HIV Protease Inhibitors for the Treatment of Multiple Myeloma

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Abstract: Outcomes in multiple myeloma (MM) patients have improved in recent years owing to the introduction of new drugs. Among them, proteasome inhibitors and immunomodulatory imide drugs have become central in the management of newly diagnosed and relapsed MM. However, resistance to these classes of agents develops in most patients and ultimately leads to death from relapsed/refractory disease. A need exists for new classes of antimyeloma drugs, especially ones that are active in the multirefractory setting. The conventional drug development process, which involves extensive preclinical and clinical testing prior to assessment of clinical activity, has fallen short in delivering adequately safe and active novel drug candidates. HIV protease inhibitors such as nelfinavir are safe, US Food and Drug Administration–approved agents that have been shown to have potent antimyeloma activity in both preclinical models and patients with refractory disease. The repurposing of HIV protease inhibitors for treatment of MM is promising in light of their antimyeloma activity in conjunction with their global availability, established safety, and relatively low cost. This review will summarize the preclinical and clinical data available on HIV protease inhibitors for the treatment of refractory MM.

Keywords
Drug repurposing, HIV protease inhibitors, multiple myeloma, proteasome inhibitors

Myeloma Cell Biology

Multiple myeloma (MM) is a cytogenetically heterogeneous clonal plasma cell proliferative disorder that accounts for 1% of all cancers and approximately 10% of hematologic malignancies. The median age of diagnosis is 69 years—with more than three-quarters of diagnoses made in patients older than 55 years—and nearly two-thirds of patients are male. The 2-year survival for MM is currently 87%. This percentage has risen over the last decades owing to the introduction of newer therapies, such as proteasome inhibitors (PIs) and immunomodulatory imide drugs (IMiDs). MM may manifest with hypercalcemia, renal failure, anemia, and lytic bone lesions (CRAB symptoms), or may be detected at an asymptomatic stage.
MM cells produce high amounts of monoclonal immunoglobulin (M-Ig), of which approximately 30% is defective and requires proteolytic degradation and recycling to maintain cellular viability.6,7 To sustain high protein turnover, MM plasma cells have evolved and adapted the endoplasmic reticulum toward high protein production and the ubiquitin proteasome system toward effective protein degradation.6,7 This extraordinarily active route is tightly controlled by the unfolded protein response (UPR) pathway, a complex and highly conserved transcriptional network that balances protein production, folding, and destruction, and that serves to resolve unwanted endoplasmic reticulum stress.8 The UPR consists of 3 regulatory branches, namely IRE1α/XBP1, PERK/eIF2α/ATF4, and ATF6 (these stand for inositol-requiring enzyme 1 α/X-box binding protein 1; PKR-like endoplasmic reticulum kinase/elongation initiation factor, subunit α/activating transcription factor 4; and activating transcription factor 6; respectively).9 In addition to its role in the UPR pathway, XBP1 transcription factor is also a major plasma cell differentiation factor.10

Owing to overproduction of M-Ig and a high rate of protein synthesis, MM cells are dependent on the proteasome to clear misfolded proteins. Consequently, proteasome inhibition in MM cells induces endoplasmic reticulum stress, activates the UPR, and results in apoptosis when endoplasmic reticulum stress becomes excessive.11-13 This strategy has been shown to be highly cytotoxic for MM cells14 and remains a major pillar for the treatment of newly diagnosed and relapsed MM, with 3 PIs approved by the US Food and Drug Administration (FDA) for clinical use: bortezomib (Velcade, Millennium/Takeda Oncology), carfilzomib (Kyprolis, Amgen), and ixazomib (Ninlaro, Millennium/Takeda Oncology).1 Despite the high sensitivity of MM cells to proteasome inhibiting drugs, however, resistance to PI treatment develops in a majority of patients.15-17 Widespread clonal heterogeneity and a high frequency of mutated genes in the RAS, BRAF, and IRE1α/PERK/eIF2α/ATF4, and ATF6 (these stand for inositol-requiring enzyme 1 α/X-box binding protein 1; PKR-like endoplasmic reticulum kinase/elongation initiation factor, subunit α/activating transcription factor 4; and activating transcription factor 6; respectively).9 In addition to its role in the UPR pathway, XBP1 transcription factor is also a major plasma cell differentiation factor.10

Drug Repurposing in MM

The development of new anticancer drugs is associated with increasing costs and failure rates. Therefore, alternative approaches to cancer drug discovery are being explored, among them drug repositioning, also known as drug repurposing or reprofiling.40,41 Repurposing is defined as finding new uses for already approved drugs outside the original indication.42 It offers many advantages over the development of new drug entities, including global availability and well-established safety, dosing, pharmacokinetics, and pharmacodynamics. Several databases have been created to gather the available information about promising drugs for repurposing. Two examples are the Repurposing Drugs in Oncology (ReDO) database43 and the Drug Repurposing Hub.44 The ReDO database so far includes 291 drugs that fulfill the criteria of high potential repurposing drugs, with 75% of the substances in the WHO Model List of Essential Medicines. The Drug Repurposing Hub currently contains annotations for a total of 6125 compounds, including 2369 launched drugs, 1619 drugs that reached phases 1 to 3 of clinical development, 96 compounds that were previously approved but withdrawn from use, and 2041 preclinical chemicals or compounds (https://clue.io/repurposing).

Thalidomide is the most successful example of drug repurposing in the MM field. Thalidomide was developed decades ago to treat morning sickness in pregnant women in Europe, and was rapidly withdrawn because of its association with severe limb defects in newborns. Almost any tissue or organ could be affected by the drug, and an estimated 10,000 children were exposed to the effects of thalidomide (the United States was largely spared, as the US FDA never approved it for use in pregnant women).45 In the early 1990s, thalidomide was found to have angiogenic effects and to be a potent tumor necrosis factor inhibitor.
HIV PROTEASE INHIBITORS FOR THE TREATMENT OF MULTIPLE MYELOMA

HIV Drugs as Potential Antimyeloma Therapy

HIV Drug Classes and Safety

HIV was identified in 1983. Currently 37 million people live with HIV, 70% of them in Africa. Since the outbreak of the disease in the early 1980s, efforts have been made to understand the pathogenesis of HIV, which led to the development of multiple drugs targeting different steps in the life cycle of the virus. By 2018, 23.3 million people were receiving antiretroviral therapy globally. HIV nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of agents introduced for HIV treatment as competitive substrate inhibitors, followed by HIV-Pis that specifically targeted HIV-1 protease, and further by non-nucleoside reverse-transcriptase inhibitors (NNRTIs) as noncompetitive reverse transcriptase inhibitors. HIV monotherapies, dual therapies, and especially the combination of NRTIs and PIs marked the beginning of the era of highly active antiretroviral therapy (HAART), which improves the CD4+ cell count and increases life expectancy in HIV patients.

The first-generation HIV protease inhibitor (HIV-PI) was also found to be a potent inhibitor of HIV-PI/ritonavir as for NNRTIs, and the loss of activity of HIV-Pis after long-term exposure is low in patients treated with PI/ritonavir vs regimens containing an NNRTI, which suggests a long-lasting protective effect of HIV-PI/ritonavir treatment and reinforcing the importance of HIV-Pis.

HIV-Pis are considered to be relatively safe, although some side effects have been identified. The most common class-associated side effect is gastrointestinal intolerance. Lipodystrophy and insulin resistance are also frequently reported. These problems are related to a decrease in sterol regulatory element-binding protein 1 (SREBP-1) in the cell nucleus, leading to reduced adiponectin and impaired adipocyte differentiation. HIV-Pis also inhibit glucose transporter type 4 (GLUT4), blocking glucose uptake in the adipocytes, and are thought to affect proteasome homeostasis.

Preclinical Evidence of Efficacy in Multiple Myeloma

Antitumor effects of HIV-Pis have been attributed to a variety of mechanisms, including downregulation of Akt/STAT3/ERK signaling, induction of endoplasmic reticulum stress via UPR induction, antiangiogenesis in vitro and in vivo, and other antinecancer pathways.

The precise mechanism by which HIV-Pis enhance cytotoxicity of MM cells is still under study. Nelfinavir, saquinavir, and ritonavir induce growth arrest and apoptosis in MM cell lines and isolated human MM cells by inhibiting interleukin 6 (IL-6)–mediated phosphorylation of both signal transducer and activator of transcription 3 (STAT3) and extracellular signal–related kinase 1/2 (ERK1/2), resulting in downregulation of induced myeloid leukemia cell differentiation protein (MCL1). MCL1 is an antiapoptotic member of the B-cell lymphoma 2 (BCL2) family that promotes survival and has been related to development of resistance in MM cells. Ritonavir decreases MCL1 expression while maintaining oxygen consumption rates in MM cells; however, its combination with metformin completely suppresses this and increases apoptosis mediated by inhibition of the Akt/AMPK pathways. Additionally, MM cells depend on the activity of GLUT4 for their viability and growth, and its suppression induces apoptosis with a decrease in MCL1 expression. These data suggest that ritonavir sensitizes MM cells to apoptosis by impairing glucose metabolism.

Nelfinavir has consistently shown the most potent antitumor activity of all HIV-Pis in vitro and in vivo. In MM cells, nelfinavir at clinically achievable doses impaired 26S proteasome activity and activated the UPR in vivo, increasing C/EBP homologous protein (CHOP) expression, caspase 3 cleavage, and apoptosis. In line with these results, Kawabata and colleagues showed that

alpha (TNF-α) inhibitor, which paved the way for its use as an anticancer agent in MM. Another example of drug repurposing is nelfinavir (Viracept, Agouron), a first-generation HIV protease inhibitor (HIV-PI) that has shown potent activity in the treatment of refractory MM.58,60

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the combination of nelfinavir and bortezomib enhanced cytotoxicity to MM cells, and that CHOP and PERK knockdown by small interfering RNA inhibited cell death induced by nelfinavir, suggesting that UPR activation might be an early effect of nelfinavir that contributes to cytotoxicity. Importantly, treatment with the protein synthesis inhibitor cycloheximide decreased nelfinavir-induced apoptosis, suggesting that a decrease in endoplasmic reticulum stress diminishes nelfinavir toxicity in MM cells. In a mouse xenograft model of MM, the combination of nelfinavir and bortezomib reduced tumor size and increased cell death compared with bortezomib alone. In this study, bortezomib-induced autophagy was abrogated by nelfinavir via calpain inhibition, which enhanced cell death in vitro and in vivo. Of all the HIV-PIs available, nelfinavir has the most potent antimyeloma effect in PI-resistant MM cell lines and isolated MM patient samples. The combination of nelfinavir and bortezomib is cytotoxic in bortezomib-resistant MM cells, with an efficacy comparable to bortezomib-sensitive MM cells, suggesting that nelfinavir (and other HIV-PIs) may sensitize MM cells to apoptosis regardless of the PI resistance mechanism, and thereby overcome PI resistance. Additionally, nelfinavir/bortezomib resulted in enhanced cytotoxic activity compared with bortezomib alone, leading to UPR activation, CHOP upregulation, caspase 3 cleavage, and decreased AKT phosphorylation.

Nelfinavir and lopinavir were recently shown to overcome carfilzomib-specific PI resistance via modulation of ABCB1 transporters, and are the most active HIV-PIs to induce cytotoxicity in MM cells during cotreatment with carfilzomib. The primary molecular target of nelfinavir in MM cells is still unknown. A systematic computational analysis showed that a high percentage of 126 possible nelfinavir partners belong to the kinase superfamily.

Further, considering the known antiangiogenic effects of nelfinavir in other tumors and the increased bone marrow microvascular density in MM patients, it is likely that nelfinavir could also modulate angiogenesis in MM patients, although this remains unproven. An overview of the molecular effects of nelfinavir on MM is shown in the Figure.

**Clinical Evidence of Efficacy in Multiple Myeloma**

Nelfinavir has shown clinical activity in MM as part of combination treatments for refractory MM. In a phase 1 study called SAKK 65/08 (Nelfinavir Mesylate and Bortezomib in Treating Patients With Relapsed or Progressive Advanced Hematologic Cancer), 12 patients with advanced hematologic malignancies, including MM, acute leukemia, and lymphoma, were treated with nelfinavir (2500-5000 mg/day by mouth on days 1-13) plus bortezomib (1.3 mg/m² on days 1, 4, 8, and 11) in 21-day cycles. Patients had received a median of 4 prior lines of therapy. The primary objective was to establish dose-limiting toxicity and safety. The recommended dose was established at 2500 mg by mouth twice a day. Interestingly, a dose of 1875 mg by mouth twice a day resulted in comparable plasma levels of nelfinavir, suggesting autoinduction of nelfinavir-metabolizing enzymes at high drug doses in MM patients. In this study, among a prospectively planned cohort of 6 bortezomib-refractory MM patients, 4 achieved a partial response according to International Myeloma Working Group (IMWG) criteria and 2 achieved a minimal remission. Importantly, all responding patients had progressed during prior bortezomib-containing therapy, suggesting that the nelfinavir/bortezomib combination overcomes bortezomib resistance.

A multicenter, open-label phase 2 trial called SAKK 39/13 (Nelfinavir as Bortezomib-Sensitizing Drug in Patients With Proteasome Inhibitor-Nonresponsive Myeloma: NCT02188537) evaluated the combination of nelfinavir, bortezomib, and dexamethasone in patients previously exposed or intolerant to IMIDs and refractory to bortezomib. In this study, 34 patients were treated with nelfinavir (2500 mg/day by mouth on days 1-14), bortezomib (1.3 mg/m² on days 1, 4, 8, and 11), and dexamethasone (20 mg by mouth on days 1-2, 4-5, 8-9, and 11-12), repeating the cycle every 21 days. The median treatment duration was 4.5 cycles, and the objective response rate (ORR) for the entire cohort was 65%, with 7 partial responses and 5 very good partial responses. Overall, 74% of the patients experienced a minimal response or better by IMWG criteria. Strikingly, ORRs of greater than 60% were seen in bortezomib-refractory patients with poor-risk cytogenetic features, as well as in triple-refractory patients (a 62% ORR in patients refractory to pomalidomide [Pomalyst, Celgene], lenaliomide [Revlimid, Celgene], and bortezomib), in this study. Patients in this trial had received an average of 5 prior lines of treatment, and a very short prospective survival is anticipated. However, mortality from bacterial infections was observed in this trial. We therefore suggest prophylactic antibiotic therapy when using bortezomib/nelfinavir/dexamethasone in patients who have advanced, multirefractory MM. It is unclear to what extent the mortality from infections reflects the background infection risk of the patient population or represents an effect of the drug treatment. We hypothesize that the addition of nelfinavir to bortezomib/dexamethasone greatly increases elimination or silencing of nonmalignant plasma cells and B cells, reflecting the superior activity that this regimen has on MM cells.

Recently, the combination of nelfinavir (2500 mg/day orally on days 1-14), lenalidomide (25 mg/day orally on days 1-21), and dexamethasone (20/40 mg orally on...
days 1, 8, 15, and 22) in 4-week cycles was explored in a phase 1/2, multicenter study called SAKK 39/10 (Nelfinavir and Lenalidomide/Dexamethasone in Progressive Multiple Myeloma; NCT01555281) in lenalidomide-refractory patients. In this trial, 31% of 29 patients had poor-risk cytogenetics, 93% had received at least 2 prior lines of treatment, and 63% had undergone prior autologous stem cell transplant. One-third of the patients enrolled were double-refractory (to bortezomib/lenalidomide), and the primary outcome of objective response was achieved in 55% of the patients, with no unexpected adverse events, suggesting that the addition of nelfinavir to lenalidomide is safe and effective in lenalidomide-refractory MM. The Table summarizes clinical trials with nelfinavir for MM treatment.

Clinical Use of Nelfinavir in Multiple Myeloma
Current MM treatment is based on PI and/or IMiD (mostly lenalidomide) backbones in the frontline setting, and usually involves a switch in the class of backbone drug together with a monoclonal antibody or a doublet/triplet of PI/IMiD plus a monoclonal antibody for therapy in the relapsed setting. For lenalidomide-refractory MM, the lenalidomide-based combinations mostly used for initial treatment of relapsed MM have not been systematically tested in large clinical trials. The combination of lenalidomide/dexamethasone with nelfinavir offers the only oral triplet combination to our knowledge that has shown clinical activity in more than 50% of patients in a prospective clinical trial. Based on this fact, the combination may be attractive as an entirely oral regimen in the lenalidomide-refractory setting.

As soon as patients have reached a double-refractory state (PI-refractory and lenalidomide-refractory), the prognosis is particularly poor. PFS is in the 3- to 4-month range, median survival is approximately 1 year, and the response rate to the next therapy line is approximately 30%, including approved standard therapies such as daratumumab (Darzalex, Janssen Biotech)/dexamethasone or pomalidomide/dexamethasone. Without a doubt, double-refractory MM patients require additional options for active therapy that involve alternative modes of action. Most therapies that have been tested for this patient population thus far were based on a pomalidomide backbone. Pomalidomide/dexamethasone-based doublets and
triplets yielded different response rates in phase 2 studies in double-refractory patients, ranging from 20% to 30% (with pomalidomide/dexamethasone) to 50% to 60% (with pomalidomide combined with either cyclophosphamide/pembrolizumab [Keytruda, Merck] or daratumumab/carfilzomib). The nelfinavir/bortezomib/dexamethasone combination yielded a response rate of greater than 60% in this population, which is comparable to—or even numerically higher than—today’s cutting-edge MM drug triplets. Given the well-established safety of nelfinavir and the fact that the generic formulation of this drug is available for 10% to 20% of the monthly treatment costs of pembrolizumab, daratumumab, or carfilzomib, and that likewise bortezomib is available generically, bortezomib/nelfinavir/dexamethasone appears particularly attractive for patients with double-refractory MM who do not have access to an unlimited supply of last-generation, expensive MM drugs (eg, because of inadequate insurance coverage, unaffordable copayments, or lack of drug availability in a given country). In addition, the activity of bortezomib/nelfinavir/dexamethasone in the phase 2 setting was independent from cytogenetic risk, the type of previous therapies, and whether the patient was sensitive or refractory to them.

Most patients with double-refractory MM soon reach the triple-refractory stage (PI-refractory, lenalidomide-refractory, and pomalidomide-refractory), given that pomalidomide/dexamethasone is active in only roughly 30% of these patients.16,107 These triple-refractory MM patients have been excluded from trials testing pomalidomide-based triplets for refractory MM, and very few studies have systematically analyzed the activity of drug combinations in this patient group. We currently lack an active treatment for this patient group that has activity higher than the 30% range reported for daratumumab/dexamethasone. The nuclear export inhibitor selinexor (Xpovio, Karyopharm) plus dexamethasone showed a 26% response rate in pentarefractory patients108 and a 58% response rate when combined with bortezomib/dexamethasone as a triplet in double-refractory MM.109 However, selinexor shows significant myelotoxicity that limits its use in advanced MM. The bortezomib/nelfinavir/dexamethasone combination, by contrast, is globally available and lacks myelotoxicity.

With a response rate of greater than 60% in triple-refractory (to bortezomib, lenalidomide, and pomalidomide) MM, the combination of bortezomib, nelfinavir, and dexamethasone is today among the most active drug combinations for the treatment of multirefractory MM reported in phase 2 trials.49 However, this activity is based on small patient numbers only, and nelfinavir is not approved for MM therapy. Although nelfinavir was granted an orphan drug designation by the FDA and the Swiss Agency for Therapeutic Products (Swissmedic) based on the data reported above, the agent has not been submitted for approval for this indication. Today, nelfinavir is approved exclusively for HIV therapy in many countries worldwide, including the United States, Canada, and most emerging and developing countries. Although nelfinavir had likewise been approved as HIV therapy in Europe by the European Medicines Agency and Swissmedic, a prolongation of this approval was not requested, so that nelfinavir has not been approved in any country in Europe since 2013. However, based on the published data, the life-threatening nature of double-refractory MM, and the limited options of active approved drugs

### Table. Active and Completed Clinical Trials With Nelfinavir for Multiple Myeloma Treatment

<table>
<thead>
<tr>
<th>Study ID/Name</th>
<th>Phase</th>
<th>Status</th>
<th>Indication</th>
<th>Intervention</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01164709/SAKK 65/0848</td>
<td>1</td>
<td>Completed</td>
<td>PI-refractory MM</td>
<td>Nelfinavir 2500 mg twice a day on days 1-14 + bortezomib/dexamethasone</td>
<td>83% ORR, 50% PR, 33% MR&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NCT02188537/SAKK 39/13</td>
<td>2</td>
<td>Completed</td>
<td>PI-refractory MM</td>
<td>Nelfinavir 2500 mg twice a day on days 1-14 + bortezomib/dexamethasone&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74% ORR, 15% VGPR, 50% PR, 9% MR</td>
</tr>
<tr>
<td>NCT01555281/SAKK 39/10&lt;sup&gt;105&lt;/sup&gt;</td>
<td>1/2</td>
<td>Active, not recruiting</td>
<td>Rd-refractory MM</td>
<td>Nelfinavir 1250 mg twice a day on days 1-21 + lenalidomide/dexamethasone&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55% ORR, 10% VGPR, 21% PR, 24% MR</td>
</tr>
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</table>

<sup>a</sup> Extension cohort with 6 patients.
<sup>b</sup> Bortezomib/dexamethasone: bortezomib at 1.3 mg/m² on days 1, 4, 8, and 11; dexamethasone at 20 mg on days 1-2, 4-5, 8-9, and 11-12. Cycles every 28 days.
<sup>c</sup> Lenalidomide/dexamethasone: lenalidomide at 25 mg on days 1-21; dexamethasone at 20/40 mg on days 1, 8, 15, and 22. Cycles every 21 days.

MM, multiple myeloma; MR, minimal remission; ORR, objective response rate; PI, proteasome inhibitor; PR, partial response; VGPR, very good partial response.
in this setting, nelfinavir may be used off-label for MM treatment in most countries outside of Europe, as well as on a compassionate use basis (after informed consent) for individual patients in most European countries, in agreement with their different drug regulations.

Nelfinavir today is a component of combination therapies for patients with refractory MM. Unfortunately, the clinical experience is based on only two small phase 2 trials, and clinical data are lacking for the use of nelfinavir in combination with pomalidomide, a second-generation PI (ie, carfilzomib, ixazomib), or monoclonal antibodies.

Summary

HIV-PIs induce synergistic endoplasmic reticulum stress and cytotoxicity in combination with proteasome inhibitors, and overcome proteasome inhibitor–based resistance in preclinical models of MM. Bortezomib/nelfinavir/dexamethasone is among the most active drug combinations tested in clinical phase 2 trials in the double-refractory setting and especially in the triple-refractory setting. Although nelfinavir is safe and has obtained orphan drug status for MM treatment, no approval trials in MM are under way, likely because of a lack of a promising strategy to commercially exploit the clinical potential of nelfinavir in MM. Nelfinavir is commercially available and may be used after informed consent and in the absence of alternative approved therapy options to treat individual patients with advanced multirefractory MM as part of combination therapies in off-label (United States, Canada) or compassionate use (Switzerland and other European countries) settings.

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Disclosures

The authors have no conflicts of interest to declare.

References