The Role of JAK Inhibitors in Multiple Myeloma

Matthew Ghermezi, Tanya M. Spektor, PhD, and James R. Berenson, MD

Abstract  Multiple myeloma (MM) is the most common primary malignancy of the bone marrow. No established curative treatment is currently available for patients diagnosed with MM. In recent years, new and more effective drugs have become available for the treatment of MM. Many newer drugs have been evaluated together and in combination with older agents. However, even in combination with other active MM agents, the responses are transient, and; thus, therapeutic approaches to help overcome resistance to these drugs are necessary. Recently, the Janus kinase (JAK) family of tyrosine kinases, including JAK1 and JAK2, has been shown to play a role in the pathogenesis of MM. Preclinical studies have demonstrated that the JAK1/2 inhibitor ruxolitinib, in combination with lenalidomide and dexamethasone, reduces proliferation of the MM cell lines and primary tumor cells derived from MM patients, and this inhibition is greater when these drugs are combined than with single agents. Clinically, early results from the oral treatment regimen of ruxolitinib, corticosteroids (methylprednisolone), and lenalidomide for patients with relapsed/refractory disease are encouraging in terms of safety and efficacy, and additional studies will provide further support for this promising new therapeutic approach for patients with MM.

Introduction

Multiple myeloma (MM), the most common hematologic neoplasm, arises from terminally differentiated plasma cells that accumulate in the bone marrow.1,2 Although no cure for MM exists at present, considerable progress in treatment has been made over the past few decades. Newer, more effective drugs have significantly increased the median overall survival in patients with MM.3,4

The immunomodulatory drugs (IMiDs) thalidomide (Thalomid, Celgene), lenalidomide (Revlimid, Celgene), and pomalidomide (Pomalyst, Celgene), all of which have US Food and Drug Administration (FDA) approval for use in MM, have played an important role in this improvement in survival and have been shown to lead to lasting responses in many patients.3,4 The proteasome inhibitors (PIs) that have been approved for MM—bortezomib...
(Velcade, Millennium/Takeda Oncology), carfilzomib (Kyprolis, Amgen), and ixazomib (Ninlaro, Millennium/Takeda Oncology)—are cytotoxic to MM cells and have become the foundation of MM treatment over the past decade. Lastly, newer antibody-based therapies such as elotuzumab (Empliciti, Bristol-Myers Squibb) and daratumumab (Darzalex, Janssen Biotech) have been shown to be effective in treating MM.

These newer, more effective treatment options have increased the 5-year survival rate for patients with MM from 25% in 1975 to roughly 40% in 2008. The 2-year survival rate for patients with MM increased from 69.9% in 2006 to 87.1% in 2012. These numbers show the profound positive impact that novel therapies are having on prolonging survival in patients with MM.

Although antiangiogenic effects are what initially generated interest in IMiDs, it is believed these drugs exert their antmyeloma effects by impeding cytokine production and interacting with the bone marrow and its associated tumor microenvironment. Thalidomide was the first IMiD approved for use in MM. An early study of thalidomide showed that 32% of patients with MM had a reduction in serum paraprotein levels of at least 25%. Lenalidomide has a reduced neurologic toxicity risk compared with thalidomide, as well as more potent antmyeloma activity as shown in preclinical studies. Pomalidomide has the additional benefit of showing efficacy among patients resistant to lenalidomide.

The success of PIs in MM is due to the sensitivity of MM cells to inhibition of the 26S proteasome. This proteasome plays a critical role in the pathogenesis and proliferation of the disease course of MM. Initial investigations of PIs focused on single-agent treatment in patients with relapsed/refractory MM (RRMM). In the phase 2 SUMMIT study (Study of Uncontrolled Myeloma Managed With Proteasome Inhibition Therapy) that led to the approval of bortezomib in MM, the response rate was 35%. However, phase 2 studies in patients with RRMM demonstrated that the addition of dexamethasone enhanced the activity of bortezomib. This finding established PI-based combination regimens as a major therapeutic option for MM. Recent studies have shown that triplet regimens, especially those involving a PI, an immunomodulatory agent, and a corticosteroid, are more active than doublet regimens.

In 2015, the monoclonal humanized IgGκ antibodies elotuzumab and daratumumab were approved for the treatment of patients with RRMM. Both drugs act by engaging the immune system to increase cellular toxicity directed against MM cells. Initially, elotuzumab was shown to enhance progression-free survival in patients with RRMM only when added to treatment with lenalidomide and dexamethasone. Results from a recent randomized phase 3 study showed that elotuzumab also improved response rates and PFS in patients with RRMM when added to treatment with pomalidomide and dexamethasone. The median PFS was 10.3 months with elotuzumab vs 4.7 months with pomalidomide/dexamethasone, and the overall response rate was 53% vs 26%, respectively. Unlike elotuzumab, daratumumab has been shown to display activity when used as a single agent and also enhances the activity of lenalidomide, pomalidomide, and bortezomib. In the phase 3 ELOQUENT-2 study (Phase III Study of Lenalidomide and Dexamethasone With or Without Elotuzumab to Treat Relapsed or Refractory Multiple Myeloma), treatment with elotuzumab, lenalidomide, and dexamethasone combination therapy showed an overall response rate of 79%.

In the phase 2 SIRIUS study (An Efficacy and Safety Study of Daratumumab in Patients With Multiple Myeloma Who Have Received at Least 3 Prior Lines of Therapy or Are Double Refractory to a PI and an IMiD), the overall response rate was 29% in patients treated with daratumumab monotherapy vs 82% in patients who received daratumumab in combination with lenalidomide and dexamethasone. Daratumumab was also used in combination with another PI, pomalidomide, and dexamethasone in patients with RRMM. Aside from increased neutropenia with this triplet therapy compared with the individual therapies, the responses in heavily pretreated patients with MM were rapid, deep, and durable. Also, among patients with RRMM, daratumumab in combination with bortezomib and dexamethasone resulted in significantly longer progression-free survival than bortezomib and dexamethasone alone. However, the safety profile of this combination showed increased incidences of infusion-related reactions and higher rates of thrombocytopenia and neutropenia than bortezomib and dexamethasone alone. The introduction of elotuzumab and daratumumab has demonstrated that monoclonal antibodies are an effective new drug class for the treatment of MM.

Although IMiDs, PIs, and monoclonal antibodies have all significantly improved survival in patients with MM, all of them except for ixazomib and the IMiDs are administered intravenously. Intravenous treatment often results in infusion reactions, especially when monoclonal antibodies are used. Because most combination therapies involve intravenous agents, these regimens usually are complex, require long infusions, and are inconvenient to patients owing to their high costs and less-than-ideal comfort. A more effective, less toxic, and less invasive way to deliver therapy is needed.

Ruxolitinib (Jakafi, Incyte), an orally administered inhibitor of Janus kinase (JAK) that has been approved...
by the FDA for the treatment of myelofibrosis and polycythemia vera, works by inhibiting the signaling of cytokines and growth factor receptors that use JAK1 and JAK2 for signaling. MM implicates JAK1 and JAK2 genes in its pathogenesis, much like myelofibrosis does.22 Myelofibrosis is a clonal disorder originating at the level of the hematopoietic stem cell that is characterized by bone marrow fibrosis, splenomegaly, and extramedullary hematopoiesis.22,23 In the randomized, phase 3 COMFORT-II trial (Controlled Myelofibrosis Study With Oral Janus-Associated Kinase Inhibitor Treatment-II), ruxolitinib treatment showed superiority to the best currently available therapy at the time.24 Specifically, ruxolitinib was found to rapidly reduce splenomegaly and debilitating symptoms of myelofibrosis.24 These results demonstrate the beneficial effects of ruxolitinib on quality of life compared with the current best available therapies in myelofibrosis. In contrast to ruxolitinib, the best available therapy was associated with an increase in spleen volume and a worsening of symptoms.24

The success of JAK inhibitors in myelofibrosis prompted preclinical experiments in other hematologic cancers, specifically MM, owing to similarities in their pathogenesis. Success in preclinical data using JAK inhibitors for the treatment of MM has further prompted early-phase studies. For example, ruxolitinib is being studied as part of an all-oral treatment regimen that addresses many of the current issues seen with regimens containing IMiDs, PIs, and monoclonal antibodies.

**Preclinical Studies of JAK Inhibitors in Multiple Myeloma**

JAK proteins, which promote survival and proliferation of abnormal cells in myelofibrosis, are activated in MM and other types of hematologic cancers.25,26 The activation of JAK2 has been demonstrated in several hematologic disorders and malignancies.25,26 Studies have found that JAK and its downstream transcription factors, signal transducer and activator of transcription (STAT) proteins, mediate hematopoietic cytokine receptor signaling.25,26 The JAK/STAT pathway affects cell growth, survival, and differentiation through many cellular events.25,26 Specific chromosomal translocations that result in continuous JAK2 activation are thought to contribute to the development of lymphoma, leukemia, and MM.25,26 Elevated levels of growth factors and cytokines in MM have been shown to contribute to increased JAK2 activation.27 Interleukin 6 (IL-6), a growth and survival factor for myeloma cells, is among these cytokines that activate JAK2 and ultimately augment its downstream signaling effects.27 Therefore, JAK1 and JAK2 inhibitors represent potential therapies for MM.

A recent preclinical study evaluated the anti-MM effects of INCB052793, a selective JAK1 inhibitor that is in clinical development.28 This study demonstrated that INCB052793 shows anti-MM activity alone and in combination with conventional anti-MM agents such as carfilzomib, bortezomib, lenalidomide, and dexamethasone.28 MM cell lines and tumor cells from patients with MM were both treated with INCB052793 in combination with carfilzomib, bortezomib, lenalidomide, or dexamethasone. The combination of this JAK1 inhibitor with these other agents showed a higher percentage of total cell death when compared with single agents.28 Additionally, the combination of INCB052793 with lenalidomide showed significant tumor growth inhibition among severe combined immune deficient (SCID) mice bearing the human MM tumor LAGκ-1A in vivo. Overall, this study showed that INCB052793 enhances the anti-MM efficacy of PIs, immunomodulatory agents, and glucocorticoids both in vivo and in vitro.28

Additional in vivo studies tested INCB052793 in combination with other agents that show anti-MM activity. Studies using a human MM xenograft in SCID mice showed that mice had smaller tumors when treated with INCB052793 than when treated with dexamethasone, lenalidomide, or pomalidomide as single agents.29 Although the combination of INCB052793, dexamethasone, and lenalidomide or pomalidomide did not inhibit MM cell line growth in vitro, mice receiving this treatment in vivo showed an effect on tumor growth that was greater than dexamethasone with lenalidomide or pomalidomide.29 Mice receiving the combination of INCB052793, dexamethasone, and lenalidomide or pomalidomide demonstrated the most significant reduction in tumor growth when compared with all other tested combinations.29

Furthermore, the preclinical effects of the JAK2 inhibitor TG101209 on MM cell lines have also been promising. TG101209 induced cytotoxicity in a variety of MM cell lines.31 This cytotoxicity inhibited cell cycle progression and induction of apoptosis in both MM cell lines and patient-derived plasma cells.30

Momelotinib (CYT387), an orally available inhibitor of JAK1 and JAK2, was also evaluated preclinically for the treatment of MM. It was demonstrated that momelotinib was able to prevent IL-6–induced phosphorylation of STAT3 in human myeloma cell lines.31 This JAK inhibitor reduced MM proliferation in a time- and concentration-dependent matter.31 When used in combination with conventional MM therapies such as melphalan (Evomela, Spectrum) and bortezomib, momelotinib was successful in killing tumor cells from human myeloma cell lines.31

INCB16562, another inhibitor of JAK1 and JAK2, also has been evaluated in myeloma cells.32 This agent
potently inhibited IL-6, which in turn inhibited proliferation and survival of myeloma cells dependent on IL-6 for growth, as well as IL-6–induced growth of primary bone marrow–derived plasma cells from a patient with MM. Additionally, INCB16562 nullified the protective effects of recombinant cytokines and prepared myeloma cells for death from exposure to dexamethasone, melphalan, or bortezomib. Lastly, INCB16562 reduced the growth of myeloma xenografts in mice and, when used in combination with other treatments, enhanced their antitumor activity. An important takeaway is that prolonged exposure to treatments such as dexamethasone results in drug resistance, but this can be partly overcome through the combination of JAK inhibitors, as seen with INCB16562.

The in vitro and in vivo activity of JAK1 and JAK2 inhibitors in combination with conventional MM treatments shows promise for the clinical use of this regimen. These studies provide further support for the clinical evaluation of these drug combinations for the treatment of patients with MM.

Preclinical Evaluation of Ruxolitinib in Multiple Myeloma

The activation of JAK proteins, which has been demonstrated in MM and other hematologic cancers, promotes survival and proliferation of tumor cells. Ruxolitinib is a nonselective inhibitor of JAK1 and JAK2 that inhibits adenosine triphosphate by binding to the catalytic site of the cytokine receptor kinase domain. Whereas ruxolitinib directly affects the JAK/STAT pathway and indirectly affects the RAS/RAF/mitogen-activated protein kinase pathway, bortezomib inhibits nuclear factor-κB. In combination, a signaling cascade will occur through the STAT proteins, and other pathways will also be impacted. For patients who do not respond well to currently available therapies, and who exhibit increased expression of JAK1 and JAK2, the combination of ruxolitinib and dexamethasone may be a promising alternative. In a study aiming to evaluate JAK1 and JAK2 expression in patients with MM, it was found that JAK1 was overexpressed in 27% and JAK2 was overexpressed in 57%. In the same study, MM cell lines treated with ruxolitinib and bortezomib led to 50% of cells being in late apoptosis, a reduction in antiapoptotic gene expression, and higher number of cells in sub-G0 phase. The combination of ruxolitinib, bortezomib, and lenalidomide induced death in 72% of cells, which was equivalent to the combination of bortezomib, lenalidomide, and dexamethasone that is currently used in clinical practice. The overactivity of the JAK/STAT pathway in patients with MM points to the potential of JAK to become a new therapeutic target.

In another preclinical study evaluating the proportion of tumor-stimulatory M2 macrophages in bone marrow from patients with MM, the percentage of M2 macrophages was markedly increased in the bone marrow from patients with progressive disease compared with those in complete remission. When MM tumor cells were treated in vitro with a low concentration of ruxolitinib, the percentage of M2 cells decreased. Therefore, the JAK inhibitor ruxolitinib shows inhibition of M2 macrophages, leading to reduction in tumor stimulatory M2 polarization that provides an additional mechanism through which JAK inhibitors may produce clinical benefits for patients with MM.

Interestingly, in a small study, ruxolitinib alone showed no anti-MM effects, but when combined with dexamethasone, there was an enhanced anti-MM effect compared with corticosteroid treatment alone.

Furthermore, a preclinical study evaluated the anti-MM effects of ruxolitinib in combination with lenalidomide and corticosteroids in vitro, in vivo, and in a patient with MM and polycythemia vera. Ruxolitinib inhibited the viability of cells from all MM cell lines, and also reduced the viability of primary MM tumor cells. The cytotoxic effects of ruxolitinib, lenalidomide, and dexamethasone were greater than those of ruxolitinib in combination with either lenalidomide or dexamethasone. Using a humanized SCID MM model, ruxolitinib in combination with lenalidomide and dexamethasone led to a marked reduction in tumor size and delay of tumor growth. Lastly, a patient with MM and polycythemia vera saw a sustained and ongoing reduction in serum M protein, immunoglobulin G (IgG), and 24-hour urine paraprotein levels, and ultimately achieved a partial response while receiving low doses of ruxolitinib (5 mg twice daily), lenalidomide (2.5 mg daily), and methylprednisolone (20 mg daily). This patient had previously received ruxolitinib alone while progressing from monoclonal gammopathy of undetermined significance to MM, and his disease failed to respond to lenalidomide and methylprednisolone. His disease did respond, however, when a low dose of ruxolitinib was added to the other 2 drugs.

These studies and the case report illustrate that the combination of the JAK inhibitor ruxolitinib with currently available anti-MM treatments shows promising preclinical and clinical results that should be further examined. Specifically, the combination of ruxolitinib and lenalidomide may prove to be a highly effective combination for treating patients with MM. Lenalidomide acts directly on MM cells by inducing cerelbon-mediated degradation of transcription factors that are essential for MM cell survival. The mucin 1 (MUC1) glycoprotein is responsible for lenalidomide resistance in MM cells. Notably, ruxolitinib blocks the expression of MUC1 in
MM cells. As a result, the addition of ruxolitinib may prove to be very effective at restoring lenalidomide sensitivity in patients with lenalidomide-resistant MM.

**Clinical Studies of Ruxolitinib in Multiple Myeloma**

Promising preclinical data with ruxolitinib and lenalidomide prompted a phase 1 trial to determine the safety and efficacy of ruxolitinib in combination with lenalidomide and methylprednisolone, an all-oral combination, for patients with RRMM who had previously been treated with lenalidomide/corticosteroids and a PI and showed progressive disease at study entry. A traditional 3+3 dose-escalation design was used to enroll subjects into 4 cohorts, with a planned total enrollment of 28 patients. Patients received ruxolitinib twice daily without interruption, lenalidomide daily on days 1 to 21 of a 28-day cycle, and methylprednisolone orally every other day. Patients at dose level 0 received ruxolitinib at 5 mg, lenalidomide at 5 mg, and methylprednisolone at 40 mg, whereas those at dose levels 1 and 2 received the same doses of lenalidomide and dexamethasone, plus ruxolitinib at 10 mg and 15 mg, respectively. Lastly, patients at dose level 3 received lenalidomide at 10 mg, the same dose of methylprednisolone, and ruxolitinib at 15 mg.

Initial results from this clinical trial were promising. Specifically, the clinical benefit and overall response rates for 28 enrolled patients were 46% and 38%, respectively.

Twelve patients who responded to this therapy had been refractory to lenalidomide. This novel combination treatment was well tolerated overall. The most common grade 3 or 4 adverse events included anemia (18%), thrombocytopenia (14%), and lymphopenia (14%). The most common serious adverse events included sepsis (11%) and pneumonia (11%), and were compatible with rates that have been previously reported among patients with MM.

Because of these promising results, the clinical trial has been expanded to 49 patients (NCT03110822). A major advantage of this combination treatment is the fact that it is an all-oral triplet combination, which adds another advantage when compared with most other triplet combination treatments for patients with RRMM.

**Conclusion**

Preclinical studies and preliminary clinical data show promise in targeting the JAK pathway in MM. MM inevitably develops resistance to therapy, which makes newer treatment regimens to prolong survival necessary. Although current therapies such as lenalidomide can decrease quality of life and make patients feel worse, ruxolitinib has resulted in an improvement in quality of life in studies involving patients with myeloproliferative neoplasms and can make patients feel better overall. Early results from the all-oral treatment regimen of ruxolitinib, dexamethasone, and lenalidomide for patients with RRMM are encouraging. In addition, it will be interesting to determine whether treatment with ruxolitinib in this combination reverses the fatigue and malaise frequently associated with the administration of lenalidomide. Most current treatment regimens are not administered orally, which has proven difficult for patients with MM and providers alike. An effective, all-oral regimen containing JAK inhibitors may ease administration and avoid the side effects seen with other agents, while not adding further toxicity. Hopefully, additional studies will provide further support for this promising new therapeutic approach for patients with MM.

**Disclosures**

Dr Berenson has received research support from and has served as a consultant for Incyte Corporation. He has equity interests in OncoTracker and ownership of the ‘Anti-Cancer Effects of JAK Inhibitors in Combination with Thalidomide Derivatives and Glucocorticoids’ patent (Application No. 15/314,434). Mr Ghermezi and Dr Spektor have declared no conflicts of interest.

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