Targeted therapeutics for multiple myeloma: The arrival of a risk-stratified approach

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Abstract

Multiple myeloma (MM) remains an incurable hematologic malignancy characterized by frequent early responses, inevitably followed by treatment relapse. Until recently, few effective therapies existed. Indeed, the use of alkylating agents and corticosteroids had remained the treatment of choice for almost four decades. Several novel agents for MM have now become available, including the immunomodulatory drugs thalidomide and lenalidomide, as well as the proteasome inhibitor bortezomib. Each of these agents is undergoing extensive clinical evaluation in combination with other therapies to produce unprecedented response rates in newly diagnosed and relapsed MM. Nevertheless, relapse remains universal and further therapeutics with broad activity are required. Importantly, it has become clear that pivotal genetic events are the primary harbingers of clinical outcome and novel targeted therapy approaches using existing approved drugs or novel agents, which address that disrupted signaling pathways are now in various stages of clinical testing. It seems increasingly likely that novel drug combinations, which together turn off these critical Achilles heels, will become the standard of care and that treatment will become increasingly personalized and guided by genetic testing and prognostic factors. [Mol Cancer Ther 2007;6(3):802–10]

Background

Multiple myeloma (MM) is an incurable B-cell malignancy with an incidence of 16,000 new cases per year in the United States and over 40,000 people are living with the disease (1). The disease represents the malignant culmination of the clonal expansion of plasma cells. Several premalignant stages have been described, including monoclonal gammopathy of undetermined significance and smoldering myeloma (1). The differentiation into stages of progression is most critical in distinguishing active MM and the preceding stages, as treatment is not usually started until actual complications from MM are evident, such as hypercalcemia, anemia, renal failure, or clinically evident bone destruction. The criteria for treatment initiation have been recently published as part of an international consensus effort (2). Clinical trials have failed to show any advantages for the initiation of early treatment, and given the lack of a curative strategy, delay of treatment until symptomatic progression remains a reasonable clinical strategy (3–5).

For over 35 years, the treatment for MM was based on the use of a combination of melphalan with corticosteroids (6). Multiple attempts at improving survival with the addition of other chemotherapy agents failed, and not much progress was observed until the introduction of i.v. high-dose melphalan based therapy (HDT; refs. 7, 8). The results of a randomized phase 3 study led by the Intergroup Franco-phone du Myelome lead to widespread use of HDT as an accepted modality for many young patients with MM (9).

Today, myeloma is the number one indication of high-dose chemotherapy in the United States. For patients not deemed suitable candidates for HDT, the traditional option of melphalan and prednisone in combination was still practiced (6).

Unfortunately, after successful initial treatment, even for those achieving a complete response, relapse is ubiquitous, and most patients will ultimately succumb to disease progression or complications (10). Despite the introduction of the novel agents to be discussed later, this statement unfortunately remains true; thus new, better treatments are still needed. Many treatments in the supportive care of MM have been developed, such as the use of bisphosphonates, kyphoplasty, recombinant growth factors, and aggressive antibiotics, which have dramatically altered the landscape and quality of life for MM patients (11, 12). Although these treatments may indirectly, and perhaps sometimes directly affect the clonal cells (and the ability of cells to survive), they will not be discussed further in this review.

Here, we will focus on currently recommended therapies for MM and place these in the context of advances in MM genetics and their prognostic and therapeutic significance.

Prognostic and Therapeutic Importance of MM Genetics

We will show here that the primary determinant of patient outcome is the underlying genetics of the tumor (13).
Furthermore, later in the article, we will make the case that therapy should be individualized based on these findings.

Myeloma Genetics 101

One initial event in the genesis of MM is the translocation of nonrandom partners that include cyclins, MAF family members, and fibroblast growth factor receptor 3 (FGFR3) to the immunoglobulin heavy chain switch regions on 14q32 (13, 14). These recurrent translocations have been identified in primary patient samples and between them account for ~50% of cases. The remaining 50% of MM lack translocations but instead are characterized by multiple trisomies (hyperdiploidy; refs. 15, 16). The end consequence of these genetic aberrations (translocations or hyperdiploidy) seems to be abnormal expression of one of the three D-type cyclins: cyclin D1, D2, or D3 (17), something not occurring in normal plasma cells. This seminal phase is also associated with genomic instability that often includes deletions of chromosome 17 and monosomy/deletions of chromosome 13 (13). Somatic mutation in genes such as P53, FGFR3, NRAS, and KRAS2 may arise (producing activation in the last three cases), or secondary translocation may occur by a non-B cell–mediated mechanism (13, 18). A common secondary translocation partner is MYC (19, 20). Recently, we have described mutation of the noncanonical nuclear factor-κB pathway as a common event in up to 45% of patients.1

t(11;14)(q13;q32) Clinical Implications

The t(11;14)(q13;q32) is associated with a slightly improved or neutral survival in patients treated with HDT (21–24). Arguing against a very favorable prognosis, studies of long-term survivors of myeloma have not revealed significant enrichment for t(11;14) patients (22, 24). There is an association of the t(11;14)(q13;q32) with oligosecretory or light chain–only myeloma, CD20 expression, and lymphoplasmacytic morphology (21, 25).

t(4;14) Clinical Implications

Approximately 25% of MM cell lines and 15% of patients exhibit the t(4;14), which results in translocation of the receptor tyrosine kinase FGFRI3 to the immunoglobulin heavy chain switch region locus (13, 26). Several studies have shown that this translocation results in a shorter survival whether patients were treated with conventional chemotherapy or HDT (23, 24, 27). In one study, we investigated the frequency and prognostic relevance of the t(4;14) in 128 patients with MM who received HDT (melphalan 200 mg/m²) followed by autologous stem cell transplantation (28). Overall, the t(4;14) was detected in 14 of 108 (13%) of the patients. Patients with a t(4;14) had a significantly higher relapse rate (79%) and shorter event–free survival after autologous stem cell transplantation (median 9.5 months) than patients without the t(4;14) (49%, 25.8 months; P = 0.0001). Patients with a t(4;14) also had a significantly shorter overall survival from time of HDT compared with patients without the translocation (median 18 months versus 46.3 months; P = 0.0053).

Clinical Value of Routine Genetic Testing

Sufficient information is now available to result in a strong recommendation for the adoption of routine molecular genetic testing in MM patients. Specifically, independent studies involving more than 2,000 patients have identified a poor prognosis associated with the presence of immunoglobulin heavy chain translocations with the exception of t(11;14), which seems to have a neutral or perhaps favorable prognostic influence. Because some conventional therapies perform extremely poorly for the 25% of patients with high-risk genetics, and because therapies targeting at least one of these translocations [the t(4;14)] are now in clinical trials (38), it is now strongly recommended that all newly diagnosed MM patients be tested at a minimum for the t(4;14), t(14;16), t(11;14), δ13, and −17. Finally, the work up should also include measurement of the serum β2-microglobulin.

The Arrival of Targeted Therapies and Therapeutic Intelligence

The detection of either t(4;14), t(14;16), deletion of p53 by fluorescence in situ hybridization, or deletion of chromosome 13 on metaphase analysis will define a population of 25% MM patients who are in a high-risk prognostic group and who do not generally benefit from HDT and should be steered toward more investigational therapies soon after diagnosis. We believe that the 75% of patients lacking these poor risk factors are more likely to benefit from HDT. For some patients, the presence of specific genetic markers may lend themselves to specific targeted therapies. This personalized dichotomy of therapy will be highlighted below with reference to existing and novel therapeutic outcomes.

Treatment for HDT Candidates

Induction

An optimal induction treatment for MM must provide quick disease control resulting in pain improvement, prevention, or resolution of renal failure and subsequent improvement in hematopoesis (correcting anemia) while minimizing toxicity, cost, and inconvenience (39). Generally, patients are divided at diagnosis into those suitable for HDT in whom myelotoxic drugs should be avoided until after stem cell collection; and those in whom HDT is not being considered due to age, coexistent disease, or patient preference (40). For patients who will ultimately require stem cell collection, the use of alkylators is in general avoided. As such, the standard induction treatment for such patients currently consists of a dexamethasone based regimen. Vincristine, Adriamycin, and dexamethasone was until very recently commonly used with generally high response rates of 60% to 80% but concerns about the requirement for a central venous line and the dubious value of vincristine have led to a decline in the popularity of this regimen (41). Induction therapy now more frequently uses dexamethasone alone or in combination with thalidomide, lenalidomide (12), or bortezomib (42).

Thalidomide

Thalidomide was first introduced into the treatment of MM by Singhal and colleagues in 1999 (43). Its use was rationalized by the potential antiangiogenic properties of the compound. As a single agent, it has shown activity in about one third of patients (43). This has been observed whether patients are treated at the time of relapse or even at the time of original diagnosis (44, 45). For an in-depth review of the development of thalidomide, the reader is referred to other review articles (44). The mechanism of action of thalidomide is not elucidated and likely includes many more aspects than pure inhibition of angiogenesis. Interference with the bone marrow microenvironment and regulation of other immune surveillance mechanisms have been proposed as well. For example tumor necrosis factor, interleukin-6, and WNT signaling pathways may all be disrupted (46).

Thalidomide is overall well tolerated although it does carry a unique set of toxicities (46). When used alone, the main complications include neuropsychiatric symptoms (somnolence, depersonalization, etc.), sensory peripheral neuropathy, and constipation. Many of these symptoms are dependent on dose and duration of treatment. The best dose of thalidomide is unknown. Most clinicians have used a median dose for patients of 200 mg/d. However, anecdotal experience suggests the anti-MM effect may be seen at lower doses. The escalation of thalidomide to doses higher than 200 mg is seldom needed and in general should be discouraged (47).

The impressive activity of thalidomide, even in patients with extensive prior treatment, made the use of this drug in combinations obvious, and one such combination (with dexamethasone) has now become the de facto standard of care for new diagnosis MM (see below). Rajkumar and colleagues have shown that thalidomide in combination with dexamethasone results in a 63% response rate (48, 49). It also is now apparent that the combination has an additional set of toxicities. Most notably, this results in deep venous thrombosis (DVT) in one of six patients treated (49). The mechanisms leading to this toxicity are unknown but are presumed to include endothelial damage because recent data suggest that the use of aspirin prevents this complication (50, 51). Unfortunately, there is no good evidence to conclusively support any one of the several DVT prophylactic approaches; aspirin versus anticoagulation with coumadin versus the use of low molecular weight heparin. In addition, dermatologic complications have been observed, including severe rash as in some very rare instances cases of Steven-Johnson syndrome (52).

Lenalidomide

To improve on the efficacy of thalidomide, potentially abrogate the teratogenic activity of the drug, and improve on the safety profile, several analogues have been studied. The most extensively studied is lenalidomide (53). Like thalidomide, it was first used in the setting of relapsed and refractory MM, where as a single agent was able to show antitumor activity in at least 30% of patients (53). Like thalidomide, the mechanism(s) of action are not fully elucidated. The initial set of observations has suggested that lenalidomide is associated with a lower rate of neurologic complications (both neuropsychiatric and peripheral neuropathy); however, the peripheral neuropathy was not completely eliminated. The actual prevalence of peripheral neuropathy after the use of lenalidomide is clouded because most patients enrolled in lenalidomide clinical trials have had prior exposure to bortezomib and/or thalidomide. After the extensive testing of lenalidomide as a single agent (confirming its activity), the compound was tested in combination with dexamethasone as thalidomide had been. Two large multicenter randomized phase 3 studies (MM-009 and MM-010) have recently been reported (54). In both cases, the combinations resulted in an improved survival and progression-free survival over patients treated with dexamethasone alone. Most
importantly, these trials have been able to achieve a complete response and near-complete response rate in 12% to 25% of patients, something unprecedented in the treatment of relapsed and refractory MM.

Because of the success with the combination of thalidomide and dexamethasone, the combination of lenalidomide and dexamethasone was also promptly moved for testing in the newly diagnosed patient with up-front response rates of 91% (55). Specifically, a phase 2 trial was conducted at the Mayo Clinic where patients were treated with lenalidomide at 25 mg for days 1 to 21, and dexamethasone, in a 28 days cycle (55). The response rate (partial response or better) was 91% and, likely because of all patients being on aspirin, only one episode of DVT was observed (3%). Patients experienced peripheral neuropathy in 21% of cases and cytopenias were also observed. This study led to the development of a phase 3 study in which patients are randomized to either lenalidomide in combination with standard doses of dexamethasone (40 mg p.o. qd on days 1–4, 9–12, and 17–20) versus a lenalidomide and dexamethasone in lower doses (40 mg p.o. weekly; Eastern Cooperative Oncology Group phase 3 clinical trial, principal investigator S.V. Rajkumar). This study has completed accrual (May 2006) and its results should be released soon. Another study conducted by the Southwest Oncology Group (SWOG) is comparing lenalidomide with dexamethasone versus dexamethasone as induction for new diagnosis MM. Of note, the interim analysis for toxicity results mandated a temporary closure of the clinical trial due to a higher rate of DVT in the high-dose dexamethasone arm (56). At the time of study design, the use of aspirin as DVT prophylaxis was not mandated, and the risk of DVT was much higher among patients not receiving the medication. The reopening of the study mandated all patients to receive aspirin prophylaxis (or other forms of DVT prophylaxis). Preliminary observations also show that despite the use of aspirin prophylaxis, the concomitant use of erythropoietin greatly increases the risk of thrombosis as well as higher doses of dexamethasone (56, 57).

**Bortezomib**

Bortezomib represents a first in class therapeutic proteasome inhibitor first developed for its use in MM (58, 59). Notably, very early clinical activity was noted for patients with MM, and this led to a rapid development of phase 2 clinical trials specific for MM (60). For an in-depth review of the development of bortezomib, the reader is referred to excellent recent literature (61). The use of bortezomib as a single agent in the setting of relapsed and refractory MM resulted in antitumor activity in 28% to 40% of patients. A large phase 3 randomized clinical trial comparing bortezomib with dexamethasone alone has been recently published (62). This trial showed that the use of bortezomib resulted in improvements in overall survival and progression-free survival. This observation occurred despite at least 62% being allowed to cross over from the dexamethasone arm to the bortezomib arm. The compelling nature of the data provided the background for the approval of bortezomib in the treatment of relapsed MM.

Although bortezomib is a reversible inhibitor of the proteasome, the full spectrum of activity leading to its antitumor effects is unknown. Inhibition of the survival signaling provided by nuclear factor-κB has been proposed as one of the main mechanisms. Of interest, other hematologic malignancies have also shown responsiveness to bortezomib, particularly mantle cell lymphoma, follicular lymphoma (63), and Waldenström macroglobulinemia. The former is interesting as, like MM, is a B-cell disorder primarily characterized by an ectopic aberrant expression of cyclin D1. Consequently, efforts at further characterizing a possible mechanistic link to the activity of the drug are under way. Overall, bortezomib is well tolerated with its primary toxicity profile, including sensory peripheral neuropathy, transient thrombocytopenia, fatigue, and gastrointestinal disturbances. Although it is not clear if directly precipitated by bortezomib (as opposed to myeloma alone), there is an apparent increase in the number of patients with herpes zoster.

The use of bortezomib has been explored in the new diagnosis setting as well. Its use has been tested both as a single agent, but also in combination with dexamethasone (42). Richardson (61) recently reported on the use of single-agent bortezomib for new diagnosis MM with an overall response rate of 40%. Likewise, Dispenzieri et al. (64) have used bortezomib single agent as induction in high-risk MM (defined by having chromosome 13 abnormalities or hypodiploidy in the karyotype, elevated plasma cell labeling index, or an elevated β2-microglobulin >5.5 mg/L). In the preliminary report, the response rate was nearly 70%. Jagannath et al. (42) explored the use of bortezomib (n = 50 patients) with dexamethasone added after two cycles in patients not achieving a partial response and after four cycles in patients not achieving a complete response. The overall response rate was 89%, with 18% of patients achieving a complete (or near-complete) response. A multiplicity of other bortezomib regimens are being tested in the up-front setting, including combination with thalidomide and dexamethasone, Adriamycin and dexamethasone, and cyclophosphamide (62, 65). Response rates are very high with each of these combination regimens.

**Induction Therapy: Present and Future**

The optimal up-front therapy for MM is not known and a “one size fits all” approach remains the standard. Institutionally, we have adopted the combination of lenalidomide and low-dose dexamethasone (40 mg every week) as it seems to offer the greatest balance of efficacy, convenience and toxicity, although lenalidomide is not yet approved for up-front use (66). In the meantime,
thalidomide and dexamethasone is most commonly used. Data related to the durability of response and risk factors are not known; however, as response rates are very high, this may be impossible to tease out. That said, we would predict that response duration might be significantly lower in high-risk patients. The addition of bortezomib and alkylating agents is likely to further improve response rates and durability of response. Indeed, high-risk patients seem to have equally elevated response rates to bortezomib, likely reflecting the proliferative nature of the MM (67). Again, response durations seem not to be durable. Bortezomib may, however, have a unique role in high-risk disease as provisional analysis of gene expression profiles and other genetic risk factors suggest that bortezomib-treated patients with high-risk disease have similar outcomes to low-risk populations. Early data from the clinical trial of Total Therapy III conducted at the University of Arkansas, where a bortezomib-based, seven-drug approach is used, show indications that this may reverse the early high relapse rate of high-risk MM.4

In summary, we propose that the available current data suggests that a p.o.-based induction (for instance lenalidomide and dexamethasone) should be used for low-risk patient induction, whereas for the high-risk patients, regimens that include bortezomib are a reasonable consideration.

Consolidation and HDT
The traditional method of providing consolidation of initial therapeutics consists of the application of HDT to reconstitute hematopoiesis (40). This is based on the observations that this treatment approach results in improvement in overall survival and disease progression (9, 68). Likewise, there is a suggestion of improvements in quality of life for patients being treated with this approach. This approach can now be feasibly conducted as an outpatient procedure and with a minimal mortality (usually <2%). As discussed above, patients with low-risk MM seem to do particularly well with HDT with 7- to 8-year median overall survivals. Given that this can be achieved with simple induction regimens, we would suggest for now that a p.o. induction regimen be used, HDT should be done and maintenance trials considered (with consideration of tandem HDT for patients not having maximum cytoreduction with the first HDT; for further reading on tandem stem cell transplant, please refer to ref. 98). Bortezomib can be saved for relapse. The counter-argument of course is that these patients are still not being cured and the early introduction of bortezomib will improve response rates and durability of response. This may well turn out to be true and ongoing trials will help address this issue.

For high-risk patients, transplant seems to offer only modest if any benefit as a consolidation, and we propose in these patients that alternative strategies should be pursued (24, 31, 69). For instance, the use of novel agents in combination with p.o. melphalan (discussed below) has resulted in very significant improvements in the response rate (67, 70). Alternatively, other alkylator sparing combinations are being proposed and will soon be tested in large phase 3 clinical trials. One such approach is the use of bortezomib in combination with dexamethasone after induction and stem cell collection. A clinical trial at the Eastern Cooperative Oncology Group, E1A05, will randomize patients to bortezomib and dexamethasone versus the same combination plus lenalidomide. Regardless, many such novel consolidation strategies will be further explored in clinical trials. We have summarized our thoughts on optimal induction and consolidation for both high- and low-risk disease using the best available therapies at this time and projected out 3 to 5 years.

Maintenance
The issue of post–stem cell transplantation maintenance for MM is unclear. Although intuitively rational, there is no conclusive data to support its use at the present time. One large study conducted by the University of Arkansas showed that although thalidomide given before, during, and after HDT as maintenance improved disease control, it had no effect on overall survival (10). A conflicting study by the Intergroup Francophone du Myelome shows that thalidomide maintenance significantly improves overall survival (71). Previous studies have shown that the use of a corticosteroid would improve event-free but not overall survival (72). As the issue remains unresolved, an ongoing intergroup study is addressing the role of maintenance after stem cell transplantation (MY-10).

Treatment of the Non–Stem Cell Transplantation Candidate
For those not deemed suitable candidates for stem cell transplantation, other options are generally pursued, classically the combination of melphalan and prednisone (73). The regimen produced durable response but only in about 60% of cases, with complete responses being extremely rare and most patients experiencing disease relapse. The suboptimal nature of melphalan and prednisone, as opposed HDT, prompted many to test the novel agents in combination with existing chemotherapeutic agents. The sum of these studies has shown that combination of novel agents with melphalan results in unprecedented response rates and, for the first time, very significant improvements in outcomes (70).

Melphalan and Prednisone with Thalidomide and Revlimid
Palumbo et al. (70) pioneered the use of thalidomide in combination with melphalan and prednisone for the treatment of the elderly with MM. They have achieved an overall response rate of 76% with 28% of patients being in a complete response or near-complete response category Event-free but not overall survival were improved. In a similar study, the Intergroup Francophone du Myelome has completed a randomized phase 3 study of melphalan and prednisone versus MPT (melphalan and prednisone

4 B. Barlogie, personal communication, June 2006.
with thalidomide) versus i.v. melphalan (vincristine, Adriamycin, and dexamethasone followed by stem cell collection and two courses of i.v. melphalan at doses of 100 mg/m²). The study was closed at the last interim analysis as the median survival times were 33 months for the melphalan and prednisone arm, 39 months for the i.v. melphalan arm, and not reached at 56 months for the MPT arm (74). In the same way that thalidomide and dexamethasone in combination produce DVT, the same phenomena has been observed in the application of MPT (70, 75, 76). These compelling data from the Intergroup Francophone du Myelome 99-06 study have led us to adopt MPT at the Mayo Clinic as the preferred treatment of newly diagnosed patients with MM who are not candidates for transplant.

The natural follow-up for the combination of MPT was the substitution of thalidomide by lenalidomide (77). Palumbo et al. have completed a prospective phase 2 study of this combination in elderly patients with new diagnosis MM. The combination is well tolerated, and at least in its preliminary analysis seems to be at least not more active than MPT. A current ongoing phase 1 clinical trial is also being conducted at the Mayo Clinic that evaluates the toxicity profile of this combination. The drawback difference between MPT and MPR (melphalan and prednisone with Revlimid) is the higher incidence of clinically significant cytopenias, particularly neutropenia, among recipients of MPR. Nevertheless, it is likely that in the future, MPR will ultimately prove superior and this concept will soon be tested in a prospective fashion against MPT.

**Bortezomib and Melphalan/Prednisone**

As had been tried with thalidomide and lenalidomide, bortezomib was tested in combination with melphalan and prednisone in the elderly (67). Impressively, 43% of patients were able to achieve a complete response/near-complete response category at the time of their best response. Overall, the regimen was well tolerated. One key advantage of the combination is that it seems to have very high level of activity regardless of genetic category (currently unknown for MPT or MPR). The role of chromosome 13 deletions and immunoglobulin heavy chain translocations on the rate of response was explored by Mateos et al. (67). Overall, there was no difference in the rate of response among the different genetic categories, emphasizing the possibility that bortezomib may overcome some of the negative prognostic connotations associated with genetics. The regimen is now being tested in a prospective randomized trial against melphalan and prednisone (VISTA trial), the results of which should be available in the near future. The appeal for this regimen is that, despite the need for i.v. administration, it seems not to be associated with significant risk for DVT and has a manageable peripheral neuropathy. It is thus likely that after the results of the VISTA trial are presented (in all likelihood in favor of vincristine, cyclophosphamide, melphalan, and prednisone), the combination will be tested against the winner of MPT versus MPR.

**Targeted Therapeutics**

As highlighted above, despite significant advances in therapy all MM patients still relapse; thus, the story is far from over and new drugs are needed. In that light, significant advances have been made in understanding the genetic basis for MM initiation and propagation. In particular, these genetic aberrations result in numerous signaling networks that are now identified as potential therapeutic targets. Although none of these have been definitively proven to be clinically active, we highlight here some promising agents that are currently being studies in clinical trials.

**FGFR3 Inhibition**

MM is a heterogeneous disorder, with the main determinant of this heterogeneity being the underlying genetic defect as reviewed above. The genetic classification of MM identifies subgroups of patients who have a more aggressive disease and shorter duration of remission after consolidation (27). Among these patients, the highest risk has been identified for those that harbor the high-risk molecular cytogenetic features: that is those with t(4;14), t(14;16) or −17p13.1. Overall, these patients have an estimated median survival after diagnosis of 24 to 30 months, with posttransplant salvage strategies being seldom effective (28). To overcome this hurdle, we have started the study of inhibition of one of the genes up-regulated by the t(4;14) (38, 78). One such approach is the use of a small-molecule inhibitor of FGFR3 (e.g., CHIR-258), which is now being studied in phase I clinical testing. Because of its broad receptor tyrosine kinase inhibitor activity, the trial is being conducted without prior selection for t(4;14), but ultimately products like this targeting the downstream consequences of genetic deregulation will become standard.

**Proteasome Inhibitors**

Following the marked success of bortezomib, at least two further proteasome inhibitors are now in clinical trials (79). Both are supported by extensive preclinical data and phase I trials are under way. PR-171 is a novel epoxomicin-related proteasome inhibitor, which unlike bortezomib binds irreversibly and with a high degree of specificity in vitro to the chymotrypsin-like subunit of the proteasome (79). PR-171 was able to inhibit proliferation of MM cell lines in a concentration- and time-dependent manner. Both continuous and pulse treatment with PR-171 was also able to inhibit proliferation in freshly purified patient-derived MM plasma cells. Taken together, these data indicate that PR-171 is a promising, novel proteasome inhibitor providing a rational basis for its translation into the clinic. The second proteasome inhibitor in trials is NPI-0052, which inhibits all three proteasome activities (80, 81). The data show that NPI-0052, like bortezomib, targets the proteasome but triggers a proteasome activity profile distinct from bortezomib.

**Hsp90 Inhibition**

Critical studies examining the mechanisms of resistance of patients to bortezomib identified heat shock protein 90 as a key regulator of resistance in that treatment of MM cells with bortezomib triggers significant HSP90 up-regulation.
as a stress response. A number of inhibitors of HSP90 have thus entered the clinic and are actively being evaluated, including IPI-504 (82), DMAG (83), and KOS-953. Again, preclinical data is highly supportive (e.g., KOS-953 shows in vitro activity against MM cells from bortezomib-resistant patients and extends survival of MM mice models).

**Insulin Growth Factor Inhibition**

Insulin-like growth factor is an important survival factor for malignant plasma cells. It also serves as survival signaling for many other forms of human malignancies (84–86). Strategies to disrupt signaling through the insulin-like growth factor pathway are of importance in MM as this may lead to agents in this class to either favor apoptosis or enhance cytotoxicity of concurrently administered agents (84, 86). Two main pathways have been explored, including the use of a small-molecule inhibitor of the pathway, as well as a monoclonal antibody–based blockade of the insulin-like growth factor receptor 1 (87, 88). Mitsiades et al. have shown that using a neutralizing antibody against insulin-like growth factor receptor 1, antagonist peptides, or a receptor kinase inhibitor show antitumor activity against MM with consequent induction of apoptosis and proliferation arrest (86). Furthermore, the combination of the kinase inhibitor (NVP-ADW742) resulted in significant antitumor activity when used with combination chemotherapy and tested in a human xenograft MM model (86).

In this particular study, the inhibition of insulin-like growth factor receptor 1 seemed to have a more pronounced effect in the growth inhibition of MM.

**GX015-070 and MCL-1 Inhibition**

Bcl (87) family members, particularly Bcl-XL and Mcl-1, are implicated in the survival of myeloma cells (89, 90). GX015-070 is a novel small-molecule antagonist of the BH3-binding groove of the Bcl family of proteins. We have shown that GX015-070 inhibited the binding of Bak to Mcl-1, up-regulated Bim, induced cytochrome c release, and activated capase-3 in treated human myeloma cell lines confirming the predicted mechanism of action. Consequently, GX015-070 potently inhibited the viability of 15 human myeloma cell lines studied (mean IC\textsubscript{50} 246 nmol/L), including those resistant to melphalan and dexamethasone.\textsuperscript{5} Further, in combination studies, GX015-070 enhanced the antmyeloma activity induced by melphalan, dexamethasone, or bortezomib. Taken together, these studies support the therapeutic application of GX015-070 for MM particularly in combination therapies and phase 1/2 trials are beginning.

**Farnesyl Transferase Inhibition (Tipifarnib)**

Inhibition of farnesylation results in decreased cell signaling by protein pathways dependent on this transformation (91). A prototype agent is tipifarnib (Zarnestra). Zarnestra has been tried as a single agent in the treatment of relapsed and refractory MM (91). The agent was initially proposed to be tested in MM because of the potential role of ras mutations, resulting in transformation of cells from benign to malignant (i.e., monoclonal gammopathy of undetermined significance/smoldering myeloma to MM). It has been later shown that many other cell signaling pathways are dependent on the activity of tipifarnib (92, 93). Even with a small subset of patients, no enhanced activity was noted among patients who had ras mutations (91). The agent is now being explored in combination with tubulin inhibitors and proteasome inhibitors (94).

**CD40 Ligand**

CD40 is expressed on MM cells and is engaged in the critical nuclear factor-κB pathway of cell survival. The Dana-Farber group has extensively studied monoclonal antibodies targeting these pathways, including CHIR12.12 (95) and SGN-40 (96), a humanized immunoglobulin G1. These antibodies mediate cytotoxicity against MM cells via suppression of IL-6–induced proliferative and antiapoptotic effects, as well as antibody-dependent cell-mediated cytotoxicity. Both antibodies are in clinical trials.

**AKT Inhibition**

Critical downstream targets of signaling pathways in MM include phosphatidylinositol 3-kinase and AKT (97). The AKT kinase has been shown activated in MM tumor cells and is associated with myeloma cell survival and migration (97). Novel inhibitors of AKT such as perifosine are now being studied in the clinic.

**Future Directions**

The future directions in MM therapeutics will likely be dictated by the two following factors: experience gained with the use of novel combinations and validation of therapeutic targets. Concerning the former, many issues remain to be addressed such as the net clinical worth of early introduction of these agents and the role of combinations of agents as they relate to disease risk categories. These questions need to be formally tested in rigorous controlled clinical trials. Nevertheless, the introduction of novel agents targeting critical MM pathways have already dramatically improved clinical outcomes in MM, and the advent of therapeutically rational approaches to patients who use targeted and individualized therapies are already here.

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