan 220mg/m^2 followed by peripheral stem cell transplantation in 27 patients with advanced multiple myeloma. Bone Marrow Transplant. 1999;23:1003–6.
36. Moreau P, Harousseau JL, Wijdenes J, Morineau N, Milpied N, Bataille R. A combination of anti-interleukin 6 murine monoclonal antibody with dexamethasone and high-dose melphalan induces high complete response rates in advanced multiple myeloma. Br J Haematol. 2000;109:661–4.
37. Carreras E, Rosinol L, Terol MJ, et al. Veno-occlusive disease of the liver after high-dose cytoreductive therapy with busulfan and melphalan for autologous blood stem cell transplantation in multiple myeloma patients. Biol Blood Marrow Transplant. 2007;13: 1448–54.
38. Lahuerta JJ, Mateos MV, Martinez-Lopez J, et al. Busulfan 12 mg/kg plus melphalan 140 mg/m^2 versus melphalan 200 mg/m^2 as conditioning regimens for autologous transplantation in newly diagnosed multiple myeloma patients included in the PETHEMA/ GEM2000 study. Haematologica. 2010;95:1913–20.
39. Blanes M, Lahuerta JJ, Gonzales JD, et al. Intravenous busulfan and melphalan as a conditioning regimen for autologous stem cell transplantation in patients with newly diagnosed multiple myeloma: a matched comparison to a melphalan-only approach. Biol Blood Marrow Transplant. 2013;19:69–74.
40. Giralt S, Bensinger W, Goodman M, et al. ^{166}Ho-DOTMP plus melphalan followed by peripheral blood stem cell transplantation in patients with multiple myeloma. Blood. 2003;102:2684–91.
41. Dispenzieri A, Wiseman GA, Lacy MQ, et al. A phase I study of (153) Sm-EDTMP with fixed high-dose melphalan as a peripheral blood stem cell conditioning regimen in patients with multiple myeloma. Leukemia. 2005;19:118–25.
42. Dispenzieri A, Wiseman GA, Lacy MQ, et al. A phase II study of (153) Sm-EDTMP and high-dose melphalan as a peripheral blood stem cell conditioning regimen in patients with multiple myeloma. Am J Hematol. 2010;85:409–13.
43. Mitsiades N, Mitsiades CS, Richardson PG, et al. The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. Blood. 2003; 101:2377–80.
44. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008; 359:906–17.
45. Roussel M, Moreau P, Huynh A, et al. Bortezomib and high-dose melphalan as conditioning regimen before autologous stem cell transplantation in patients with de novo multiple myeloma: a phase 2 study of the Intergroupe Francophone du Myélome (IFM). Blood. 2010;115:32–7.
46. Thompson PA, Prince HM, Seymour JF, et al. Bortezomib added to high-dose melphalan as pre-transplant conditioning is safe in patients with heavily pre-treated multiple myeloma. Bone Marrow Transplant. 2011;46:764–5.
47. Lonial S, Kaufman J, Tighiouart M, et al. A phase I/II trial combining high-dose melphalan and autologous transplant with bortezomib for multiple myeloma: a dose and schedule finding study. Clin Cancer Res. 2010;15:5079–86.

Evolving Strategies in the Initial Treatment of Multiple Myeloma

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Until the advents of novel agents, partial response (PR) or better was the established gold standard to initial therapy of multiple myeloma (MM), and treatment goals were focused on relieving symptoms, prevention of organ damage, and modest improvements in survival. With the introduction of autologous stem cell transplant (ASCT), deeper responses, including complete responses (CRs) were more frequent, and contributed to longer survival. In the era of novel therapies, ASCT remains commonly used and its impact on outcome appears superior, albeit less so than when compared with conventional therapy, and its survival benefit is yet to be established in either setting. In addition, in non-transplant candidates, novel therapies have now significantly improved the overall response rates, depth of response, and clinical benefit, to the levels previously only observed with ASCT, which now increasingly challenges the role and timing of ASCT in eligible patients. Nevertheless, the two approaches of treatment, transplant or no transplant, remain commonly accepted. With an improvement in the tolerability of newer regimens and the deferral of ASCT in transplant candidates, the debate has emerged whether the two-pathway approach to the treatment of newly diagnosed myeloma should be re-evaluated. At the same time, treatment goals are also shifting. Many believe that MM can be converted into a chronic disease and that a functional cure maybe a realistic goal, for at least a proportion of patients. This contribution will review these points of discussion and the evolving approach to treatment of newly diagnosed MM.
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EVOLVING ROLE OF AUTOLOGOUS STEM CELL TRANSPLANT IN THE ERA OF NOVEL AGENTS

The role and timing of high-dose chemotherapy with single or tandem autologous stem cell transplant (ASCT) as consolidation of initial treatment of multiple myeloma (MM) in the era of conventional therapy, as well as outcome from studies with ASCT in the era of novel agents, are reviewed in more detail by Drs Moreau and Touzeau in this issue of *Seminars in Oncology*.

Transplant studies in MM patients who received induction with novel agents provide clear evidence that consolidation with high-dose melphalan and ASCT will further improve the depth of response by increasing rates of very good partial response (VGPR) and complete response (CR). The latter convey clinical benefit as reflected by improved progression-free survival (PFS), although no difference in overall survival (OS) has been seen to date. Importantly, difference in pretransplant VGPR/CR rates between three-drug novel induction regimens and two-drug novel regimens remains statistically

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significant after ASCT,[1,2] suggesting that selection of more active induction regimens will result in higher rates of CR and VGPR after transplant. However, in view of the lack of evidence that transplant improves OS and with mounting evidence from recent studies that extended treatment with novel regimens without transplant can result in similar overall survival and with less toxicity,[3–5] the role of transplant has been challenged. In the context of these studies, which provided delayed transplant as an option, the critical question has emerged as to whether all or only a subgroup of patients may benefit from ASCT and if so, where in the treatment sequence should transplant be incorporated.

Retrospective analyses and non-randomized trials have been informative but not definitive. In a large, retrospective analysis of 290 newly diagnosed MM patients treated with immunomodulatory drug (IMiD)-based induction therapy, there was no OS difference at 4 years between those who underwent early versus late transplant, where the cut-off for transplant was set at 12 months.[6] In a post hoc analysis of the Eastern Cooperative Oncology Group (ECOG) E4A03 clinical trial which randomized patients to lenalidomide plus low-dose versus high-dose dexamethasone,[7] the survival for those who underwent early transplant appeared higher among all age groups, including those over the age of 70.[8] On the other hand, in the phase II portion of the trial with lenalidomide, bortezomib, and dexamethasone (RVD), which achieved a remarkable 100% rate of PR or better in both the phase I and phase II portions of the study, similar rates of PFS and OS were observed for patients undergoing transplant versus those who did not at 18 months of follow-up.[5] Likewise, the phase I/II trial of carfilzomib, lenalidomide, and dexamethasone (CRd), which was designed to continue extended CRd treatment also in transplant candidates, showed not only very high response rates with a 60% rate of stringent CRs but also a favorable PFS of 94% at 24 months of follow-up.[4,9] The results of these two trials support the notion that prolonged treatment with a combination of a proteasome inhibitor and IMiDs is not only feasible but may prolong disease-free survival and provide outcomes similar to that observed with transplant used as intensification. However, these promising results were generated in small, single-arm trials, with relatively short follow-up, and their results should be viewed as hypothesis-generating, rather than as evidence that ASCT is not needed. In addition, there is still a question whether durability of CR achieved with novel regimens is as good as CR achieved with high-dose melphalan and ASCT, with many believing that even in the era of active regimens such as RVD and CRd, patients may benefit further from transplant used as intensification. On this assumption, a CRd trial is currently enrolling transplant candidates to receive CRd induction for four cycles followed by ASCT followed by an extended CRd treatment as in the original CRd trial design.

Moreover, a definite answer to the timing of ASCT in the treatment of MM is anticipated to come from the Dana Farber Cancer Group and the Inter-Groupe Francophone du Myeloma (IFM) international trial, which is designed to evaluate the role of early versus delayed transplant incorporating RVD consolidation and maintenance. Specifically, based on the results of two independent RVD trials,[5,10] patients receive RVD induction followed by randomization to transplant followed by RVD consolidation followed by lenalidomide maintenance versus extended RVD treatment followed by lenalidomide maintenance. The primary endpoint of the study is PFS, with secondary endpoints including OS, response rate, toxicity, and quality of life, as well as cost assessment. In addition, incorporated into the study design is extensive correlative science which may help determine whether certain subgroups of patients could be identified as not needing transplant early, or, conversely those who might benefit from initial use of transplant.

A similar question was asked in the recently completed randomized phase III trial conducted by Palumbo and colleagues.[11] In this trial, transplant eligible patients received four cycles of induction therapy with lenalidomide plus low-dose dexamethasone (Rd), following which they were randomized to either consolidation with six cycles of melphalan, prednisone, and lenalidomide (MPR) or tandem ASCT. Each arm then underwent a second randomization to lenalidomide maintenance versus observation. At a median follow-up of 45 months, results demonstrated a superior PFS in the transplant arm compared to conventional treatment. Although there was no OS benefit in the transplant group, it is likely that longer follow-up is needed to detect this difference. Nevertheless, the study appears to support a role for ASCT in the era of novel agents but is limited by the absence of a proteasome inhibitor in either arm. Moreover, the results are perhaps not surprising, as MPR is not as effective as other current novel regimens, and that alternative novel agent-based therapies such as RVD or CRd may be better choices than MPR for non–transplant-based therapy.

The Role of Transplant in the Era of Novel Agents–The Bottom Line

In view of these results and other trials, ASCT remains part of the standard of care for treatment of MM in younger patients and should be considered in all transplant-eligible patients. While more myeloma specialists are now adopting the practice of early stem cell collection and deferring transplant until the time of relapse in standard-risk patients, this should ideally be

done in the context of a clinical trial as the evidence for this approach remains an area of active research. The decision for early transplant is influenced by different factors, including patient's age, tolerability of the induction regimen, potential toxicities of extended treatment, depth of response to initial therapy, comorbidities, and patient's preference. In the current era of personalized therapy, it is likely we soon will be able to identify subgroups of patients who may benefit from early transplant or those in which transplant may not be necessary or can reasonably be delayed. The evaluation of minimal residual disease by multiparametric flow cytometry and possibly by other methods, including polymerase chain reaction (PCR), may become an increasingly useful tool in this context, and is discussed in more detail subsequently.

THE ROLE OF DEPTH OF RESPONSE IN INITIAL TREATMENT

Initially with ASCT and then with the emergence of more active and better tolerated novel regimens alone and in combination with ASCT, the ability to achieve CRs has progressively increased over time. Since the achievement of deeper responses appears to be associated with improved clinical benefit, a link between rate of CR and long-term outcome has been proposed. Formal analyses have showed statistical correlation between rates of CR and PFS in a number of studies and with OS in a smaller number of reports. Recently, some investigators have proposed a shift of treatment goals to overall strategies which give the best chance for the achievement of the deepest and most durable responses in both ASCT and non-ASCT patients. However the issue is far from settled and some of the controversies stem not least from different definitions of CR used in prior studies,[12,13] now mostly resolved with the acceptance of the consensus International Myeloma Working Group (IMWG) response criteria. These include a new category of stringent CR (sCR), which builds upon the original CR and near CR (nCR) used in the modified European Bone Marrow Transplant (EBMT) criteria.[14,15] In addition, lack of consistency in how to take into account time and duration of achievement of CR may have contributed to some of the controversy.[16] This section will review the role of CR as a predictor and as a surrogate marker of response to therapy.

The Role of CR in the Era of Conventional Therapy

The importance of achieving a CR in newly diagnosed multiple myeloma (NDMM) patients had already been proposed in the era of conventional therapy. Although varying definitions of CR were used in prior studies,[12,13] this association seemed evident in the setting of ASCT with only one large non-transplant study demonstrating a survival benefit for patients who achieved CR[17] while no association in others.[18–20] This lack of correlation in the non-transplant setting is not surprising given the low rates of CR seen with conventional drugs. In the setting of ASCT consolidation, however, increased CR rates post-transplant translated in multiple analyses into longer PFS,[12,21] and in some retrospective studies, into superior OS.[22–24] In a large meta-analysis reporting outcomes of 4,990 ASCT patients, there was a clear association between maximal response (CR/nCR/VGPR) post-SCT and OS and event-free survival (EFS)/PFS.[24]

The Role of CR in the Era of Novel Agents

CR in Patients Receiving ASCT

There is extensive evidence in NDMM treated with novel-based induction regimens including thalidomide (THAL), bortezomib (BTZ), and lenalidomide (LEN) followed by ASCT with or without consolidation that CR achievement post-SCT improves survival.[25–31] Although novel agent–based regimens yield high pretransplant VGPR and CR rates, the correlation of post-induction response with outcome, especially improved OS, has been mostly reported based on outcome from single-arm prospective and retrospective series.[24,32,33] Probably the best evidence comes from analysis of randomized trial IFM 2005-01 of induction treatment with VAD versus VD followed by transplant, which showed a correlation between VGPR and PFS. Regardless of type of induction, but also the achievement of VGPR prior to ASCT had greater impact on PFS than the achievement of VGPR post-SCT.[34]

There are more randomized studies in which post-SCT CR achievement was associated with EFS and OS benefit, including TT2 with randomization to THAL,[35] and the PETHEMA study, which compared BTZ/THAL/DEX (VTD) versus TD versus VBMCP/VBAD/B. In the latter, VTD induction resulted in a higher pre- and post-SCT CR and a significantly longer PFS. Correlation between post-induction CR and survival was not reported.[27] Similarly, in the GIMEMA phase III trial assessing VTD versus TD induction followed by double ASCT, and then VTD versus TD consolidation resulted in sequential improvement of CR in the VTD versus the TD arm; this correlated with longer PFS. Again, an impact of post-induction responses was not reported.[26] Using landmark analysis, HOVON reported that the achievement of CR was superior with novel induction regimens with THAL, doxorubicin (DOX), and DEX (TAD) and BTZ, DOX, and DEX (PAD), but not VAD.[28,29] A meta-analysis of data pooled from the PETHEMA, GIMEMA, HOVON-65/GMMG-HD4, and

IFM 2005-01 trials discussed above demonstrated improved CR and VGPR rates with BTZ-DEX–containing regimens that were associated with longer PFS, if achieved at 4 months post-SCT but not post-induction.[36]

On the other hand, the results from several trials cast a doubt on significance of achievement of CR, either pre- and/or post-transplant. The previously described IFM 2005-01 trial of induction with VAD versus BTZ-DEX (VD) failed initially to show statistical correlation between depth of response and survival.[37] However, later updates from this study showed statistically significant correlation with both pre- and post-transplant VGPR.[34] Another landmark study of vTD versus VD showed statistically higher CR rates in the three- versus two-drug regimen; however, there was no statistical difference in PFS and OS between two arms, likely related to the relatively short follow-up.[2]

CR in Transplant-Ineligible Patients

Novel drugs have been incorporated into the treatment of non-transplant candidates, most commonly in association with melphalan-prednisone (MP), resulting consistently in higher overall response rates, and rates of CR, PFS, and OS, as reviewed by Cerrato and Palumbo in this issue of *Seminars*. Initially, because some of MPT versus MP studies showed no difference in PFS and OS, despite higher CR rates in MPT arms, some questioned whether CR rate is a good surrogate endpoint for survival in transplant-ineligible patients. Subsequently, the first evidence of role of depth of response in transplant-ineligible patients came from the phase III VISTA trial, which demonstrated that VMP is superior to MP. In this study, the achievement of CR versus PR by modified EBMT criteria in the VMP arm was associated with significantly longer time to progression (TTP) but no significant difference in OS.[38] Furthermore, TTP was similarly prolonged among patients achieving CR versus VGPR by IMWG criteria. Interestingly, CR duration appeared similar among patients achieving early CR before cycle 5 and later during VMP treatment.[38] In a more recent phase III trial, which evaluated the four-drug regimen of VMP plus THAL (VMPT) followed by maintenance with BTZ-THAL (VMPT-VT) compared with VMP alone in untreated elderly patients, a higher CR rate in the VMPT-VT arm was associated with statistically significant prolongation of PFS, and per a recent update, prolongation of OS.[39,40] However, there was no formal analysis of outcome based on depth of response. In a phase III trial in the elderly of reduced-intensity induction with BTZ, THAL, and prednisone (VTP), compared with BTZ, melphalan, and prednisone (VMP) followed by BTZ-based maintenance,[41] immunophenotypic (IP) remissions, representing MRD-negative disease in a subset of patients with CR, were associated with better outcome. Median PFS was not reached in the MRD (-) cases compared with 31 months for MRD (+) cases. No formal analysis was presented for patients achieving or not achieving CR. However, an estimated 3-year PFS of was 90% for those in IP remission, but only 39% for CR, 34% for nCR, and 29% for PR. Finally, a meta-analysis performed to assess the impact of CR on PFS and OS in 1,175 elderly patients enrolled in the prospective non-transplant studies treated with the regimens MP, MPT, VMP, or VMPT showed an OS benefit for patients who achieved CR.[42] Patients who achieved CR versus VGPR had higher rates of 3-year PFS (67% *v* 27%) and OS (91% *v* 70%). Similar benefit was seen in patients older than 75 years of age, supporting the use of CR as a surrogate endpoint for long-term outcome in the elderly, despite concerns from MPT studies. LEN-based regimens have not been included in this meta-analysis, in part because of lower CR rates in the elderly compared to other BTZ-based combinations. Thus, there is limited information on correlation between CR and time to event from studies with LEN in the non-transplant setting. In a phase I/II study of LEN plus MP in the elderly, 1-year EFS and OS were 92% and 100%, for all patients, although EFS appeared longer in those achieving ≥VGPR. Correlation with CR was not reported. The evidence from randomized trials is lacking. In untreated patients 65 years or younger assigned to MP, MPR, or MPR followed by LEN maintenance (MPR-R), induction arms with LEN yielded higher VGPR (29.4% *v* 9.1%) and longer median duration of CR (31 *v* 22 months).[43] However, at 30 months median follow-up, despite more than a tripling of the rate of VGPR, MPR without LEN maintenance did not improve median PFS or 3-year OS versus MP. Given the small numbers of CR attained, induction alone without continued therapy was not enough to impact time to event, as the only arm with an improved PFS was MPR followed by LEN maintenance.

Recent Frontline Regimens With Superior Depth and Duration of Response

A number of recent phase I/II studies in NDMM, which enrolled both transplant-eligible and -ineligible patients, reported superior rates of nCR/CR and CR/sCR reaching 80% and 60%, respectively, which are dramatically better than results seen historically in similar patient populations. In the phase I/II study of LEN, BTZ, and DEX (RVD) for NDMM, response rates of 57% ≥nCR and 74% ≥VGPR were associated with a PFS rate 75% at 18 months,

and an overall response rate of 100% PR or better for all evaluable patients (n = 66).[5] In a large randomized phase II trial (EVOLUTION), in which three- and four-drug combinations including LEN (R), BTZ (V), DEX (D), and cyclophosphamide (C) were assessed in NDMM, patients were assigned to VDCR, VDR (reflecting a different dose and schedule of DEX compared to RVD), VCD, and VCD-modified followed by maintenance V in all arms.[44] Although CR/VGPR rates and the corresponding 1-year PFS were not statistically different between arms, PFS appeared to reflect high rate of deep responses in all arms. Interestingly, the best performing regimens in this study from the standpoint of both outcome and minimal residual disease proved to be VRD and VCD modified. Most recently, a phase I/II study assessed carfilzomib (CFZ), LEN, and DEX (CRd) in NDMM patients, again including both transplant-eligible and -ineligible patients.[4] After a median of 12 cycles, 62% of patients achieved at least nCR and 42% sCR, and in 36 patients completing eight or more cycles, 78% reached at least nCR and 61% sCR. At a median 22 months of CRd treatment, the response rates further increased, including sCR at 55%. After a median of 25 months of follow-up these high CR rates are associated with an exceptional 2-year PFS of 94%, with OS of 98%.[9]

These results, although not from randomized trials, further support a notion that an improved depth of response may yield superior PFS and OS, for RVD and CRd clearly exceed historical standards. Certainly, these encouraging results need to be validated in larger randomized trials, including a soon to start comparison of CRd versus RVD phase III trial as part of a cooperative group effort. Nevertheless, it can be concluded that a combination of an active regimen with long-term tolerability, allowing for prolonged treatment, may yield both high rates of overall response as well as CR and time to event benefit that equal or surpass that of historical results from studies with sequential therapy including induction with an active novel regimen, followed by ASCT, and post-SCT consolidation and/or maintenance.

Role of CR in the Relapsed/Refractory Setting

The impact of achievement of CR on survival also appears to be important in the relapsed setting, mostly since the introduction into clinical practice of novel agents, which in turn may further validate the importance of this parameter in NDMM. In the APEX trial comparing BTZ with high-dose DEX, CR rates were associated with a longer median treatment-free interval and time to alternative therapy versus VGPR/PR and there was a trend towards longer TTP and OS.[45] In a pooled analysis of the MM-009 and MM-010 phase III studies of LEN/DEX versus high-dose DEX, achievement of CR/VGPR was associated with improved response duration and TTP, but prolonged OS was only seen when comparing CR to PR.[46,47] At 48 months follow-up, the median response duration, TTP, and OS were longer in patients with CR/VGPR than in those with PR.[38] In a recently published trial, time to progression was significantly longer with VTD than TD in relapsed myeloma and was associated with higher CR/nCR rate (45% *v* 25%; $P = .001$). At 24 months there was a trend to also longer OS in VTD versus TD arm (71% *v* 65%; $P = .093$).[48] It is anticipated that the results from a number of ongoing randomized trials in relapsed myeloma, including results from the ASPIRE trial of CRd versus Rd, may generate additional information on the correlation between the depth of response and time to event in this setting.

Limitations of CR

The impact of tumor biology. Attainment of CR is not only a function of drug activity but also of tumor biology.[16] Few clinical predictors of CR have been previously identified in the context of specific clinical trials. IgA isotype, elevated lactate dehydrogenase (LDH) levels, and the presence of cytogenetic abnormalities have been associated with higher CR rates and paradoxically shorter OS.[25] This is likely reflective of higher tumor proliferative activity with greater sensitivity to cytotoxic chemotherapy but subsequently rapid relapse rates. The Arkansas group demonstrated that importance of achieving CR may differ between standard versus high-risk disease. Gene expression profile (GEP) data from 668 uniformly treated patients on TT2 showed a correlation of CR with prolonged EFS and OS, whereas patients with low-risk disease had similar survival regardless of CR attainment.[49]

CR duration. More recently, it has been shown that not only achievement but also durability of CR is predictive of outcomes. A retrospective analysis of three phase III trials of alkylator-based induction in transplant-ineligible patients demonstrated that duration of response from initial therapy was a major predictor of OS.[50] This also has been demonstrated by the Arkansas group, both in the context of TT2 and TT3. In TT2, a 3-year sustained CR after start of treatment was associated with prolonged OS compared to patients who never achieved CR or achieved CR but relapsed within 3 years of starting therapy.[51] In TT3, the addition of BTZ was associated with improved CR durability and improved EFS and a trend toward improved OS compared to TT2 with or without THAL.[52] CR rates post-SCT were similar between the two studies; however, the 2-year sustained CR rate was superior (91% *v* 81%) in TT3

versus TT2, which translated into improved EFS and a trend toward improved OS. A landmark analysis revealed that both failure to achieve CR and, especially, loss of CR were independently associated with inferior survival.[53] Furthermore, data from TT2 also indicate that durability of CR rather than CR achievement may be a better predictor of PFS/OS in high-risk patients.[54] Thus, in high-risk disease, CR may not be a surrogate marker for an improved outcome and in this group of patients the ultimate strategy should be focused on maintaining CR.[16]

MRD as an emerging predictor of outcome. The role, significance, and assessment of MRD in MM are evolving rapidly. As association between depth of response and long-term outcomes appears to be clearly emerging, the need to examine patients beyond sCR level of response is valid. Although the optimal measurement tool or combination of tools that is also clinically feasible remains to be defined prospectively, multi-parametric flow (MPF) and molecular response (MR) by PCR are approaching the forefront. Identification of MRD by MFC has been widely demonstrated to be of clinical importance and may soon be incorporated into the routine evaluation of all patients. This is supported by several large phase III trials that demonstrate a correlation between MRD (-) disease at 100 days post-ASCT and survival.[55–57] In one study, MRD(-), immunofixation (IF)(-) patients and MRD(-), IF(+) patients had significantly longer PFS than MRD(+), IF (-) patients suggesting that MPF may be a more sensitive method than immunofixation for detection of residual clones and have stronger correlation with outcomes.[57] In another study, MRD (+) disease in addition to cytogenetics at 100 days post-ASCT was predictive of loss of CR status.[58] Several trials in the elderly also have demonstrated that immunophenotypic CR by MPF was the strongest predictor of TTP, PFS,[59] and OS.[41]

MR with PCR assessment of immunoglobulin gene rearrangements for MRD also has been examined in the context of prospective studies and in general has been considered to be more sensitive than MPF. However, when compared directly in a group of 130 patients who achieved CR/VGPR post-induction or SCT on the GEM2000/2005 trial, MR provided similar prognostic value to IP remissions.[60] More recently, comparable results for MPF and MR-based evaluation of MRD were shown, with slightly higher proportion of patients recorded as MRD(-) by PCR-based methods.[61]

While these methods have already proved to be feasible surrogate markers and are soon to become primary endpoints in clinical trials, we still need to overcome several challenges before incorporating them into clinical practice. While MPF may be more applicable in the clinical setting, and has already been reported as feasible in a number of European studies,[62] it is currently only available in several centers in the United States and its techniques have not yet been standardized among the different institutions. Furthermore, the data provided by MPF are highly time-dependent, in addition to variability of the quality of the aspirate. Because of these constraints, PCR-based techniques may eventually emerge as superior for evaluation of MRD. Finally, the ultimate role of MRD assessment should not be limited to predicting treatment outcome, but ultimately to aide in monitoring of the disease and detection of early relapse, which still needs additional and prospective validations.

The Role of CR—Conclusion

In summary, CR has been associated with improved outcomes in NDDM, both in the setting of ASCT and in non-transplant candidates and also in relapsed/refractory myeloma. Its association at any point of initial treatment with PFS is very strong. Although it is still not clear how important is the depth of response prior to transplant, studies to date suggest that selecting an induction regimen with high probability of achieving CR is important. On the other hand, there is no evidence to support a change of initial choice of induction treatment in an attempt to achieve pre-transplant VGPR, nCR, or CR, despite reports of association of pretransplant depth of response with improved time to event. Furthermore, it is not established whether the achievement of CR has the same prognostic value if attained with novel regimens alone versus with contribution of ASCT. There is also not enough evidence for CR to be used as a surrogate endpoint for PFS in NDDM, given that duration of CR, rather than achievement of CR, may better predict survival. With the development of more sophisticated methods for detection of MRD, attention may soon shift beyond CR/sCR to MRD as an endpoint. At this time, there are not enough data to use MRD as a surrogate endpoint of treatment or as a substitute for cure. However, rapid progress in this area is being made and we can anticipate that MRD may soon be accepted as a surrogate endpoint, which may hasten advances towards discovering a cure.

NON-TRANSPLANT AND TRANSPLANT TREATMENT PATHWAYS: ARE WE READY TO SHIFT TO A DIFFERENT PARADIGM?

In the era of conventional therapy where response rates to induction therapy were poor, and the goal of treatment was achievement of PR and prevention of organ damage, patients were stratified to those who are transplant eligible and consolidated with high-dose melphalan/SCT and those who were transplant ineligible and received oral melphalan

with prednisone (MP). Therefore, efforts to approve new agents in newly diagnosed myeloma were mostly focused on the non-transplant population. Because MP was considered a standard of care in the pre–novel agent era, many studies in non-transplant candidates were designed to compare MP plus novel agent versus MP alone. Combination regimens such as melphalan-prednisone-thalidomide (MPT), bortezomib-melphalan-prednisone (VMP), and melphalan-prednisone-lenalidomide followed by lenalidomide maintenance (MPR-R), have now proved to be superior to MP in large randomized phase III trials (for details see Ceratto and Palumbo in this issue). To improve further outcome beyond novel triplet regimens with MP–backbone, two novel agents were added to MP (VMPT) followed by bortezomib and thalidomide maintenance (VT). The study has shown higher response rates, PFS, and, at recent update, also OS when compared to VMP.[39,40] Although these results show an improvement compared to triplet MP-based regimens, it appears that median PFS and OS is still shorter than the best results achieved with strategies involving transplant in younger patients. Is this because of transplant or because we are limiting our evaluations in non-transplant patients to MP-based regimens? What if we use established non-MP based regimens such as VD, Rd, VDT, RVD, or CRd in transplant-ineligible patients? We already have learned that VD has comparable activity, PFS, and OS to VMP from a completed randomized study conducted in the US community-based oncology centers.[63] Comparison of MPT with Rd, which has completed enrollment, awaits final reports. If this study shows at least comparability of Rd to MPT, there would be opportunity to use Rd in place of MP-based regimen as comparator in upcoming randomized trials in non-transplant candidates. But even if both VD and RD are established as comparable to MP-based novel triplets, an argument can be made to also use three-drug regimens in the elderly, with the presumption that these regimens may be less tolerated in this population of patients. And indeed, outcome in the VDT arm was not different from that in the VD arm in a randomized study in elderly patients in the community setting.[64] However, if we bring more active, and at the same time better tolerated regimens to the elderly, this could improve the outcome in this population as well. The results from the RVD trial[5] and from the CRd trial[4] in patients aged 65 years and older[65] indicate that we can do better in this patient population. It is possible that with such regimens, randomized trials in NDMM can be designed for all age groups, stratified by transplant eligibility, and break from the current practice of separate studies for transplant and non-transplant candidates.

SUMMARY AND FUTURE DIRECTIONS

The achievement of CR to initial therapy in multiple myeloma is now possible in the majority of patients due to more effective targeted agents and integration of extended sequential therapy to the treatment algorithm. Furthermore, attainment of CR at any time point during treatment in both transplant eligible and ineligible patients is associated with improved outcome; it thus is likely to be established as a goal of therapy in all patients with NDMM. Because of significant improvement in outcomes and achievement of high rates of CR without transplant, the role of ASCT is being re-evaluated. Although ASCT still may be needed and can be considered a standard of care in all eligible patients, we need to establish whether to perform ASCT in all eligible patients early in the course of therapy or in a subgroup of patients with defined characteristics. Rational, biologically derived combination approaches with novel agents incorporating newer drugs and first in class antibodies provide the prospect of yet further improvements in outcome.[66,67] In addition, as we refine the definition of CR by incorporating modern methodologies for detection of MRD in those in clinical CR and improve our surrogate endpoints, in the near future we may be able to identify patients who are possibly fundamentally cured (ie, enjoy durable CR lasting many years) and in whom treatment may be tailored accordingly.

REFERENCES

1. 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;376:2075–85.
2. 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;118:5752–8.
3. Rajkumar SV, Hayman SR, Lacy MQ, et al. Combination therapy with lenalidomide plus dexamethasone (Rev/Dex) for newly diagnosed myeloma. Blood. 2005;106:4050–3.
4. 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;120:1801–9.
5. Richardson PG, Weller E, Lonial S, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood. 2010;116:679–86.
6. Kumar SK, Lacy MQ, Dispenzieri A, et al. Early versus delayed autologous transplantation after immunomodulatory agents-based induction therapy in patients

with newly diagnosed multiple myeloma. Cancer. 2012;118:1585–92.
7. 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.
8. Siegel DSd, Jacobus S, Rajkumar SV, et al. Outcome with lenalidomide plus dexamethasone followed by early autologous stem cell transplantation in the ECOG E4A03 randomized clinical trial. ASH Annual Meeting Abstracts. 2010;116:38.
9. Jakubowiak AD, Griffith KA. Treatment outcome with the combination of carfilzomib, lenalidomide, and low-dose dexamethasone (CRd) for newly diagnosed multiple myeloma (NDMM) after extended follow-up. J Clin Oncol. 2013;31(supp; absrt)8543.
10. Roussel M, Avet-Loiseau H, Moreau P, et al. Frontline therapy with bortezomib, lenalidomide, and dexamethasone (VRD) induction followed by autologous stem cell transplantation, VRD consolidation and lenalidomide maintenance in newly diagnosed multiple myeloma patients: primary results of the IFM 2008 phase II study. ASH Annual Meeting Abstracts. 2010;116:624.
11. Boccadoro M, Francesca MG, Di Raimondo F, et al. Melphalan/prednisone/lenalidomide (MPR) versus high-dose melphalan and autologous transplantation (MEL200) plus lenalidomide maintenance or no maintenance in newly diagnosed multiple myeloma (MM). J Clin Oncol. 2013;31 (suppl; abstr 8509).
12. 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;335:91–7.
13. 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;102:1115–23.
14. Durie BG, Harousseau JL, Miguel JS, et al. International uniform response criteria for multiple myeloma. Leukemia. 2006;20:1467–73.
15. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med. 2003;348:2609–17.
16. Rajkumar SV, Gahrton G, Bergsagel PL. Approach to the treatment of multiple myeloma: a clash of philosophies. Blood. 2011;118:3205–11.
17. Kyle RA, Leong T, Li S, et al. Complete response in multiple myeloma: clinical trial E9486, an Eastern Cooperative Oncology Group study not involving stem cell transplantation. Cancer. 2006;106:1958–66.
18. Blade J, Lopez-Guillermo A, Bosch F, et al. Impact of response to treatment on survival in multiple myeloma: results in a series of 243 patients. Br J Haematol. 1994;88:117–21.
19. Oivanen T, Kellokumpu-Lehtinen P, Koivisto AM, et al. Response rate and survival after conventional chemotherapy for multiple myeloma by hospitals with different inclusion rates of patients to the trials. A Finnish Leukemia Group study. Eur J Haematol. 1999;63: 225–30.
20. Durie BG, Jacobson J, Barlogie B, et al. Magnitude of response with myeloma frontline therapy does not predict outcome: importance of time to progression in Southwest Oncology Group chemotherapy trials. J Clin Oncol. 2004;22:1857–63.
21. Child JA, Morgan GJ, Davies FE, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J Med. 2003;348:1875–83.
22. Lahuerta JJ, Mateos MV, Martinez-Lopez J, et al. Influence of pre- and post-transplantation responses on outcome of patients with multiple myeloma: sequential improvement of response and achievement of complete response are associated with longer survival. J Clin Oncol. 2008;26:5775–82.
23. Wang M, Delasalle K, Feng L, et al. CR represents an early index of potential long survival in multiple myeloma. Bone Marrow Transplant. 2010;45:498–504.
24. van de Velde HJ, Liu X, Chen G, et al. Complete response correlates with long-term survival and progression-free survival in high-dose therapy in multiple myeloma. Haematologica. 2007;92:1399–406.
25. Barlogie B, Tricot G, Rasmussen E, et al. Total therapy 2 without thalidomide in comparison with total therapy 1: role of intensified induction and posttransplantation consolidation therapies. Blood. 2006;107:2633–8.
26. Cavo M, Pantani L, Petrucci MT, et al. Bortezomib-thalidomide-dexamethasone is superior to thalidomide-dexamethasone as consolidation therapy after autologous hematopoietic stem cell transplantation in patients with newly diagnosed multiple myeloma. Blood. 2012;120: 9–19.
27. 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;120:1589–96.
28. Lokhorst HM, van der Holt B, Zweegman S, et al. A randomized phase 3 study on the effect of thalidomide combined with adriamycin, dexamethasone, and high-dose melphalan, followed by thalidomide maintenance in patients with multiple myeloma. Blood. 2010;115: 1113–20.
29. 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;30:2946–55.
30. Chanan-Khan AA, Giralt S. Importance of achieving a complete response in multiple myeloma, and the impact of novel agents. J Clin Oncol. 2010;28: 2612–24.
31. Terpos E, Apperley JF, Samson D, et al. Autologous stem cell transplantation in multiple myeloma: improved survival in nonsecretory multiple myeloma but lack of influence of age, status at transplant, previous treatment and conditioning regimen. A single-centre experience in 127 patients. Bone Marrow Transplant. 2003;31:163–70.
32. Jakubowiak AJ, Kendall T, Al-Zoubi A, et al. Phase II trial of combination therapy with bortezomib,

pegylated liposomal doxorubicin, and dexamethasone in patients with newly diagnosed myeloma. J Clin Oncol. 2009;27:5015–22.
33. Dytfeld D, Griffith KA, Friedman J, et al. Superior overall survival of patients with myeloma achieving very good partial response or better to initial treatment with bortezomib, pegylated liposomal doxorubicin, and dexamethasone, predicted after two cycles by a free light chain- and M-protein-based model: extended follow-up of a phase II trial. Leuk Lymphoma. 2011;52:1271–80.
34. Moreau P, Attal M, Pegourie B, et al. Achievement of VGPR to induction therapy is an important prognostic factor for longer PFS in the IFM 2005-01 trial. Blood. 2011;117:3041–4.
35. Barlogie B, Tricot G, Anaissie E, et al. Thalidomide and hematopoietic-cell transplantation for multiple myeloma. N Engl J Med. 2006;354:1021–30.
36. Sonneveld P, Goldschmidt H, Rosinol L, et al. Bortezomib (btz) versus non-btz-based induction prior to ASCT in multiple myeloma (MM): a meta-analysis of phase 3 trials. Clin Lymphoma Myeloma Leuk. 2013;13 (suppl 1):O–11.
37. 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;28:4621–9.
38. Harousseau JL, Palumbo A, Richardson PG, et al. Superior outcomes associated with complete response in newly diagnosed multiple myeloma patients treated with nonintensive therapy: analysis of the phase 3 VISTA study of bortezomib plus melphalan-prednisone versus melphalan-prednisone. Blood. 2010;116:3743–50.
39. Palumbo A, Bringhen S, Rossi D, et al. Bortezomib-melphalan-prednisone-thalidomide followed by maintenance with bortezomib-thalidomide compared with bortezomib-melphalan-prednisone for initial treatment of multiple myeloma: a randomized controlled trial. J Clin Oncol. 2010;28:5101–9.
40. Palumbo AB, Bringhen S, Rossi S, et al. Overall survival benefit for bortezomib-melphalan-prednisone-thalidomide followed by maintenance with bortezomib_thalidomide (VMPT_VT) versus bortezomib-melphalan-prednisone (VMP) in newly diagnosed multiple myeloma patients. Blood (ASH Annual Meeting Abstracts). 2012;120:200.
41. Mateos MV, Oriol A, Martinez-Lopez J, et al. Bortezomib, melphalan, and prednisone versus bortezomib, thalidomide, and prednisone as induction therapy followed by maintenance treatment with bortezomib and thalidomide versus bortezomib and prednisone in elderly patients with untreated multiple myeloma: a randomised trial. Lancet Oncol. 2010;11:934–41.
42. Gay F, Larocca A, Wijermans P, et al. Complete response correlates with long-term progression-free and overall survival in elderly myeloma treated with novel agents: analysis of 1175 patients. Blood. 2011;117:3025–31.
43. Palumbo A, Hajek R, Delforge M, et al. Continuous lenalidomide treatment for newly diagnosed multiple myeloma. N Engl J Med. 2012;366:1759–69.
44. 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: 4375–82.
45. Niesvizky R, Richardson PG, Rajkumar SV, et al. The relationship between quality of response and clinical benefit for patients treated on the bortezomib arm of the international, randomized, phase 3 APEX trial in relapsed multiple myeloma. Br J Haematol. 2008;143:46–53.
46. Weber DM, Chen C, Niesvizky R, et al. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. N Engl J Med. 2007;357:2133–42.
47. Dimopoulos M, Spencer A, Attal M, et al. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. N Engl J Med. 2007;357:2123–32.
48. Garderet L, Iacobelli S, Moreau P, et al. Superiority of the triple combination of bortezomib-thalidomide-dexamethasone over the dual combination of thalidomide-dexamethasone in patients with multiple myeloma progressing or relapsing after autologous transplantation: the MMVAR/IFM 2005-04 randomized phase III trial from the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol. 2012;30:2475–82.
49. Haessler J, Shaughnessy JD Jr, Zhan F, et al. Benefit of complete response in multiple myeloma limited to high-risk subgroup identified by gene expression profiling. Clin Cancer Res. 2007;13:7073–9.
50. Kumar S, Zhang L, Dispenzieri A, et al. Response duration with initial therapy is a major predictor of overall survival in multiple myeloma: analysis from multiple phase III ECOG clinical trials [abstract]. Blood (ASH Annual Meeting Abstracts). 2008;112:5129.
51. Barlogie B, Anaissie E, Haessler J, et al. Complete remission sustained 3 years from treatment initiation is a powerful surrogate for extended survival in multiple myeloma. Cancer. 2008;113:355–9.
52. Pineda-Roman M, Zangari M, Haessler J, et al. Sustained complete remissions in multiple myeloma linked to bortezomib in total therapy 3: comparison with total therapy 2. Br J Haematol. 2008;140:625–34.
53. Hoering A, Crowley J, Shaughnessy JD, Jr, et al. Complete remission in multiple myeloma examined as time-dependent variable in terms of both onset and duration in Total Therapy protocols. Blood. 2009;114: 1299–305.
54. Barlogie B, Pineda-Roman M, van Rhee F, et al. Thalidomide arm of Total Therapy 2 improves complete remission duration and survival in myeloma patients with metaphase cytogenetic abnormalities. Blood. 2008;112:3115–21.
55. De Tute R, Rawstron A, Child JA, et al. Impact of minimal residual disease and induction therapy on outcome post ASCT: insights from the MRC Myeloma IX trial. Clin Lymphoma Myeloma Leuk. 2013;13(suppl 1):16.
56. Child JA, Rawstron A, De Tute R, et al. Experience from the MRC myeloma IX study. Clin Lymphoma Myeloma Leuk. 2013;13(suppl 1):2.

57. 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;112:4017–23.
58. Paiva B, Gutierrez NC, Rosinol L, et al. High-risk cytogenetics and persistent minimal residual disease by multiparameter flow cytometry predict unsustained complete response after autologous stem cell transplantation in multiple myeloma. Blood. 2012;119:687–91.
59. Paiva B, Martinez-Lopez J, Vidriales MB, et al. Comparison of immunofixation, serum free light chain, and immunophenotyping for response evaluation and prognostication in multiple myeloma. J Clin Oncol. 2011;29:1627–33.
60. Redondo EF, Martinez-Lopez J, Garcia-Sanz R, et al. Under scope of the current redefinition process of optimal response in multiple myeloma: assessment of molecular response by fluorescent PCR of Ig genes has similar applicability and prognosis impact to immunophenotypic response. (A GEM/PETHEMA study). Blood (ASH Annual Meeting Abstracts). 2011;118:3951.
61. Martinez-Lopez JG-SR, Pepin F. Prognostic value of deep sequencing method for minimal residual disease (MRD) detection in multiple myeloma. J Clin Oncol. 2013 (suppl; abstr 8511).
62. 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;31:2540–7.
63. Niesvizky RF, 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. Blood (ASH Annual Meeting Abstracts). 2011;118:478.
64. 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. ASH Annual Meeting Abstracts. 2010;116:619.
65. Dytfeld DJ, Griffith J, Kent, A. Treatment with CRd - carfilzomib, lenalidomide, and low-dose dexamethasone for elderly patients with newly diagnosed multiple myeloma enrolled in a phase 1/2 study. Clinical Lymphoma & Myeloma (14th International Myeloma Abstract Workshop Abstract Book), Apr 3-7, 2013, Kyoto, Japan, Vol 13 (Suppl 1):0–10.
66. Richardson PG, Mitsiades C, Schlossman R, et al. New drugs for myeloma. Oncologist. 2007;12:664–89.
67. Richardson PG, Lonial S, Jakubowiak AJ, et al. Monoclonal antibodies in the treatment of multiple myeloma. Br J Haematol. 2011;154:745–54.

New Developments in Post-transplant Maintenance Treatment of Multiple Myeloma

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Treatment of multiple myeloma (MM) has evolved significantly over the past two decades with high-dose chemotherapy and autologous stem cell transplant (ASCT), incorporating novel therapies such as proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) during induction and post-transplant maintenance therapies. We reviewed the evolution of maintenance therapy from traditional chemotherapy, interferon (IFN), and prednisone to the current use of thalidomide, lenalidomide, and bortezomib in post-transplant maintenance setting. Based on existing literature, either thalidomide or lenalidomide can be recommended for maintenance therapy post-transplant resulting in improved progression- free survival (PFS) and overall survival (OS). Thalidomide is less tolerated than lenalidomide and does not improve survival in patient subgroups who had achieved at least a very good partial response (VGPR) or who had chromosome 13 deletion. Thalidomide maintenance maybe even detrimental in patients with high-risk cytogenetics. Alternatively, lenalidomide maintenance improves PFS in all subgroups of patients including those achieving at least a VGPR and those with high-risk cytogenetics, and improves OS in one other study. Bortezomib maintenance improves PFS and OS as part of induction and maintenance when compared to thalidomide maintenance and it is uncertain as to whether this improvement was due to bortezomib used during induction. The future research in maintenance therapy may include incorporation of current novel agents and testing new oral agents such as pomalidomide, or ixazomib or antibody therapy with elotuzumab.
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SHORT REVIEW OF PRIOR RESEARCH OF MAINTENANCE IN MYELOMA

Multiple myeloma (MM) was first recognized in the 19th century and was described as "mollities ossium" accompanied by the presence of Bence Jones protein in urine.[1] The median overall survival (MOS) was short: a few months due to the lack of effective treatment at that time. By the 1950s, the first effective treatment for MM was established with alkylating agent–based therapy, including melphalan, which improved the MOS to 36 months from the time of diagnosis.[2] Over the past 15 years, many advances in MM treatment, including autologous stem cell transplant (ASCT) and novel agents (proteasome inhibitors [PIs] and immunomodulatory drugs [IMiDs]), have further improved the MOS.[3] However, MM is not a curable disease. Virtually all MM patients eventually relapse or have progressive disease. Thus, strategies to improve on remission duration have been considered. Consolidation therapy for MM to enhance outcome and prolong response after induction therapy is discussed by Cavo et al in this issue of *Seminars in Oncology*. Maintenance therapy has been explored to prolong survival by sustaining disease control following induction chemotherapy. The first clinical trial of maintenance therapy was led by SWOG. Published in 1975, it consisted of 96 patients responding to 12 months of various melphalan combination regimens who were randomized to one of three maintenance treatments: carmustine with prednisone, continued melphalan with prednisone, or no chemotherapy.[4] No differences in relapse, remission duration, or OS were found among these three groups. A second randomized control trial with 185 patients who responded initially to melphalan-prednisone (MP) confirmed no survival benefit in patients randomized to MP maintenance.[5] An increased incidence of secondary acute leukemia was reported in patients with prolonged melphalan

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treatment.[6,7] The focus of maintenance therapy was therefore moved away from cytotoxic chemotherapy to IMiDs. Interferon (IFN) and glucocorticoids have been tested in the maintenance setting and proved to have survival benefit but were associated with significant toxicities and poor tolerability.

Interferon

IFNs are cytokines produced naturally by cells in response to viral challenge. IFN has both antiproliferative and immunomodulating effects and was first shown to have anti-myeloma properties as a single agent in 1979.[8] Subsequent trials using IFN as maintenance therapy revealed different results: some showed a progression-free survival (PFS) benefit and a trend in improved OS. However, most trials did not demonstrate a significant OS benefit.[1] Two meta-analyses[9,10] showed a significant improvement in PFS and OS. The Myeloma Trialists' Collaborative Group conducted a meta-analysis using individual patient data from 24 IFN maintenance trials They reported a superior PFS (33% *v* 24%, $P < .00001$) and OS (53% *v* 49%, $P = .01$) in patients treated with IFN maintenance (2–6 million units [MU] x 2–3 times per week) versus observation at 3 years of follow-up.[9] The MOS benefit was 4 months. Fritz et al performed a meta-analysis based on 13 trials of IFN maintenance therapy versus observation and confirmed a PFS benefit of 4.4 months ($P < .01$) and an OS benefit of 7 months ($P < .01$).[10] The routine use of IFN maintenance therapy is limited by significant adverse events (AEs). Almost all patients experience flu-like symptoms, including fever, chills, myalgias, headaches, and malaise. Other major toxicities include depression, arrhythmias, liver function abnormalities, anorexia, nausea, diarrhea, or hematologic toxicities. The toxic side effects of IFN maintenance therapy caused discontinuation of treatment in up to 37% of patients in clinical trials.[11]

Glucocorticoids

Glucocorticoids were found to have significant anti-myeloma effect as single agents[12,13] or as part of combination therapy due to additive or synergistic activity.[14,15] The first randomized maintenance trial using glucocorticoids was published in 1998 by Salmon et al from SWOG.[16] Eighty-nine newly diagnosed MM patients who achieved remission were randomized to one of two maintenance arms: IFN (3 MU three times weekly) plus 50 mg prednisone (IFN/P) versus IFN alone until relapse. Patients who received IFN/P maintenance had an improved PFS (19 *v* 9 months, $P = .008$) without OS benefit (57 *v* 46 months from the start of maintenance, $P = .36$). The role of glucocorticoids alone as maintenance therapy was evaluated in the subsequent SWOG 9210 trial.[17] One hundred twenty-five patients achieving at least a 25% tumor reduction were randomized to receive either 50 mg or 10 mg of prednisone on alternate days until disease progression. The patients receiving 50 mg alternate-day prednisone as maintenance had a significantly better PFS (14 *v* 5 months, $P = .003$) and OS (37 *v* 26 months, $P = .05$) from the time of maintenance randomization when compared with 10 mg every other day. A comparison of dexamethasone (20 mg daily for 4 days per month) with IFN maintenance (3 MU 3 x weekly) showed a similar duration of remission and OS between the two arms.[18] Another randomized trial comparing dexamethasone versus observation showed an improved median PFS (2.8 years *v* 2.1 years, $P = .0002$) without OS benefit (4.1 years *v* 3.8 years, $P = .4$).[19] Glucocorticoids have not shown a consistent benefit in improving OS. Long-term maintenance therapy with glucocorticoids led to grade 3 and higher AEs, including increased infection, weight gain, myopathy, myalgia, change in mood, and Cushingoid features in one quarter of the SWOG 9210 trial patients.

RECENT UPDATES ON ROLE OF MAINTENANCE AFTER TRANSPLANT

A major advance in MM treatment has been made since the introduction of IMiDs (thalidomide, lenalidomide) and PIs (bortezomib). These novel agents have recently become the center of interest for maintenance therapy.

Thalidomide

Thalidomide, initially marketed as a sedative and antiemetic medicine in 1950s, was banned in early 1960s after causing severe congenital deformities.[20] In the late 1990s, thalidomide was found to have anti-angiogenesis and immunomodulatory effects, and exhibited an anti-tumor effect against refractory MM.[21,22] Rajkumar et al demonstrated the superior efficacy of thalidomide and dexamethasone compared to dexamethasone alone in newly diagnosed MM patients and established a new standard of care in 2006.[23] Thalidomide became the focus of interest for maintenance studies because of the lack of severe hematologic toxicity and its oral formulation.

There are seven trials with thalidomide maintenance with or without corticosteroids after ASCT published to date.[24–30] Attal et al reported the benefit of thalidomide maintenance after ASCT.[24] In this Intergroup Francophone du Myélome (IFM) 99 study, 597 patients were randomized to three groups: thalidomide until progression plus pamidronate, pamidronate alone, or no maintenance at 2 months post tandem ASCT. Significantly more patients on the thalidomide arm achieved complete

responses (CRs) or very good partial responses (VGPRs) (67% *v* 57% *v* 55%, $P = .03$). Thalidomide maintenance improved the event-free survival (EFS) at 3 years post randomization (52% *v* 37% *v* 36%, $P < .009$), and the OS (4 years post diagnosis: 87% *v* 74% *v* 77%, $P < .04$). Subgroup analyses showed that thalidomide maintenance did not benefit patients who achieved a VGPR or CR at randomization or patients with the chromosome 13 deletion cytogenetic abnormality. The subgroup analyses were limited by small number of patients as only 55 patients with chromosome 13 deletion and 100 patients with VGPR or CR received thalidomide maintenance.

Barlogie et al reported superior CR rates (62% *v* 43%) and EFS (56% *v* 44%) with thalidomide maintenance for patients receiving Total Therapy 2 (TT2).[25] TT2 consisted of four cycles of induction therapy, tandem ASCT, four cycles of consolidation therapy, and 1 year of maintenance with IFN and dexamethasone. Patients randomized to the thalidomide arm received thalidomide 100 mg daily for the first year, then 50 mg every other day until disease progression or AEs occurred. The OS survival benefit was not obvious at the initial report; however, with a longer follow-up of 87 months, a significantly improved OS ($P = .04$) was found in thalidomide maintenance group.[31] Patients with metaphase-defined cytogenetic abnormalities benefited more with thalidomide maintenance (OS at 5 years, 56% *v* 43%; $P = .02$) with earlier segregation of the survival curves at 2 to 3 years, compared to 7 years for those without cytogenetic abnormalities.[32] There is a limitation to evaluating the exact benefit of thalidomide maintenance in TT2 because only the thalidomide maintenance group received thalidomide during induction and the control group did not receive thalidomide induction. The survival benefit in the thalidomide group could be derived from thalidomide induction or the inclusion of thalidomide in both induction and maintenance phases.

In the Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON)-50 study,[27] 556 patients were randomized to two arms: induction with vincristine, doxorubicin, and dexamethasone (VAD) followed by ASCT and IFN maintenance (arm A) or induction with thalidomide, doxorubicin, and dexamethasone (TAD) followed by ASCT and thalidomide 50 mg daily maintenance (arm B). Thalidomide maintenance significantly improved best overall response rate (88% *v* 79%, $P = .005$), EFS (34 months *v* 22 months, $P < .001$), PFS (34 months *v* 25 months, $P < .001$) without significant difference in OS (73 months *v* 60 months, $P = .077$). The HOVON-50 study arm A did not receive thalidomide as a part of induction therapy and the EFS and PFS benefit may not be due solely to thalidomide maintenance.

Two studies[26,28] evaluated maintenance therapy with thalidomide (100–200 mg daily) for 1 year[26] or until progression,[28] respectively, with prednisone (every other day). Both trials showed a superior PFS with thalidomide/prednisone maintenance. The OS benefit was seen in one trial (3-year OS, 86% *v* 75%; $P = .004$)[26] and not the other (4-year OS, 68% *v* 60%; $P = .18$).[28]

Another trial evaluating the role of thalidomide with corticosteroid maintenance was recently reported by the Brazilian Multiple Myeloma Study Group (BMMSG/GEMOH).[30] One hundred eight patients were randomized to receive maintenance with dexamethasone or dexamethasone with thalidomide (200 mg daily) for 12 months or until progression. At a median follow-up of 27 months, the 2-year PFS was superior in the dexamethasone with thalidomide maintenance arm (64% *v* 30%, $P = .002$) compared to dexamethasone alone. The addition of thalidomide to dexamethasone as maintenance improved the PFS mainly in patients who did not achieve at least a VGPR post ASCT. There was no significant difference in 2-year OS (85% *v* 70%, $P = .27$).

Recently, the British Medical Research Council (MRC) Myeloma IX study examined thalidomide maintenance for transplant eligible and non–transplant-eligible patients.[29] Patients were randomized to thalidomide therapy (50–100 mg) or to no maintenance. In the transplant group, thalidomide maintenance resulted in a significant longer PFS (30 *v* 23 months, $P < .001$). There were no significant OS difference between arms (HR=0.91, $P = .4$) at a median follow-up from maintenance randomization of 38 months. Patients with adverse cytogenetics [t(4;14), t(14;16), t(14;20), del 17p, del 1p32, gain 1q21] attained a similar PFS (9 *v* 12 months, $P = .49$) but a worse OS ($P = .009$). This study suggests that thalidomide maintenance benefited only low-risk myeloma patients.

A meta-analysis of six thalidomide maintenance trials was recently published and confirmed the improvement of both PFS (HR = 0.65, $P < .01$) and a trend toward significant improvement in OS (HR = 0.83, $P = .07$).[35] The OS improvement was more prominent in subgroups using corticosteroids with thalidomide as maintenance (HR = 0.70, $P = .02$). More frequent venous thrombosis (risk difference, 0.024; $P < .05$) and peripheral neuropathy occurred with thalidomide maintenance. The side effects of thalidomide limited long-term use, with the median duration of thalidomide maintenance varying from 7–24 months in the trials discussed above.

Lenalidomide

Lenalidomide, a derivative of thalidomide is a more potent stimulator of T-cell proliferation, and

interleukin (IL)-2 and IFN-γ production.[33,34] Lenalidomide decreases the secretion of IL-6, tumor necrosis factor (TNF)-α, and IL-1β in a dose-dependent manner. Given the less risk of neurotoxicity (up to 17% incidence of mild to moderate peripheral neuropathy with full-dose lenalidomide) compared to thalidomide and the efficacy of at lower doses, lenalidomide became the next logical choice for maintenance therapy studies. Palumbo et al reported the feasibility and efficacy of using lenalidomide as consolidation-maintenance therapy in a pilot phase II trial,[35] that served as the foundation for two phase III post ASCT lenalidomide maintenance trials recently published.[36,37] In both trials, study drug assignments were unblinded early because of a dramatic, significantly improved PFS or time to progression (TTP), the primary endpoints.

The IFM-2005-02 trial examined 614 MM patients who had received VAD or bortezomib/dexamethasone induction, followed by a single or double (21%) ASCT and two cycles of lenalidomide consolidation and who were then randomized to lenalidomide maintenance or placebo.[37] The patients on the lenalidomide arm took 10 mg lenalidomide daily (increased to 15 mg after 3 months if tolerated) until disease progression or unacceptable AEs. Lenalidomide consolidation increased the CR rate from 14% to 20% ($P < .001$) and the VGPR rate from 58% to 67% ($P < .001$). The best response during lenalidomide maintenance improved but not significantly (CR rate, 25% *v* 22%, $P = .4$; VGPR rate, 77% *v* 70%, $P = .08$). At a median follow-up of 45 months (36 months after randomization), the median PFS was significantly improved in the lenalidomide maintenance arm (41 *v* 24 months, HR = 0.5; $P < .001$). The probability of surviving free of progression for 3 years post randomization was 59% versus 35% favoring the lenalidomide maintenance group. The PFS benefit was seen in all patient subgroups who received lenalidomide maintenance therapy, independent of response status at randomization (VGPR/CR or not), β_2-microglobulin level, or presence or absence of 13q deletion. The 3-year OS after randomization was similar in both groups (80% in the lenalidomide group and 84% in the placebo group, HR = 1.25; $P = .29$). There were more patients with adverse cytogenetic disease [t(4;14) or deletion 17p] in the lenalidomide maintenance group ($P < .01$).

In the Cancer and Leukemia Group B (CALGB) 100104 trial, 460 MM patients received induction therapy (multiple regimens), a single ASCT, and randomization to lenalidomide maintenance (10 mg daily for 3 months, then increased to 15 mg if tolerated) or to placebo.[36] The median TTP was significantly prolonged in the lenalidomide maintenance group (46 months *v* 27 months, HR = 0.48; $P < .001$). When the primary endpoint (TTP) was met, 86 of 128 eligible patients in the placebo group crossed over and received lenalidomide maintenance therapy. Based on an intent-to-treat analysis, at a median follow-up of 34 months, the OS was significantly improved with lenalidomide maintenance compared to placebo (85% *v* 77%, $P = .03$) despite the cross-over. In a subgroup analysis, there was evidence that induction therapy with lenalidomide was associated with improved OS in the lenalidomide maintenance group as compared with the placebo group ($P = .03$). CALGB 100104 patients received different induction therapies when compared to the IFM 05-02 patients. Twenty-nine percent of CALGB 100104 patients received thalidomide-based and 35% received lenalidomide-based induction therapy whereas majority of patients (96%) in IFM-2005-02 received VAD or bortezomib and dexamethasone. In the IFM-2005-02 trial, both experimental and control arms received 2 cycles of lenalidomide consolidation post- transplant; whereas no consolidation was given post-transplant in CALGB 100104 trial. The IFM 05-02 study stopped lenalidomide maintenance therapy at an estimated 32 months of therapy. The reasons for stopping therapy are described in the next section. The difference in patient population, induction therapies, length of maintenance treatment and trial design may explain the difference of OS benefit between the IFM-2005-02 and CALGB 100104 trials. In addition, poor risk cytogenetic profiles including 17p deletion and t (4:14) were more common in the lenalidomide group (p=0.006) in the IFM-2005-02 trial which may have adversely affected the survival.

A major concern during maintenance therapy is any toxicity that limits its long-term use, the ability to receive future treatment after progression or results in life-threatening illness. In both the IFM and CALGB studies, grade 3 or 4 AEs were mostly hematologic events. The incidences of grade 4 neutropenia were 13% for the CALGB lenalidomide arm and 3% for the placebo arm. The incidences of grade 4 thrombocytopenia were 5 and 3% for the lenalidomide and placebo arms, respectively. The IFM aggregated grade 3 and 4 AEs. For neutropenia the incidences for the lenalidomide and placebo arms were 51% and 18%, respectively. The aggregated grade 3 and 4 thrombocytopenia AEs were 14% and 7%, respectively, for the lenalidomide and placebo arms. There were more non-hematologic grade 3 toxicities on the CALGB lenalidomide arm and no difference in grade 4 and 5 AEs when compared with placebo. There were more thromboembolic events (6% v 2%) on the IFM lenalidomide arm when compared to placebo. The maintenance discontinuation rate due to AEs was 10% and 1% for lenalidomide and placebo, respectively, in the CALGB study and 27% and 15%, respectively, in

the IFM study. Both the IFM-2005-02 and CALGB 100104 trials reported an increased risk of second primary malignancies (SPM). In the IFM-2005-02, new hematologic malignancies and solid tumors were reported in 4% and 3% of patients treated with lenalidomide maintenance as compared to the placebo group who developed 2% hematologic malignancies and 1% solid tumors, respectively. In the CALGB 100104 study, 3.5% and 4% of patients in the lenalidomide maintenance arm developed new hematologic malignancies and solid tumors, as compared to the control group who developed 0.4% and 2.2% new hematologic malignancies and solid tumors, respectively. The second hematologic malignancies were acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), Hodgkin lymphoma (HL), and non-Hodgkin lymphoma (NHL). Compared to the placebo arms, the IFM-2005-02 lenalidomide arm had an increase in ALL and HL without increase in AML/MDS, whereas the CALGB 100104 lenalidomide arm had an increase in AML/MDS without an increase in ALL and HL. Despite the increase in SPM, lenalidomide maintenance therapy reduced the risk of MM relapse by 50% in both trials and prolonged the OS in CALGB 100104 trial. Cavallo et al recently reported the decrease in the risk of progression with lenalidomide maintenance post-transplant or post-melphalan/prednisone/lenalidomide (MPR) therapy.[38] At a median follow-up of 38 months, the PFS for transplant and MPR patients receiving lenalidomide maintenance versus patients not receiving maintenance was 66% versus 47%, respectively (P = .002). There is no difference in OS at the time of this report.

Bortezomib

Bortezomib is a PI that induces high response rates in both relapse/refractory and newly diagnosed MM patients.[39–41] Bortezomib induction therapy improves the survival of MM patients with selected high-risk cytogenetic abnormalities.[42,43] Bortezomib has been studied as post ASCT consolidation therapy.[44,45] In the transplant setting, there is one bortezomib phase III randomized maintenance trial published to date. The HOVON and the German Multicenter Myeloma Group (GMMG) randomized 613 newly diagnosed MM patients to VAD induction, high-dose chemotherapy and ASCT and maintenance therapy with thalidomide 50 mg daily for 2 years (arm A), or to bortezomib, doxorubicin, and dexamethasone (PAD), high-dose chemotherapy and ASCT and maintenance with bortezomib 1.3 mg/m^2 intravenously every 2 weeks for 2 years.[46] At a median follow-up of 40 months, the quality of response was significantly improved in arm B: the near CR/CR rate was 38% in arm A versus 50% in arm B, the VGPR or better rate was 61% in arm A versus 75% in arm B. The 3 year PFS and OS were significantly higher in arm B with a PFS of 48% versus 42% in arm A (HR, 0.81, p=0.047) and OS of 78% in arm B and 71% in arm A (HR, 0.74, p=0.048). Seventy-eight percent of patients in the VAD arm started thalidomide maintenance therapy as compared to 65% patients in the PAD arm who started bortezomib maintenance therapy. Completion of maintenance was achieved in 27% of the thalidomide maintenance arm and 47% of the bortezomib maintenance arm. Discontinuation of maintenance therapy was due to progressive disease (PD) (31%), toxicity (31%), and other reasons (2%) in the thalidomide maintenance group versus PD (29%), toxicities (9%), or other reasons (9%) in the bortezomib maintenance arm. The study demonstrated that PAD/ASCT/bortezomib maintenance is superior to the VAD/ASCT/thalidomide maintenance even in patients with renal impairment or with adverse fluorescence in situ hybridization (FISH)-defined poor-risk cytogenetics [t(4;14), amplification of 1q21, and del 17p]. Due to the study design, it is difficult to conclude whether the benefit is due to the bortezomib-containing induction, maintenance, or both.

COMBINED NOVEL THERAPIES FOR MAINTENANCE

Combination of novel therapies using bortezomib and thalidomide (VT) as maintenance therapy has been evaluated in both non-transplant and transplant patients. In the non-transplant setting, two randomized control trials were reported to date with marginal benefit.[47,48] These studies compared two groups of patients randomized to receive either bortezomib, melphalan, prednisone, and thalidomide (VMPT) induction followed by VT maintenance (bortezomib 1.3 mg/m^2 every 2 weeks plus thalidomide 50 mg daily) for 2 years or VMP induction without maintenance and reported a better CR rate (38% v 24%, P < .001) and 3-year PFS (56% v 41%, P = .008) in patients received VT maintenance. The 3-year OS was similar (89% v 87%, P = .77). After a longer follow-up (median follow-up of 47.2 months), median OS was significantly better in the VMPT-VT arm compared with the VMP arm (not reached v 58.2 months).[48] However, due to the study design, it is unclear the benefit in CR and PFS was purely due to VT maintenance or including thalidomide in induction. Palumbo et al compared two maintenance therapies with 3 years of bortezomib (one cycle every 3 months) plus thalidomide 50 mg daily (VT) or prednisone 50 mg every other day (VP) and found no statistical significant difference in both PFS (39 v

32 months, $P = .1$) and OS (5-year OS 69% *v* 50%, $P = .1$).[49] Rosinnol et al recently reported the result of VT maintenance in transplant setting.[50] The patients were randomized to three groups to receive maintenance therapies for 3 years with either VT (bortezomib one cycle every 3 months and thalidomide 100 mg daily), T (thalidomide 100 mg daily) or IFN (3 MU 3 times/wk). The maintenance therapies increased CR rate by 15%-19% without difference among each group. The PFS was significantly longer with VT compared with T and IFN with no increased toxicities and OS were similar among three maintenance groups.

BISPHOSPHONATES

The MRC myeloma IX study also evaluated the effect of zoledronic acid on the reduction of skeletal events and improvement of clinical outcomes.[51,52] The study randomized 1,970 newly diagnosed MM patients to zoledronic acid (4 mg IV every 3-4 weeks) or clodronic acid (1,600 mg per day orally). Bisphosphonates were started during induction therapy and continued until disease progression or adverse event. Patients received bisphosphonates for a median of 350 days (range, 137-632). At a median follow-up of 3.7 years, zoledronic acid significantly improved PFS by 12% (95% CI, 2-20), reduced mortality by 16% (HR 0.84, $P = .0118$) and extended MOS by 5.5 months (50 months *v* 44.5 months, $P = .04$). Both bisphosphonates were generally well tolerated with similar incidence of acute renal failure. Zoledronic acid was associated with higher rates of osteonecrosis of the jaw (4%) than clodronic acid (< 1%).

RECOMMENDATIONS IN VIEW OF EXISTING EVIDENCE

The International Myeloma Working Group (IMWG) recently published consensus recommendations regarding maintenance therapy in multiple myeloma.[53] Offering maintenance therapy to MM patients has to be individualized based on the patient's age, performance status, prior treatment and tolerance of the novel agents, and cytogenetic/FISH risk stratification. Thalidomide maintenance post ASCT is an option that increases PFS consistently and OS marginally. The dose should be limited to 50–100 mg daily with the duration of therapy limited to 1 year or less to limit significant neurotoxicity. Patients with FISH-defined poor-risk cytogenetics had an inferior outcome with thalidomide maintenance compared to control.[29] Thalidomide maintenance did not benefit patients who achieved at least a VGPR at randomization or patients with del (13)[24]. Lenalidomide maintenance post ASCT is well tolerated and active in most risk groups. Lenalidomide maintenance improved PFS or TTP significantly in both the IFM and CALGB trials, and OS in the CALGB trial. The starting dose should be 10 mg daily with the dose adjusted between 5 mg and 15 mg depending on tolerability. The duration of maintenance is to continue until PD or unacceptable toxicity. In contrast to thalidomide, lenalidomide maintenance improved PFS in all stratified subgroups of patients including those achieved at least a VGPR and those with high-risk cytogenetics (del(13q)).[37] Due to the concern for second primary malignancies, the question has been raised as to whether a shorter duration of lenalidomide maintenance therapy (eg, 2 or 3 years) would still provide the same survival benefit. Biweekly bortezomib maintenance therapy is feasible for up to 2 years.[46] It remains to be determined if the PFS and OS benefit observed in the HOVON/GMMG trial is attributed to bortezomib induction, maintenance, or the combination. The combination of bortezomib and thalidomide as maintenance is feasible and showed improved PFS compared to thalidomide alone.[50] The incorporation of bortezomib in maintenance therapy may overcome the poor impact of high risk cytogenetics.

Further trials using novel agents in combination as part of induction, consolidation, ASCT, and maintenance should improve MM PFS, OS, and long-term outcome. Using lenalidomide and bortezomib as standards for maintenance therapy will allow for future study comparisons. Clinical trials that incorporating oral IMiDs such as pomalidomide or proteasome inhibitors such as ixazomib (MLN9708) or oprozomib will define optimal approaches to maintenance therapy.

REFERENCES

1. Cherry BM, Korde N, Kwok M, Roschewski M, Landgren O. Evolving therapeutic paradigms for multiple myeloma: back to the future. Leuk Lymphoma. 2013;54:451–63.
2. Alexanian R, Bergsagel DE, Migliore PJ, Vaughn WK, Howe CD. Melphalan therapy for plasma cell myeloma. Blood. 1968;31:1–10.
3. Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008;111:2516–20.
4. Remission maintenance therapy for multiple myeloma. Arch Intern Med. 1975;135:147–52.
5. Belch A, Shelley W, Bergsagel D, et al. A randomized trial of maintenance versus no maintenance melphalan and prednisone in responding multiple myeloma patients. Br J Cancer. 1988;57:94–9.
6. Sieber SM, Adamson RH. Letter: Maintenance therapy in myeloma: risk versus benefit. Br Med J. 1975;2:557.
7. Bergsagel DE, Bailey AJ, Langley GR, MacDonald RN, White DF, Miller AB. The chemotherapy on plasma-

cell myeloma and the incidence of acute leukemia. N Engl J Med. 1979;301:743–8.
8. Mellstedt H, Aahre A, Bjorkholm M, et al. Interferon therapy in myelomatosis. Lancet. 1979;2:697.
9. Myeloma Trialists' Collaborative Group. Interferon as therapy for multiple myeloma: an individual patient data overview of 24 randomized trials and 4012 patients. Br J Haematol. 2001;113:1020–34.
10. Fritz E, Ludwig H. Interferon-alpha treatment in multiple myeloma: meta-analysis of 30 randomised trials among 3948 patients. Ann Oncol. 2000;11:1427–36.
11. Schaar CG, Kluin-Nelemans HC, Te Marvelde C, et al. Interferon-alpha as maintenance therapy in patients with multiple myeloma. Ann Oncol. 2005;16:634–9.
12. Alexanian R, Yap BS, Bodey GP. Prednisone pulse therapy for refractory myeloma. Blood. 1983;62: 572–7.
13. Kumar S, Lacy MQ, Dispenzieri A, et al. Single agent dexamethasone for pre-stem cell transplant induction therapy for multiple myeloma. Bone Marrow Transplant. 2004;34:485–90.
14. Barlogie B, Smith L, Alexanian R. Effective treatment of advanced multiple myeloma refractory to alkylating agents. N Engl J Med. 1984;310:1353–6.
15. Rosinol L, Oriol A, Mateos MV, et al. Phase II PETHEMA trial of alternating bortezomib and dexamethasone as induction regimen before autologous stem-cell transplantation in younger patients with multiple myeloma: efficacy and clinical implications of tumor response kinetics. J Clin Oncol. 2007;25:4452–8.
16. Salmon SE, Crowley JJ, Balcerzak SP, et al. Interferon versus interferon plus prednisone remission maintenance therapy for multiple myeloma: a Southwest Oncology Group Study. J Clin Oncol. 1998;16:890–6.
17. Berenson JR, Crowley JJ, Grogan TM, et al. Maintenance therapy with alternate-day prednisone improves survival in multiple myeloma patients. Blood. 2002;99:3163–8.
18. Alexanian R, Weber D, Dimopoulos M, Delasalle K, Smith TL. Randomized trial of alpha-interferon or dexamethasone as maintenance treatment for multiple myeloma. Am J Hematol. 2000;65:204–9.
19. Shustik C, Belch A, Robinson S, et al. A randomised comparison of melphalan with prednisone or dexamethasone as induction therapy and dexamethasone or observation as maintenance therapy in multiple myeloma: NCIC CTG MY.7. Br J Haematol. 2007;136: 203–11.
20. Speirs AL. Thalidomide and congenital abnormalities. Lancet. 1962;1:303–5.
21. D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci U S A. 1994;91:4082–5.
22. Singhal S, Mehta J, Desikan R, et al. Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med. 1999;341:1565–71.
23. Rajkumar SV, Blood E, Vesole D, Fonseca R, Greipp PR. Phase III clinical trial of thalidomide plus dexamethasone compared with dexamethasone alone in newly diagnosed multiple myeloma: a clinical trial coordinated by the Eastern Cooperative Oncology Group. J Clin Oncol. 2006;24:431–6.
24. Attal M, Harousseau JL, Leyvraz S, et al. Maintenance therapy with thalidomide improves survival in patients with multiple myeloma. Blood. 2006;108: 3289–94.
25. Barlogie B, Tricot G, Anaissie E, et al. Thalidomide and hematopoietic-cell transplantation for multiple myeloma. N Engl J Med. 2006;354:1021–30.
26. Spencer A, Prince HM, Roberts AW, et al. Consolidation therapy with low-dose thalidomide and prednisolone prolongs the survival of multiple myeloma patients undergoing a single autologous stem-cell transplantation procedure. J Clin Oncol. 2009;27:1788–93.
27. Lokhorst HM, van der Holt B, Zweegman S, et al. A randomized phase 3 study on the effect of thalidomide combined with adriamycin, dexamethasone, and high-dose melphalan, followed by thalidomide maintenance in patients with multiple myeloma. Blood. 2010;115: 1113–20.
28. Stewart KA, Trudel S, Bahlis NJ, et al. A randomized phase 3 trial of thalidomide and prednisone as maintenance therapy after ASCT in patients with MM with a quality-of-life assessment: the National Cancer Institute of Canada Clinicals Trials Group Myeloma 10 Trial. Blood. 2013;121:1517–23.
29. Morgan GJ, Gregory WM, Davies FE, et al. The role of maintenance thalidomide therapy in multiple myeloma: MRC Myeloma IX results and meta-analysis. Blood. 2012;119:7–15.
30. Maiolino A, Hungria VT, Garnica M, et al. Thalidomide plus dexamethasone as a maintenance therapy after autologous hematopoietic stem cell transplantation improves progression-free survival in multiple myeloma. Am J Hematol. 2012;87:948–52.
31. 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;28:1209–14.
32. Barlogie B, Pineda-Roman M, van Rhee F, et al. Thalidomide arm of Total Therapy 2 improves complete remission duration and survival in myeloma patients with metaphase cytogenetic abnormalities. Blood. 2008;112:3115–21.
33. Kagoya Y, Nannya Y, Kurokawa M. Thalidomide maintenance therapy for patients with multiple myeloma: meta-analysis. Leuk Res. 2012;36:1016–21.
34. Corral LG, Haslett PA, Muller GW, et al. Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha. J Immunol. 1999;163:380–6.
35. Palumbo A, Gay F, Falco P, et al. Bortezomib as induction before autologous transplantation, followed by lenalidomide as consolidation-maintenance in untreated multiple myeloma patients. J Clin Oncol. 2010;28:800–7.
36. McCarthy PL, Owzar K, Hofmeister CC, et al. Lenalidomide after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366:1770–81.
37. Attal M, Lauwers-Cances V, Marit G, et al. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366:1782–91.

38. Cavallo F HI, Gay F. Lenalidomide maintenance significantly reduces the risk of progression in newly diagnosed young multiple myeloma patients enrolled in RV-MM-PI-209 trial. Haematologica. 2012:1142a.
39. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med. 2003;348:2609–17.
40. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005;352:2487–98.
41. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008;359: 906–17.
42. Mateos MV. Role of bortezomib for the treatment of previously untreated multiple myeloma. Exp Rev Hematol. 2008;1:17–28.
43. 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;28:4630–4.
44. 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;376:2075–85.
45. Barlogie B, Anaissie E, van Rhee F, et al. Incorporating bortezomib into upfront treatment for multiple myeloma: early results of Total Therapy 3. Br J Haematol. 2007;138:176–85.
46. 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;30:2946–55.
47. Palumbo A, Bringhen S, Rossi D, et al. Bortezomib-melphalan-prednisone-thalidomide followed by maintenance with bortezomib-thalidomide compared with bortezomib-melphalan-prednisone for initial treatment of multiple myeloma: a randomized controlled trial. J Clin Oncol. 2010;28:5101–9.
48. Palumbo A, Bringhen S, Rossi D, et al. Overall Survival Benefit for Bortezomib-Melphalan-Prednisone-Thalidomide Followed by Maintenance with Bortezomib-Thalidomide (VMPT-VT) Versus Bortezomib-Melphalan-Prednisone (VMP) in Newly Diagnosed Multiple Myeloma Patients. ASH Annual Meeting Abstracts. 2012;120:200.
49. Mateos MV, Oriol A, Martinez-Lopez J, et al. Maintenance therapy with bortezomib plus thalidomide or bortezomib plus prednisone in elderly multiple myeloma patients included in the GEM2005MAS65 trial. Blood. 2012;120:2581–8.
50. Rosinnol L, Oriol A, Teruel AI, et al. Maintenance Therapy After Stem-Cell Transplantation for Multiple Myeloma with Bortezomib/Thalidomide Vs. Thalidomide Vs. alfa2b-Interferon: Final Results of a Phase III Pethema/GEM Randomized Trial. ASH Annual Meeting Abstracts. 2012;120:334.
51. 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: 1989–99.
52. Morgan GJ, Child JA, Gregory WM, et al. Effects of zoledronic acid versus clodronic acid on skeletal morbidity in patients with newly diagnosed multiple myeloma (MRC Myeloma IX): secondary outcomes from a randomised controlled trial. Lancet Oncol. 2011;12:743–52.
53. Ludwig H, Durie BG, McCarthy P, et al. IMWG consensus on maintenance therapy in multiple myeloma. Blood. 2012;119:3003–15.

Role of Consolidation Therapy in Transplant Eligible Multiple Myeloma Patients

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The role of high-dose therapy and autologous stem-cell transplantation (ASCT) in the treatment of multiple myeloma (MM) has continued to evolve in recent years. The novel agents thalidomide, bortezomib, and lenalidomide have been successfully incorporated into induction therapy in preparation for ASCT and are currently being investigated also as post-ASCT consolidation and maintenance therapy. Consolidation treatment is generally short term and aims to increase the frequency and depth of response obtained with the previous treatment phases, including novel agent-based induction therapy and ASCT. This review will focus on recent trials of novel agents as post-ASCT consolidation therapy, offering an overview of pros and cons of this new treatment strategy in the ASCT sequence for MM patients.
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Over the last two decades, high-dose therapy (HDT) and autologous stem cell transplantation (ASCT) has been the mainstay of upfront treatment for younger patients with newly diagnosed multiple myeloma (MM) based on the increased rate of complete response (CR) and prolonged overall survival (OS) reported in comparison with conventional chemotherapy in several phase III studies.[1,2] A systematic review and meta-analysis of randomized clinical trials comparing ASCT with standard-dose therapy has confirmed a significant benefit in prolonging progression-free survival (PFS), but not OS, with a single ASCT.[3] Conflicting results also have been reported by various groups who compared a single versus double ASCT, mainly due to heterogeneity across different trials.[4]

The role of ASCT in the treatment of MM has continued to evolve in recent years.[5,6] The choice of induction therapy has shifted from conventional chemotherapy to newer regimens incorporating the immunomodulatory derivatives (IMiDs) thalidomide or lenalidomide, and/or the proteasome inhibitor, bortezomib.[6] Novel agent–based induction therapies have affected unprecedented rates of CR that rival those previously seen with conventional chemotherapy and subsequent ASCT. Excellent activity shown by IMiDs and/or bortezomib before ASCT has led to their investigational use as consolidation and maintenance therapy after autotransplantation.[7,8] Although the terms consolidation and maintenance are often used synonymously in the transplant setting, the rationale supporting these two strategies is different. Consolidation treatment is generally short-term and aims to increase the frequency and depth of response obtained with the previous treatment phases, including HDT and ASCT. Maintenance therapy is given for a prolonged time period with the goal of decreasing the risk of relapse, while ensuring a good quality of life. This review will focus on post-ASCT consolidation therapy, offering an overview of pros and cons reported in studies so far available in MM.

ASCT AS CONSOLIDATION THERAPY IN THE PRE–NOVEL AGENT ERA

Before the novel agent era, the probability of achieving CR for transplant-eligible MM patients primarily treated with conventional induction regimens, such as vincristine-doxorubicin-dexamethasone (VAD) or high-dose dexamethasone (HD-dex) alone, was below 5%. Based on these disappointing results, ASCT has been traditionally considered the standard strategy to further increase the CR rate. Data from several randomized trials confirmed that the enhanced frequency of CR obtained with ASCT in comparison with conventional chemotherapy,

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resulted in longer PFS, while OS benefit was inconsistent.[3] Subsequent efforts to improve the results of ASCT relied upon the existence of a dose-response relationship for melphalan. To further increase the cytotoxic dose intensity, administration of two sequential courses of melphalan requiring double, or tandem, ASCT was explored in several pilot studies. Following demonstration that such a procedure was feasible and effective, five randomized trials addressed the question of single versus double ASCT as upfront therapy for MM.[9–13] Results of these trials were not homogeneous. In particular, while extended event-free survival (EFS) with double ASCT was observed in most of the studies, an OS benefit was not consistently shown.[9–13] A meta-analysis of data pooled from controlled clinical trials confirmed that double ASCT was associated with improved response rates and EFS in comparison with a single ASCT.[4] In two studies of double ASCT, post hoc analyses of several subgroups of patients suggested that the second autotransplant was of major benefit for those patients who failed to achieve either CR or at least very good partial response (VGPR) after the first ASCT.[9,10] However, a major limitation of these studies was their lack of power to demonstrate the equivalence of one versus two transplants for patients achieving high-quality responses after the first course of HDT. With the recent incorporation of novel agents into the transplantation sequence, the role of single versus double ASCT still remains undetermined and needs to be prospectively evaluated in randomized clinical trials. Two of these studies are currently ongoing in Europe and the United States, one headed by the European Myeloma Network and the other chaired by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN).

NOVEL AGENTS AS CONSOLIDATION THERAPY AFTER ASCT

Rationale

The novel agents thalidomide, lenalidomide, and bortezomib have been successfully combined with one another and/or with cytotoxic drugs to form various doublet, triplet, and quadruplet regimens that have been widely investigated as induction therapy before ASCT. Doublet therapies incorporating thalidomide-dexamethasone (TD)[14,15] or bortezomib-dexamethasone (VD)[16] were superior to VAD or HD-dex alone in terms of increased overall response rate, including CR (generally, $<10\%$ with novel agents). In two phase III studies comparing triplet thalidomide-based regimens with VAD, the rate of CR or at least VGPR with the addition of doxorubicin or cyclophosphamide to TD was slightly higher than that previously seen with TD.[17,18] In comparison with TD or VD, triplet bortezomib-based therapies further enhanced the rate of CR to the 20%–30% range, including CR plus VGPR rates averaging approximately 60%.[19–22] Based on these favorable results, a three-drug regimen incorporating bortezomib combined with an IMiD or a cytotoxic drug, like cyclophosphamide or doxorubicin, is currently considered the standard of care in preparation for ASCT.[6,23] High-dose melphalan requiring ASCT is complementary with novel agents and further increases the rate of CR and VGPR, even in the face of high tumor cell mass reduction affected by newer induction regimens. In several phase III studies the gain offered by novel agents incorporated into the ASCT sequence in terms of enhanced high-quality responses translated into extended PFS[19,21,22] and, albeit less frequently, OS.[22] Achievement of conventionally defined CR after induction therapy and ASCT is associated with improved prognosis and represents a major endpoint of current treatment strategies incorporating autotransplantation upfront.[24] However, increasing the depth of response up to the level of undetectable minimal residual disease (MRD) and maintaining a sustained CR are even stronger predictors of favorable long-term outcomes than attainment of CR per se.[25,26] To reach these objectives, over the last years the novel agents have been extensively investigated as part of post-ASCT consolidation and maintenance strategies.

The impact on clinical outcomes of consolidation therapy for transplant-eligible MM patients is discussed below and summarized in Table 1.

Conventional Chemotherapy With or Without Thalidomide

The use of consolidation therapy after ASCT was pioneered by Barlogie et al as part of Total Therapy 2 (TT2), an intensified treatment program that was primarily aimed at evaluating in a randomized fashion the role of thalidomide incorporated into double ASCT.[27] In addition, TT2 introduced post-transplant consolidation therapy, initially with DCEP (dexamethasone plus 4-day continuous infusions of cyclophosphamide, etoposide and cisplatin) for four cycles versus DCEP alternating with CAD (4-day continuous infusions of cyclophosphamide, doxorubicin, dexamethasone) for eight cycles and, in a later phase, with D-PACE (dexamethasone plus 4-day continuous infusions of cisplatin, doxorubicin, cyclophosphamide, etoposide) for four cycles. Pulsing HD-dex was offered as an alternative consolidation strategy to those patients who failed platelet recovery or response to DCEP induction. In a post hoc analysis, the outcomes of patients randomized to the non–thalidomide-based arm of TT2 were compared with those of patients enrolled in the previous

Table 1. Studies of Post-ASCT Novel Agent–Based Consolidation Therapy: Impact on Outcomes

Reference	Type of Trial	Treatment Scheme	No. of Patients	Response Rate	EFS or PFS	OS
Bortezomib-based						
Cavo[7]	Phase III	VTD *v* TD consolidation	160 *v* 161	CR/nCR pre consolidation: 63% *v* 55% (P = NS) CR/nCR post consolidation: 73% *v* 61% (P = .020)	3-yr PFS 60% *v* 48% P = .042	3-yr OS 90% *v* 88% P = NS
Mellqvist[28]	Phase III	Bortezomib consolidation *v* no consolidation	187 *v* 183	≥VGPR pre consolidation: 40% *v* 39% (P = NS) ≥VGPR post consolidation: 71% *v* 57% (P = .009)	Median PFS 27 m *v* 20 m P = .05	3-yr OS 80% *v* 80% P = NS
Leleu[29]	Retrospective comparison	VTd consolidation *v* no consolidation	121 *v* 96	CR post consolidation: 52% *v* 30% (P = .001)	Median TTP not reached *v* 25 m (P = .005)	4-yr OS 84% *v* 91% P = NS
Ladetto[30]	Phase II	VTD consolidation	39	CR pre VTD: 15% CR post VTD: 49%	Median PFS 60 m	3-yr OS 89%
Lenalidomide-based						
Attal[31]	Phase III	Len consolidation + Len maintenance *v* Len consolidation + placebo	307 *v* 307	CR pre consolidation: 58% CR post consolidation: 69% P < .001	NR after consolidation	NR after consolidation
Roussel[32]	Phase II	RVD consolidation	31	sCR/CR pre VRD: 42% sCR/CR post VRD: 48%	NR	NR

Abbreviations: VTD, bortezomib, thalidomide, dexamethasone; TD, thalidomide, dexamethasone; CR, complete response; nCR, near complete response; PFS, progression-free survival; OS, overall survival; ns, not significant; VGPR, very good partial response; VTd, bortezomib, thalidomide, low dose dexamethasone; TTP, time to progression; Len, lenalidomide; NR, not reported; VRD, bortezomib, lenalidomide, dexamethasone sCR, stringent complete response; VRD, bortezomib, lenalidomide, dexamethasone.

TT1 program that did not include thalidomide and post-ASCT consolidation therapy.[27] Despite similar rates of CR with the two treatments in the overall patient population, TT2 was associated with a higher 5-year probability of EFS (43%) and sustained CR (45%) than TT1 (28% and 32%, respectively; $P < .001$ for both comparisons). In comparison with TT1, TT2 extended both EFS and OS for patients whose tumors lacked chromosomal abnormalities. Among patients who were enrolled in TT2 and had abnormal metaphases in their bone marrow plasma cells, those receiving post-ASCT consolidation chemotherapy had a longer OS (measured from a 6-month landmark after the second autotransplantation) at 4 years (76%) compared with those treated with HD-dex (34%) ($P < .020$). The 4-year OS estimate for patients who were enrolled in TT1 and did not have cytogenetic changes was 69%, suggesting that consolidation chemotherapy in TT2 improved the outcome of the high-risk cytogenetic subgroup to the level obtained with TT1 in the low-risk group. However, results of this retrospective analysis should be cautiously interpreted due to differences between studies with respect to the treatment program that included a more intensive induction chemotherapy in TT2 and the lack of post-transplant consolidation therapy in TT1.

Bortezomib

A phase III study was designed to evaluate the role of bortezomib as single agent consolidation therapy after ASCT in patients not previously exposed to the proteasome inhibitor.[28] A total of 370 patients were randomized three months after a single ASCT to receive no consolidation therapy or standard-dose bortezomib given twice-weekly for the first two 3-week cycles and then once weekly on days 1, 8, and 15 for four additional 4-week cycles. The rate of at least VGPR at the time of randomization was in the 39% range in both treatment arms. After bortezomib consolidation therapy, the probability of achieving CR plus VGPR was 71%, a value significantly higher than the 57% seen in the control group ($P = .008$). As a result, median PFS measured from the time of randomization was 27 months for bortezomib-treated patients compared to 20 months ($P = .05$) for those randomly assigned to the no consolidation arm.

Bortezomib Combined With Thalidomide

Bortezomib combined with thalidomide and dexamethasone (VTD) has been reported to yield profound and quick tumor cell mass reduction before ASCT. Based on these data, several groups have explored the activity of this triplet regimen as consolidation therapy after a single or double ASCT.

In a large phase III study designed to compare VTD versus TD as induction therapy prior to ASCT, 321 patients who were initially randomized to the VTD (n = 160) or TD (n = 161) arms of the study were planned to receive two 35-day cycles of the same triplet or doublet regimens as consolidation following double autotransplantation.[7] In both arms, thalidomide and dexamethasone as part of consolidation therapy were given at the dose of 100 mg daily and 320 mg per cycle, respectively, while bortezomib, 1.3 mg/m^2 once per week on days 1, 8, 15, and 22, was incorporated into VTD. At the landmark of starting consolidation therapy, the rates of CR were similar in the two treatment groups. However, after consolidation the frequency of CR was significantly higher with VTD (61%) than TD (47%) ($P = .012$). Overall, the probability of upgrading from less than CR before consolidation to CR after consolidation therapy was two times higher for the VTD-treated group compared with TD (31% *v* 17%, $P = .030$). With a median follow-up of 30 months from start of consolidation therapy, the estimated 3-year PFS rate was significantly longer with VTD versus TD (60% *v* 48%, $P = .042$), a gain retained across subgroups of patients with poor prognosis and confirmed in a multivariate analysis.

The activity of VTD consolidation after a single ASCT preceded by VTD induction was recently reported by another group.[29] Consolidation was given for two 3-week cycles and included standard-dose, twice-weekly, bortezomib combined with thalidomide, 100 mg daily. Clinical outcomes of these patients were retrospectively compared with those of a control group who received the same treatment without consolidation therapy. Results confirmed the benefits of consolidation therapy in terms of increased rate of CR (52% *v* 30%, $P = .001$) and reduced probability of relapse (21% *v* 45%, $P = .001$), although no difference in PFS was seen between the two groups.

An additional phase II study was designed to enroll patients who had achieved at least a VGPR after double ASCT and had an available molecular marker to detect MRD.[30] Thirty-nine bortezomib-naïve patients who met these criteria were included and received four 35-day cycles of bortezomib (1.6 mg/m^2 on days 1, 8, 15, and 22), thalidomide (up to the maximum daily dose of 200 mg) and dexamethasone (20 mg on days 1–4, 8–11, and 15–18). The CR rate increased from 15% after double ASCT to 49% after VTD consolidation therapy. MRD, as evaluated by qualitative polymerase chain reaction (PCR) using tumor-clone-specific primers, was undetectable in a single patient (3%) before the start of consolidation therapy and in six patients of 37 (16%) who were assessed after consolidation. In these latter patients, consolidation therapy yielded a quantitative tumor cell mass reduction in the range of approximately four natural logarithms. Patients

achieving a low residual tumor mass measured by quantitative PCR had a significantly longer PFS in comparison with those who failed this objective.

Lenalidomide

Lenalidomide is the ideal agent to be used as maintenance therapy due to the oral formulation, the lack of neurological toxicity and its dual mechanism of action, including immunomodulation. In a phase III trial, patients with nonprogressive disease after a single ASCT were randomized to receive consolidation therapy with lenalidomide followed by lenalidomide maintenance or lenalidomide consolidation followed by placebo.[31] In the consolidation phase of the study, lenalidomide was given at 25 mg/day for 3 weeks every 28 days, for a total of two cycles. Although the primary study endpoint was PFS for patients randomized to lenalidomide maintenance or placebo, results of consolidation therapy were briefly reported. Overall, consolidation with standard-dose lenalidomide improved the rate of CR plus VGPR, which increased from 58% before consolidation to 69% after consolidation therapy ($P < .0001$).

Lenalidomide Combined With Bortezomib

In a phase II study, lenalidomide (25 mg/d for 21 days) was combined with standard-dose, twice-weekly, bortezomib and dexamethasone to form a triplet regimen (RVD) that was given as induction therapy before, and consolidation after, a single ASCT.[32] The primary study endpoint was the best response achieved after two 3-week cycles of RVD as consolidation therapy. The rate of CR, including stringent CR, observed among 31 patients who were enrolled was 42% after ASCT and 48% after RVD consolidation. Overall, consolidation therapy upgraded responses in 26% of patients, but only one of them had undetectable MRD assessed by flow cytometry.

Ongoing Studies Exploring the Role of Novel Agent–Based Consolidation Therapy

The role of novel agents as (part of) consolidation therapy after ASCT needs to be prospectively explored in the context of randomized clinical trials before routine consolidation can be recommended. One of these trials is currently ongoing in Europe and is headed by the European Myeloma Network (EMN02 study). Patients with newly diagnosed MM are randomized to an intensive therapy arm including single or double ASCT upfront or to a non-intensive treatment comprising bortezomib-melphalan-prednisone (VMP) eventually followed by salvage ASCT at the time of relapse. In both arms a second randomization to receive two cycles of RVD consolidation or no consolidation is planned after ASCT upfront or VMP. In the United States, the ongoing BMT CTN 0702 trial aims to evaluate the role of a second ASCT as consolidation therapy after the first transplant versus four cycles of RVD versus no consolidation.

TOXICITIES RELATED TO CONSOLIDATION THERAPY

Based on data so far reported, consolidation treatment appears to be generally safe and well tolerated, regardless of the class and number of agents used as part of the treatment program.

The most common adverse event reported with the use of thalidomide- and bortezomib-based treatment is peripheral neuropathy (PN), a complication that can impair patients' quality of life. Thalidomide-induced PN (TiPN) is typically dose-dependent (more prevalent with daily doses higher than 200 mg) and duration-dependent (more likely to occur after 6–12 months of treatment). Bortezomib-induced PN (BiPN) is more frequently sensory, often painful, rarely presenting with motor signs. It is dose-dependent and reaches a plateau at a threshold dose ranging between 30 and 45 mg/m^2. Because consolidation is short-term and treatment intensity is frequently lower in comparison with other phases of the ASCT sequence, in many studies the frequency and severity of treatment-emergent or -worsening PN was low. In a study of bortezomib as single agent, grade 2 or higher PN was observed in 5% of patients. In two studies ~~of~~ investigating the triplet VTD regimen with standard-dose bortezomib, either once or twice a week, and thalidomide 100 mg daily, the rate of grade 3–4 PN did not exceed 1%.[7,29] In an additional study in which four cycles of VTD as consolidation were planned using higher doses of bortezomib (1.6 mg/m^2, once per week) and thalidomide (up to 200 mg/daily), grade 3–4 neuropathic pain was reported in 13% of cases.[30] Grade 2 PN was observed in 13% of patients treated with two cycles of RVD,[32] while 8% of patients receiving two VTD cycles had grade 2–3 neurological toxicity.[7]

Myelosuppression is the most common toxicity related to lenalidomide therapy. In a study designed to administer ~~4~~ two cycles of RVD consolidation with bortezomib at the standard dose and lenalidomide at 25 mg daily for 3 weeks, grade 3–4 neutropenia and thrombocytopenia were seen in 17% and 10% of patients, respectively.[32] As expected, severe thrombocytopenia (grade 3–4) was observed more frequently in comparison with that reported with the triplet VTD regimen (5.5% all grades).[7] Notably, no major infectious complications were reported with RVD.[32]

Table 2. Studies of Post-ASCT Novel Agent–Based Consolidation Therapy: Grade 3-4 Toxicities

Reference	Type of Trial	Treatment Scheme	No. of Patients	Hematologic Toxicity	Nonhematologic Toxicity
Cavo[7]	Phase III	VTD *v* TD	160 *v* 161	NR	Infections: 1.2% *v* 3.1% (P = NS) HZV: 0.6% *v* 0.6% (P = NS) GI: 1.8% *v* 0.6% (P = NS) PN: 0.6% *v* 0% (P = NS) DVT: 0.6% *v* 0.6% (P = NS)
Mellqvist[28]	Phase III	Bortezomib consolidation *v* no consolidation	187 *v* 183	NR	PN grade > 2: 5% *v* 1% (P < .04)
Ladetto[30]	Phase II	VTD	39	Thrombocytopenia 5%	Infections: 12% HZV: 5% GI: 7% PN: 7% Fatigue: 8%
Roussel[32]	Phase II	RVD	31	Neutropenia: 17% Thrombocytopenia: 10%	Grade 2 PN: 13%

Abbreviations: DVT, deep vein thrombosis; GI, gastrointestinal; HZV, herpes zoster virus; NR, not reported; PN, peripheral neuropathy; TD, thalidomide, dexamethasone; VRD, bortezomib, lenalidomide, dexamethasone; VTD, bortezomib, thalidomide, dexamethasone.

In one study designed to compare no consolidation with six cycles of bortezomib as single-agent consolidation therapy, the planned number of bortezomib infusions was 20 and the median number of infusions actually received was 19, corresponding to a median given dose of 90% (calculated as the total given dose divided by the total planned dose for each patient).[28] In another study of VTD versus TD consolidation, 93% of the patients in the VTD arm received the planned doses of bortezomib and thalidomide; in TD arm 97% of patients received the planned dose of thalidomide.[7]

The impact of consolidation therapy on patients' quality of life was prospectively evaluated in a phase III study.[28] In comparison with no consolidation, fatigue was reported more frequently after the first two cycles of bortezomib given twice weekly and was no more registered when the once-weekly schedule was used.

The major toxicities of consolidation therapy reported in the above-mentioned trials are summarized in Table 2.

CONCLUSION AND OPEN ISSUES

Overall, all available studies demonstrate that novel agent–based consolidation therapy enhances the frequency and depth of response achieved with the previous treatment phases, including induction with novel agents and either single or double ASCT. In several trials, the depth of response was improved up to the molecular level, a finding previously seen only after allogeneic stem cell transplantation. Enhanced rates and quality of responses offered by consolidation therapy translated into an extended PFS, suggesting that this treatment phase contributed to the improved clinical outcomes seen on an intention-to-treat analysis of the entire ASCT sequence.[19] Nevertheless, the role of consolidation needs to be formally demonstrated before this treatment strategy is routinely recommended.

Notably, in several trials the superior activity of a particular induction regimen was retained despite re-administration of the same therapy as post-ASCT consolidation, suggesting that a switch from one class to another class of novel agents is not necessary moving from induction to consolidation therapy. As previously demonstrated in the induction phase, it is likely that combining two different agents with different mechanisms of action, like a proteasome inhibitor with an IMiD, may help to maximize the activity of consolidation therapy.

Consolidation treatment appears to be generally safe, with a substantial reduction of toxic events in comparison with those frequently seen with the same regimen in the induction phase, a finding possibly related to a reduction in treatment intensity and changes in the schedule of the drugs. Recent availability of subcutaneous bortezomib, as well as of novel proteasome inhibitors that have little neurological toxicity, would potentially allow a higher dose-intensity and/or more prolonged consolidation therapy. Whether more intensive consolidation regimens might ultimately result in improved activity and reduced toxicity compared with previous ones remains an open issue.

Additional, not yet addressed, ~~is~~ issues include the choice of the best consolidation therapy, the need, if any, to use consolidation therapy in all patients or, by the opposite, to plan a risk- and/or response-adapted consolidation strategy and the interface of consolidation with subsequent maintenance therapy. All of these issues should be addressed in the context of future prospective randomized trials designed to further improve long-term clinical outcomes, while retaining a good quality of life.

REFERENCES

1. 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(2):91–7.
2. Child JA, Morgan GJ, Davies FE, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J Med. 2003;348(19):1875–83.
3. Koreth J, Cutler CS, Djulbegovic B, et al. High-dose therapy with single autologous transplantation versus chemotherapy for newly diagnosed multiple myeloma: a systematic review and meta-analysis of randomized controlled trials. Biol Blood Marrow Transplant. 2007;13(2):183–96.
4. Kumar A, Kharfan-Dabaja MA, Glasmacher A, Djulbegovic B. Tandem versus single autologous hematopoietic cell transplantation for the treatment of multiple myeloma: a systematic review and meta-analysis. J Natl Cancer Inst. 2009;101(2):100–6.
5. Cavo M, Baccarani M. The changing landscape of myeloma therapy. N Engl J Med. 2006;354(10):1076–8.
6. Cavo M, Rajkumar SV, Palumbo A, et al. International Myeloma Working Group consensus approach to the treatment of multiple myeloma patients who are candidates for autologous stem cell transplantation. Blood. 2011;117(23):6063–73.
7. Cavo M, Pantani L, Petrucci MT, et al. Bortezomib-thalidomide-dexamethasone is superior to thalidomide-dexamethasone as consolidation therapy after autologous hematopoietic stem cell transplantation in patients with newly diagnosed multiple myeloma. Blood. 2012;120(1):9–19.
8. Ludwig H, Durie BG, McCarthy P, et al. IMWG consensus on maintenance therapy in multiple myeloma. Blood. 2012;119(13):3003–15.
9. Attal M, Harousseau JL, Facon T, et al. Single versus double autologous stem-cell transplantation for multiple myeloma. N Engl J Med. 2003;349(26):2495–502.

10. 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(17): 2434–41.
11. Segeren CM, Sonneveld P, van der Holt B, et al. Overall and event-free survival are not improved by the use of myeloablative therapy following intensified chemotherapy in previously untreated patients with multiple myeloma: a prospective randomized phase 3 study. Blood. 2003;101(6):2144–51.
12. Goldschmidt H. Single vs double high-dose therapy in multiple myeloma: second analysis of the GMMG-HD2 trial. Haematologica (10th International Myeloma Workshop Meeting Abstracts). 2005;90(s1):S38.
13. Fermand J. High dose therapy supported with autologous stem cell transplantation in multiple myeloma: long term follow-up of the prospective studies of the MAG group. Haematologica (10th International Myeloma Workshop Meeting Abstracts). 2005;90(S1):S40.
14. Cavo M, Zamagni E, Tosi P, et al. Superiority of thalidomide and dexamethasone over vincristine-doxorubicindexamethasone (VAD) as primary therapy in preparation for autologous transplantation for multiple myeloma. Blood. 2005;106(1):35–9.
15. Rajkumar SV, Blood E, Vesole D, Fonseca R, Greipp PR. Phase III clinical trial of thalidomide plus dexamethasone compared with dexamethasone alone in newly diagnosed multiple myeloma: a clinical trial coordinated by the Eastern Cooperative Oncology Group. J Clin Oncol. 2006;24(3):431–6.
16. 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(30): 4621–9.
17. Lokhorst HM, van der Holt B, Zweegman S, et al. A randomized phase 3 study on theeffect of thalidomide combined with adriamycin, dexamethasone, and high-dose melphalan, followed by thalidomide maintenance in patients with multiple myeloma. Blood. 2010;115 (6):1113-2.
18. Morgan GJ, Davies FE, Gregory WM, et al. Cyclophosphamide, thalidomide, and dexamethasone as induction therapy for newly diagnosed multiple myeloma patients destined for autologous stem-cell transplantation: MRC Myeloma IX randomized trial results. Haematologica. 2012;97(3):442–50.
19. 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(9758):2075–85.
20. Moreau P, Avet-Loiseau H, Facon T, et al. Bortezomib plus dexamethasone versus reduced-dose bortezomib, thalidomide plus dexamethasone as induction treatment prior to autologous stem cell transplantation in newly diagnosed multiple myeloma. Blood. 2011;118: 5752–8.
21. Rosiñol 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(8):1589–96.
22. 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;20;30(24): 2946–55.
23. Ludwig H, Avet-Loiseau H, Bladé J, et al. European perspective on multiple myeloma treatment strategies: update following recent congresses. Oncologist. 2012;17(5):592–606.
24. Chanan-Khan A, Giralt S. Importance of achieving a complete response in multiple myeloma, and the impact of novel agents. J Clin Oncol. 2010;28(15): 2612–24.
25. Barlogie B, Anaissie E, Haessler J, et al. Complete remission sustained 3 years from treatment is a powerful surrogate for extended survival in multiple myeloma. Cancer. 2008;113(2):355–9.
26. 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(20):2540–7.
27. Barlogie B, Tricot G, Rasmussen E, et al. Total therapy 2 without thalidomide in comparison with total therapy 1: role of intensified induction and posttransplantation consolidation therapies. Blood. 2006;107(7): 2633–8.
28. 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 III trial. Blood. 2013;121 (23):4647–54.
29. Leleu X, Fouquet G, Hebraud B, et al. Consolidation with Vtd significantly improves the complete remission rate and time to progression following Vtd induction and single autologous stem cell transplantation in multiple myeloma. Leukemia 2013 Apr 5 [Epub ahead of print].
30. Ladetto M, Pagliano G, Ferrero S, et al. Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma. J Clin Oncol. 2010;28(12):2077–84.
31. Attal M, Cances-Lauwers V, Marit G, et al. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366:1782–91.
32. Roussel M, Robillard N, Moreau P, et al. Bortezomib, lenalidomide, and dexamethasone (VRD) consolidation and lenalidomide maintenance in frontline multiple myeloma patients: updated results of the IFM 2008 phase II VRD intensive program. ASH Annual Meeting Abstracts. 2011;118(21):1872.

Novel Generation of Agents With Proven Clinical Activity in Multiple Myeloma

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The activity observed with proteasome inhibitors and immunomodulatory drugs (IMIDs) in multiple myeloma (MM) has prompted the development of second- and third-generation agents with similar, but not exactly the same, mechanisms of action as their predecessors. This review summarizes the mechanism of action and the available data on the clinical activity of novel proteasome inhibitors (carfilzomib, oprozomib, ixazomib, and marizomib) and novel IMIDs (pomalidomide), stressing the similarities and differences with bortezomib, and with thalidomide and lenalidomide, respectively. In summary, these novel agents have shown clinical activity as single agents and in combination with dexamethasone, with similar or even higher efficacy than their parental drugs; moreover, they may even overcome resistance, indicating that there are some differences in their mechanisms of action and resistance. These data indicate that both the inhibition of the proteasome and the modulation of the immune system are good strategies to target MM tumor cells and this, along with the absence of complete cross-resistance observed among these drugs, open new avenues to optimize their use through the most appropriate sequencing and combinations.
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The outcome of multiple myeloma (MM) patients has dramatically improved in recent years and this has been possible essentially due to the introduction of several classes of agents, mainly proteasome inhibitors and immunomodulatory drugs (IMIDs).[1] Nevertheless, MM is still considered an incurable disease in the vast majority of patients and the classical pattern of evolution of the disease is of subsequent responses/relapses, with each relapse generally being of shorter duration than the previous ones. In this scenario, there is a need for novel drugs, either with novel mechanisms of action, or agents based on the mechanisms of action that have already been demonstrated to be effective in the treatment of MM. Although several agents directed against novel targets have been developed, their activity as single agents is generally limited and most of them need to be combined with others with a broader spectrum of activity to have efficacy. By contrast, the pleiotropic activity of proteasome inhibitors and of IMIDs has clearly demonstrated clinical activity and this has led to the development of second- and third-generation derivatives with several aims: to maintain or even increase the activity of the parental drugs, to decrease toxicity, and to find a more convenient schedule or route of administration.

This article reviews the rationale for the use of these novel proteasome inhibitors and IMIDs and the clinical results currently available with these agents.

PROTEASOME INHIBITORS

Biological Rationale for Their Anti-myeloma Effects

The discovery of the catalytic activity of the proteasome[2] along with the synthesis of bortezomib (PS-341),[3] the first-in-class proteasome inhibitor and the demonstration of its preclinical[4,5] and clinical[6–8] activity in MM, has been one of the major milestones in the treatment of MM patients in the last years.

The proteasome is an intracellular enzyme complex responsible for the degradation of most of the intracellular proteins. It has three important catalytic

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subunits: the β1 (caspase-like), β2 (trypsin-like), and β5 (chymotrypsin-like), that, in selected conditions, such as after exposure to interferon-γ (IFN-γ) or tumor necrosis factor-α (TNF-α), may be replaced with β1i (LMP2), β2i (MECL1 or LMP10), and β5i (LMP7) to form what is called the immunoproteasome.[9] When a cell needs to eliminate a protein, it becomes polyubiquitinated by a specialized set of enzymes, and is then recognized by the proteasome and degraded into small peptides.[10]

This pathway is of key importance in cellular homeostasis and its inhibition has been associated with several biological processes that lead to an anti-myeloma effect.[11–13] Among the main consequences responsible for this anti-tumor activity, it is important to highlight the blockade of the degradation of cyclin- or cyclin-dependent kinase (CDK)-inhibitors and several anti-apoptotic and tumor-suppressor proteins. Proteasome inhibition also prevents the clearance of misfolded proteins, inducing endoplasmic reticulum (ER) stress and activation of the unfolded protein response.[14,15] Finally, proteasome inhibitors block the nuclear factor-κB (NF-κB) transcription factor pathway, by preventing the degradation of the IκB (inhibitor of NF-κB) after its polyubyquitination by IKK (IκB kinase).[16]

Development of Proteasome Inhibitors: From Bortezomib to Second-Generation Proteasome Inhibitors

Bortezomib was the first proteasome inhibitor introduced into the clinic. It is a boronic acid derivative that reversibly inhibits the chymotrypsin- and caspase-like activities of both the constitutive proteasome and the immunoproteasome.[17,18] Although the intravenous route is the most common way of administration, subcutaneous administration may be equally effective and less toxic, and both routes are currently approved.[19]

Bortezomib has represented an excellent example of a novel drug that was quickly moved from the bench to the beside and, now, after a decade of experience in clinical practice, the investigation continues in order to optimize its use not only in terms of efficacy but safety and tolerability as well.

The initial phase I trial with bortezomib as monotherapy in patients with relapsed and/or refractory hematologic malignancies showed a clinical benefit in the nine patients with plasma cell dyscrasias included in the trial including one with a durable complete remission (CR), who also was the first myeloma patient ever treated with bortezomib.[20] This activity against relapsed/refractory myeloma was then further evaluated in two multicenter phase II trials, the Study of Uncontrolled Multiple Myeloma Managed with Proteasome Inhibition Therapy (SUMMIT) and the Clinical Response and Efficacy Study of Bortezomib in the Treatment of Relapsing Multiple Myeloma (CREST).[6,21] Patients on the SUMMIT trial received an initial dose of 1.3 mg/m^2, while the smaller CREST study also explored a lower dose of 1.0 mg/m^2. On the SUMMIT trial, where most of the patients included had disease refractory to the last line of treatment, a partial response (PR) or better was seen in 27% of the 193 evaluable patients. In addition, 10% of patients achieved CR or near-CR, with a median time to progression (TTP) of 7 months, approximately double that with their previous line of therapy.[6] The patients included in the CREST trial had relapsed or refractory disease after front-line therapy and received bortezomib either alone at 1.3 mg/m^2 or with the addition of dexamethasone; the response rate was 50%. On the other hand, the cohort of patients who received bortezomib at 1.0 mg/m^2 achieved an overall response rate of 38%. Considering this result, most of the following trials have used bortezomib as an intravenous push at 1.3 mg/m^2. However, it is interesting to note that the efficacy observed with 1.0 mg/m^2 was balanced with a lower likelihood of developing some adverse events and this finding has been considered in the management of bortezomib for dose-reduction schedules.[21]

The data observed in the SUMMIT trial were the rationale for an accelerated approval of bortezomib for relapsed/refractory myeloma patients, and led to a randomized phase III trial, the Assessment of Proteasome Inhibition for Extending Remissions, or APEX study.[7] This trial included patients who had relapsed after no more than three prior lines of therapy, and were randomized to receive bortezomib as monotherapy at 1.3 mg/m^2 as an intravenous push on days 1, 4, 8, and 11 followed by a 10-day rest period or high-dose dexamethasone as monotherapy. The first report of the results already observed a significant benefit for bortezomib arm, with a ≥PR rate of 38%, including 9% CRs, compared with 18% and $<1\%$ for the dexamethasone arm. Continued therapy led to an improvement in the responses rate on the bortezomib arm up to 43%, while no significant benefit was observed in patients on the dexamethasone arm. The median TTP was 6.22 months with bortezomib and 3.49 with dexamethasone, and this benefit also translated into overall survival (OS), with a median of 29.8 and 23.7 months for bortezomib and dexamethasone, respectively.[8] These data supported the full approval of bortezomib for patients with relapsed and/or refractory myeloma who had received at least one prior therapy, and it was registered as an antineoplastic agent for intravenous use only at a dose of 1.3 mg/m^2 given as a 3- to 5-second bolus intravenous injection via peripheral or central intravenous catheter, followed by a standard saline flush;

in addition, it is indicated to maintain at least a 72 hours rest period between doses in order to allow a restoration of the proteasome function towards baseline.

The aforementioned three trials clearly supported modulation of the proteasome function as an attractive therapeutic option; moreover, preclinical studies showed that bortezomib could enhance the sensitivity to other agents and, in many cases, even overcome drug resistance.[22] The first preclinical data showed a synergistic and/or additive effect between bortezomib and corticosteroids, and accordingly, in the SUMMIT and CREST studies, patients with suboptimal responses to single-agent bortezomib received dexamethasone at 20 mg on the day on and after each dose of bortezomib. With this approach, improvements in the quality of response were seen in up to one third of such patients, and other trials have shown that with the addition of corticosteroids, response rates improve to 60% or more, without increases in toxicity.[6,21]

The second step was to combine bortezomib with other agents, such as pegylated liposomal doxorubicin. A phase III randomized trial showed that this combination was able to improve the median TTP as compared to bortezomib alone by approximately 3 months (9.3 months *v* 6.5 months). Based on these results, bortezomib plus pegylated doxorubicin was approved for relapsed and/or refractory myeloma patients.[23] Bortezomib has also been combined with alkylating agents, including cyclophosphamide, melphalan, and bendamustine. Melphalan-based combinations with bortezomib have been widely used, ranging from the doublet to four-drug programs, such as bortezomib, melphalan, prednisone, and thalidomide.[24] The next step was to combine bortezomib with the IMiDs, thalidomide and lenalidomide. These combinations have resulted in an overall response rate up to 60%–70% and, notably, they have shown activity even in patients who had previously relapsed or progressed through bortezomib plus dexamethasone, or lenalidomide plus dexamethasone.[25–27]

Based on the positive results obtained with bortezomib in relapsed and/or refractory myeloma patients, several groups moved to use it upfront, both in young patients candidates to autologous stem cell transplant and in elderly patients. Four randomized trials have evaluated the role of bortezomib-based combinations as induction therapy in transplant candidate myeloma patients, revealing a high efficacy (>80% response rate, with 20%–30% CRs) that increased after autologous stem cell transplant, confirming the results of numerous previous pilot studies with bortezomib-based combinations.[28–31] In patients who are not candidates to autologous stem cell transplant, bortezomib in combination with melphalan and prednisone also has proved to be superior to conventional therapy, with high overall and CR rates (81% and 30%, respectively), and a significantly longer TTP (24 months) and OS (60% at 3 years) as compared with conventional schemes. This combination, bortezomib plus melphalan and prednisone, was the last approval obtained for bortezomib, in this case for untreated MM patients not eligible for stem cell transplantation.[32]

As far as tolerability is concerned, all these trials contributed also to establish the pattern of adverse events (AEs) of bortezomib and their management. In the SUMMIT trial, the most significant AEs reported were thrombocytopenia, fatigue, peripheral neuropathy, and neutropenia.[6] It should be noted that in the CREST trial, the cohort of patients receiving bortezomib at a dose of 1 mg/m^2 experienced a lower likelihood of developing some adverse events such as diarrhea, vomiting, and neuropathy.[21] The APEX trial, including more than 600 patients, was optimal to define the toxicity profile; the most significant side effects of all grades were diarrhea (57%), nausea (57%), fatigue (42%), constipation (42%), neuropathy (36%), vomiting (35%), anorexia (35%), and thrombocytopenia (35%). Although the majority of these AEs were grade 1 or 2, thrombocytopenia, gastrointestinal toxicity, and peripheral neuropathy have been more extensively studied because they are probably the most significant, especially peripheral neuropathy. Thus, the frequency of grade 3–4 thrombocytopenia and gastrointestinal symptoms are approximately of 25% and 20%, respectively.[7] Concerning bortezomib-related peripheral neuropathy, its incidence of grade 3–4 ranged from 8%–15% and no significant differences in incidence, severity, or outcome have been reported between newly diagnosed and relapsed and/or refractory patients. However, patients who received bortezomib-containing therapy as initial induction did experience less neuropathic pain and fewer symptoms, which resolved or improved more quickly than in those with relapsed disease. Moreover, the new combination schemes using weekly doses of bortezomib have shown a significant reduction in the incidence of peripheral neuropathy, which is now between 5%–8%.[33,34]

Based on these positive results, bortezomib as a proteasome inhibitor is considered part of the backbone of the treatment of MM patients and its introduction in the setting of relapsed and newly diagnosed patients translated into prolonged OS. However, myeloma remains as an incurable disease and patients finally have subsequent relapses. In addition, some patients become bortezomib-refractory, which is why other proteasome inhibitors were necessary to increase the treatment armamentarium and also to

Table 1. Biological Features of the Most Relevant Proteasome Inhibitors in MM

Proteasome Inhibitor	Type	Catalytic Activity Inhibition			Pattern of Inhibition	Route
		CT-L	C-L	T-L		
Bortezomib (PS-341)	Boronic acid	X	X		Reversible	IV/SC
Carfilzomib (PX-171)	Epoxyketone	X			Irreversible	IV
Oprozomib (ONX-0912)	Epoxyketone	X			Irreversible	PO
Ixazomib (MLN-9708)	Boronic acid	X	X		Reversible	IV/PO
Marizomib (NPI-0052)	Salinosporamide	X	X	X	Irreversible	IV

Abbreviations: CT-L, chymotrypsin-like; C-L, caspase-like; T-L, trypsin-like; IV, intravenous; SC, subcutaneous; PO, oral.

be able to rescue bortezomib-refractory patients. For this reason, novel proteasome inhibitors emerged, belonging to the same chemical family of boronic acids (ixazomib [MLN-9708]) or to different structural families such as epoxyketones (carfilzomib and oprozomib) or salinosporamides (marizomib) (Table 1). These agents differ in the biological properties, as they target different catalytic subunits of the proteasome as compared to bortezomib, being either selective of the chymotrypsin-like activity such as carfilzomib or oprozomib, or having a broader pattern of inhibition, as is the case of marizomib. Another difference is the reversibility of the inhibition, and, in this regard, carfilzomib and oprozomib irreversibly inhibit this activity. Finally, some of these novel agents have the potential to be orally bioavailable, including ixazomib and oprozomib. The next section reviews the clinical results with these novel proteasome inhibitors (Table 2).

Efficacy and Safety Results With the Novel Proteasome Inhibitors

Carfilzomib

Carfilzomib is a second-generation proteasome inhibitor widely introduced in the clinic following bortezomib and, in fact, the US Food and Drug Administration (FDA) granted accelerated approval to carfilzomib injection for the treatment of patients with MM who have received at least two prior therapies, including bortezomib and an immunomodulatory agent, and have demonstrated disease progression on or within 60 days of the completion of the last therapy.

It has a quite different pattern of proteasome inhibition as compared with bortezomib. In this regard, carfilzomib induces an irreversible inhibition of the proteasome, and, therefore, the proteasomal activity of the cells is only restored when they synthesize new proteasomes. Morever, also in contrast to bortezomib, carfilzomib has a great specificity against the β5 (chymotrypsin-like) subunit, with very little or no inhibitory effect in the other catalytic subunits. These biological differences may lead to differences in their clinical activity and, in fact, preclinical experiments have demonstrated that carfilzomib may overcome bortezomib resistance.[35]

Regarding the preclinical rationale for choosing one schedule of administration, Demo et al[36] observed in preclinical in vivo experiments that carfilzomib was well tolerated when administered for either 2 or 5 consecutive days and the anti-tumor efficacy of this drug delivered on 2 consecutive days was greater than that of bortezomib administered on its usual clinical dosing schedule with 2 days rest after each dose. This may be due to the higher efficacy of a sustained inhibition of the proteasome for 48 hours, and led to the choice of the 2 consecutive days weekly schedule for most of the clinical studies.

The first phase I study with carfilzomib was performed in patients with advanced hematological malignancies, including 29 patients with MM.[37] Carfilzomib was given during 5 consecutive days of a 14-day cycle at escalating doses. The maximum tolerated dose (MTD) was determined to be 15 mg/m^2 and antitumor activity was observed at doses greater than 11 mg/m^2; one PR and one minor response (MR) were observed in MM. The most significant nonhematologic toxicities included fatigue, nausea, and diarrhea in more than a third of patients, and most were grade 1 or 2 in severity. No grade 3 or 4 peripheral neuropathies were reported.

The first trial that evaluated the administration of carfilzomib, twice weekly on consecutive days for 3 weeks in 28-day cycles, was performed by Alsina et al (PX-171-002).[38] It was a dose-escalating phase I trial that included 28 patients with relapsed/refractory MM. The minimal effective dose (MED) was 15 mg/m^2 with maximum (80%) proteasome inhibition achieved in peripheral blood and mononuclear cells at this dose. The MTD was not reached at 27 mg/m^2. Seven of the 26 evaluable patients achieved a response: five PRs and two MRs. Regarding safety, a reversible grade 2 creatinine increase was reported in some patients and was associated with a rapid

Table 2. Most Relevant Clinical Trials With Novel Proteasome Inhibitors in Monotherapy in Relapsed/Refractory MM

Drug	Trial	Phase	n	Dose	Schedule	ORR (≥PR)
Carfizomib (PR-171)	PX-171-001[37]	1	10 MM	MTD: 15 mg/m^2	1-5 /14d	10%
	PX-171-002[38]	1	28 MM	Recommended dose: 20 mg/m^2 initially 27 mg/m^2 from C1D8	1-2, 8-9, 15-16/28d	19%
	PX-171-003A0[39]	2	46	20 mg/m^2	1-2, 8-9, 15-16/28d	17%
	PX-171-003A1[110]	2	266	20 mg/m^2 in C1 27 mg/m^2 from C2	1-2, 8-9, 15-16/28d	24%
	PX-171-004[41,43]	2	129 Btz-naïve pts	C-1: 20 mg/m^2 C-2: 20 mg/m^2 in C1 27 mg/m^2 from C2	1-2, 8-9, 15-16/28d	C-1: 42%, C-2:52%
			35 Btz-treated pts	20 mg/m^2	1-2, 8-9, 15-16/28d	17%
	PX-171-005[45]	2	39, renal impairment)	15 mg/m^2 in C1 20 mg/m^2 in C2 27 mg/m^2 from C3	1-2, 8-9, 15-16/28d	25%
Ixazomib (MLN-9708)	C16004[111]	1	36	MTD: 2.97 mg/m^2	1, 8, 15/28d	11%
	C16003[112]	1	57	MTD: 2 mg/m^2	1, 4, 8, 11/21d	13%
Marizomib (NPI-0052)	NPI-0052-101, NPI-0052-102[63]	1	34	MTD: 0.4 mg/m^2 in 1h inf. & 0.5 mg/m^2 in 2h inf.	1, 4, 8, 11/21d	14%

Abbreviations: ORR, overall response rate; PR, partial response; Btz, bortezomib; pts, patients; MTD, maximum tolerated dose.

decline in M-protein without evidence of tumor lysis syndrome. Cyclic thrombocytopenia was rapidly reversible and painful peripheral neuropathy was not reported.

Based on the data obtained in these studies, two phase II trials were initiated in patients with relapsed and refractory MM. PX-171-003-A0 included 46 MM patients relapsing after at least two prior lines of therapy. All were also refractory to the last line of therapy.[39] They received carfilzomib at 20 mg/m^2 on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle for up to 12 cycles. The overall response rate (ORR) was 17% and the clinical benefit rate (CBR) including MR was 24%. The good results obtained in this part of the trial prompted the expansion into the PX-171-003-A1, which included 266 patients who had previously received bortezomib, an IMiD, and an alkylator.[40] The dose and schedule of treatment were identical to that in the PX-171-003-A0 trial in the first cycle, but then dose was escalated to 27 mg/m^2 from cycle 2. The ORR (≥PR) was 24% with a median duration of response of 7.8 months and a median OS of 15.6 months. A second trial, PX-171-004, was started in relapsed/refractory MM, and patients were stratified according to whether or not they had been exposed previously to bortezomib. The schema of treatment was identical to that described for the PX-171-003 trial, but 129 patients were included in two cohorts; a first cohort received 20 mg/m^2 of carfilzomib for the 12 cycles of the trial and a second received the dose escalated to 27 mg/m^2 from cycle 2. Among the bortezomib-naïve patients, 42% and 52% achieved ≥PR in the 20- and 27-mg/m^2 cohorts, respectively.[41] The median TTP was 8.3 months and median duration of response was 13.1 months in cohort 1, while the median TTP and duration of response for cohort 2 have not been reached. These figures compare favorably with the 43% ORR and 6.2 months of TTP observed with bortezomib in a similar population in the APEX trial, despite the fact that the PX-171-004 trial included more patients refractory to prior therapies.[7,8] It also indicates a dose-response relationship for the drug, and this observation was confirmed and quantified using a statistically rigorous, multivariate analysis including 430 patients from phase II studies, showing that the dose response relationship was also apparent in the magnitude of response (PR or better) across study participants.[42] Thirty-five patients were previously exposed to bortezomib, but not necessarily refractory to it, and after treatment with carfilzomib at 20 mg/m^2, 17% of them achieved a PR or better.[43] This is similar to what was observed in the PX-171-003 in which 19% of patients refractory to bortezomib in their last line of therapy obtained ≥PR,[40] probably indicating a non-complete cross-resistance between these two proteasome inhibitors. It is important to remark that the MTD for single-agent carfilzomib has not been defined in the relapsed setting. In fact, the drug is being tested in a dose-escalation trial with doses up to 56 mg/m^2 that have proven to be safe.

The role of prognostic factors, such as high-risk cytogenetic abnormalities, has been evaluated in the PX-171-003 and -004 trials.[44] Although numbers are still small, response rates were almost identical in patients with standard- and high-risk cytogenetic abnormalities, showing a trend towards to a higher response rate for t(4;14) but a lower rate for t(14;16), as well as a shorter duration of response for the subgroup of patients with del 17p13.

The PX-171-005 trial was designed to evaluate the activity and safety of this novel proteasome inhibitor in patients with renal insufficiency.[45] Carfilzomib was administered at a dose of 15 mg/m^2 intravenously on days 1, 2, 8, 9, 15, and 16 every 28 days for cycle 1, escalating to 20 mg/m^2 in cycle 2 and 27 mg/m^2 from cycle 3, with the possibility of adding dexamethasone in case of suboptimal response (patients failing to achieve PR by cycle 2 or CR by cycle 4). Thirty-nine patients with different degrees of renal impairment were enrolled. Pharmacokinetic and pharmacodynamic parameters were similar across all groups. Carfilzomib was undetectable in plasma within 3 hours and did not accumulate after cycle 2, and PR or better was achieved in 25% of patients with a 7.9-month median duration of response.

Regarding safety, a pooled analysis of the toxicity profile of 526 patients receiving carfilzomib in monotherapy in these three phase II trials (PX-171-003, 004, and 005) has been reported recently.[46] The most frequent AEs (present in ≥30% of patients) were fatigue (55%), anemia (47%), nausea (45%), thrombocytopenia (36%), dyspnea (35%), diarrhea (33%), and pyrexia (30%). The grade 3 AEs present in ≥10% of patients were mostly hematological: thrombocytopenia (23%), anemia (22%), lymphopenia (18%), and neutropenia (10%). Interestingly, only 14% developed peripheral neuropathy (with only 1.3% grade 3). Moreover, dose modifications or discontinuations were required in only 5 patients (1%). Renal AEs (mainly grade 2) were reported in 174 (33%) patients, but carfilzomib was discontinued because of a renal AE in only 21 patients (4%), which is in line with the results of the PX-171-005 trial performed in patients with renal impairment.[45] Concerning cardiac toxicity, cardiac failure events were reported in 7% of patients regardless of causality. Cardiac events resulting in treatment discontinuation included congestive heart failure (2%), cardiac arrest (1%), and myocardial ischemia (<1%). The extent to which cardiac events were due to patients' baseline comorbidities, toxicity from prior treatments, effects of MM, carfilzomib itself, or

a combination of these factors could not be determined. Rates and causes of death were consistent with those observed in heavily pretreated patients with end-stage MM. The conclusion of this analysis is that single-agent carfilzomib has an acceptable safety profile in heavily pretreated patients with relapsed and refractory MM.

Based on all these trials and in order to support regulatory approvals around the world, a phase III randomized trial (Focus) has just completed its recruitment in Europe, comparing carfilzomib versus best supportive care in patients with relapsed and refractory MM who have received three or more prior therapies.

Apart from all these studies of carfilzomib monotherapy, several combinations are currently being explored, both in the relapsed and the upfront settings. The PX-171-006 trial has studied the combination of carfilzomib with lenalidomide and low-dose dexamethasone in 52 relapsed refractory patients. It showed excellent tolerability that allowed the continued administration of the combination for up to 18 cycles and an ORR of 78% including 18% CR/near-CR, 22% very good partial response (VGPR), and 38% PR.[47] Fifty-three newly diagnosed patients also have been treated with this combination in a recently reported phase I/II study.[48] Successful stem cell harvest was achieved in all the patients in which it was attempted. After a median of 12 cycles, 62% of patients achieved at least near-CR and 42% stringent CR. Responses were rapid and improved during treatment. In 36 patients completing eight or more cycles, 78% achieved at least near-CR and 61% stringent CR. With a median follow-up of 13 months, the 24-month progression-free survival estimate was 92%. Regarding the toxicity profile, low rates of neutropenia (12% of grade 3–4) were observed, and 24% of patients had peripheral neuropathy that was limited to grade 1/2 in all cases. Another combination that is being explored is carfilzomib plus thalidomide and dexamethasone in untreated patients; so far, the ORR is 84% (stringent CR + CR = 21%), but some safety concerns have been raised around the occurrence of two tumor lysis syndromes, five cardiac events, and grade 1/2 peripheral neuropathy (probably thalidomide-related) in 35% of patients.[49] Other drugs such as histone deacetylase inhibitors (vorinostat or panobinostat), pomalidomide, or alkylators are being investigated with carfilzomib in different combinations. Finally, a randomized phase III trial (Aspire) comparing the efficacy and safety of lenalidomide plus low-dose dexamethasone with or without carfilzomib in patients with relapsed or progressive MM has already completed enrollment.[50]

Oprozomib (ONX-0912 and previously PR-047) is another second-generation proteasome inhibitor[51] that is a structural analog of carfilzomib and is orally bioavailable. It has demonstrated high levels of proteasome inhibition and an acceptable safety profile in a phase 1 trial in patients with advanced solid tumors,[52] and the preliminary results of a phase Ib trial in chronic lymphocytic leukemia and MM (nine patients included) has shown one PR and one MR among the MM patients. The main AEs were gastrointestinal toxicity and thrombocytopenia.[53]

MLN9708 (Ixazomib)

MLN9708 is other second-generation proteasome inhibitor. It is a dipeptidilic boronic acid that is rapidly hydrolyzed in water and converts into MLN2238, the active form that potently, reversibly, and selectively inhibits the proteasome. As compared with bortezomib, MLN9708/MLN2238 has a shorter proteasome dissociation half-life (18 *v* 110 minutes), a larger blood volume distribution at steady state, and greater pharmacodynamic effects in tissues. MLN2238 is active, even in bortezomib-resistant cells and a head-to-head analysis of MLN2238 versus bortezomib showed a significantly longer survival time in tumor-bearing mice treated with MLN2238 than mice receiving bortezomib.[54]

MLN9708 is the first proteasome inhibitor used in the clinics that is orally bioavailable. Several phase I studies have evaluated the safety of MLN9708 in different patient populations and by using different routes of administration (oral or intravenous). Moreover, the preliminary pharmacokinetic (PK) results indicate that the administration of MLN9708 in a flat dose is feasible, which makes it very convenient for oral administration.[55]

Two studies are evaluating the oral administration of MLN9708 in monotherapy in relapsed/refractory MM patients previously exposed to proteasome inhibitors. One of them (C16004), including to date 32 patients, uses a weekly administration (days 1, 8, and 15 of 28-day cycles) of the drug. The MTD has not yet been reached at 2.94 mg/m^2 and 11% of patients had ≥PR (one VGPR, one PR, eight stable disease [SD]).[56] The second one (C16003) is administering MLN9708 in a biweekly schedule (days 1, 4, 8, and 11 of 21-day cycles).[57] In the dose-escalating phase of this trial, 26 patients were included and the MTD was established at 2 mg/m^2. Thirty more patients have been included in the dose-expansion phase thus far. Preliminary results showed an ORR of 13% (one CR, five PRs, one MR, 28 SD).

Regarding toxicity, in the two oral studies in MM, the most common all-grades AEs were fatigue (30%–40%), thrombocytopenia (30%–40%), nausea (30%), diarrhea (25%), vomiting (20%), and less frequently rash and neutropenia. Between 14%–21% of patients had AEs resulting in dose reductions and in 6%–11% the drug had to be discontinued. This indicates a

similar toxicity profile to that previously observed with bortezomib. Interestingly, only 10% of the patients reported peripheral neuropathy, and in all of them it was grade 1–2; moreover, all of these patients had residual peripheral neuropathy at the time of entry in the trial.

Other studies are currently studying the activity of this drug in different combinations in newly diagnosed MM. This is the case of the combination with melphalan and prednisone (C16006) or with lenalidomide and low-dose dexamethasone (C16005 and C16008). The preliminary results of the first of these trials in 65 patients were recently reported, with a 88% ≥PR rate and a safety profile similar to that already reported with this agent: grade 3 any-drug–related AEs in 40% of patients, including erythematous rash, and nausea and vomiting (5% each). Low rates of peripheral neuropathy were observed, with one patient (3%) experiencing grade 3 and three patients (9%) grade 2 peripheral neuropathy.[58]

The results of the combination with melphalan and prednisone, based on the weekly administration of MLN9708, have been recently reported, with all 15 patients evaluable for response achieving ≥PR (three CRs, six VGPRs, and six PRs) and a good tolerability, similar to that already mentioned.[59]

Marizomib

Marizomib (NPI-0052 or salinosporamide A) is a non-peptide novel proteasome inhibitor that was isolated from the marine actimomycete *Salinispora tropica*. It differs structurally from other proteasome inhibitors that are peptide mimetics, and these structural differences translate into significant differences in proteasome inhibition, toxicology, and efficacy profiles between these two classes of inhibitors.[60,61] It is a potent inhibitor of the three catalytic subunits of the proteasome, which is different than what has been described for bortezomib (quite specific of the β1 and β5), and carfilzomib (β5-specific). Moreover, similar to carfilzomib and different from bortezomib, it has an irreversible pattern of inhibition. This explains, at least in part, the synergy observed in an in vivo model with the combination of bortezomib + NPI-0052.[62] Although it is orally active, the trials performed to date have used the intravenous formulation.

Three phase I trials in advanced solid tumors or refractory lymphoma (NPI-0052-100), in MM (NPI-0052-101), and in advanced malignancies (NPI-0052-102) are currently recruiting patients. Trials 101 and 102 included 44 and 25 MM patients, respectively.[63] Marizomib was given intravenously on days 1, 4, 8, and 11 of 21-day cycles. Dexamethasone was administered to all patients in the first trial and only in case of suboptimal response in the second one. One third of the patients had received previous bortezomib and more than 50% of them were bortezomib-refractory. Nineteen percent of patients achieved a PR and 57% SD. In the bortezomib-refractory population, the response rate was similar (17% ≥PR and 67% SD), indicating that NPI-0052 may overcome bortezomib resistance due to its different mechanism of action. The most frequent AEs were fatigue, insomnia, nausea, diarrhea, constipation, headache, and pyrexia, but most of them were grade 1/2. No significant peripheral neuropathy was observed, suggesting that the bortezomib-induced peripheral neuropathy may be an off-target effect not related to proteasome inhibition.

Novel Immunomodulatory Agents: Pomalidomide

Mechanism of Action of Immunomodulatory Agents

Following the discovery of the anti-myeloma activity of thalidomide, several analogs (lenalidomide-CC-5013 and pomalidomide-CC-4047) with similar structure were developed. Although the mechanisms underlying their effect has been extensively studied, they are not yet clearly understood. In this regard, recent studies suggest that they bind to cereblon, a molecule that forms an E3 ubiquitin ligase complex with damaged DNA binding protein 1 (DDB1) and Cul4A,[64,65] and the absence of cereblon is associated with resistance to IMIDs. Several mechanisms have been associated with the antitumor activity of lenalidomide, such as a decrease in interferon regulatory factor 4 (IRF4) levels,[66,67] the induction of several CDK inhibitors (p15, p16, p21, and p27),[68,69] or the induction of p21 WAF-1 expression through an LSD1-mediated epigenetic mechanism.[70] This agent also disrupts the interaction between tumor cells and their microenvironment by decreasing interleukin-6 and vascular endothelial growth factor (VEGF) levels.[68,71] However, the most specific effect of this group of drugs is the immunomodulatory effect, which is mediated through the augmentation of natural killer (NK) cytotoxicity,[72,73] the inhibition of regulatory T cells,[74] and the restoration of the immune synapse formation.[75]

Although thalidomide, lenalidomide, and pomalidomide share a common basic structure and mechanism, they differ in their potency related to their cytotoxic, immunomodulatory, and anti-angiogenic properties.[76]

Clinical Results With Thalidomide and Lenalidomide

Thalidomide was the first IMiD introduced into the treatment of MM patients and its therapeutic efficacy

was initially shown in 1999[77] in relapsed/refractory MM with an ORR of 37% (2% CR, 12% near-CR) and a 2-year event-free survival (EFS) and OS rates of 20% and 48%, respectively. A review of phase II studies using thalidomide as monotherapy in 1629 MM patients shows an ORR of 29%.[78] In combination with dexamethasone, the PR rate increases to 35% and 55% (mean, 47%), and extends the progression-free survival (PFS) as compared with placebo/dexamethasone (PFS at 1 year: 46.5% *v* 31%, $P = .004$).[79] Even higher response rates (55%–76%) have been reported upon adding cyclophosphamide, melphalan, or etoposide. In fact, the oral combination of thalidomide plus cyclophosphamide and dexamethasone is widely used in this setting, and in the experience of the Spanish Myeloma Group (GEM), this combination results in a response rate of 60%, including 10% CRs, and it yields durable responses (57% EFS at 2 years).[80] Thalidomide was subsequently moved to the first line of therapy and, at present, its use in combination with bortezomib plus dexamethasone is one of the standard induction regimens in young patients.[30,81,82] In elderly patients, thalidomide in combination with MP (melphalan + prednisone) or cyclophoshamide and adjusted dose of dexamethasone represent also two standards of care for this patient population.[83,84] One of the most significant issues of thalidomide is the toxicity profile, especially peripheral neuropathy. This thalidomide-related AE led to a high rate of discontinuation of treatment, especially in elderly patients.

At present, thalidomide is being replaced by the next IMiD, Lenalidomide, which is more potent and less toxic. Initial results from phase I and II studies conducted in relapsed/refractory patients showed that lenalidomide, used as a single agent, has relatively low activity with response rates ranging from 17%–29%.[85,86] However, its activity was markedly increased when lenalidomide was combined with dexamethasone. Two phase III trials (one in the United States and the other in Europe) compared lenalidomide plus dexamethasone versus dexamethasone alone in relapsed/refractory MM. In both studies, the lenalidomide arm was associated with a significantly higher response rate (≥PR, mean 60% *v* 22%), CR rate (15% *v* 2%), and longer time to progression (median, 11.1 *v* 4.7 months). Moreover, treatment with lenalidomide/dexamethasone is associated with longer median OS (35 *v* 31 months) in spite of the fact that 42% of patients from the dexamethasone arm crossed over.[87,88] The activity of this regimen was independent of the number of lines of therapy, including transplant and previous use of thalidomide as well as age. These trials supported the approval of lenalidomide plus dexamethasone in relapsed and/or refractory MM patients. Lenalidomide also has been evaluated in relapsed/refractory MM patients in combination with anthracyclines, such as doxorubicin plus dexamethasone with a response rate of 87% (23% CRs)[89] or liposomal doxorubicin, vincristine, and dexamethasone (DVd-R) with a response rate (≥PR) of 75%, including 29% CR/near-CR. It also has been combined with cyclophosphamide and dexamethasone (Len-Cy-Dex) showing a response rate of 65%.[90] Lenalidomide also has been moved to the upfront setting, as an induction regimen in young and elderly patients and also as maintenance therapy. In spite of these positive results, as occurs with the proteasome inhibitors, patients became lenalidomide-refractory and other new IMiDs were needed. Pomalidomide emerged as the next-generation IMiD. The next sections review the clinical trials that have been reported with this agent with or without dexamethasone in MM (Table 3).

Initial Clinical Results With Pomalidomide

The first study in MM was a phase I study of pomalidomide alone in relapsed/refractory MM patients, who had previously received at least two cycles of treatment (CC-4047-MM-001). Twenty-four relapsed or refractory patients with a median number of three prior lines of treatment were treated with oral pomalidomide at escalating doses of 1, 2, and 5 mg, in two different schedules: daily[91] and every other day.[92] The MTD was defined at 2 mg for the daily schedule. The drug was tolerated with no serious nonhematologic adverse events. Three patients developed a deep venous thrombosis (DVT). Thirteen of the 24 evaluable patients (54%) experienced at least a PR and four patients (17%) entered CR with a PFS of 9.7 months and a median OS of 22.5 months. In the second cohort, 20 patients received pomalidomide on alternate days. MTD was defined as 5 mg. No thrombotic events were observed. Ten percent of patients had a CR, and ≥PR was achieved in 50% of subjects. PFS was 10.5 months and median OS was 33 months. The most common AEs in both cohorts were hematological (neutropenia, which was the main dose-limiting toxicity, and thrombocytopenia). As indicated, DVT was only observed in the once-daily dosing.

An alternative schedule of pomalidomide alone and in combination with dexamethasone given during 21 days of a 28-day cycle was explored in the phase Ib/II trial CC-4047-MM-002. In the phase I portion of the study, 38 patients who had received at least two prior therapy regimens including lenalidomide and bortezomib and were refractory to the last regimen were enrolled. Twenty-four of the patients were refractory to both bortezomib and lenalidomide. The median of prior lines of

Table 3. Most Relevant Clinical Trials With Pomalidomide +/− Dexamethasone in MM

Reference	Phase	n	Dose	Schedule	Dex	Response ≥PR	DOR (mo)	PFS (mo)	OS (mo)
C-4047-MM-001									
Schey[91]	1	24	MTD:2 mg	1-28 (daily)	No	54%		9.7	22.5
Streetly[92]	1	20	MTD: 5 mg	1-28 (every other day)	No	50%		10.5	33
CC-4047-MM-002 richardson[93,94]	1b	38	MTD: 4 mg	1-21	Yes†	22% + Dex: 2 PR	6.5	3.7	17
	2	108	4 mg	1-21	No	13%	7.7	4.6	14.4
	2	113	4 mg	1-21	40 mg/wk	34%	8.3	2.6	13.6
Lacy[95,102]	2	60	2 mg	1-28	40 mg/wk	65%	21	13	40
Lacy[101,102]	2	34*	2 mg	1-28	40 mg/wk	32%	9	4.7	27
Lacy[102]	2	60*	4 mg	1-28	40 mg/wk	37%		7.9	93%‡
	2	35*	2 mg	1-28	40 mg/wk	26%	16	6.5	17
Lacy[102,103]	2	35*	4 mg	1-28	40 mg/wk	29%	3	3.3	9
Leleu[104,105]	2	43*	4 mg	1-21	40 mg/wk	35%	11.4	6.3	
	2	41*	4 mg	1-28	40 mg/wk	34%	7.9	6.3	

Abbreviations: PR, partial response; DOR, duration of response; PFS, progression-free survival; OS, overall survival; MTD, maximum tolerated dose; Dex, dexamethasone.

* Lenalidomide-refractory patients.
† Dex added in 20 non-responding patients.
‡ OS @ 6 months.

therapy was six (range, 2–17).[93] The MTD was 4 mg and this was the dose selected for the phase II portion of the study. Twenty-two percent of patients achieved ≥PR (one CR, six PRs) with an estimated median duration of response of 28.1 weeks, and estimated median PFS of 16.1 weeks. In 20 patients, dexamethasone was added due to lack of response to pomalidomide, and two PRs and seven MRs were observed with the combination. Neutropenia and anemia were the most common grade 3/4 toxicities. The phase II part of this trial randomized 221 patients with a median number of five prior therapies (range, 2–13) to receive pomalidomide alone at 4 mg on days 1–21 of a 28-day cycle (n = 108) or pomalidomide at the same dose plus low-dose dexamethasone (40 mg/wk) (n = 113).[94] Responses (≥PR) were seen in 13% of patients in the pomalidomide-alone arm, and in 34% in the pomalidomide + dexamethasone arm, including 1% CR in each arm. Median PFS was 4.6 versus 2.6 months and median OS was 14.4 versus 13.6 months for pomalidomide + dexamethasone versus pomalidomide, respectively. These results demonstrate the potentiation of pomalidomide with dexamethasone, and therefore this combination will be the one further pursued in the next trials. Responses were similar in the subgroup of patients refractory to both lenalidomide and bortezomib, but with slightly lower median PFS and OS. The most frequent grade 3/4 AEs were neutropenia (38% *v* 47%), febrile neutropenia (2% *v* 2%), thrombocytopenia (19% *v* 21%), anemia (21% *v* 17%), pneumonia (19% *v* 8%), and fatigue (10% *v* 8%). All grades of peripheral neuropathy, DVT, and renal failure occurred in 7% versus 10%, 2% versus 1%, and 2% versus 1% of patients.

Another phase II study evaluating the administration of pomalidomide at 2 mg every day continuously plus low-dose dexamethasone in a less heavily pretreated population of patients with relapsed/refractory MM who had only received one to three prior regimens was simultaneously performed.[95,96] Sixty patients were treated with pomalidomide, achieving an ORR of 65% (including three CRs and 17 VGPRs) and a PFS of 13 months. These results are quite similar to those observed with lenalidomide + dexamethasone in two phase III trials that showed 60% ≥PR (15% CR) and a TTP of 11.2 months.[87,88,97] Nevertheless, it has to be noted that in the pomalidomide study two thirds of the patients had received previous IMIDs.

Clinical Results of Pomalidomide in Lenalidomide-Refractory Patients

An important question with these novel derivatives is whether they are able to overcome the resistance to the first-in-class agents or not. In fact, regarding IMIDs, there are some preclinical and retrospective clinical data suggesting that pomalidomide may overcome lenalidomide resistance.[98–100] To address this question, several trials have explored the activity of pomalidomide + dexamethasone in lenalidomide-refractory patients (Table 3). These trials have shown quite comparable responses, with approximately one third of patients achieving ≥PR. The first trial was an expansion of the aforementioned trial conducted by Lacy et al,[95] and treated 34 lenalidomide-refractory patients with pomalidomide 2 mg on days 1–28.[101] ORR was 32% with a PFS of 4.7 months. Lacy also performed a second trial[102] based on the MTD of 4 mg previously reported in the phase Ib/II trial performed by Richardson et al.[93] Sixty patients were included with a response rate of 37% and a PFS of 7.9 months.

Finally, two phase II trials have been performed to evaluate different doses or schemas of administration in patients refractory to both lenalidomide and bortezomib. The first used the continuous dose of pomalidomide (28/28), and two cohorts of patients were included. One received 2 mg and the other 4 mg of pomalidomide.[102,103] Thirty-five patients were treated in each arm with similar response rates (26% *v* 29%) but superior PFS (6.5 *v* 3.3 months) and 6 months OS (76% *v* 67%) for the 2-mg cohort. Myelosuppression was again the most common toxicity and discontinuations due to AEs were more frequent in the 4-mg cohort (3% *v* 16%). Another phase II trial randomized patients to receive pomalidomide (oral 4 mg daily) and dexamethasone (oral 40 mg weekly) in two different schedules: 21/28 or 28/28.[104,105] Eighty-four patients were enrolled: 43 in arm 21/28 and 41 in arm 28/28, with a median number of prior lines of therapy of five (range, 1–13). The ORR was 35% in arm 21/28 and 34% in arm 28/28. The median PFS was 6.3 (range, 4.1–9.1) months in either arm, and the median durations of response were 11.4 (range, 3.7–13.6) months and 7.9 (range, 4.0- not reported) months in arms 21/28 and 28/28, respectively. The activity observed in all patients in these two studies suggests that pomalidomide may overcome, at least partially, the resistance to both lenalidomide and bortezomib.

All of these trials have led to the phase III randomized trial (Nimbus) comparing pomalidomide + dexamethasone versus high-dose dexamethasone in 455 refractory MM patients who have failed to respond to both bortezomib and lenalidomide. Obviously all patients had received previous lenalidomide and bortezomib and 93% and 78% were lenalidomide- and bortezomib-refractory, respectively. The ORR was significantly better for the pomalidomide arm (21% *v* 3%) and the PFS was double for patients receiving the IMID (3.6 *v* 1.8 months; hazards ratio [HR] = 0.45, $P < .001$). There was also a significant advantage in OS, despite the fact that almost one third of patients in the high-dose dexamethasone arm received pomalidomide after progression (not reached *v* 7.8 months; HR = 0.53, $P < .001$). Regarding toxicity, both arms were comparable and only a higher incidence of grade 3/4 neutropenia was observed in patients receiving pomalidomide (42% *v* 15%).[106]

What Is the Optimal Dose and Schedule of Pomalidomide + Dexamethasone?

Regarding the optimal dosing, no direct comparison has been performed with all four schedules of treatment (2 *v* 4 mg and 21 *v* 28 days of treatment). Nevertheless, some conclusions may be obtained from the two studies previously mentioned. The study of Lacy et al[102] suggests that 4 mg administered continuously (28/28) seemed to be too toxic, with an inferior duration of response, PFS, and OS than 2 mg in this same schedule. Nevertheless, in the French study from Leleu et al,[104,105] the incorporation of a 1-week rest period to the 4-mg dose improved the safety profile and induced a better duration of response than the continuous dosing. Thus a dose of 4 mg on days 1–21 followed by a 1-week rest period has been chosen as the standard for subsequent randomized trials.

Combinations of Pomalidomide in Relapsed/Refractory MM

Several trials are currently exploring the activity of pomalidomide and dexamethasone in combination with several anti-myeloma agents in previously treated MM patients. This is the case of the combination of pomalidomide + cyclophosphamide + prednisone administered during six cycles and then maintenance with pomalidomide and dexamethasone until progression. It has resulted in an ORR of 51% (6% CR) in 55 patients and 41% ≥PR in lenalidomide-refractory patients. The main grade 3 AEs were neutropenia (16%), rash (7%), and infections (9%).[107] Clarithromycin also has been combined in 98 patients, 54% of them lenalidomide- and bortezomib-refractory, with and ORR of 57% (7% stringent CR) and a PFS of 8.6 months.[108] Finally, a combination with the proteasome inhibitor carfilzomib has recently been reported with 50% ≥PR in 32

relapsed/refractory patients. PFS was 7.4 months and the OS at 1 year was 90%.[109] All of these data indicate that pomalidomide, similar to the first-generation IMIDs, is a good partner for combination with several agents.

CONCLUSION

Second- and third-generation proteasome inhibitors and IMIDs, when used as monotherapy, display similar activity to their respective parental drugs in relapsed refractory MM patients. Some of them, ie, carfilzomib, ixazomib, and pomalidomide, are currently being explored in combination with several novel and approved anti-myeloma agents both in the relapsed and the newly diagnosed settings.

Regarding proteasome inhibitors, several of them with different properties have been designed and, in fact, these biological differences translate into different clinical efficacy and toxicity. In this regard, the activity of carfilzomib in relapsed MM patients is similar or possibly higher than that previously observed with bortezomib in a less heavily treated population. By contrast, ixazomib and marizomib are in earlier stages of development and it is still premature to draw definite conclusions about their activity. As far as toxicity is concerned, there are clear differences, as the novel drugs have not shown significant peripheral neuropathy, a side effect that if not correctly managed limits the possibility of administration of bortezomib and may be related to off-target effects of bortezomib.

Pomalidomide seems to be quite similar to lenalidomide in terms of efficacy and toxicity. Again the activity shown in relapsed patients compares favorably with that previously observed with lenalidomide, and it has a favorable toxicity profile, with neutropenia being the most frequent AE.

A very important piece of information that is derived from these studies is that the second- and third-generation compounds have only partial cross-resistance with their parental drugs. This suggests the presence of different mechanisms of action and of resistance for these novel drugs, despite having the same basic molecular structure and the same scientific rationale for their use.

These data indicate that both the inhibition of the proteasome as well as the modulation of the immune system are good strategies to target MM and this, along with the absence of complete cross-resistance observed among these drugs, and the promising efficacy and safety of several combinations that are currently being tested with a wide variety of novel and conventional agents, opens new avenues to optimize their use through the appropriate sequencing and combinations.

REFERENCES

1. Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008;111(5): 2516–2520.
2. Arrigo AP, Tanaka K, Goldberg AL, Welch WJ. Identity of the 19S 'prosome' particle with the large multifunctional protease complex of mammalian cells (the proteasome). Nature. 1988;331(6152): 192–4.
3. Adams J, Palombella VJ, Sausville EA, et al. Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res. 1999;59(11): 2615–22.
4. Hideshima T, Richardson P, Chauhan D, et al. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res. 2001;61(7):3071–6.
5. LeBlanc R, Catley LP, Hideshima T, et al. Proteasome inhibitor PS-341 inhibits human myeloma cell growth in vivo and prolongs survival in a murine model. Cancer Res. 2002;62(17):4996–5000.
6. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med. 2003;348(26):2609–17.
7. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005;352(24):2487–98.
8. Richardson PG, Sonneveld P, Schuster M, et al. Extended follow-up of a phase 3 trial in relapsed multiple myeloma: final time-to-event results of the APEX trial. Blood. 2007;110(10):3557–60.
9. Rivett AJ, Hearn AR. Proteasome function in antigen presentation: immunoproteasome complexes, peptide production, and interactions with viral proteins. Curr Protein Pept Sci. 2004;5(3):153–61.
10. Ciechanover A, Schwartz AL. The ubiquitin-proteasome pathway: the complexity and myriad functions of proteins death. Proc Natl Acad Sci U S A. 1998;95 (6):2727–30.
11. Mitsiades N, Mitsiades CS, Poulaki V, et al. Molecular sequelae of proteasome inhibition in human multiple myeloma cells. Proc Natl Acad Sci U S A. 2002;99 (22):14374–9.
12. Hideshima T, Mitsiades C, Akiyama M, et al. Molecular mechanisms mediating antimyeloma activity of proteasome inhibitor PS-341. Blood. 2003;101(4): 1530–4.
13. Hideshima T, Richardson PG, Anderson KC. Targeting proteasome inhibition in hematologic malignancies. Rev Clin Exp Hematol. 2003;7(2):191–204.
14. Carvalho P, Goder V, Rapoport TA. Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins. Cell. 2006;126(2):361–73.
15. Raasi S, Wolf DH. Ubiquitin receptors and ERAD: a network of pathways to the proteasome. Semin Cell Dev Biol. 2007;18(6):780–91.
16. Karin M. How NF-kappaB is activated: the role of the IkappaB kinase (IKK) complex. Oncogene. 1999;18 (49):6867–74.

17. Adams J. Development of the proteasome inhibitor PS-341. Oncologist. 2002;7(1):9–16.
18. Altun M, Galardy PJ, Shringarpure R, et al. Effects of PS-341 on the activity and composition of proteasomes in multiple myeloma cells. Cancer Res. 2005;65 (17):7896–901.
19. 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(5):431–40.
20. Orlowski RZ, Stinchcombe TE, Mitchell BS, et al. Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. J Clin Oncol. 2002;20(22):4420–7.
21. Jagannath S, Barlogie B, Berenson J, et al. A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br J Haematol. 2004;127(2): 165–172.
22. Rajkumar SV, Richardson PG, Hideshima T, Anderson KC. Proteasome inhibition as a novel therapeutic target in human cancer. J Clin Oncol. 2005;23(3):630–9.
23. Orlowski RZ, 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(25):3892–901.
24. Mitsiades N, Mitsiades CS, Richardson PG, et al. The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. Blood. 2003;101(6):2377–80.
25. Richardson PG, Weller E, Lonial S, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood. 2010;116(5):679–86.
26. Jakubowiak AJ, Griffith KA, Reece DE, et al. Lenalidomide, bortezomib, pegylated liposomal doxorubicin, and dexamethasone in newly diagnosed multiple myeloma: a phase 1/2 Multiple Myeloma Research Consortium trial. Blood. 2011;118(3):535–43.
27. Richardson PG, Weller E, Jagannath S, et al. Multicenter, phase I, dose-escalation trial of lenalidomide plus bortezomib for relapsed and relapsed/refractory multiple myeloma. J Clin Oncol. 2009;27(34): 5713–5719.
28. 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 (30):4621–9.
29. Sonneveld P, Schmidt-Wolf I, van der Holt B, et al. HOVON-65/GMMG-HD4 Randomized phase III trial comparing bortezomib, doxorubicin, dexamethasone (PAD) vs VAD followed by high-dose melphalan (HDM) and maintenance with bortezomib or thalidomide in patients with newly diagnosed multiple myeloma (MM). ASH Annual Meeting Abstracts. 2010;116(21):40.
30. 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(9758):2075–85.
31. Rosinol L, Cibeira MT, Mateos MV, et al. A phase III PETHEMA/GEM study of induction therapy prior autologous stem cell transplantation (ASCT) in multiple myeloma: superiority of VTD (bortezomib/thalidomide/dexamethasone) over TD and VBMCP/VBAD plus bortezomib. ASH Annual Meeting Abstracts. 2010;116(21):307.
32. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008;359(9):906–17.
33. Mateos MV, Oriol A, Martinez-Lopez J, et al. Bortezomib, melphalan, and prednisone versus bortezomib, thalidomide, and prednisone as induction therapy followed by maintenance treatment with bortezomib and thalidomide versus bortezomib and prednisone in elderly patients with untreated multiple myeloma: a randomised trial. Lancet Oncol. 2010;11(10):934–41.
34. Palumbo A, Bringhen S, Rossi D, et al. Bortezomib-melphalan-prednisone-thalidomide followed by maintenance with bortezomib-thalidomide compared with bortezomib-melphalan-prednisone for initial treatment of multiple myeloma: a randomized controlled trial. J Clin Oncol. 2010;28(34):5101–9.
35. Kuhn DJ, Chen Q, Voorhees PM, et al. Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. Blood. 2007;110(9): 3281–90.
36. Demo SD, Kirk CJ, Aujay MA, et al. Antitumor activity of PR-171, a novel irreversible inhibitor of the proteasome. Cancer Res. 2007;67(13):6383–91.
37. O'Connor OA, Stewart AK, Vallone M, et al. A phase 1 dose escalation study of the safety and pharmacokinetics of the novel proteasome inhibitor carfilzomib (PR-171) in patients with hematologic malignancies. Clin Cancer Res. 2009;15(22):7085–91.
38. Alsina M, Trudel S, Furman RR, et al. A phase I single-agent study of twice-weekly consecutive-day dosing of the proteasome inhibitor carfilzomib in patients with relapsed or refractory multiple myeloma or lymphoma. Clin Cancer Res. 2012;18(17):4830–40.
39. Jagannath S, Vij R, Stewart AK, et al. An open-label single-arm pilot phase II study (PX-171-003-A0) of low-dose, single-agent carfilzomib in patients with relapsed and refractory multiple myeloma. Clin Lymphoma Myeloma Leuk. 2012;12(5):310–8.
40. 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(14):2817–25.

41. 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(24):5661–70.
42. Squifflet P, Michiels S, Siegel DS, Vij R, Ro S, Buyse ME. Multivariate modelling reveals evidence of a dose-response relationship in phase 2 studies of single-agent carfilzomib. ASH Annual Meeting Abstracts. 2011;118(21):1877.
43. Vij R, Siegel DS, Jagannath S, et al. An open-label, single-arm, phase 2 study of single-agent carfilzomib in patients with relapsed and/or refractory multiple myeloma who have been previously treated with bortezomib. Br J Haematol. 2012;158(6):739–48.
44. Jakubowiak AJ, Siegel DS, Singhal S, et al. Unfavorable cytogenetic characteristics do not adversely impact response rates in patients with relapsed and/or refractory multiple myeloma treated with single-agent carfilzomib on the 003 (A1) study. ASH Annual Meeting Abstracts. 2011;118 (21):1875.
45. Badros AZ, Vij R, Martin T, et al. Carfilzomib in multiple myeloma patients with renal impairment: pharmacokinetics and safety. Leukemia. 2013 Aug;27(8):1707–14.
46. Singhal S, Siegel DS, Martin T, et al. Integrated safety from phase 2 studies of monotherapy carfilzomib in patients with relapsed and refractory multiple myeloma (MM): an updated analysis. ASH Annual Meeting Abstracts. 2011;118(21):1876.
47. Wang M, Bensinger W, Martin T, et al. Interim results from PX-171-006, a phase (Ph) II multicenter dose-expansion study of carfilzomib (CFZ), lenalidomide (LEN), and low-dose dexamethasone (loDex) in relapsed and/or refractory multiple myeloma (R/R MM). ASCO Meeting Abstracts. 2011;29(15 suppl): 8025.
48. 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 (9):1801–9.
49. Sonneveld P, Hacker E, Zweegman S, et al. Carfilzomib combined with thalidomide and dexamethasone (CARTHADEX) as induction treatment prior to high-dose melphalan (HDM) in newly diagnosed patients with multiple myeloma (MM). A trial of the European Myeloma Network EMN. ASH Annual Meeting Abstracts. 2011;118(21):633.
50. Moreau P, Palumbo AP, Stewart AK, et al. A randomized, multicenter, phase (Ph) III study comparing carfilzomib (CFZ), lenalidomide (LEN), and dexamethasone (Dex) to LEN and Dex in patients (Pts) with relapsed multiple myeloma (MM). ASCO Meeting Abstracts. 2011;29(15 suppl):TPS225.
51. Zhou HJ, Aujay MA, Bennett MK, et al. Design and synthesis of an orally bioavailable and selective peptide epoxyketone proteasome inhibitor (PR-047). J Med Chem. 2009;52(9):3028–38.
52. Papadopoulos KP, Mendelson DS, Tolcher AW, et al. A phase I, open-label, dose-escalation study of the novel oral proteasome inhibitor (PI) ONX 0912 in patients with advanced refractory or recurrent solid tumors. ASCO Meeting Abstracts. 2011;29(15 suppl): 3075.
53. Savona MR, Berdeja JG, Lee SJ, et al. A phase 1b dose-escalation study of split-dose oprozomib (ONX0912) in patients with hematologic malignancies. ASH Annual Meeting Abstracts. 2012;120(21):203.
54. Chauhan D, Tian Z, Zhou B, et al. In vitro and in vivo selective antitumor activity of a novel orally bioavailable proteasome inhibitor MLN9708 against multiple myeloma cells. Clin Cancer Res. 2011;17(16): 5311–21.
55. Gupta N, Saleh M, Venkatakrishnan K. Flat-dosing versus BSA-based dosing for MLN9708, an investigational proteasome inhibitor: population pharmacokinetic (PK) analysis of pooled data from 4 phase-1 studies. ASH Annual Meeting Abstracts. 2011;118 (21):1433.
56. Kumar S, Bensinger WI, Reeder CB, et al. Weekly dosing of the investigational oral proteasome inhibitor MLN9708 in patients with relapsed and/or refractory multiple myeloma: results from a phase 1 dose-escalation study. ASH Annual Meeting Abstracts. 2011;118(21):816.
57. Richardson PG, Baz R, Wang L, et al. Investigational agent MLN9708, an oral proteasome inhibitor, in patients (Pts) with relapsed and/or refractory multiple myeloma (MM): results from the expansion cohorts of a phase 1 dose-escalation study. ASH Annual Meeting Abstracts. 2011;118(21):301.
58. Kumar SK, Berdeja JG, Niesvizky R, et al. A phase 1/2 study of weekly MLN9708, an investigational oral proteasome inhibitor, in combination with lenalidomide and dexamethasone in patients with previously untreated multiple myeloma (MM). ASH Annual Meeting Abstracts. 2012;120(21):332.
59. Berdeja JG, Richardson PG, Lonial S, et al. Phase 1/2 study of oral MLN9708, a novel, investigational proteasome inhibitor, in combination with lenalidomide and dexamethasone in patients with previously untreated multiple myeloma (MM). ASH Annual Meeting Abstracts. 2011;118(21):479.
60. Chauhan D, Hideshima T, Anderson KC. A novel proteasome inhibitor NPI-0052 as an anticancer therapy. BrJCancer. 2006;95(8):961–5.
61. Chauhan D, Catley L, Li G, et al. A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. Cancer Cell. 2005;8(5):407–19.
62. Chauhan D, Singh A, Brahmandam M, et al. Combination of proteasome inhibitors bortezomib and NPI-0052 trigger in vivo synergistic cytotoxicity in multiple myeloma. Blood. 2008;111(3):1654–64.
63. Richardson PG, Spencer A, Cannell P, et al. Phase 1 clinical evaluation of twice-weekly marizomib (NPI-0052), a novel proteasome inhibitor, in patients with relapsed/refractory multiple myeloma (MM). ASH Annual Meeting Abstracts. 2011;118(21):302.
64. Ito T, Ando H, Suzuki T, et al. Identification of a primary target of thalidomide teratogenicity. Science. 2010;327(5971):1345–50.

65. Zhu YX, Braggio E, Shi CX, et al. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. Blood. 2011;118(18):4771–9.
66. Li S, Pal R, Monaghan SA, et al. IMiD immunomodulatory compounds block C/EBP{beta} translation through eIF4E down-regulation resulting in inhibition of MM. Blood. 2011;117(19):5157–65.
67. Lopez-Girona A, Heintel D, Zhang LH, et al. Lenalidomide downregulates the cell survival factor, interferon regulatory factor-4, providing a potential mechanistic link for predicting response. Br J Haematol. 2011;154(3):325–36.
68. Hideshima T, Chauhan D, Shima Y, et al. Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. Blood. 2000;96(9):2943–50.
69. Gandhi AK, Kang J, Capone L, et al. Dexamethasone synergizes with lenalidomide to inhibit multiple myeloma tumor growth, but reduces lenalidomide-induced immunomodulation of T and NK cell function. Curr Cancer Drug Targets. 2010;10(2):155–67.
70. Escoubet-Lozach L, Lin IL, Jensen-Pergakes K, et al. Pomalidomide and lenalidomide induce p21 WAF-1 expression in both lymphoma and multiple myeloma through a LSD1-mediated epigenetic mechanism. Cancer Res. 2009;69(18):7347–56.
71. Gupta D, Treon SP, Shima Y, et al. Adherence of multiple myeloma cells to bone marrow stromal cells upregulates vascular endothelial growth factor secretion: therapeutic applications. Leukemia. 2001;15(12):1950–61.
72. Chang DH, Liu N, Klimek V, et al. Enhancement of ligand-dependent activation of human natural killer T cells by lenalidomide: therapeutic implications. Blood. 2006;108(2):618–21.
73. Wu L, Adams M, Carter T, et al. lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CD20+ tumor cells. Clin Cancer Res. 2008;14(14):4650–7.
74. Galustian C, Meyer B, Labarthe MC, et al. The anticancer agents lenalidomide and pomalidomide inhibit the proliferation and function of T regulatory cells. Cancer Immunol Immunother. 2009;58(7):1033–45.
75. Ramsay AG, Johnson AJ, Lee AM, et al. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. J Clin Invest. 2008;118(7):2427–37.
76. Davies F, Baz R. Lenalidomide mode of action: linking bench and clinical findings. Blood Rev. 2010;24 Suppl 1:S13-9.
77. Barlogie B, Desikan R, Eddlemon P, et al. Extended survival in advanced and refractory multiple myeloma after single-agent thalidomide: identification of prognostic factors in a phase 2 study of 169 patients. Blood. 2001;98(2):492–4.
78. Glasmacher A, Hahn C, Hoffmann F, et al. A systematic review of phase-II trials of thalidomide monotherapy in patients with relapsed or refractory multiple myeloma. BrJHaematol. 2006;132(5):584–93.
79. Fermand J-P, Jaccard A, Macro M, et al. A randomized comparison of dexamethasone + thalidomide (Dex/Thal) vs Dex + placebo (Dex/P) in patients (pts) with relapsing multiple myeloma (MM). ASH Annual Meeting Abstracts. 2006;108(11):3563.
80. Garcia-Sanz R, Gonzalez-Porras JR, Hernandez JM, et al. The oral combination of thalidomide, cyclophosphamide and dexamethasone (ThaCyDex) is effective in relapsed/refractory multiple myeloma. Leukemia. 2004;18(4):856–63.
81. 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(22):5752–8.
82. 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(8):1589–96.
83. Fayers PM, Palumbo A, Hulin C, et al. Thalidomide for previously untreated elderly patients with multiple myeloma: meta-analysis of 1685 individual patient data from 6 randomized clinical trials. Blood. 2011;118(5):1239–47.
84. Morgan GJ, Davies FE, Gregory WM, et al. Cyclophosphamide, thalidomide, and dexamethasone (CTD) as initial therapy for patients with multiple myeloma unsuitable for autologous transplantation. Blood. 2011;118(5):1231–8.
85. Richardson PG, Schlossman RL, Weller E, et al. Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma. Blood. 2002;100(9):3063–7.
86. Richardson PG, Blood E, Mitsiades CS, et al. A randomized phase 2 study of lenalidomide therapy for patients with relapsed or relapsed and refractory multiple myeloma. Blood. 2006;108(10):3458–64.
87. Weber DM, Chen C, Niesvizky R, et al. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. N Engl J Med. 2007;357(21):2133–42.
88. Dimopoulos M, Spencer A, Attal M, et al. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. N Engl J Med. 2007;357(21):2123–32.
89. Knop S, Gerecke C, Liebisch P, et al. Lenalidomide, adriamycin, and dexamethasone (RAD) in patients with relapsed and refractory multiple myeloma: a report from the German Myeloma Study Group DSMM (Deutsche Studiengruppe Multiples Myelom). Blood. 2009;113(18):4137–43.
90. 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(3):268–9.

91. Schey SA, Fields P, Bartlett JB, et al. Phase I study of an immunomodulatory thalidomide analog, CC-4047, in relapsed or refractory multiple myeloma. J Clin Oncol. 2004;22(16):3269–76.
92. Streetly MJ, Gyertson K, Daniel Y, Zeldis JB, Kazmi M, Schey SA. Alternate day pomalidomide retains anti-myeloma effect with reduced adverse events and evidence of in vivo immunomodulation. Br J Haematol. 2008;141(1):41–51.
93. Richardson PG, Siegel D, Baz R, et al. A phase 1/2 multi-center, randomized, open label dose escalation study to determine the maximum tolerated dose, safety, and efficacy of pomalidomide alone or in combination with low-dose dexamethasone in patients with relapsed and refractory multiple myeloma who have received prior treatment that includes lenalidomide and bortezomib. ASH Annual Meeting Abstracts. 2010;116(21):864.
94. Richardson PG, Siegel DS, Vij R, et al. Randomized, open label 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. ASH Annual Meeting Abstracts. 2011;118(21):634.
95. Lacy MQ, Hayman SR, Gertz MA, et al. Pomalidomide (CC4047) plus low-dose dexamethasone as therapy for relapsed multiple myeloma. J Clin Oncol. 2009;27 (30):5008–14.
96. Mikhael JR, Hayman SR, Laumann K, et al. Long term outcomes of pomalidomide and dexamethasone in patients with relapsed multiple myeloma: analysis 4 years after the original cohort. ASH Annual Meeting Abstracts. 2011;118(21):2942.
97. Dimopoulos MA, Chen C, Spencer A, et al. Long-term follow-up on overall survival from the MM-009 and MM-010 phase III trials of lenalidomide plus dexamethasone in patients with relapsed or refractory multiple myeloma. Leukemia. 2009;23(11):2147–52.
98. Madan S, Lacy M, Dispenzieri A, et al. Efficacy of retreatment with immunomodulatory compounds in patients receiving initial therapy for newly diagnosed multiple myeloma. ASH Annual Meeting Abstracts. 2010;116(21):1964.
99. Madan S, Lacy MQ, Dispenzieri A, et al. Efficacy of retreatment with immunomodulatory drugs (IMiDs) in patients receiving IMiDs for initial therapy of newly diagnosed multiple myeloma. Blood. 2011; 118(7):1763–5.
100. Ocio EM, Fernandez-Lazaro D, San-Segundo L, et al. Reversibility of the resistance to lenalidomide and pomalidomide and absence of cross-resistance in a murine model of MM. ASH Annual Meeting Abstracts. 2011;118(21):134.
101. Lacy MQ, Hayman SR, Gertz MA, et al. Pomalidomide (CC4047) plus low dose dexamethasone (Pom/dex) is active and well tolerated in lenalidomide refractory multiple myeloma (MM). Leukemia. 2010;24(11): 1934–9.
102. Lacy MQ, LaPlant BR, Laumann K, et al. Pomalidomide and dexamethasone in relapsed myeloma: results of 225 patients treated in five cohorts over three years. ASH Annual Meeting Abstracts. 2011;118 (21):3963.
103. Lacy MQ, Allred JB, Gertz MA, et al. Pomalidomide plus low-dose dexamethasone in myeloma refractory to both bortezomib and lenalidomide: comparison of two dosing strategies in dual-refractory disease. Blood. 2011;118(11):2970–5.
104. Leleu X, Attal M, Arnulf B, et al. High response rates to pomalidomide and dexamethasone in patients with refractory myeloma, final analysis of IFM 2009-02. ASH Annual Meeting Abstracts. 2011;118 (21):812.
105. Leleu X, Attal M, Moreau P, et al. Phase 2 study of 2 modalities of pomalidomide (CC4047) plus low-dose dexamethasone as therapy for relapsed multiple myeloma. IFM 2009-02. ASH Annual Meeting Abstracts. 2010;116(21):859.
106. 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. ASH Annual Meeting Abstracts. 2012;120(21): LBA-6.
107. Palumbo A, Larocca A, Montefusco V, et al. Pomalidomide cyclophosphamide and prednisone (PCP) treatment for relapsed/refractory multiple myeloma. ASH Annual Meeting Abstracts. 2012;120(21):446.
108. Mark TM, Boyer A, Rossi AC, et al. ClaPD (clarithromycin, pomalidomide, dexamethasone) therapy in relapsed or refractory multiple myeloma. ASH Annual Meeting Abstracts. 2012;120(21):77.
109. Shah JJ, Stadtmauer EA, Abonour R, et al. A multicenter phase I/II trial of carfilzomib and pomalidomide with dexamethasone (Car-Pom-d) in patients with relapsed/refractory multiple myeloma. ASH Annual Meeting Abstracts. 2012;120(21):74.
110. diCapua Siegel DS, Martin T, Wang M, et al. Results of PX-171-003-A1, an open-label, single-arm, phase 2 (Ph 2) study of carfilzomib (CFZ) in patients (pts) with relapsed and refractory multiple myeloma (MM). ASH Annual Meeting Abstracts. 2010;116(21):985.
111. Kumar S, Bensinger W, Reeder CB, et al. Weekly dosing of the investigational oral proteasome inhibitor MLN9708 in patients (pts) with relapsed/refractory multiple myeloma (MM): a phase I study. ASCO Meeting Abstracts. 2012;30(15 suppl):8034.
112. Lonial S, Baz RC, Wang M, et al. Phase I study of twice-weekly dosing of the investigational oral proteasome inhibitor MLN9708 in patients (pts) with relapsed and/or refractory multiple myeloma (MM). ASCO Meeting Abstracts. 2012;30(15 suppl):8017.

Novel Agents for Multiple Myeloma to Overcome Resistance in Phase III Clinical Trials

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The incorporation of novel agents such as bortezomib and lenalidomide into initial therapy for multiple myeloma has improved the response rate of induction regimens. Also, these drugs are being increasingly used in the peri-transplant setting for transplant-eligible patients, and as part of consolidation and/or maintenance after front-line treatment, including in transplant-ineligible patients. Together, these and other strategies have contributed to a prolongation of progression-free survival (PFS) and overall survival (OS) in myeloma patients, and an increasing proportion are able to sustain a remission for many years. Despite these improvements, however, the vast majority of patients continue to suffer relapses, which suggests a prominent role for either primary, innate drug resistance, or secondary, acquired drug resistance. As a result, there remains a strong need to develop new proteasome inhibitors and immunomodulatory agents, as well as new drug classes, which would be effective in the relapsed and/or refractory setting, and overcome drug resistance. This review will focus on novel drugs that have reached phase III trials, including carfilzomib and pomalidomide, which have recently garnered regulatory approvals. In addition, agents that are in phase II or III, potentially registration-enabling trials will be described as well, to provide an overview of the possible landscape in the relapsed and/or refractory arena over the next 5 years.
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The last decade has in some ways been a golden era for novel therapeutic drug development in multiple myeloma. It started with the approval of the proteasome inhibitor bortezomib for relapsed and refractory myeloma in May 2003, based on positive findings from a pivotal phase II study.[1] This was followed by approvals of bortezomib for relapsed myeloma after at least one prior therapy, first as a single agent in March 2005,[2] and then in combination with pegylated liposomal doxorubicin in May 2007.[3] By June 2008, bortezomib was approved for initial therapy of myeloma based on a randomized study with bortezomib incorporated into a regimen with melphalan and prednisone.[4] Immunomodulatory drugs (IMiDs) entered the fray against myeloma when thalidomide, which had been used for many years off-label in the relapsed and/or refractory setting,[5] was approved with dexamethasone as induction therapy in May 2006.[6,7] Shortly thereafter, in June 2006, lenalidomide with high-dose dexamethasone was approved for patients with relapsed disease after at least one prior therapy.[8,9] Most recently, the second-generation proteasome inhibitor carfilzomib gained regulatory approval for relapsed and refractory disease in July 2012,[10] and the third-generation immunomodulator pomalidomide was approved for the same population in February 2013.[11]

Beyond just the approval of these novel agents, two important trends have emerged in the myeloma field, which include moving novel agents first approved in later lines of therapy into the upfront setting, and combining the various drug classes into more effective regimens. Examples of the former include the recent success of regimens such as lenalidomide with low-dose dexamethasone,[12] and bortezomib either with dexamethasone[13] or with thalidomide and dexamethasone,[14] in outperforming older induction regimens to establish new standards of care. Examples of the latter trend to combine

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proteasome inhibitors and IMiDs include bortezomib with thalidomide and dexamethasone,[14,15] which also may provide superior outcomes in the relapsed setting,[16] and regimens such as bortezomib with lenalidomide and dexamethasone.[17,18] Moreover, combinations of the most recent generation of agents in each class are being tested as well, as evidenced by studies of carfilzomib with lenalidomide and dexamethasone,[19,20] bortezomib with pomalidomide and dexamethasone,[21] and carfilzomib with pomalidomide and dexamethasone,[22] among others. While some of these have not yet reached the phase III setting, and their full impact on clinical outcomes in myeloma are yet to be determined, it is clear that those that have been part of the first wave of novel drugs have made a very positive impact on prognosis in this disease. Several studies indicate that novel agents have improved outcomes, especially in newly diagnosed[23] but also in relapsed patients,[23,24] and have added to the benefits of traditional approaches such as stem cell transplantation[25,26] to the point that survival has been doubled in some settings.[23–27] Moreover, an increasing proportion of patients remain in complete remission for prolonged periods of time, prompting some to consider the possibility that at least a fraction of myeloma patients may already be functionally cured of their disease.[26,28,29]

Despite these encouraging findings, and the likelihood that the recently approved agents will find their way into earlier lines of therapy, the vast majority of patients with multiple myeloma will still eventually relapse after front-line therapy. As a result, there remains a need to develop new proteasome inhibitors and immunomodulatory agents, and especially new drug classes, which would be effective in the relapsed and/or refractory setting. These agents would be especially useful if they could overcome drug resistance that may have emerged due to prior therapy, and if their use could be guided by biomarkers that identify patients who would be most likely to benefit. This contribution will review some of the current drug classes and agents that could possibly meet some of these criteria, and will update the reader on their progress towards the goal of incorporating them into our armamentarium against multiple myeloma.

DEACETYLASE INHIBITORS

Histone deacetylases (HDACs), along with histone acetyl transferases, regulate acetylation of a wide variety of cellular proteins, including histones. Through these modifications, HDACs influence pathways involved in many key processes in myeloma cells, including gene expression, cell cycle progression, DNA replication and repair, and protein folding through chaperone functions, among others (reviewed recently[30,31]). Deacetylase inhibitors have shown activity against preclinical models of myeloma through a number of important mechanisms. These include cell cycle arrest through increased expression of $p21^{WAF1}$, decreased expression of the interleukin (IL)-6 receptor, and retinoblastoma protein dephosphorylation, as well as apoptosis through increased expression of Bax.[32,33] Additional mechanisms include cleavage of Bid, as well as of poly (ADP)ribose polymerase (PARP) by calpains, inhibition of stromal cell IL-6 production,[33] induction of caspases,[34] and suppression of members of the insulin-like growth factor (IGF)/IGF-1 receptor (IGF-1R) pathway, DNA synthesis and repair enzymes, and expression of proteasome subunits and therefore of proteasome activity.[35] Deacetylase inhibitors have been validated in a number of combinations with both conventional and novel agents preclinically against multiple myeloma.[32–35] Perhaps the strongest rationale has been provided for combination regimens with proteasome inhibitors, based in part on the reduction of proteasome subunit expression by HDAC inhibitors,[35] which would sensitize cells to agents like bortezomib. In addition, proteasome and deacetylase inhibitors activate apoptosis synergistically by inducing oxidative injury and mitochondrial dysfunction.[36] Proteasome inhibition induces formation of aggresomes, aggregates of ubiquitin-conjugated proteins that protect cells from the toxic effects of these proteins, while HDAC inhibitors in general, and HDAC-6 inhibition in particular, disrupt this, thereby enhancing cell killing.[37,38] Finally, recent studies identified signaling through the IGF-1/IGF-1R pathway as an important contributor to bortezomib resistance,[39] and the ability of HDAC inhibitors to suppress IGF-1/IGF-1R signaling[35] is another rationale for combining them. Taken together, these multiple cooperative mechanisms provided strong support for the possibility that a regimen of a proteasome and deacetylase inhibitor could achieve chemosensitization, and possibly also overcome chemoresistance.

Vorinostat

Vorinostat (suberoylanilide hydroxamic acid, SAHA; Zolinza, Merck, Whitehouse Station, NJ) was evaluated first as a single agent in multiple myeloma in a phase I study that was abbreviated by the sponsor, and therefore did not identify a maximum tolerated dose (MTD).[40] Common drug-related adverse events (AEs) included fatigue, anorexia, dehydration, diarrhea, and nausea, and one minor response (MR) was seen among 10 evaluable patients. The combination of vorinostat and bortezomib was then studied in two phase I trials, the first

of which administered bortezomib at 1.0 or 1.3 mg/m^2 on days 1, 4, 8, and 11 of every 21-day cycle, along with vorinostat at 100–500 mg on days 1–8 of each 21-day cycle.[41] Nonhematologic toxicities included diarrhea (seen in 52%), nausea (48%), fatigue (35%), peripheral neuropathy (57%), and increased creatinine (30%), while hematologic toxicities included thrombocytopenia (52%), anemia (30%), and neutropenia (17%). Also, QT interval prolongation was noted in two patients who were treated at one level above what was ultimately defined as the MTD. Of 21 evaluable patients, nine (42%) experienced at least a partial response (PR) and, interestingly, none experienced an improvement with the addition of dexamethasone. In the second phase I study, bortezomib was dosed from 0.7–1.3 mg/m^2, while vorinostat was dosed for 14 days at 200 mg twice daily, 400 mg daily, or 300 mg twice daily.[42] Toxicities of any grade seen in at least one quarter of patients included nausea (74%), diarrhea (74%), fatigue (68%), thrombocytopenia (59%), vomiting (59%), peripheral neuropathy (29%), fever (29%), and constipation (27%). Nine of 34 patients (27%) achieved a PR, while two patients had MRs (6%), and another 20 had stable disease (SD) (59%). Notably, of seven patients whose disease was bortezomib-refractory, one experienced a PR while the other six had SD, and the duration of response was 120 days among all patients who had SD or better.

The encouraging data obtained from the phase I combination studies led to the design and completion of a phase II trial designed to determine if vorinostat could overcome resistance to bortezomib. Patients with at least two prior lines of therapy whose disease was refractory to bortezomib, and either refractory or ineligible for thalidomide and/or lenalidomide, received bortezomib and vorinostat, and dexamethasone could be added after four cycles if progression or SD was seen.[43] An overall response rate (ORR) (PR or better) of 17% was reported, while the clinical benefit response (CBR) rate (MR or better) was 31%, with a duration of response of 6.3 months. Progression-free survival (PFS) was 3.13 months, while the median overall survival (OS) was 11.2 months. Also, a phase III randomized study comparing single-agent bortezomib with placebo to the combination of bortezomib with vorinostat has been completed and reported.[44] While the ORR (PR or better) and the CBR rate (MR or better) both were significantly better for the combination regimen (Figure 1), the response duration was not, at 8.5 months for bortezomib and vorinostat, compared to 8.4 months for bortezomib alone. Moreover, PFS was 7.63 months for the combination versus 6.83 months for bortezomib alone, and while this represented a statistically significant difference ($P = 0.01$),

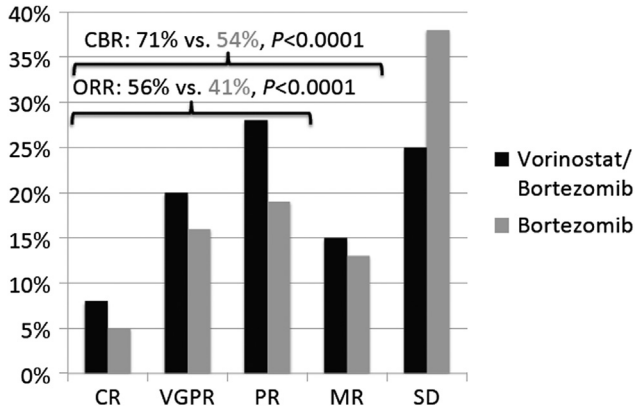

Figure 1. Response rates seen in patients treated on the VANTAGE 088 trial for relapsed myeloma with either bortezomib, or the combination of vorinostat and bortezomib. Abbreviations: CR, complete response; CBR, clinical benefit response; MR, minor response; ORR, overall response rate; PR, partial response; SD, stable disease; VGPR, very good partial remission.

it translated to a benefit of only 24 days, which was not clinically meaningful. Finally, though not mature, the OS data were comparable for the two arms, while a number of hematologic and non-hematologic toxicities were increased by the addition of vorinostat. These findings do suggest the possibility that a subset of patients may benefit from the regimen of vorinostat and bortezomib, and that if they could be identified prospectively using a molecular signature, this could still be a valuable therapeutic approach. For now, however, further development of vorinostat in the multiple myeloma setting has been put on hold.

Panobinostat

Panobinostat (LBH589, Novartis Corp, New York, NY), like vorinostat, inhibits a number of the known human deacetylases, including those in classes I, II, and IV, and has activity preclinically against myeloma both alone, and in combinations, including with bortezomib, through analogous mechanisms.[45–48] A phase II study of single-agent panobinostat given three times weekly for each week of a 3-week cycle described grade 3 or 4 toxicities that occurred in at least 5% of patients as including neutropenia (32%), thrombocytopenia (26%), anemia (18%), back pain (8%), hypercalcemia (8%), hypokalemia (8%), fatigue (5%), and pneumonia (5%).[49] Among the 38 patients evaluated, one PR and one MR were seen, demonstrating that, as was the case for vorinostat, single-agent deacetylase inhibitors probably do not have a significant role in relapsed and/or refractory myeloma, despite one case report of a near-CR after panobinostat.[50] The combination of panobinostat and bortezomib was studied in a phase Ib trial,

which allowed addition of dexamethasone starting with cycle 2 if a suboptimal response was seen.[51] Based on this study, the MTD of panobinostat was identified as 20 mg given three times weekly for 2 weeks, along with the standard dose and schedule of bortezomib. Among 47 patients in the dose-escalation phase, 36 (76%) achieved at least an MR or better, and 75% of 12 evaluable patients in the dose-expansion cohort did as well. Also of note, 11/19 (58%) patients who had previously bortezomib-refractory disease responded to the combination. As a result of these very encouraging data, a phase randomized III trial comparing bortezomib and dexamethasone with or without panobinostat (Table 1) is underway. While data about the primary endpoints have not yet been reported, preliminary presentations of planned interim analyses of up to 525 blinded patients focusing on toxicity have indicated a comparable safety profile to that expected of bortezomib and dexamethasone.[52,53]

PROTEASOME INHIBITORS

Bortezomib is the first proteasome inhibitor to reach the clinic, and garnered approvals as a single agent in the relapsed and/or refractory setting based on exciting data from phase I through III trials,[1,2,54] and was subsequently approved as part of front-line

Table 1. Phase III Trials of Novel Agents in Relapsed/Refractory Multiple Myeloma*

Trial	Clinicaltrials.gov Identifier	Regimens Control *v* Experimental	Projected Accrual	Estimated Completion
Novel proteasome inhibitors				
ASPIRE	NCT01080391	Lenalidomide/dexamethasone *v* Carfilzomib/lenalidomide/dexamethasone	780	12/2013
FOCUS	NCT01302392	Best supportive care *v* Carfilzomib	302	6/2014
ENDEAVOR	NCT01568866	Bortezomib/dexamethasone *v* Carfilzomib/dexamethasone	888	1/2015
TOURMALINE-MM1	NCT01564537	Lenalidomide/dexamethasone *v* MLN9708/lenalidomide/dexamethasone	703	2/2019
Novel immunomodulatory agents				
NIMBUS	NCT01311687	High-dose dexamethasone *v* Pomalidomode/dexamethasone	426	5/2013
OPTIMISMM	NCT01734928	Bortezomib/dexamethasone *v* Pomalidomide/bortezomib/dexamethasone	782	1/2015
Novel deacetylase inhibitors				
VANTAGE 088		Bortezomib *v* Vorinostat/bortezomib	637	12/2011
PANORAMA-1	NCT01023308	Bortezomib/dexamethasone *v* Panobinostat/bortezomib/dexamethasone	762	6/2013
Novel monoclonal antibodies				
ELOQUENT-2	NCT01239797	Lenalidomide/dexamethasone *v* Elotuzumab/lenalidomide/dexamethasone	640	3/2014
Novel signal transduction inhibitors				
AB06002	NCT01470131	Bortezomib/dexamethasone *v* Masitinib/bortezomib/dexamethasone	300	4/2013
ADMYRE	NCT01102426	Dexamethasone *v* Plitidepsin/dexamethasone	250	6/2014
PERIFOSINE 339	NCT01002248	Bortezomib/dexamethasone *v* Perifosine/bortezomib/dexamethasone	450	9/2014

* Data are based on a search of clinicaltrials.gov performed on February 23, 2013, using the terms "multiple myeloma" and "relapse" and "phase 3." The "estimated completion" column provides the date when data about the primary endpoint will be mature, as provided by the study sponsors. Studies within each drug category are arranged based on when they may be expected to report their primary endpoint data. Phase III single-center trials, studies of nontherapeutic interventions, and those that did not incorporate a novel agent were excluded.

therapy with melphalan and prednisone.[4] By validating the proteasome as a target for cancer therapy, bortezomib also spurred interest in the possibility that other drugs targeting the proteasome, and indeed the entire ubiquitin-proteasome pathway, could play a role in our armamentarium against multiple myeloma as well. A number of inhibitors of the constitutive and/or immunoproteasomes are under study preclinically and clinically,[55,56] and carfilzomib and ixazomib have reached the phase III setting for multiple myeloma.

Carfilzomib

Carfilzomib (Kyprolis, Onyx Pharmaceuticals, South San Francisco, CA) is a peptide epoxy-ketone that binds the N-terminal threonine active site of the β5 subunit of the proteasome in an irreversible manner, possibly providing a more durable inhibition of the proteasome than reversible agents such as bortezomib.[57,58] In models of multiple myeloma, carfilzomib induced apoptosis in part through the c-Jun-N-terminal kinase (JNK), and activated both the intrinsic and extrinsic caspase pathways.[59] Notably, carfilzomib was effective against cell lines and primary samples that were resistant to conventional and novel drugs, including bortezomib, acted synergistically with other agents such as dexamethasone,[59] and showed in vivo anti-tumor activity.[60] In addition to its anti-myeloma effects, carfilzomib also may have the benefit of suppressing bone resorption and promoting bone anabolic activities,[61] and may be more specific than bortezomib for the proteasome,[62] possibly contributing to a more favorable toxicity profile.

Phase I studies of carfilzomib evaluated the safety and toxicity of this drug on two schedules, including dosing 5 days in a row followed by 9 days off,[63] or two consecutive days for 3 weeks on and 1 week off, which translated to dosing on days 1, 2, 8, 9, 15, and 16 of every 28-day cycle.[64] On the more intensive schedule, the MTD was 15 mg/m^2, with dose-limiting toxicities (DLTs) including febrile neutropenia and thrombocytopenia.[63] Activity was seen against mantle cell lymphoma, Waldenström's macroglobulinemia, and multiple myeloma, with the latter including a response in a patient whose disease had previously been refractory to bortezomib. With twice-weekly consecutive-day dosing, an MTD was not identified, and the highest dose level tested administered carfilzomib at 20 mg/m^2 on days 1 and 2 of cycle 1, and then 27 mg/m^2 on subsequent days of that cycle, and on all later dosing days.[64] As was the case for the earlier phase I trial, the latter also showed evidence of activity against multiple myeloma and non-Hodgkin lymphoma, and this schedule was selected for further evaluation in the phase II setting.

One combination regimen incorporating carfilzomib that has garnered particular attention is that with lenalidomide and dexamethasone. For patients with relapsed or progressive myeloma,[20] no MTD was defined, and the highest level was recommended for further study. This consisted of carfilzomib at 20 mg/m^2 on days 1 and 2 of cycle 1, followed by 27 mg/m^2 on all subsequent days of cycle 1 and later cycles, along with lenalidomide at 25 mg on days 1–21, and dexamethasone at 40 mg on days 1, 8, 15, and 22. Treatment-emergent AEs that occurred in at least 10% of patients, and that reached grade 3 or 4 severity, included neutropenia (40%), thrombocytopenia (33%), anemia (18%), lymphopenia (18%), hyperglycemia (15%), hyponatremia (15%), and hypophosphatemia (15%), with no grade 3 or 4 neuropathic events. The ORR including patients with at least a PR was 63%, and clinical benefit with at least an MR was seen in 75%, while response duration and PFS were 11.8 and 10.2 months, respectively. A similar regimen also has been studied in the frontline setting,[19] where carfilzomib was escalated to the highest planned dose level of 36 mg/m^2 with standard-dose lenalidomide and low-dose dexamethasone. Addition of a proteasome inhibitor to an immunomodulatory agent could have the ability to overcome lenalidomide resistance through a number of mechanisms. Cereblon expression has been found to be important for the effects of lenalidomide and other immunomodulatory drugs (IMiDs), and low expression may be associated with resistance.[65,66] Since the abundance of most cellular proteins is regulated in part through the ubiquitin-proteasome pathway, inhibition of the proteasome should increase cereblon levels, which could enhance the activity of lenalidomide. Also, cereblon may itself inhibit the proteasome by binding to the β4 subunit,[67] which is a distinct target from the β5 subunit to which carfilzomib predominantly binds, possibly providing a mechanism for synergistic proteasome inhibition. Finally, resistance to lenalidomide also has been associated with activation of signaling through the Wnt/β-catenin pathway,[68] possibly through upregulation of CD44 and adhesion-mediated drug resistance.[69] β-catenin is also a target for the ubiquitin-proteasome pathway,[70] but it may be cleared in part through aggresomes.[71] Thus, it is possible that proteasome inhibition directs β-catenin to the aggresome/lysosome pathway, leading to decreased signaling throught the Wnt/β-catenin pathway, thereby overcoming lenalidomide resistance.

Due to the encouraging data with carfilzomib in the phase I setting, phase II studies were initiated targeting patients with relapsed and refractory disease. Regulatory approval of carfilzomib was based

on the outcomes from the PX-171-003-A1 trial, in which patients received dosing at 20 mg/m^2 during cycle 1, and then 27 mg/m^2 starting in cycle 2. Among 257 patients who were evaluable for efficacy, of whom 95% had disease that was refractory to their most recent line of therapy, and 80% were either refractory or intolerant to lenalidomide and bortezomib, an ORR of 24% was reported. Responses were also sustained, with a duration of response of 7.8 months, and a median OS of 15.6 months.[10] Common AEs that reached grade 3 or 4 severity, and were seen in at least 5% of patients, included thrombocytopenia (29%), anemia (24%), lymphopenia (20%), neutropenia (11%), pneumonia (9%), hyponatremia (8%), fatigue (8%), leukopenia (7%), hypophosphatemia (6%), and upper respiratory tract infection (5%). Peripheral neuropathy of any grade was seen in only 33 patients (12%), including only three events at grade 3 (1%) and none at grade 4. A second study of carfilzomib, PX-171-004, evaluated patients with relapsed and/or refractory myeloma who were bortezomib-naive, and included two cohorts, the first of which received dosing at 20 mg/m^2 throughout, while the second used stepped up dosing in cycle 2 at 27 mg/m^2,[72] as had been the case for PX-171-003-A1. The toxicity profile was comparable to the prior phase II study, with again a low rate of peripheral neuropathy. Notably, there was a trend towards a better response rate in the latter cohort (42% *v* 52%), response durability (median duration of response of 13.1 months *v* not reached), and time to progression (TTP; median of 8.3 months *v* not reached) with the latter approach. An additional phase II study of note focused on patients with relapsed and/or refractory disease who had been exposed to bortezomib,[73] and reported a response rate of 17.1%, indicating the presence of some cross-resistance between bortezomib and carfilzomib, while duration of response was >10.6 months and TTP was 4.6 months. Additional information about phase II studies with carfilzomib can be found in the article by Mateos et al in this issue.

Several phase III trials that will provide further insights into the role of carfilzomib are currently underway (Table 1). The ASPIRE study comparing lenalidomide and low-dose dexamethasone with or without carfilzomib for patients with relapsed myeloma who have received one to three prior lines of therapy has already completed enrollment. Positive data from this trial would lead to full approval of carfilzomib for patients in the relapsed setting, supporting the earlier approval of single-agent carfilzomib in relapsed and refractory myeloma. In addition, the FOCUS study is comparing carfilzomib to best supportive care in patients with relapsed and refactory myeloma who have undergone at least three prior lines of treatment.[74] FOCUS also has completed enrollment, and encouraging findings could support the approval of carfilzomib in Europe. Finally, the ongoing ENDEAVOR study for patients with one to three prior lines of therapy and relapsed myeloma is comparing bortezomib and dexamethasone as a salvage regimen to carfilzomib and dexamethasone. Notably, in this study, carfilzomib is being administered at 20 mg/m^2 on days 1 and 2 of cycle 1, and then 56 mg/m^2 for all later doses. This dosing is based on results from a phase Ib study indicating that carfilzomib can be safely administered at doses up to 56 mg/m^2 as an infusion over 30 minutes,[75] as opposed to the standard 2–10 minutes. AEs with this approach were similar to those in other carfilzomib studies, including fatigue (36%), headache (36%), thrombocytopenia (36%), anemia (32%), cough (32%), dyspnea (32%), insomnia (27%), upper respiratory tract infection (27%), nausea (23%), and hypertension (18%). Responses were seen in patients with relapsed and/or refractory myeloma, including two very good PRs. Moreover, a recent phase II study using this approach in patients with relapsed or refractory multiple myeloma corroborated the encouraging safety signal,[76] and noted a response rate of 58% among patients who received at least four cycles of therapy or who progressed during their first four cycles. ENDEAVOR will therefore determine if this higher dose carfilzomib regimen has a role to play in therapy of relapsed myeloma.

Ixazomib

Both of the currently approved proteasome inhibitors are administered as injections, with bortezomib delivered either through an intravenous or subcutaneous route,[77] while carfilzomib is delivered intravenously. Ixazomib (Millennium: The Takeda Oncology Co, Cambridge, MA), on the other hand, also known as MLN9708, is the first orally available proteasome inhibitor to reach the clinic. This drug, which is rapidly metabolized in vivo to the active agent, MLN2238, is characterized by a shortened proteasome dissociation half-life, which may allow it to more rapidly redistribute from off-target tissues to tumor cell proteasomes, and induce greater antitumor activity.[78] In models of multiple myeloma, ixazomib activated apoptosis through both caspase 8 and caspase 9, induced the endoplasmic reticulum stress response while inhibiting nuclear factor kappa B, and showed synergistic anti-tumor activity in combination with dexamethasone and lenalidomide.[79] Recent studies also suggest a role for modulation of micro RNA 33b by ixazomib in its mechanism of action.[80]

As a single agent in the relapsed and/or refractory setting, the MTD of ixazomib given on the bortezomib schedule of days 1, 4, 8, and 11 every 21 days was 2.0 mg/m^2.[81] Drug-related AEs included fatigue (45%), thrombocytopenia (30%), nausea (26%), diarrhea (25%), vomiting (23%), and rash (23%), while neuropathy (8%) was rare. Among 36 response-evaluable patients, six had at least an MR (17%), while 22 patients had SD (61%). A second study has been evaluating ixazomib given once weekly, and has reported similar drug-related AEs, though with a lesser incidence of rash and no DLTs as of yet.[82] Finally, a phase I/II study is being conducted with ixaomib in combination with lenalidomide and dexamethasone for patients with previously untreated multiple myeloma.[83] An ORR of 88% has been seen to date, including 18% in CR and 40% with a very good PR, while tolerability has been comparable to what would be expected of single-agent ixazomib, as well as lenalidomide with low-dose dexamethasone.[12] Based on the latter data, a phase III study in the relapsed and/or refractiory setting is ongoing comparing lenalidomide and low-dose dexamethasone with or without the addition of ixazomib on days 1, 8, and 15 of every 28-day cycle. Successful completion of this study with supportive data could lead to the regulatory approval of this oral proteasome inhibitor.

IMMUNOMODULATORY AGENTS

Thalidomide and lenalidomide are the first two members of the IMiD family to obtain regulatory approvals for treatment of multiple myeloma, and they have contributed significantly to the improvements seen recently in patient outcomes.[84–86] Other IMiDs are also under development, with pomalidomide being the agent that has advanced furthest, having been approved on an accelerated basis in February 2013 for patients with relapsed and refractory myeloma who have had at least two prior lines of therapy that included bortezomib and lenalidomide.

Pomalidomide

Pomalidomide is a third-generation IMiD that was previously known as CC-4047, and while Actimid (Celgene Corp, Summit, NJ) was its trade name in the past, the current name is Pomalyst (Celgene). Like other agents in this class of drugs, pomalidomide has multiple mechanisms of action, including modulating and stimulating the host immune system, inhibiting angiogenesis and production of stromal cell cytokines that would normally make the microenvironment more permissive for myeloma cells, and also directly suppressing tumor cell proliferation and activating programmed cell death.[84–86] While structurally similar to thalidomide and lenalidomide, pomalidomide has been shown in a number of assays to be more potent than its predecessors,[87,88] which in part prompted hopes that it could help to overcome resistance that had emerged after therapy with either thalidomide or lenalidomide.

The first phase I study of pomalidomide in patients with relapsed or refractory multiple myeloma found it to be well tolerated from the standpoint of serious non-hematologic AEs but did report neutropenia and deep vein thrombosis.[89] An MTD of 2 mg/d was identified, and MR or better was seen in 67% of patients, while 54% experienced at least a PR. Correlative studies showed an associated increase in serum levels of the IL-2 receptor and of IL-12, supporting the possibility of T-cell costimulation as a mechanism of action. Pomalidomide at 2 mg daily was then combined with low-dose dexamethasone, and this regimen was found to be well tolerated and active against relapsed myeloma,[90] with a similar ORR of 63%, including CR in three patients (5%), and very good PR in 17 (28%). Response durability was also documented, with a PFS of 11.6 months, which was not significantly reduced in patients with high-risk cytogenetic features. This approach also was shown to be effective against myeloma that was relapsed and refractory,[91] though, as would be expected, the response rates were lower in this group that had more resistant disease, with 32% of patients having a PR or better.

One area of controversy that arose early in the development of pomalidomide was with respect to its most appropriate dose and schedule. Pomalidomide was given at either 2 mg or 4 mg continuously with low-dose dexamethasone in patients with myeloma that was refractory to both bortezomib and lenalidomide in a non-randomized study.[92] Myelsuppression was the most commonly seen toxicity, while MRs or better were seen in 49% of patients who received 2 mg dosing, and 43% who received 4 mg dosing. Interestingly, the OS rates at 6 months for these two groups were 78% and 67%, suggesting that there was no advantage for the 4-mg dose over the 2-mg dose. Also, two different schedules of pomalidomide with low-dose dexamethasone have been studied in such patients with so-called double-refractory myeloma, comparing pomalidomide at 4 mg given for 21 days of each 28-day cycle, or pomalidomide with continuous dosing throughout the cycle.[93] This randomized study suggested that the median time to the first response could be longer with dosing for only 21 days, but the response rates were comparable (Table 2), and most of the measures of response durability were either similar, or favored 21-day dosing followed by 1 week off. Finally, the appropriate dose was likely settled by a phase I study of patients who had refractory myeloma after prior therapy with both lenalidomide and

Table 2. Outcomes Data From the IFM 2009-02 of Pomalidomide With Low-Dose Dexamethasone in Relapsed/Refractory Multiple Myeloma*

Outcome Measure, n (%)	Pomalidomide 21/28 Days (n = 43)	Pomalidomide 28/28 Days (n = 41)	Total Population (N = 84)
Overall response rate (at least PR)	15 (35%)	14 (34%)	29 (35%)
CR rate	1 (2%)	2 (5%)	3 (4%)
Very good PR rate	1 (2%)	1 (2%)	2 (2%)
PR rate	13 (30%)	11 (27%)	24 (27%)
SD rate	19 (44%)	21 (51%)	40 (48%)
Progressive disease rate	5 (12%)	3 (7%)	8 (10%)
Not evaluable	4 (9%)	3 (7%)	7 (8%)
Median time to first response (mo)	2.7	1.1	1.8
Median response duration (mo)	6.4	8.3	7.3
One-year relapse-free survival (%)	42%	47%	44%
Median time to progression (mo)	5.5	4.6	5.4
Median PFS (mo)	5.4	4.4	4.6
Median OS (mo)	14.9	14.8	14.9

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; OS, overall survival; PFS, progression-free survival.
*Data are from Leleu et al.[93]

comparing all of the doses and schedules, the dose that was taken forward into registration studies was 4 mg for 3 consecutive weeks of each 28-day cycle.

Accelerated approval of pomalidomide was recently granted based on the findings of the MM-002 phase II study, which randomized patients to either pomalidomide alone or to pomalidomide with low-dose dexamethasone.[95] Grade 3 and 4 AEs for the two arms were predominantly hematologic, including neutropenia, thrombocytopenia, anemia, or leukopenia, while non-hematologic events included pneumonia, fatigue, back pain, and dyspnea. The response rate for pomalidomide alone was 9%, while PR or better was seen in 30% of patients who received the combination. Median duration of response was 7.4 months for pomalidomide with dexamethasone, while it had not yet been reached with pomalidomide alone, suggesting that while dexamethasone was improving the response rate, it was in patients with biologically more aggressive disease that was not likely to remain in remission. However, other durability measures tended to favor the combination, including PFS, which was and OS was significantly superior for the combination (Table 3), and these data may support the approval of this treatment regimen in Europe.

A confirmatory phase III study of pomalidomide is underway, which is comparing pomalidomide with bortezomib and dexamethasone to bortezomib and dexamethasone in patients with one to three prior lines of therapy (Table 1). This is based in part on data from a phase I study of the three-drug regimen, which did not detect DLTs within the planned dosing cohorts,[21] and noted a PR or better rate of 73%. Encouraging findings from the international phase III study would support full approval of pomalidomide. Other interesting combinations based on pomalidomide that are being studied include carfilzomib with pomalidomide and dexamethasone, which has reported a 50% ORR in patients with lenalidomide-refractory disease,[22] and pomalidomide with clarithromycin and dexamethasone, which induced a PR or better in 54% of patients with relapsed or refractory myeloma. The interested reader is referrred to the contribution in this issue of *Seminars* by Mateos et al for additional data on pomalidomide.

Table 3. Progression-Free and Overall Survival Data From the NIMBUS Study in Relapsed/Refractory Multiple Myeloma*

Outcomes by Group	Pomalidomide + Low-Dose Dexamethasone (n = 302)	High-Dose Dexamethasone (n = 153)	Hazard Ratio	*P* Value
Median PFS				
Intent-to-treat population	3.6 mo	1.8 mo	0.45	<.001
Refractory to bortezomib	3.6 mo	1.8 mo	0.47	<.001
Refractory to lenalidomide	3.7 mo	1.8 mo	0.38	<.001
Refractory to bortezomib & lenalidomide	3.2 mo	1.7 mo	0.48	<.001
Median OS				
Intent-to-treat population	Not reached	7.8 mo	0.53	<.001
Refractory to bortezomib	Not reached	8.1 mo	0.56	.037
Refractory to lenalidomide	Not reached	8.6 mo	0.39	.003
Refractory to bortezomib & lenalidomide	Not reached	7.4 mo	0.56	.003

Abbreviations: PFS, progression-free survival; OS, overall survival.
* Data from Dimopoulos et al.[96]

MONOCLONAL ANTIBODIES

No monoclonal antibodies have yet been approved for the treatment of multiple myeloma, though this is likely to change in the near future, since a number of such agents are in clinical trials and showing encouraging signs of activity. Several of these antibodies have been raised against cell surface proteins, such as elotuzumab, which recognizes CS1, daratumumab, which is directed against CD38, and lorvotuzumab mertansine, which targets CD138. Other antibodies target cytokines that are important to the plasma cell in its microenvironment, such as siltuximab, which neutralizes IL-6, and tabalumab, which recognizes B-cell–activating factor. A number of excellent reviews have recently been published that detail the properties and preclinical as well as known clinical activity of these antibodies.[31,97–99] Two of these, siltuximab and elotuzumab, have reached potential registration-enabling studies, and greater detail about these agents is provided below.

Siltuximab

Signaling through the IL-6 pathway has been shown to play a key role in myeloma pathobiology, including in processes such as plasma cell proliferation, survival, and chemotherapy resistance, as well as osteoclast activation, providing a strong rationale to target IL-6 with monoclonal antibodies.[100,101] Preclinical studies with siltuximab (Janssen Pharmaceuticals, Titusville, NJ) revealed activity as a single agent against both IL-6–dependent and –independent cell lines and primary samples, and it enhanced the cytotoxicity of bortezomib in an additive to synergistic manner.[102] This occurred in part through inhibition of bortezomib-mediated induction of anti-apoptotic heat shock protein 70 and myeloid cell leukemia 1. Additional studies showed that siltuximab sensitized models of myeloma to corticosteroid-induced cell death,[103] as well as to alkylating agents such as melphalan.[104]

Based in part on the strong rationale outlined above, siltuximab was studied in a phase I dose-escalating trial for patients with relapsed and refractory myeloma.[105] Treatment was well tolerated, and decreases were seen in the IL-6 surrogate marker C-reactive protein, but no responses were seen among the 12 patients treated. The excellent safety profile of single-agent siltuximab was then confirmed in a subsequent phase I study in patients with a variety of hematologic malignancies, including B-cell non-Hodgkin lymphoma, myeloma, as well as Castleman's disease, in which DLTs were not seen.[106] Notably, activity was seen against multiple myeloma, with five patients treated for at least one year showing benefit, including two CRs,[106] and the possibility to achieve CR with single-agent siltuximab also has been reported from another study.[107] These findings prompted a phase II study of siltuximab in patients with myeloma, which included one cohort in which siltuximab was used first and dexamethasone could be added later if an inadequate response was seen, while a second cohort gave the two agents together.[108] Siltuximab alone showed no activity in this heavily pretreated population, among whom 83% were relapsed and refractory to their last line of therapy. However, when combination therapy with siltuximab and

dexamethasone was given, 23% of patients achieved at least an MR, including in patients whose disease was previously refractory to a corticosteroid-containing regimen. Response durations were reasonable as well, with a median PFS of 3.7 months, median TTP of 4.4 months, and a median OS of 20.4 months. Finally, the results of a randomized phase II study were recently reported, which compared the efficacy of bortezomib with placebo to bortezomib with siltuximab in relapsed myeloma patients with up to three prior lines of therapy who were bortezomib-naïve.[109] While the ORR was superior for the combination compared to bortezomib alone (55% achieved at least a PR *v* 47%), as was the CR rate (11% *v* 7%), significant differences in long-term outcomes were not seen. Progression-free survival, for example, which was the primary endpoint, was 245 days for the combination in 142 patients, while for bortezomib with placebo it was 232 days in 144 patients. Also, OS slightly favored patients in the bortezomib + placebo arm, at 1,121 days, compared with 1,068 days for the bortezomib/siltuximab arm. A number of factors likely contributed to the negative outcome of this study, including the use of what was later identified as a suboptimal dose and schedule for siltuximab, a greater rate of discontinuations due to AEs on the siltuximab arm, and the influence of subsequent therapies on outcome. However, due to these disappointing findings, further development of siltuximab in multiple myeloma has been halted.

Elotuzumab

Elotuzumab (Bristol-Myers Squibb, New York, NY) targets CS1, which was noted to be highly expressed on more than 97% of primary patient plasma cells,[110] although it also has been found on natural killer (NK) cells, NK-like T cells, and $CD8^+$ T cells.[111] Consistent with the possibility that this protein plays a role in cellular adhesion, eloutuzmab inhibited binding of myeloma cells to stromal cells.[110] It exerted an antibody-dependent cytotoxic effect both alone[110] and in the presence of effector NK cells.[111] Also, elotuzumab exerted enhanced activity when it was added to a variety of conventional and novel agents, including bortezomib[110,112] and lenalidomide,[110] and it showed anti-tumor activity in vivo.[110,111]

As a single agent, with elotuzumab adminstered intravenously every 2 weeks from 0.5 to 20 mg/kg, no MTD was identified in the phase I study, while common AEs included cough, headache, back pain, fever, and chills.[113] CS1 on marrow plasma cells was found to be saturated at 10 and 20 mg/kg, but stable disease was the best response, and was seen in nine patients (27%). In combination with bortezomib, elotuzumab again was well tolerated without an MTD within the tested range, while frequent grade 3 and 4 AEs were lymphopenia and fatigue.[114] PR or better was seen in 48% of 27 evaluable patients and, interestingly, although only three patients had bortezomib-refractory disease, two responded, and the overall median TTP was an encouraging 9.5 months. The most impressive clinical activity in a phase I setting was obtained when elotuzumab was combined with lenalidomide and low-dose dexamethasone, which likewise found no DLTs or MTD.[115] Some myelosuppression was seen, with neutropenia in 36% of patients and thrombocytopenia in 21%, and two patients did have serious infusion-related toxicities during the first treatment cycle only. A PR or better was seen in 82% of patients, including 21/22 (95%) who were lenalidomide-naïve, 15/16 (94%) who had been exposed to thalidomide, and 10/12 (83%) of those whose disease was refractory to their most recent therapy. To obtain additional information to guide a phase III trial, a randomized phase II study was then started comparing lenalidomide and dexamethasone with elotuzumab at either 10 or 20 mg/kg.[116] Common toxicities in this larger study were lymphopenia (in 19%), neutropenia (18%), thrombocytopenia (16%), anemia (12%), leukopenia (10%), hyperglycemia (10%), pneumonia (7%), diarrhea (7%), fatigue (7%), and hypokalemia (6%), while infusion reactions occurred in 12% of patients. Notably, while the ORR and PFS were excellent in both arms (Table 4), there was a trend towards better results in both of these endpoints with the 10-mg/kg dose. As a result, the ongoing phase III study comparing lenalidomide/low-dose dexamethasone with or without elotuzumab (Table 1) is using this lower dose, and has already reached its accrual goal.

SIGNAL TRANSDUCTION INHIBITORS

The major drug classes being tested in myeloma remain within the catgories of deacetylase inhibitors, proteasome inhibitors, immunomodulatory agents, and monoclonal antibodies. However, a number of other agents with activity as inhibitors of signal transduction pathways important to the pathobiology of multiple myeloma also are being evaluated in radomized phase III trials that could lead to new drug approvals.

Masitinib

Masitinib, also known as AB1010 (AB Science, Paris, France) (and KINAVET-CA1 for canine use), is a novel phenylaminothiazole-type tyrosine kinase inhibitor that targets the stem cell factor receptor c-Kit, as well as the platelet-derived growth factor receptor (PDGFR), the intracellular Lyn kinase, and fibroblast growth factor receptor (FGFR) 3.[117] It was first reported to delay TTP of recurrent or non-

Table 4. Response Rate and Response Durability Data From a Phase II Trial of Elotuzumab With Lenalidomide and Low-Dose Dexamethasone in Patients With Relapsed/Refractory Multiple Myeloma*

	Elotuzumab 10 mg/kg (n = 36)	Elotuzumab 20 mg/kg (n = 36)	Overall (N = 73)
Median PFS (mo)	26.9	18.6	25.0
ORR, n (%)	33 (92%)	28 (76%)	61 (84%)
Selected subgroup analyses			
ORR and PFS in patients with 1 prior line of therapy	NR	NR	91% 25.0 mo
ORR and PFS in patients with 2 or more prior lines of therapy	NR	NR	78% 21.3 mo
ORR and PFS in patients with prior treatment with thalidomide	NR	NR	82% 26.9 mo

Abbreviations: NR, not reported; ORR, overall response rate; PFS, progression-free survival.
*Data are from reference (116).

resectable grade II or III mast cell tumors in canines.[118] A later phase I human study determined that 12 mg/kg/d was safe for human dosing, and also reported stable disease in 29% of patients with imatinib-resistant gastrointestinal stromal tumors (GIST).[119] Activity in this disease was later confirmed in the first-line for patients with GIST, who experienced a median PFS of 41.3 months.[120] Among other human malignancies, masitinib is active against systemic and cutaneous mastocytosis,[121] as well as mast cell leukemia.[122] Preclinical studies documenting the activity of masitinib either alone, or in combination with other agents, have not yet appeared in the peer-reviewed literature. However, c-Kit is expressed in myeloma and may play a role in plasma cell proliferation,[123] and FGFR-3, especially in the setting of the 4;14 translocation, is known to contribute to high-risk features of this disease.[124] Also, since signaling through Lyn kinase[125,126] and PDGFR[127] may play roles in myeloma proliferation and angiogenesis, it is certainly possible that masitinib may have activity against this disease. To determine if this could be the case, a phase III trial comparing bortezomib and dexamethasone to masitinib with bortezomib and dexamethasone is currently underway (Table 1).

Plitidespin

Plitidepsin (Aplidin, PharmaMar, Madrid, Spain) is a marine-derived cyclodepsipeptide that has shown activity against myeloma in both the syngeneic 5T33MM murine mouse model,[128] and in human myeloma cell lines and primary samples.[129] In the latter, plitidepsin activated the p38 and JNK kinases, and also induced Fas/CD95 translocation to lipid rafts, as well as caspase activation. A phase II clinical trial of plitidepsin has been completed targeting patients with relapsed and refractory multiple myeloma, which administered this drug at 5 mg/m^2 as a 3-hour infusion every 2 weeks, with the possibility to later add oral dexamethasone if a suboptimal response was seen. Common hematologic toxicities included grade 3 and 4 anemia (in 29% of patients), thrombocytopenia (18%), and neutropenia (18%). Non-hematologic toxicities included elevations of laboratory studies such as the alanine (28%) or aspartate (10%) aminotransferases, creatinine (4%) or creatine kinase (6%), and alkaline phosphatase or total bilirubin (2% each), as well as fatigue (16%), myalgia (4%), or either nausea, muscle weakness, anorexia, vomiting, or dyspnea (2% each).[130] The ORR (including at least MRs) was reported as 13% with plitidepsin alone, which rose to 22% in the 19 patients who also received dexamethasone. Time to progression and PFS for plitidepsin alone was 2.3 months, which rose to 4.2 and 3.8 months, respectively, in the subgroup who received added dexamethasone. In the ongoing phase III study (Table 1), plitidepsin with dexamethasone is being compared to dexamethasone alone for patients with relapsed or relapsed and refractory disease that has been treated with at least three but not more than six prior regimens.

Perifosine

Perifosine ([octadecyl-(1,1-dimethyl-piperidinio-4-yl)-phosphate]; KRX-0401, Keryx Biopharmaceuticals, Inc, New York, NY) is an alkylphospholipid

that was found to induce cytotoxicity in myeloma cell lines and patient samples, overcome drug resistance, and enhance the activity of other anti-myeloma agents.[131] This occurred in part through activation of the JNK pathway, and in association with inhibition of activation of anti-apoptotic Akt.[131] Since activation of Akt by bortezomib is a proposed mechansim of resistance to this proteasome inhibitor,[131,132] perifosine could be a directed strategy to enhance proteasome inhibitor sensitivity, and possibly overcome drug resistance. Additional mechanisms of action for perifosine may include downregulation of Survivin,[133] while recruitment of death receptors and associated signaling molecules into lipid rafts may play a role as well.[134,135] Two combination approaches to myeloma therapy incorporating perifosine have been reported, including one study with lenalidomide and dexamethasone,[136] and another with bortezomib, which allowed later addition of dexamethasone.[137] The latter has formed the basis for an ongoing phase III study comparing bortezomib/dexamethasone to the same regimen with added perifosine (Table 1). Eligible patients include those who have had one to four prior lines of therapy, and are relapsed and/or refractory, providing that their disease was not refractory to a bortezomib-containing regimen. Selection of the latter strategy was based in part on the findings from the phase I trial, which recommended a perifosine dose of 50 mg daily for further study in combination with bortezomib. Toxicities seen in at least 25% of patients included nausea (63%), diarrhea (57%), fatigue (43%), musculoskeletal pain (42%), upper respiratory tract infection (33%), anorexia (33%), peripheral neuropathy (29%), vomiting (29%), and coughing (25%). More significant, grade 3 or 4 events in at least 10% of patients included thrombocytopenia (23%), neutropenia (15%), anemia (14%), pneumonia (12%), musculoskeletal pain (11%), and bleeding (10%). Responses, including at least an MR, were seen in 41% of patients overall, including in 13/20 (65%) who were bortezomib relapsed, and 17/53 (32%) who were bortezomib-refractory. These encouraging findings formed the rationale for the randomized study, the results of which are eagerly awaited.

CONCLUSIONS

The recent approvals of carfilzomib and pomalidomide for patients with relapsed and refractory myeloma after at least two prior lines of therapy are likely harbingers of their future adoption into the relapsed setting for patients with one or more prior therapies. Moreover, other new agents that represent new drug classes, such as panobinostat and elotuzumab, may be on the cusp of approval, since registration-enabling studies have already been fully enrolled, and hopefully positive data will be reported soon. Further in the future, even more novel drugs are showing promise, including other monoclonal antibodies such as daratumumab,[138] and new drug classes such as kinesin spindle protein inhibitors.[139,140] If they continue to demonstrate encouraging activity in the refractory setting, they too may soon become incorporated into the treatment algorithm for relapsed disease. These will give patients and caregivers facing decisions on treatment of relapsed myeloma an ever wider and better array of treatment options, which will likely induce a greater response rate and deeper response quality than our currently available agents and, most importantly, improve quality of life and OS.

Despite this encouraging picture, many challenges remain for development of drugs in the relapsed setting. With the increasing efficacy of front-line therapy,[23–27] and the greater tendency to use maintenance after either stem cell transplant[141,142] or standard dose approaches,[143,144] fewer patients will have relapsed disease. Patients will tend to either stay in remission, which will certainly be welcome, or will develop disease that is refractory and more chemotherapy resistant, which will slow drug development. The latter may prove to be an argument that will allow continued use of the accelerated approval pathway for myeloma, without which all new drug applications would likely need to come from large, randomized phase III studies that slow the time to wide availability of new drugs. Another matter is that of the economics of therapy, since while current analyses have suggested that agents such as bortezomib, thalidomide, and lenalidomide alone, and in combination, are likely cost-effective,[145,146] there is agreement that more studies are needed in this area.[147]

All of these arguments support the need for a greater understanding of the molecular mechanisms that support the pathobiology of multiple myeloma in the relapsed setting. It is likely that drug resistance is mediated by a finite set of pathways whose relative contributions will vary in individual patients in a manner that could be determined through the use of validated biomarkers. If so, this would allow genomic and proteomic analyses to be performed on primary samples from patients with relapsed myeloma to determine which targets need to be suppressed or activated to restore sensitivity to drugs that were used successfully in a prior line of therapy, or to maximize the benefits of the available new drug options. For example, if lenalidomide resistance emerged due to decreased expression of Cereblon,[65,66] current data argue that pomalidomide alone may be less successful for such patients, while pomalidomide with a proteasome inhibitor to

increase Cereblon levels could be of value. In contrast, if lenalidomide resistance were instead mediated by induction of the Wnt/β-catenin pathway,[68] pomalidomide could be more successful, or lenalidomide could be reused with approaches that suppress Wnt/β-catenin, such as antibodies that target CD44, or all-*trans* retinoic acid, which reduces CD44 expression.[69] By so personalizing therapy, we would optimize patient outcomes by targeting the vulnerabilities of each person's myeloma, minimize toxicities by limiting exposure of patients to agents to which their disease would be unlikely to respond, save valuable healthcare resources, and speed new drug development.

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REFERENCES

1. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med. 2003;348(26):2609–17.
2. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005;352(24):2487–98.
3. Orlowski RZ, 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(25):3892–901.
4. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008;359(9):906–17.
5. Singhal S, Mehta J, Desikan R, et al. Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med. 1999;341(21):1565–71.
6. Rajkumar SV, Blood E, Vesole D, Fonseca R, Greipp PR. Phase III clinical trial of thalidomide plus dexamethasone compared with dexamethasone alone in newly diagnosed multiple myeloma: a clinical trial coordinated by the Eastern Cooperative Oncology Group. J Clin Oncol. 2006;24(3):431–6.
7. Rajkumar SV, Rosinol L, Hussein M, et al. Multicenter, randomized, double-blind, placebo-controlled study of thalidomide plus dexamethasone compared with dexamethasone as initial therapy for newly diagnosed multiple myeloma. J Clin Oncol. 2008;26(13):2171–7.
8. Weber DM, Chen C, Niesvizky R, et al. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. N Engl J Med. 2007;357(21):2133–42.
9. Dimopoulos M, Spencer A, Attal M, et al. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. N Engl J Med. 2007;357(21):2123–32.
10. 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(14):2817–25.
11. Vij R, Richardson PGG, Jagannath S, et al. Pomalidomide (POM) with or without low-dose dexamethasone (LoDEX) in patients (pts) with relapsed/refractory multiple myeloma (RRMM): outcomes in pts refractory to lenalidomide (LEN) and/or bortezomib (BORT). J Clin Oncol. 2012;30(Suppl):abstr 8016.
12. 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(1):29–37.
13. 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(30):4621–9.
14. 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(9758):2075–85.
15. 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(22):5752–8.
16. Garderet L, Iacobelli S, Moreau P, et al. Superiority of the triple combination of bortezomib-thalidomide-dexamethasone over the dual combination of thalidomide-dexamethasone in patients with multiple myeloma progressing or relapsing after autologous transplantation: the MMVAR/IFM 2005-04 Randomized Phase III Trial from the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol. 2012;30(20):2475–82.
17. Richardson PG, Weller E, Jagannath S, et al. Multicenter, phase I, dose-escalation trial of lenalidomide plus bortezomib for relapsed and relapsed/refractory multiple myeloma. J Clin Oncol. 2009;27(34):5713–9.
18. Richardson PG, Weller E, Lonial S, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood. 2010;116(5):679–86.
19. 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 (9):1801–9.
20. Niesvizky R, Martin TG, 3rd, Bensinger W, et al. Phase Ib dose-escalation study (PX-171-006) of carfilzomib, lenalidomide, and low-dose dexamethasone in relapsed or progressive multiple myeloma. Clin Cancer Res. 2013. In press.
21. Richardson PG, Hofmeister CC, Siegel D, et al. MM-005: a phase 1, multicenter, open-label, dose-escalation study to determine the maximum tolerated dose for the combination of pomalidomide, bortezomib, and low-dose dexamethasone in subjects with relapsed or refractory multiple myeloma. ASH Annual Meeting Abstracts. 2012;120(21):727.
22. Shah JJ, Stadtmauer EA, Abonour R, et al. A multicenter phase I/II trial of carfilzomib and pomalidomide with dexamethasone (Car-Pom-d) in patients with relapsed/refractory multiple myeloma. ASH Annual Meeting Abstracts. 2012;120(21):74.
23. Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008;111 (5):2516–20.
24. Brenner H, Gondos A, Pulte D. Expected long-term survival of patients diagnosed with multiple myeloma in 2006-2010. Haematologica. 2009;94(2): 270–5.
25. 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(7):1209–14.
26. Usmani SZ, Crowley J, Hoering A, et al. Improvement in long-term outcomes with successive Total Therapy trials for multiple myeloma: are patients now being cured? Leukemia. 2013;27(1):226–32.
27. Anderson KC. The 39th David A. Karnofsky Lecture: bench-to-bedside translation of targeted therapies in multiple myeloma. J Clin Oncol. 2012;30(4):445–52.
28. Alexanian R, Delasalle K, Wang M, Thomas S, Weber D. Curability of multiple myeloma. Bone Marrow Res. 2012;2012:916479.
29. Othus M, Barlogie B, Leblanc ML, Crowley JJ. Cure models as a useful statistical tool for analyzing survival. Clin Cancer Res. 2012;18(14):3731–6.
30. Hideshima T, Richardson PG, Anderson KC. Mechanism of action of proteasome inhibitors and deacetylase inhibitors and the biological basis of synergy in multiple myeloma. Mol Cancer Ther. 2011;10 (11):2034–42.
31. Mahindra A, Laubach J, Raje N, Munshi N, Richardson PG, Anderson K. Latest advances and current challenges in the treatment of multiple myeloma. Nat Rev Clin Oncol. 2012;9(3):135–43.
32. Lavelle D, Chen YH, Hankewych M, DeSimone J. Histone deacetylase inhibitors increase p21(WAF1) and induce apoptosis of human myeloma cell lines independent of decreased IL-6 receptor expression. Am J Hematol. 2001;68(3):170–8.
33. Mitsiades N, Mitsiades CS, Richardson PG, et al. Molecular sequelae of histone deacetylase inhibition in human malignant B cells. Blood. 2003;101 (10):4055–462.
34. Catley L, Weisberg E, Tai YT, et al. NVP-LAQ824 is a potent novel histone deacetylase inhibitor with significant activity against multiple myeloma. Blood. 2003;102(7):2615–22.
35. Mitsiades CS, Mitsiades NS, McMullan CJ, et al. Transcriptional signature of histone deacetylase inhibition in multiple myeloma: biological and clinical implications. Proc Natl Acad Sci U S A.. 2004;101 (2):540–5.
36. Pei XY, Dai Y, Grant S. Synergistic induction of oxidative injury and apoptosis in human multiple myeloma cells by the proteasome inhibitor bortezomib and histone deacetylase inhibitors. Clin Cancer Res. 2004;10(11):3839–52.
37. Nawrocki ST, Carew JS, Pino MS, et al. Aggresome disruption: a novel strategy to enhance bortezomib-induced apoptosis in pancreatic cancer cells. Cancer Res. 2006;66(7):3773–81.
38. Hideshima T, Bradner JE, Wong J, et al. Small-molecule inhibition of proteasome and aggresome function induces synergistic antitumor activity in multiple myeloma. Proc Natl Acad Sci U S A. 2005;102(24):8567–872.
39. Kuhn DJ, Berkova Z, Jones RJ, et al. Targeting the insulin-like growth factor-1 receptor to overcome bortezomib resistance in preclinical models of multiple myeloma. Blood. 2012;120(16):3260–370.
40. Richardson P, Mitsiades C, Colson K, et al. Phase I trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) in patients with advanced multiple myeloma. Leuk Lymphoma. 2008;49(3):502–7.
41. Badros A, Burger AM, Philip S, et al. Phase I study of vorinostat in combination with bortezomib for relapsed and refractory multiple myeloma. Clin Cancer Res. 2009;15(16):5250–7.
42. Weber DM, Graef T, Hussein M, et al. Phase I trial of vorinostat combined with bortezomib for the treatment of relapsing and/or refractory multiple myeloma. Clin Lymphoma Myeloma Leuk. 2012;12(5):319–24.
43. Siegel DS, Dimopoulos MA, Yoon S-S, et al. Vantage 095: vorinostat in combination with bortezomib in salvage multiple myeloma patients: final study results of a global phase 2b trial. ASH Annual Meeting Abstracts. 2011;118(21):480.
44. Dimopoulos MA, Jagannath S, Yoon S-S, et al. Vantage 088: vorinostat in combination with bortezomib in patients with relapsed/refractory multiple myeloma: results of a global, randomized phase 3 trial. ASH Annual Meeting Abstracts. 2011;118(21):811.
45. Catley L, Weisberg E, Kiziltepe T, et al. Aggresome induction by proteasome inhibitor bortezomib and alpha-tubulin hyperacetylation by tubulin deacetylase (TDAC) inhibitor LBH589 are synergistic in myeloma cells. Blood. 2006;108(10) (3441-349).
46. Maiso P, Carvajal-Vergara X, Ocio EM, et al. The histone deacetylase inhibitor LBH589 is a potent antimyeloma agent that overcomes drug resistance. Cancer Res. 2006;66(11):5781–9.
47. Ocio EM, Vilanova D, Atadja P, et al. In vitro and in vivo rationale for the triple combination of

panobinostat (LBH589) and dexamethasone with either bortezomib or lenalidomide in multiple myeloma. Haematologica. 2010;95(5):794–803.
48. Sanchez E, Shen J, Steinberg J, et al. The histone deacetylase inhibitor LBH589 enhances the anti-myeloma effects of chemotherapy in vitro and in vivo. Leuk Res. 2011;35(3):373–9.
49. Wolf JL, Siegel D, Goldschmidt H, et al. Phase II trial of the pan-deacetylase inhibitor panobinostat as a single agent in advanced relapsed/refractory multiple myeloma. Leuk Lymphoma. 2012;53(9):1820–3.
50. Schmitt S, Ho AD, Goldschmidt H. The oral histone deacetylase inhibitor LBH589 is a potential and promising therapeutic agent in multiple myeloma after at least two lines of chemotherapy including bortezomib or lenalidomide. Onkologie. 2010;33(4):183–6.
51. San-Miguel JF, Richardson PGG, Sezer O, et al. A phase lb study of oral panobinostat and IV bortezomib in relapsed or relapsed and refractory multiple myeloma. J Clin Oncol. 2011;29(Suppl):abstr 8075.
52. San-Miguel JF, Moreau P, Yoon S-S, et al. Phase III study of panobinostat with bortezomib and dexamethasone in patients with relapsed multiple myeloma (PANORAMA 1). J Clin Oncol. 2012;30(Suppl):abstr e18572.
53. San-Miguel JF, de Moraes Hungria VT, Yoon S-S, et al. Update on a phase III study of panobinostat with bortezomib and dexamethasone in patients with relapsed multiple myeloma: PANORAMA 1. ASH Annual Meeting Abstracts. 2011;118(21):3976.
54. Orlowski RZ, Stinchcombe TE, Mitchell BS, et al. Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. J Clin Oncol. 2002;20(22):4420–7.
55. Shah JJ, Orlowski RZ. Proteasome inhibitors in the treatment of multiple myeloma. Leukemia. 2009;23 (11):1964–79.
56. Moreau P, Richardson PG, Cavo M, et al. Proteasome inhibitors in multiple myeloma: 10 years later. Blood. 2012;120(5):947–59.
57. Kirk CJ. Discovery and development of second-generation proteasome inhibitors. Semin Hematol. 2012;49(3):207–14.
58. Kortuem KM, Stewart AK. Carfilzomib. Blood. 2013;121(6):893–7.
59. Kuhn DJ, Chen Q, Voorhees PM, et al. Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. Blood. 2007;110 (9):3281–90.
60. Demo SD, Kirk CJ, Aujay MA, et al. Antitumor activity of PR-171, a novel irreversible inhibitor of the proteasome. Cancer Res. 2007;67(13):6383–91.
61. Hurchla MA, Garcia-Gomez A, Hornick MC, et al. The epoxyketone-based proteasome inhibitors carfilzomib and orally bioavailable oprozomib have anti-resorptive and bone-anabolic activity in addition to anti-myeloma effects. Leukemia. 2013;27(2):430–40.
62. Arastu-Kapur S, Anderl JL, Kraus M, et al. Non-proteasomal targets of the proteasome inhibitors bortezomib and carfilzomib: a link to clinical adverse events. Clin Cancer Res. 2011;17(9):2734–43.
63. O'Connor OA, Stewart AK, Vallone M, et al. A phase 1 dose escalation study of the safety and pharmacokinetics of the novel proteasome inhibitor carfilzomib (PR-171) in patients with hematologic malignancies. Clin Cancer Res. 2009;15(22):7085–91.
64. Alsina M, Trudel S, Furman RR, et al. A phase I single-agent study of twice-weekly consecutive-day dosing of the proteasome inhibitor carfilzomib in patients with relapsed or refractory multiple myeloma or lymphoma. Clin Cancer Res. 2012;18(17):4830–40.
65. Zhu YX, Braggio E, Shi CX, et al. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. Blood. 2012;118 (18) (4771-479).
66. Lopez-Girona A, Mendy D, Ito T, et al. Cereblon is a direct protein target for immunomodulatory and antiproliferative activities of lenalidomide and pomalidomide. Leukemia. 2012;26(11):2326–35.
67. Lee KM, Lee J, Park CS. Cereblon inhibits proteasome activity by binding to the 20S core proteasome subunit beta type 4. Biochem Biophys Res Commun. 2012;427(3):618–22.
68. Bjorklund CC, Ma W, Wang ZQ, et al. Evidence of a role for activation of Wnt/beta-catenin signaling in the resistance of plasma cells to lenalidomide. J Biol Chem. 2011;286(13):11009–120.
69. Bjorklund CC, Aukerman SL, Antonia Lopez-Girona A, et al. Targeting the Wnt/β-catenin signaling pathway and CD44-mediated adhesion as a rational approach to overcome lenalidomide resistance in multiple myeloma. Blood. 2011:118 (abstr 928).
70. Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J. 1997;16(13):3797–804.
71. Sukhdeo K, Mani M, Hideshima T, et al. beta-catenin is dynamically stored and cleared in multiple myeloma by the proteasome-aggresome-autophagosome-lysosome pathway. Leukemia. 2012;26(5):1116–9.
72. 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(24) (5661-570).
73. Vij R, Siegel DS, Jagannath S, et al. An open-label, single-arm, phase 2 study of single-agent carfilzomib in patients with relapsed and/or refractory multiple myeloma who have been previously treated with bortezomib. Br J Haematol. 2012;158(6):739–48.
74. Hajek R, Bryce R, Ro S, Klencke B, Ludwig H. Design and rationale of FOCUS (PX-171-011): A randomized, open-label, phase 3 study of carfilzomib versus best supportive care regimen in patients with relapsed and refractory multiple myeloma (R/R MM). BMC Cancer. 2012;12:415.
75. Badros AZ, Papadopoulos KP, Zojwalla N, Lee JRJ, Siegel DS. A phase 1b study of 30-minute infusion carfilzomib 20/45 and 20/56 mg/m2 plus 40 mg weekly dexamethasone in patients with relapsed and/or refractory (R/R) multiple myeloma. ASH Annual Meeting Abstracts. 2012;120(21):4036.
76. Lendvai N, Landau H, Lesokhin A, et al. Phase II study of infusional carfilzomib in patients with

relapsed or refractory multiple myeloma. ASH Annual Meeting Abstracts. 2012;120(21):947.
77. 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(5):431–40.
78. Kupperman E, Lee EC, Cao Y, et al. Evaluation of the proteasome inhibitor MLN9708 in preclinical models of human cancer. Cancer Res. 2010;70(5):1970–80.
79. Chauhan D, Tian Z, Zhou B, et al. In vitro and in vivo selective antitumor activity of a novel orally bioavailable proteasome inhibitor MLN9708 against multiple myeloma cells. Clin Cancer Res. 2011;17(16):5311–21.
80. Tian Z, Zhao JJ, Tai YT, et al. Investigational agent MLN9708/2238 targets tumor-suppressor miR33b in MM cells. Blood. 2012;120(19):3958–67.
81. Richardson PG, Baz R, Wang L, et al. Investigational agent MLN9708, an oral proteasome inhibitor, in patients (pts) with relapsed and/or refractory multiple myeloma (MM): results from the expansion cohorts of a phase 1 dose-escalation study. ASH Annual Meeting Abstracts. 2011;118(21):301.
82. Kumar S, Bensinger W, Reeder CB, et al. Weekly dosing of the investigational oral proteasome inhibitor MLN9708 in patients with relapsed and/or refractory multiple myeloma: results from a phase 1 dose-escalation study. ASH Annual Meeting Abstracts. 2011;118(21):816.
83. Kumar SK, Berdeja JG, Niesvizky R, et al. A phase 1/2 study of weekly MLN9708, an investigational oral proteasome inhibitor, in combination with lenalidomide and dexamethasone in patients with previously untreated multiple myeloma (MM). ASH Annual Meeting Abstracts. 2012;120(21):332.
84. Latif T, Chauhan N, Khan R, Moran A, Usmani SZ. Thalidomide and its analogues in the treatment of multiple myeloma. Exp Hematol Oncol. 2012;1(1):27.
85. Andhavarapu S, Roy V. Immunomodulatory drugs in multiple myeloma. Expert Rev Hematol. 2013;6(1):69–82.
86. Saini N, Mahindra A. Novel immunomodulatory compounds in multiple myeloma. Expert Opin Investig Drugs. 2013;22(2):207–15.
87. Schafer PH, Gandhi AK, Loveland MA, et al. Enhancement of cytokine production and AP-1 transcriptional activity in T cells by thalidomide-related immunomodulatory drugs. J Pharmacol Exp Ther. 2003;305(3):1222–32.
88. Hideshima T, Chauhan D, Shima Y, et al. Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. Blood. 2000;96(9):2943–50.
89. Schey SA, Fields P, Bartlett JB, et al. Phase I study of an immunomodulatory thalidomide analog, CC-4047, in relapsed or refractory multiple myeloma. J Clin Oncol. 2004;22(16):3269–76.
90. Lacy MQ, Hayman SR, Gertz MA, et al. Pomalidomide (CC4047) plus low-dose dexamethasone as therapy for relapsed multiple myeloma. J Clin Oncol. 2009;27(30):5008–14.
91. Lacy MQ, Hayman SR, Gertz MA, et al. Pomalidomide (CC4047) plus low dose dexamethasone (Pom/dex) is active and well tolerated in lenalidomide refractory multiple myeloma (MM). Leukemia. 2010;24(11):1934–9.
92. Lacy MQ, Allred JB, Gertz MA, et al. Pomalidomide plus low-dose dexamethasone in myeloma refractory to both bortezomib and lenalidomide: comparison of 2 dosing strategies in dual-refractory disease. Blood. 2011;118(11):2970–5.
93. 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.
94. Richardson PG, Siegel D, 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.
95. Vij R, Hofmeister CC, Richardson PG, et al. Pomalidomide (POM) with low-dose dexamethasone (LoDEX) in patients with relapsed and refractory multiple myeloma (RRMM): outcomes based on prior treatment exposure. ASH Annual Meeting Abstracts. 2012;120(21):4070.
96. 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. ASH Annual Meeting Abstracts. 2012;120(21):LBA-6.
97. Danylesko I, Beider K, Shimoni A, Nagler A. Monoclonal antibody-based immunotherapy for multiple myeloma. Immunotherapy. 2012;4(9):919–38.
98. Yang J, Yi Q. Therapeutic monoclonal antibodies for multiple myeloma: an update and future perspectives. Am J Blood Res. 2011;1(1):22–33.
99. Tai YT, Anderson KC. Antibody-based therapies in multiple myeloma. Bone Marrow Res. 2011;2011:924058.
100. Trikha M, Corringham R, Klein B, Rossi JF. Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence. Clin Cancer Res. 2003;9(13):4653–65.
101. Bommert K, Bargou RC, Stuhmer T. Signalling and survival pathways in multiple myeloma. Eur J Cancer. 2006;42(11):1574–80.
102. Voorhees PM, Chen Q, Kuhn DJ, et al. Inhibition of interleukin-6 signaling with CNTO 328 enhances the activity of bortezomib in preclinical models of multiple myeloma. Clin Cancer Res. 2007;13(21):6469–78.
103. Voorhees PM, Chen Q, Small GW, et al. Targeted inhibition of interleukin-6 with CNTO 328 sensitizes pre-clinical models of multiple myeloma to dexamethasone-mediated cell death. Br J Haematol. 2009;145(4):481–90.
104. Hunsucker SA, Magarotto V, Kuhn DJ, et al. Blockade of interleukin-6 signalling with siltuximab enhances melphalan cytotoxicity in preclinical models of multiple myeloma. Br J Haematol. 2011;152(5):579–92.
105. van Zaanen HC, Lokhorst HM, Aarden LA, et al. Chimaeric anti-interleukin 6 monoclonal antibodies

in the treatment of advanced multiple myeloma: a phase I dose-escalating study. Br J Haematol. 1998;102(3):783–90.
106. Kurzrock R, Voorhees PM, Casper C, et al. Long-term safety in a phase 1 study of siltuximab (CNTO 328), an anti-interleukin-6 monoclonal antibody, in patients with B-cell non-Hodgkin's lymphoma, multiple myeloma, or Castleman's disease. ASH Annual Meeting Abstracts. 2011;118(21):3959.
107. Chari A, Pri-Chen H, Jagannath S. Complete remission achieved with single agent CNTO 328, an anti-IL-6 monoclonal antibody, in relapsed and refractory myeloma. Clin Lymphoma Myeloma Leuk. 2013. In press.
108. Voorhees PM, Manges RF, Sonneveld P, et al. A phase 2 multicentre study of siltuximab, an anti-interleukin-6 monoclonal antibody, in patients with relapsed or refractory multiple myeloma. Br J Haematol. 2013. In press.
109. Orlowski RZ, Gercheva L, Williams C, et al. Phase II, randomized, double blind, placebo-controlled study comparing siltuximab plus bortezomib versus bortezomib alone in pts with relapsed/refractory multiple myeloma. J Clin Oncol. 2012;30(Suppl):abstr 8018.
110. Tai YT, Dillon M, Song W, et al. Anti-CS1 humanized monoclonal antibody HuLuc63 inhibits myeloma cell adhesion and induces antibody-dependent cellular cytotoxicity in the bone marrow milieu. Blood. 2008;112(4):1329–37.
111. 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(9): 2775–84.
112. van Rhee F, Szmania SM, Dillon M, et al. Combinatorial efficacy of anti-CS1 monoclonal antibody elotuzumab (HuLuc63) and bortezomib against multiple myeloma. Mol Cancer Ther. 2009;8(9):2616–24.
113. Zonder JA, Mohrbacher AF, Singhal S, et al. A phase 1, multicenter, open-label, dose escalation study of elotuzumab in patients with advanced multiple myeloma. Blood. 2012;120(3):552–9.
114. Jakubowiak AJ, Benson DM, Bensinger W, et al. Phase I trial of anti-CS1 monoclonal antibody elotuzumab in combination with bortezomib in the treatment of relapsed/refractory multiple myeloma. J Clin Oncol. 2012;30(16):1960–5.
115. Lonial S, Vij R, Facon T, et al. Phase I trial of elotuzumab, lenalidomide, and low-dose dexamethasone in patients with relapsed or refractory multiple myeloma. J Clin Oncol. 2011;29(Suppl):abstr 8076.
116. Richardson PG, Jagannath S, Moreau P, et al. A phase 2 study of elotuzumab (Elo) in combination with lenalidomide and low-dose dexamethasone (Ld) in patients (pts) with relapsed/refractory multiple myeloma (R/R MM): updated results. ASH Annual Meeting Abstracts. 2012;120(21):202.
117. Dubreuil P, Letard S, Ciufolini M, et al. Masitinib (AB1010), a potent and selective tyrosine kinase inhibitor targeting KIT. PLoS One. 2009;4(9):e7258.
118. Hahn KA, Ogilvie G, Rusk T, et al. Masitinib is safe and effective for the treatment of canine mast cell tumors. J Vet Intern Med. 2008;22(6):1301–9.
119. Soria JC, Massard C, Magne N, et al. Phase 1 dose-escalation study of oral tyrosine kinase inhibitor masitinib in advanced and/or metastatic solid cancers. Eur J Cancer. 2009;45(13):2333–41.
120. Le Cesne A, Blay JY, Bui BN, et al. Phase II study of oral masitinib mesilate in imatinib-naive patients with locally advanced or metastatic gastro-intestinal stromal tumour (GIST). Eur J Cancer. 2010;46 (8):1344–51.
121. Paul C, Sans B, Suarez F, et al. Masitinib for the treatment of systemic and cutaneous mastocytosis with handicap: a phase 2a study. Am J Hematol. 2010;85(12):921–5.
122. Georgin-Lavialle S, Lhermitte L, Suarez F, et al. Mast cell leukemia: identification of a new c-Kit mutation, dup(501-502), and response to masitinib, a c-Kit tyrosine kinase inhibitor. Eur J Haematol. 2012;89 (1):47–52.
123. Lemoli RM, Fortuna A. C-kit ligand (SCF) in human multiple myeloma cells. Leuk Lymphoma. 1996;20(5-6):457–64.
124. Kalff A, Spencer A. The t(4;14) translocation and FGFR3 overexpression in multiple myeloma: prognostic implications and current clinical strategies. Blood Cancer J. 2012;2:e89.
125. Hallek M, Neumann C, Schaffer M, et al. Signal transduction of interleukin-6 involves tyrosine phosphorylation of multiple cytosolic proteins and activation of Src-family kinases Fyn, Hck, and Lyn in multiple myeloma cell lines. Exp Hematol. 1997;25 (13):1367–77.
126. Iqbal MS, Tsuyama N, Obata M, Ishikawa H. A novel signaling pathway associated with Lyn, PI 3-kinase and Akt supports the proliferation of myeloma cells. Biochem Biophys Res Commun. 2010;392(3): 415–20.
127. Coluccia AM, Cirulli T, Neri P, et al. Validation of PDGFRbeta and c-Src tyrosine kinases as tumor/vessel targets in patients with multiple myeloma: preclinical efficacy of the novel, orally available inhibitor dasatinib. Blood. 2008;112(4):1346–56.
128. Caers J, Menu E, De Raeve H, et al. Antitumour and antiangiogenic effects of Aplidin in the 5TMM syngeneic models of multiple myeloma. Br J Cancer. 2008;98(12):1966–74.
129. Mitsiades CS, Ocio EM, Pandiella A, et al. Aplidin, a marine organism-derived compound with potent antimyeloma activity in vitro and in vivo. Cancer Res. 2008;68(13):5216–25.
130. Mateos MV, Cibeira MT, Richardson PG, et al. Phase II clinical and pharmacokinetic study of plitidepsin 3-hour infusion every two weeks alone or with dexamethasone in relapsed and refractory multiple myeloma. Clin Cancer Res. 2010;16(12):3260–9.
131. Hideshima T, Catley L, Yasui H, et al. Perifosine, an oral bioactive novel alkylphospholipid, inhibits Akt and induces in vitro and in vivo cytotoxicity in human multiple myeloma cells. Blood. 2006;107 (10):4053–62.
132. Shi YY, Small GW, Orlowski RZ. Proteasome inhibitors induce a p38 mitogen-activated protein kinase (MAPK)-dependent anti-apoptotic program involving

MAPK phosphatase-1 and Akt in models of breast cancer. Breast Cancer Res Treat. 2006;100(1):33–47.
133. Hideshima T, Catley L, Raje N, et al. Inhibition of Akt induces significant downregulation of survivin and cytotoxicity in human multiple myeloma cells. Br J Haematol. 2007;138(6):783–91.
134. Gajate C, Mollinedo F. Edelfosine and perifosine induce selective apoptosis in multiple myeloma by recruitment of death receptors and downstream signaling molecules into lipid rafts. Blood. 2007;109 (2):711–9.
135. David E, Sinha R, Chen J, Sun SY, Kaufman JL, Lonial S. Perifosine synergistically enhances TRAIL-induced myeloma cell apoptosis via up-regulation of death receptors. Clin Cancer Res. 2008;14(16): 5090–8.
136. Jakubowiak AJ, Richardson PG, Zimmerman T, et al. Perifosine plus lenalidomide and dexamethasone in relapsed and relapsed/refractory multiple myeloma: a phase I Multiple Myeloma Research Consortium study. Br J Haematol. 2012;158(4):472–80.
137. Richardson PG, Wolf J, Jakubowiak A, et al. Perifosine plus bortezomib and dexamethasone in patients with relapsed/refractory multiple myeloma previously treated with bortezomib: results of a multicenter phase I/II trial. J Clin Oncol. 2011;29 (32):4243–9.
138. Plesner T, Lokhorst H, Gimsing P, Nahi H, Lisby S, Richardson PG Daratumumab. A CD38 monoclonal antibody in patients with multiple myeloma - data from a dose-escalation phase I/II study. ASH Annual Meeting Abstracts. 2012;120(21):73.
139. Shah JJ, Zonder JA, Cohen AD, et al. The novel KSP inhibitor ARRY-520 is active both with and without low-dose dexamethasone in patients with multiple myeloma refractory to bortezomib and lenalidomide: results from a phase 2 study. ASH Annual Meeting Abstracts. 2012;120(21):449.
140. Shah JJ, Weber DaM, Thomas SaK, et al. Phase 1 study of the novel kinesin spindle protein inhibitor ARRY-520 + carfilzomib in patients with relapsed and/or refractory multiple myeloma. ASH Annual Meeting Abstracts. 2012;120(21):4082.
141. McCarthy PL, Owzar K, Hofmeister CC, et al. Lenalidomide after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366(19):1770–81.
142. Attal M, Lauwers-Cances V, Marit G, et al. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;366(19): 1782–91.
143. Palumbo A, Hajek R, Delforge M, et al. Continuous lenalidomide treatment for newly diagnosed multiple myeloma. N Engl J Med. 2012;366(19):1759–69.
144. Mateos MV, Oriol A, Martinez-Lopez J, et al. Maintenance therapy with bortezomib plus thalidomide or bortezomib plus prednisone in elderly multiple myeloma patients included in the GEM2005MAS65 trial. Blood. 2012;120(13):2581–8.
145. Messori A, Maratea D, Nozzoli C, Bosi A. The role of bortezomib, thalidomide and lenalidomide in the management of multiple myeloma: an overview of clinical and economic information. Pharmacoeconomics. 2011;29(4):269–85.
146. Gaultney JG, Redekop WK, Sonneveld P, Uyl-de Groot CA. Novel anticancer agents for multiple myeloma: a review of the evidence for their therapeutic and economic value. Expert Rev Anticancer Ther. 2012;12(6):839–54.
147. Gaultney JG, Franken MG, Tan SS, et al. Real-world health care costs of relapsed/refractory multiple myeloma during the era of novel cancer agents. J Clin Pharm Ther. 2013;38(1):41–7.

The Future of Drug Development and Therapy in Myeloma

Sagar Lonial and Lawrence H. Boise

The treatment options and outcomes for patients with myeloma has dramatically improved over the past decade, due in large part to the availability of improved anti-myeloma treatments including high dose therapy, thalidomide, bortezomib, and lenalidomide. Many of the currently active agents are effective because of their impact on normal plasma cell biology, suggesting that targeting the plasma cell, rather than malignant cell biology has led to more effective therapy. Additionally, the use of combination therapy, with agents that are synergistic when combined, has led to deeper responses, and these have likely also contributed to improvements not only in progression free but also overall survival. With the wealth of new agents coming into the myeloma space, it is incumbent upon us as investigators to utilize efficient study designs with novel statistical approaches in order to rapidly test and evaluate new drugs. These concepts as well as a few selective promising targets in early development will be reviewed in the current discussion.
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Treatment options for myeloma have dramatically changed over the past 10 years due in large part to the development of new agents that are significantly more effective than historical treatment options of alkylating agents and corticosteroids. Many of the treatment advances are a consequence of an improved understanding of malignant and normal plasma cell biology, which has resulted in the identification of targets and pathways that are critical for myeloma cell survival. In this review we will discuss many of the key questions in current myeloma therapy, including the roles of combinations versus sequenced therapy, new potential targets, novel clinical trial designs, and genomics in allowing for the rapid development of new treatments, with an eye towards personalized medicine.

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Conflicts of interest: Dr. Lonial is a Consultant for Millennium, Celgene, Novartis, BMS, Onyx, and Janssen. Dr. Boise is a Consultant for Onyx.

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RATIONAL APPROACH TO DRUG DEVELOPMENT

Decades of clinical investigation prior to the late 1990s resulted in the development and refinement of ways to deliver combinations of alkylating agents and corticosteroids in all phases of myeloma treatment. The initial choice of melphalan was somewhat fortuitous but could not be shown to improve survival when compared with other chemotherapy-based approaches,[1] until the introduction of high-dose melphalan followed by autologous bone marrow or stem cell transplant.[2] While high-dose therapy did improve outcomes compared with conventional therapy, when post relapse therapy consisted largely of alkylating agents, again, limited benefit was noted.[3] The use initially of thalidomide and then bortezomib radically changed the level of response among relapsed patients, and then in earlier stages of treatment.[4] While the mechanisms of action for these two agents appear to be very different (cereblon binding[5,6] and proteasome inhibition,[7] respectively), they both have normal plasma cell biology as a common theme. Proteasome function is critical for normal and malignant plasma cell survival as a consequence of continued protein production by plasma cells even when in a transformed or malignant phenotype, though malignant plasma cells may be more susceptible to the effects of persistant proteasome inhibition due to deregulation of other

survival pathways. This concept of plasma cell dependence upon proteasome function is best exemplified by the recent use of bortezomib in the setting of antibody-mediated solid organ rejection, where normal plasma cells are the source of antibody-mediated rejection.[8,9] In a similar fashion, cereblon, a known target for the immunomodulatory agents (ImIDs) thalidomide, lenalidomide, and pomalidomide, is equally expressed between normal plasma cell, monoclonal gammopathy of undetermined significance (MGUS), smoldering, and symptomatic myeloma, suggesting its presence and function are not limited to malignant plasma cells.[9a] As such, the use of proteasome inhibitors (PIs) and ImIDs appears to be effective as a consequence of targeting their role in the normal plasma cell biology maintained in the myeloma plasma cell rather than targeting "oncogenic" transformation. To continue along this line, the most active agents in myeloma that we currently use, thalidomide, lenalidomide, pomalidomide, bortezomib, carfilzomib, and corticosteroids, all target normal plasma cell biology that renders myeloma cells more susceptible to these types of treatment approaches compared to other tumor types.

However, normal plasma cell biology is not sufficient to account for the fate and survival of a transformed cell that is no longer sufficiently regulated by normal homeostatic mechanisms. Thus, targeting malignant plasma cell biology is also essential and is likely different depending on the specific genetic or genomic transformative events that have occurred to generate a specific malignant clone. These can include events such as chromosomal translocations [t(4:14), FGFR3 or MMSET activation, t(14;16), maf overexpression], deletion of 17p (p53 deletion), and mutations such as n-ras or k-ras.[10,11] The associated biologic changes with these events provide a survival advantage for the malignant clone, which is distinct from normal plasma cell biology. It is in this area that we have the greatest work to do when it comes to drug development, because these targets may not represent single-agent response-inducing drugs in a large and heterogeneous patient population. However, when combined with the PI/ImID backbone approaches, they may represent unique and effective ways to circumvent drug resistance by addressing specific oncogenic transformative events allowing for eradication of the malignant clone. If these oncogenic changes contribute to or are unrelated to PI/ImID resistance is unknown, and may depend on the specific mutation. They may be associated with enhanced genomic instability, which is known to contribute to clonal evolution and generalized drug resistance. It is in this area, as investigators, that we need to target our clinical trials to subsets of patients who harbor specific oncogenic abnormalities not based on normal biology but rather as a consequence of genomic aberrations and instability. In the quest to cure or induce long-term remissions for patients, effective strategies of treatment will likely require targeting normal plasma cell biology in conjunction with targeting acquired mutations resulting in oncogenic transformation.

SINGLE-AGENT VERSUS COMBINATION APPROACHES

In the practice of oncology, the question of sequential administration of chemotherapy versus combination chemotherapy is one that is often asked and frequently debated. In solid tumors such as metastatic breast cancer or lung cancer, combinations rarely lead to improvements in overall response rate, and are often associated with increased toxicity. The relative lack of increased efficacy when agents are combined, and poorer overall adverse event profile, results in a lack of benefit as assessed by the objective outcomes of progression-free survival (PFS) or overall survival. However, there are numerous examples in oncology where combination therapy does offer significant benefit over sequential therapy both in the context of curative or palliative therapy such as R-CHOP for non-Hodgkin lymphoma, ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) for Hodgkin lymphoma, or FCR (fludarabine, cyclophosphamide, rituximab) for chronic lymphocytic leukemia. Thus while combination therapy does have its limitations, these are often a direct result of poorly understood biology and tolerability-based assessments rather than conceptual concerns over combinations.[1] In myeloma, the story of combination versus sequential therapy has been historically dominated by the use of largely ineffective combinations of chemotherapy (M2 regimen, vincristine, BCNU [carmustine], cyclophosphamide, melphalan, prednisone) when compared to the standard melphalan and prednisone (MP) regimen.[1] Given the low overall response rates (ORR) for any of these historical regimens, it is not surprising that the use of sequential therapy was equivalent (or equally ineffective) to the use of combination therapy, when it consisted of historical agents with modest activity. Current combinations of agents, specifically combinations of PIs and ImIDs (RVD,[12] VTD [bortezomib/thalidomide/dexamethasone][13]) have resulted in heretofore unexpected ORRs, depth of response, and duration of response. In a series of patients from the Intergroupe Francophone du Myelome (IFM) group treated with RVD (lenalidomide, bortezomib, dexamethasone) induction, single autologous transplant, followed by consolidation and maintenance therapy, PFS at 2 years is approximately 88% with greater than very good

partial response (VGPR) rates of 84%.[14] In another series reported by Nooka et al, patients receiving RVD followed by either early or delayed autologous transplant had an estimated 90% survival at 4 years.[15] These small experiences are being tested in large randomized phase III clinical trials, and represent the power of combination therapy in the induction setting. In the relapsed disease setting, trials testing combination therapy over single agents were first established as demonstrating superiority in the context of bortezomib versus bortezomib + liposomal doxorubicin[16] and more recently we have tested more intense three-drug combinations versus two-drug combinations in the relapsed setting as well. In a series from Garderet et al,[17] the duration of remission for a group of early relapse patients who were treated with VTD versus TD (thalidomide/dexamethasone) in the relapsed setting was significantly longer than any duration of remission noted to date in a relapse trial. Based on these observations, it is clear that in a disease such as multiple myeloma, the use of rationale combinations based on synergistic interactions between drugs, not simply combining agents that each have limited activity, is able to demonstrate significant clinical benefit. This is in sharp counter distinction with many solid tumors, where the use of combination therapy is associated with increased toxicity favoring a sequential approach. This does not appear to be the case with myeloma. With the evaluation of new agents in the relapsed disease setting being tested as part of three drugs used sequentially versus three-drug combinations, we will have additional opportunities to more fully evaluate the benefit of combination therapy not only in newly diagnosed patients but also in patients with relapsed disease.

INNOVATIVE CLINICAL TRIAL DESIGNS

Standard approaches for phase I studies have used what is now known as the modified Fibonacci dose escalation schema or the "3+3" dose-escalation design. While this approach is easy to understand and easy from the clinical perspective to execute, there are considerable concerns that this type of phase I dose escalation results in too many patients exposed to lower doses of study drugs, and that when reasonable doses are finally achieved, there is little power in the three or six patients evaluated at that dose cohort to provide any significant confidence on the safety of the maximum tolerated dose (MTD). Of late, there have been several attempts to use more statistical-based models that incorporate Bayesian approaches to dose escalation that have the potential to more rapidly escalate through low-dose cohorts, reduce the number of patients treated with subtherapeutic doses of study drug, and at the same time to increase the number tested at clinically significant doses to increase the predictive power of the final MTD to be an effective dose.[18] Additionally, these Bayesian approaches also have the potential to speed up enrollment over traditional phase I designs, and for this reason are being tested and used more frequently.[19–22] However, while there may be numerous advantages to these modified phase I designs, it is not clear they are more efficient from either the investigator or patient perspective. Many phase I studies now in myeloma or other diseases are multi-site studies, and thus several patients may be ready at different locations to begin dosing. While the 3+3 design may not be ideal, it does allow sites to anticipate the dose and timing for enrolling the next patient with greater ease. Additionally, standard phase I dosing allows the principal investigator of a study to anticipate how many patients will be enrolled in the phase I portion of the study making the actual conduct of the study and the budget process significantly easier. This can be enhanced by following the phase I dose-escalation portion of any given trial with a phase II study that is used for confirmation of the initial safety signal, and one that provides additional power around the efficacy signal as well.[23] As with all study designs, there may be types of studies where one design is superior to others and clinical experience and situation dictate the optimal approach. It has been our experience that when running early-phase single-agent studies, the use of Bayesian phase I dose escalation is likely preferable from the perspective of reducing the fraction of under-dosed patients, and in order to evaluate fewer patients at lower dose cohorts.[21] However, when combination trials are designed where the goal is the evaluate how to best combine two agents whose single-agent dose and safety is known, a standard 3+3 design may be more efficient, especially if the trial is a multicenter trial.

Another area of significant discussion in myeloma and in oncology in general regards the design of phase II studies. It has been the case that most active agents in myeloma were tested in a larger phase II setting[24–26] as a prelude to a larger confirmatory phase III randomized clinical trial. These large phase II studies (typically 200–300 patients) have tended to be single arm trials with the goal of establishing efficacy and toxicity in a large patient population with relapsed and refractory disease. This is different from what is often done in other oncology areas, where the proof of principle is first evaluated in the context of a randomized phase II study, where half the patients receive study drug, and the other do not. This is done in an attempt to eliminate bias when interpreting a single-arm study in the relapsed setting, and also provides the opportunity to avoid committing a large number of patients to a phase III study when the new agent does not have sufficient activity

for approval. From the clinical perspective, the advantage of the large phase II study is a better and more robust experience with safety and efficacy. This is of even greater advantage when the agent being tested clearly has significant activity, and is clearly a biologic and clinical advance over currently available treatments. However, when an agent has marginal activity, or the benefit from early-phase studies does not clearly demonstrate a major advantage over existing therapies, the use of a randomized phase II design where only half the patients receive study drug can offer a quick way to assess the efficacy of the new drug. Additionally, the use of randomized phase II studies is of benefit when testing an endpoint such as PFS, while the use of single-arm studies are only suited to evaluate overall response rate when compared with historical controls. For the approval of bortezomib and carfilzomib, single-arm studies were sufficient due to high levels of activity in the context of refractory disease where few treatment options were available, and toxicity of existing agents is quite high. Both approaches likely have some benefit depending on the agent, class of drugs, and absolute benefit for patients.

INCORPORATING NEW DRUGS AND TARGETS WITH EXISTING EFFECTIVE AGENTS

It is clear that there are two classes of agents, which are very active in treating plasma cell disorders, PIs, and ImIDs. When combined, virtually all patients will respond, so how are new agents and targets best incorporated? To decipher this puzzle, one needs to go back to a model that accounts for agents that target basic plasma cell biology (PIs and ImIDs), and those that target oncogenic targets (translocations, mutations, expression of epigenetically silenced genes). Thus, while combinations such as RVD are very active, to enhance durability of response and depth of response, one would need to additionally target specific oncogenic drivers of malignant plasma cell survival. While antibodies may be able to be applied in a more broad fashion (they represent normal plasma cell biology and rarely have overlapping toxicity with existing agents), the use of agents such as KSP (kinesin spindle protein) inhibitors, poly (ADP-ribose) polymerase (PARP) inhibitors, and nuclear transport inhibitors will likely depend in part on the specifics of a patients myeloma. In this manner, one could imagine four- or five-drug induction regimens that use PIs, ImIDs, corticosteroids, and an antibody, and the fifth drug would depend on a tumor's specific biology. Alternatively, one could define a series of "HCVAD" type (cyclophosphamide, vincristine, doxorubicin, dexamethasone, cytarabine, methotrexate) regimens[27] with alternating A and B blocks where the agents that are alternated are combinations of patient-specific oncogenically targeted agents in combinations with PIs, ImIDs, and antibodies. Thus, the broad use of agents that target specific biology would not be in global unselected patients, as we currently do trials but rather would be more focused on niche patient populations when added to standard myeloma regimens.

FUTURE DRUGS AND TARGETS IN DEVELOPMENT

Targeting Nuclear Export Signals

Cargo transported from the nucleus to the cytoplasm are exported through the nuclear pore complex (NPC) and while small proteins can pass freely through the NPC, larger ones must be assisted by a transport receptor.[28] Transport receptors belong to the karyopherin-β family of proteins including chromosome maintenance protein-1 (CRM1)/exportin-1 (XPO1).[28] CRM1 recognizes a leucine-rich export signal in the cargo protein and when complexed to RanGTP will export proteins into the cytoplasm. CRM1 has been shown to be overexpressed in many tumors.[29] Interestingly many of the cargo proteins exported by CRM1 are tumor-suppressor proteins and can contribute to tumorigenesis through the export of proteins including p53 and pRb.[29,30] Moreover, CRM1 was identified as a promising target in myeloma cells via an RNAi screen of 6,722 druggable targets.[31] CRM1 ranked in the top 50 targets in this screen. Additionally, topoisomerase IIα is a cargo protein of CRM1 and its nuclear export can result in resistance to doxorubicin and etoposide in human myeloma cell lines.[32] Together these findings point to the promise of targeting CRM1 as a therapeutic strategy in cancer, especially myeloma. Early studies focused on the use of leptomycin B, which inhibits CRM1 through covalent modification of the reactive site cysteine residue (528).[30] While active in preclinic models, leptomycin B was shown to be too toxic for clinical use in a phase I study.[33] A new series of CRM1 inhibitors referred to as selective inhibitors of nuclear export (SINE) has been developed and has shown promise in preclinical models, including in myeloma.[34–36] KPT-330 an orally available SINE is in phase I trials in both solid tumors and hematologic malignancies.

Kinesin Spindle Protein Inhibitors

The use of anti-mitotics in myeloma has been hindered by significant toxicity and questionable activity. Anti-mitotics were widely used in myeloma as part of the VAD (vincristine, doxorubicin, and dexamethasone) combination that was a standard of care prior to the introduction of PIs and ImIDs. However the role of vincristine in this combination

was brought in to question.[37] Taxanes also had little activity or significant toxicity. An Eastern Cooperative Oncology Group (ECOG) study of docetaxel in relapsed/refractory myeloma was not positive[38] and an additional study with paclitaxel as upfront treatment in myeloma showed a 29% objective response rate and median survival that was comparable to melphalan-prednisone.[39] However, dose-limiting toxicity was significant in this study. Toxicity of anti-microtubule agents is due to inhibition of non-mitotic actions of microtubules in post-mitotic cells.[40] Therefore anti-mitotics that do not function through inhibition of microtubules are desirable for therapy as they should have an enhanced therapeutic index. One such agent that has been developed to function in this fashion is ARRY-520.[41] ARRY-520 is an inhibitor of KSP. KSP is essential for spindle assembly and equal segregation of sister chromatids; therefore, inhibition of KSP results in metaphase arrest but does not alter non-mitotic effects of microtubules.[42] A recent study demonstrated that ARRY-520 induces mitotic arrest and apoptosis in human myeloma cell lines.[43] Mitotic arrest and cell death correlated with loss of Mcl-1, an anti-apoptotic protein that is essential for myeloma cell survival. Consistent with this model, silencing of the Mcl-1 inhibitor, Noxa, also results in ARRY-520 resistance while silencing of Mcl-1 sensitizes cells. Clinical trials with ARRY-520 are underway in myeloma and recently presented data from a phase II study suggests activity in patients that are refractory to bortezomib and ImIDs.[44] A phase I study of the combination of ARRY-520 and carfilzomib,[45] and a combination with bortezomib are underway.

Bromodomain Inhibitors

Epigenetic targeting is an area of great interest in oncology as a method by which to impact the expression, or lack thereof, of important genes following malignant transformation. However, much of the effort in this area has been focused on the process of "writing" or "erasing" markings on histones. The bromodomains represent a new area for epigenetic targeting that is responsible for the "reading" pattern of histone acetylation, and thus represents a different target than the traditional histone deacetylase inhibitor or histone methyltransferase inhibitors, which are currently used to treat various hematologic malignancies.[46] Recently identified inhibitors of the bromodomain, targeting the BET subfamily, have been shown both to inhibit inflammation (I-BET762) significantly and to promote tumor cell differentiation (JQ1) in the context of murine models.[47] In the context of myeloma, data using JQ1 have demonstrated the ability to modulate the activation of c-myc, a transcription factor known to be activated in both early- and late-stage myelomas. Data from Delmore et al have identified JQ1 as an agent that directly displaces BET bromodomains from binding chromatin, thereby blocking the effects of myc activation without directly inhibiting myc.[48] The net result of this pharmacologic intervention is inhibition of proliferation and interaction, via BRD4, with IgH enhancers, and ultimately indirect inhibition of myc effects without direct blockade of the transcription factor itself.[48] Clinical studies are currently underway to further test the clinical impact of this strategy.

CONCLUSION

The development of new, more effective treatment options for patients with myeloma in all phases of treatment, have had a dramatic impact on the depth of response, duration of remission, and overall survival of patients. To make further improvements, specific attention needs to be paid not only to the transformative changes (oncogenic changes) but also to better understanding how long-lived plasma cells survive and resist the effects of standard treatment. To test these approaches, novel clinical trials may be useful to minimize the exposure of low doses of new agents to patients, but these novel trial designs need to be balanced with approaches that efficiently and safely enroll patients in the context of multicenter trials. Finally, advances have occurred in large part due to the use of PIs and ImIDs, but new targets and agents are needed to better understand differences among a heterogeneous group of patients classified pathologically as multiple myeloma. Efficient drug development coupled with clinical trials and correlative mechanistic studies will help to bring these new agents to our patients, and eventually overcome treatment resistance.

REFERENCES

1. Combination chemotherapy versus melphalan plus prednisone as treatment for multiple myeloma: an overview of 6,633 patients from 27 randomized trials. Myeloma Trialists' Collaborative Group. J Clin Oncol. 1998;16:3832-3842.
2. 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;335:91–7.
3. Kumar SK, Therneau TM, Gertz MA, et al. Clinical course of patients with relapsed multiple myeloma. Mayo Clin Proc. 2004;79:867–74.
4. Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008;111:2516–20.
5. Zhu YX, Braggio E, Shi CX, et al. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. Blood. 2011;118:4771–9.

6. Ito T, Ando H, Suzuki T, et al. Identification of a primary target of thalidomide teratogenicity. Science. 2010;327:1345–50.
7. Chauhan D, Hideshima T, Mitsiades C, Richardson P, Anderson KC. Proteasome inhibitor therapy in multiple myeloma. Mol Cancer Ther. 2005;4:686–92.
8. Sadaka B, Alloway RR, Shields AR, Schmidt NM, Woodle ES. Proteasome inhibition for antibody-mediated allograft rejection. Semin Hematol. 2012;49:263–9.
9. Guthoff M, Schmid-Horch B, Weisel KC, Haring HU, Konigsrainer A, Heyne N. Proteasome inhibition by bortezomib: effect on HLA-antibody levels and specificity in sensitized patients awaiting renal allograft transplantation. Transplant Immunol. 2012;26: 171–5.

9a. Zhu YX, Braggio E, Shi CX, et al. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. Blood. 2011;118(18):4771–9.

10. 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;109:3489–95.
11. Chapman MA, Lawrence MS, Keats JJ, et al. Initial genome sequencing and analysis of multiple myeloma. Nature. 2011;471:467–72.
12. Richardson PG, Weller E, Lonial S, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood. 2010;116(5):679–86.
13. Kaufman JL, Nooka A, Vrana M, Gleason C, Heffner LT, Lonial S. Bortezomib. thalidomide, and dexamethasone as induction therapy for patients with symptomatic multiple myeloma: a retrospective study. Cancer. 2010;116:3143–51.
14. Roussel M, Robillard N, Moreau P, et al. Bortezomib, lenalidomide, and dexamethasone (VRD) consolidation and lenalidomide maintenance in frontline multiple myeloma patients: updated results of the IFM. 2008 Phase II VRD intensive program. ASH Annual Meeting Abstracts. 2011;118:1872.
15. Nooka A, Langston A, Waller EK, et al. Early versus delayed autologous stem cell transplant (ASCT) in patients receiving induction therapy with lenalidomide, bortezomib, and dexamethasone (RVD) for newly diagnosed multiple myeloma (MM). In: American Society of Clinical Oncology, 2013 June. Chicago, IL: ASCO; 2013:8540.
16. Orlowski RZ, 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;25:3892–901.
17. Garderet L, Iacobelli S, Moreau P, et al. Superiority of the triple combination of bortezomib-thalidomide-dexamethasone over the dual combination of thalidomide-dexamethasone in patients with multiple myeloma progressing or relapsing after autologous transplantation: the MMVAR/IFM. 2005-04 randomized phase III trial from the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol. 2012;30:2475–82.
18. Yuan Y, Yin G. Bayesian hybrid dose-finding design in phase I oncology clinical trials. Stat Med. 2011;30: 2098–108.
19. Tighiouart M, Rogatko A, Babb JS. Flexible Bayesian methods for cancer phase I clinical trials. Dose escalation with overdose control. Stat Med. 2005;24:2183–96.
20. Harvey RD, Owonikoko TK, Lewis CM, et al. A phase 1 Bayesian dose selection study of bortezomib and sunitinib in patients with refractory solid tumor malignancies. Br J Cancer. 2013;108:762–5.
21. Rogatko A, Schoeneck D, Jonas W, Tighiouart M, Khuri FR, Porter A. Translation of innovative designs into phase I trials. J Clin Oncol. 2007;25:4982–6.
22. Lonial S, Kaufman J, Tighiouart M, et al. A phase I/II trial combining high-dose melphalan and autologous transplant with bortezomib for multiple myeloma: a dose- and schedule-finding study. Clin Cancer Res. 2010;16:5079–86.
23. Jakubowiak AJ, Griffith KA, Reece DE, et al. Lenalidomide, bortezomib, pegylated liposomal doxorubicin, and dexamethasone in newly diagnosed multiple myeloma: a phase 1/2 Multiple Myeloma Research Consortium trial. Blood. 2011;118:535–43.
24. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med. 2003;348:2609–17.
25. 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.
26. Richardson PG, Siegel DS, Vij R, et al. Randomized, open label 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. ASH Annual Meeting Abstracts. 2011;118:634.
27. Dimopoulos MA, Weber D, Kantarjian H, Delasalle KB, Alexanian R. HyperCVAD for VAD-resistant multiple myeloma. Am J Hematol. 1996;52:77–81.
28. Wente SR, Rout MP. The nuclear pore complex and nuclear transport. Cold Spring Harb Perspect Biol. 2010;2:a000562.
29. Turner JG, Dawson J, Sullivan DM. Nuclear export of proteins and drug resistance in cancer. Biochem Pharmacol. 2012;83:1021–32.
30. Kau TR, Way JC, Silver PA. Nuclear transport and cancer: from mechanism to intervention. Nat Rev Cancer. 2004;4:106–17.
31. Tiedemann RE, Zhu YX, Schmidt J, et al. Identification of molecular vulnerabilities in human multiple myeloma cells by RNA interference lethality screening of the druggable genome. Cancer Res. 2012;72:757–68.
32. Turner JG, Marchion DC, Dawson JL, et al. Human multiple myeloma cells are sensitized to topoisomerase II inhibitors by CRM1 inhibition. Cancer Res. 2009;69:6899–905.
33. Newlands ES, Rustin GJ, Brampton MH. Phase I trial of elactocin. Br J Cancer. 1996;74:648–9.
34. Acharya C, Zhong MY, Calle Y, et al. CRM1 inhibition abrogates osteoclast formation and bone resorption

via inhibition of RANKL-induced NFκB while sparing osteoblastogenesis: further therapeutic implication in multiple myeloma. ASH Annual Meeting Abstracts. 2012;120:1835.
35. Schmidt J, Braggio E, Chesi M, et al. Genome wide studies in multiple myeloma identify XPO1/CRM-1 as a critical target validated using the selective inhibitor of nuclear export (SINE) KPT-276. ASH Annual Meeting Abstracts. 2012;120:573.
36. Tai Y-T, Landesman Y, Acharya C, et al. CRM1 blockade by novel inhibitors of nuclear export (SINEs) inhibits multiple myeloma cell growth, osteoclastogenesis, and myeloma-induced osteolysis. ASH Annual Meeting Abstracts. 2012;120:326.
37. Alexanian R, Dimopoulos MA, Delasalle K, Barlogie B. Primary dexamethasone treatment of multiple myeloma. Blood. 1992;80:887–90.
38. Friedenberg WR, Graham D, Greipp P, Blood E, Winston RD. The treatment of multiple myeloma with docetaxel (an ECOG study). Leuk Res. 2003;27:751–4.
39. Miller HJ, Leong T, Khandekar JD, Greipp PR, Gertz MA, Kyle RA. Paclitaxel as the initial treatment of multiple myeloma: an Eastern Cooperative Oncology Group study (E1A93). Am J Clin Oncol. 1998;21:553–6.
40. Scripture CD, Figg WD, Sparreboom A. Peripheral neuropathy induced by paclitaxel: recent insights and future perspectives. Curr Neuropharmacol. 2006;4:165–72.
41. Carter BZ, Mak DH, Woessner R, et al. Inhibition of KSP by ARRY-520 induces cell cycle block and cell death via the mitochondrial pathway in AML cells. Leukemia. 2009;23:1755–62.
42. Blangy A, Lane HA, d'Herin P, Harper M, Kress M, Nigg EA. Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo. Cell. 1995;83:1159–69.
43. Tunquist BJ, Woessner RD, Walker DH. Mcl-1 stability determines mitotic cell fate of human multiple myeloma tumor cells treated with the kinesin spindle protein inhibitor ARRY-520. Mol Cancer Ther. 2010;9:2046–56.
44. Shah JJ, Zonder JA, Cohen A, et al. The novel KSP inhibitor ARRY-520 Is active both with and without low-dose dexamethasone in patients with multiple myeloma refractory to bortezomib and lenalidomide: results from a phase 2 study. ASH Annual Meeting Abstracts. 2012;120:449.
45. Shah JJ, Weber DM, Thomas SK, et al. Phase 1 study of the novel kinesin spindle protein inhibitor ARRY-520 + carfilzomib in patients with relapsed and/or refractory multiple myeloma. ASH Annual Meeting Abstracts. 2012;120:4082.
46. Chung CW. Small molecule bromodomain inhibitors: extending the druggable genome. Prog Med Chem. 2012;51:1–55.
47. Prinjha RK, Witherington J, Lee K. Place your BETs: the therapeutic potential of bromodomains. Trends Pharmacol Sci. 2012;33:146–53.
48. Delmore JE, Issa GC, Lemieux ME, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell. 2011;146:904–17.