HEMA-TIMES
UNDERSTANDING THE MECHANISMS OF ACTION
OF MULTIPLE MYELOMA THERAPIES
& THEIR CLINICAL IMPLICATIONS

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INTRODUCTION

Multiple myeloma (MM) is a plasma cell malignancy based in the bone marrow, in which standard treatments have historically included corticosteroids (e.g. dexamethasone and prednisone) and cytotoxic drugs (e.g. melphalan, vincristine, cyclophosphamide, and doxorubicin). However, the treatment paradigm is shifting away from classical chemotherapy to therapies that more directly target the unique plasma cell biology. This may be either through pathways unique to the plasma cell itself or the tumour microenvironment upon which they are dependent. Significant improvements in overall survival and remission duration in multiple myeloma (MM) are largely due to the advent of novel therapeutic agents, including proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs). Newer agents, including those that affect the epigenome, and antibody-based therapies that directly target the plasma cell are also coming into play. As the therapeutic landscape widens, it will be important to study these agents and their mechanisms of action (MoA).

This paper will focus on the MoA of existing and new agents, in order to promote a better understanding of how they work, and what their implications are in the Canadian clinical setting.
I. MECHANISM OF ACTION: PROTEASOME INHIBITORS

The proteasome serves an important cellular function, enabling the clearance of abnormal or mutant proteins. Tumour cells are heavily reliant on this clearance mechanism and are sensitive to proteasome inhibition, leading to an antiproliferative and proapoptotic effect mediated through multiple pathways including the induction of endoplasmic reticulum stress, activation of caspases, and reactive oxygen species. 

Proteasome inhibitors (PIs) work by blocking the proteasome, which then produces conflicting regulatory signals, and interferes with critical cellular functions. Cancer cells cannot process this signal overload, which causes apoptosis. Normal cells, on the other hand, can recover since they are less sensitive to the proapoptotic effects (See Figure 1).

Proteasome inhibition has emerged as an important therapeutic strategy in multiple myeloma (MM). Since the publication of the first bortezomib trials 10 years ago, this PI has contributed substantially to the survival benefit in MM patients over the past decade. While this agent is still highly relevant in the Canadian landscape, there are also a number of second generation PIs currently in development.

II. PROTEASOME INHIBITORS: AGENTS

First Generation Proteasome Inhibitor: Bortezomib

Bortezomib, a first-in-class drug, has significantly improved the response and survival of patients with multiple myeloma over the last decade. It is a ‘dipeptidyl boronic acid-based specific, reversible PI that targets the chymotrypsin- and caspase-like active sites, with minimal effect on trypsin-like activity.’ Initially, it was approved by Health Canada for relapsed/refractory MM, however over the years it has expanded its indication and is now available to all patients in the front-line setting as well. This approval was based on multiple phase III trials that proved its efficacy and safety in both transplant eligible and transplant ineligible patients.

Two bortezomib options are currently available: branded agent, Velcade, and a generic version of bortezomib (recently Health Canada approved). Differences do exist between these options and should be considered when prescribing. The main difference is that generic bortezomib does not have as many approved indications and is not approved for subcutaneous administration.

Velcade is available for administration via IV as well as subcutaneous (SC), while with generic bortezomib only the IV option is available. The option for subcutaneous delivery has numerous advantages and allows more patients to benefit from the use of bortezomib, especially those who have poor venous access. There is also increased safety with this method of administration for patients that have a higher risk of developing, or pre-existing, peripheral neuropathy. A large phase III study was completed where Velcade SC vs Velcade IV were compared to determine safety and efficacy. This study validated the use of the more convenient and less toxic SC route of administration.
Second Generation Proteasome Inhibitors: Carfilzomib, Ixazomib Citrate, Oprozomib and Marizomib

Carfilzomib is an ‘intravenous, irreversible tetrapeptide epoxyketone, second-generation PI.’ It was granted FDA approval on July 20, 2012 and is expected to be submitted for Health Canada approval in the near future as it has demonstrated activity in the relapsed/refractory setting. Its irreversible binding properties and higher affinity for the proteasome translates into ‘superior biological activity and cytotoxicity’ in bortezomib-resistant cell lines in vitro and in vivo. It appears to have a good toxicity profile as well. According to the phase III ENDEAVOR trial, carfilzomib doubles PFS in relapsed myeloma, compared to bortezomib. This is a multi-centre, open-label, randomized trial for patients with relapsed multiple myeloma, comparing carfilzomib plus dexamethasone vs. bortezomib plus dexamethasone (NCT01568866). However despite these findings, OS data has not yet matured, thus bortezomib is still upheld as the ‘gold standard’ with respect to this important clinical endpoint. Additionally, the recently published Phase III ASPIRE trial compared carfilzomib with lenalidomide and dexamethasone vs. lenalidomide and dexamethasone alone. Results showed a positive PFS and favourable risk-benefit profile when adding a proteasome inhibitor (carfilzomib) to an immunomodulatory agent (lenalidomide) (NCT01080391).

Ixazomib citrate was the first oral PI to be developed. Ixazomib has shown improved pharmacokinetic and pharmacodynamic parameters compared with bortezomib, in addition to similar efficacy in the control of myeloma growth and prevention of bone loss. Ixazomib was found to overcome bortezomib resistance and to trigger synergistic anti-myeloma activity with dexamethasone, lenalidomide, and histone deacetylase inhibitors. Two phase I studies have evaluated ixazomib administered as monotherapy in patients with relapsed and/or refractory MM. A recent combination phase 3 trial (TOURMALINE MM-1) comparing ixazomib, lenalidomide and dexamethasone vs. placebo, lenalidomide and dexamethasone, found the ixazomib group had a longer PFS than those in the placebo arm. Other phase 3 trials are currently underway.

Oprozomib and Marizomib are other novel PIs being investigated in early phase trials. Oprozomib is an oral, irreversible, tripeptide epoxyketone that exerts its activity by inhibiting chymotrypsin-like activity of the proteasome. It is the oral equivalent of carfilzomib and demonstrates similar activity (both antiangiogenic and proapoptotic) in vitro and in vivo. Marizomib is an intravenous infusion that functions by blocking the activity of proteasomes and by doing so, it disrupts processes related to the growth and survival of cancer cells.

III. CLINICAL IMPLICATIONS OF PROTEASOME INHIBITORS

• The introduction of Velcade was practice changing in multiple myeloma. The ability of bortezomib to be used in combination with a wide variety of other classes of drugs will likely continue to make it a standard in clinical practice, especially as many physicians are experienced and comfortable with managing patients on bortezomib. It has set a high standard for the second generation PIs.

• Second generation PIs offer advantages such as ease of administration (for oral agents), and potentially lower toxicity profiles. However, long-term overall survival data has yet to mature. Future research will include incorporation of second generation agents in first-line and relapsed combination regimens.
I. MECHANISM OF ACTION: IMMUNOMODULATORY AGENTS

Thalidomide (see Figure 2) was first used as an anti-cancer agent when it was found to have anti-angiogenic and immunomodulatory properties. Thalidomide analogues are approximately 50000-fold more potent at TNF\(\alpha\) inhibition in vitro compared with thalidomide, and are also much more potent than thalidomide in their ability to co-stimulate T cells.

Immunomodulatory (IMiDs) agents are given their name due to their action on the immune system. They possess anti-myeloma properties including: immune-modulation, anti-angiogenic, anti-inflammatory and anti-proliferative effects. It appears that anti-proliferative effects and down-regulation of crucial cytokines are the most important anti-MM attributes. Much research remains to be done regarding the complex interplay of immunomodulatory cytokines occurring in vivo, in order to understand what is necessary to facilitate optimal manipulation of these drugs for MM treatment.\(^{xx}\)

IMiDs function by binding to cereblon (CRBN), which is a protein encoded by the CRBN gene in humans. Cereblon expression is required for the anti-myeloma activity of IMiDs.\(^{xxi}\) CRBN depletion is directly cytotoxic to myeloma cells and the acquired loss of CRBL expression in the MM1.S cell line is associated with resistance to IMiDs.\(^{xxii}\) CRBN expression is able to predict resistance to IMiD monotherapy and is a predictive biomarker for survival outcomes.\(^{xxiii}\) Recent findings suggest that additional proteins, including IKZF1 and IKZF3, among others, are also required for IMiD activity.\(^{xxiv}\) Without these proteins, this class of drugs may be ineffective due to the development of resistance.

Although significant research is ongoing regarding the role of cereblon and associated proteins and their interaction with iMIDS, currently routine use of the level of cereblon to guide iMID use is premature. Standardized assay are not available to measure cereblon levels or activity and further research is required to determine threshold levels and mutations which may effect levels and resistance.

Lenalidomide and pomalidomide are small-molecule IMiDs, and are anti-tumour drugs derived by adding an amino group to the fourth carbon of the phthaloyl ring of thalidomide, (lenalidomide see Figure 3, pomalidomide see Figure 4) which improves potency and decreases toxicity.\(^{xxv}\)
II. IMiDS: AGENTS

Lenalidomide

Lenalidomide is a 4-amino-glutamyl analogue of thalidomide that lacks the neurologic side effects of sedation and neuropathy and has emerged as a drug with activity against various hematological and solid malignancies. In Canada, it is approved for clinical use in patients that have received at least one prior line of therapy for multiple myeloma.\(^{xxvi}\)

Lenalidomide is administered orally, which makes it extremely convenient. In the FIRST trial, lenalidomide was given to newly diagnosed, transplant-ineligible patients and was able to prolong PFS and OS. A consistent benefit was seen across most subgroups, showing a biomarker of response.\(^{xxvii}\) No unexpected toxicities were reported in this trial. Furthermore, in a double-blind, multicenter, randomized study comparing melphalan-prednisone-lenalidomide (MPR) induction followed by lenalidomide maintenance (MPR-R) with MPR or melphalan-prednisone (MP), MPR-R significantly improved PFS in patients with newly diagnosed MM who were ineligible for transplantation, again supporting both the upfront and continuous use of lenalidomide.\(^{xxviii}\) It should be noted that the loss of or decreased cereblon expression at the mRNA or protein level is associated with resistance to lenalidomide.\(^{xxix}\) Cereblon may prove to be an important functional marker to identify responsiveness to IMiDs but the story remains complicated and is not ready for routine clinical use.

In the transplant-eligible setting a number of recent trials have examined lenalidomide as a component of therapy. While routinely used in the upfront setting in the US it is still not approved for this indication in Canada. That said, based on a growing number of studies, maintenance lenalidomide post-transplant is now funded in most provinces. A Phase 3 study comparing lenalidomide to placebo in the maintenance setting showed a significantly longer time to disease progression and improved overall survival for lenalidomide, despite elevated toxicity levels.\(^{xxx}\) A similar finding in terms of extended PFS was discovered in the randomized Phase 3 IFM 2005-02 study, which also compared lenalidomide to a placebo. Overall survival in this study was not reached.\(^{xxxi}\) A third recently published Italian study also demonstrated the advantage of maintenance lenalidomide after induction therapy and further highlighted the importance of early transplant.\(^{xxxii}\) This improvement in outcome seems to have come at a cost of some toxicity. The notable late effect of second primary malignancies seen in the IFM 2005-02 trial adds a new dimension to the care of patients treated in this fashion.

There is clear evidence that lenalidomide maintenance improves PFS, with an evolving suggestion that OS may also be improved. Further follow-up will be required to formally assess the latter outcome. In addition, recent results from a phase II trial showed that lenalidomide-bortezomib-dexamethasone induction and consolidation followed by lenalidomide maintenance post-ASCT produces high-quality responses (including a substantial number of MRD negative cases by flow cytometry) and was well tolerated in newly diagnosed MM patients.\(^{xxxiii}\) Moving forward, longer follow-up of these initial studies will better inform the risk-benefit of this newly adopted strategy in Canada, weighing the potential benefit of longer term disease control with the risks of chronic chemotherapy such as thromboembolic disease, immune suppression and second primary malignancies. Furthermore, the evolving role of triplet based combinations with both proteasome inhibitors and IMiDs remains to be seen; no doubt heavily influenced by the cost of these cocktails.
Pomalidomide

Pomalidomide is the newest immunomodulatory drug, and a promising new agent. As aforementioned, it was developed by chemical modification of thalidomide, however has a reduced amount of toxicity. The MoA for this agent is not completely understood, but the drug functions similarly to lenalidomide in terms of anti-angiogenic effects, immunomodulation, the effect on the myeloma microenvironment and the protein cereblon.\textsuperscript{xxxiv}

It has shown positive activity in patients with relapsed/refractory MM in phase II studies. This includes patients who were heavily pre-treated, and/or disease refractory to lenalidomide and bortezomib, and those with high-risk cytogenetic or molecular markers.\textsuperscript{xxxv} In the Phase III MM-003 trial, the results confirmed significant PFS and OS benefits for pomalidomide plus low-dose dexamethasone vs. high-dose dexamethasone alone. The most notable adverse event with pomalidomide was a DLT of grade 4 neutropenia.\textsuperscript{xxxvi}

III. CLINICAL IMPLICATIONS OF IMiDS

• Lenalidomide has established itself as a standard treatment for multiple myeloma in the relapsed setting. It is also now funded in a number of provinces for use as a maintenance agent in the post-transplant setting. There is excitement around its possible use in the upfront therapy of transplant ineligible patients.

• Pomalidomide is currently available for patients who have failed both lenalidomide and bortezomib.

• Research continues to demonstrate the benefits and expand the indications for use of IMiDs in terms of treatment sequencing as well as in combination with other drugs.
MONOCLONAL ANTIBODIES (mAbs)

I. MECHANISM OF ACTION: mAbs

Monoclonal antibodies (mAbs), which specifically target antigens present in tumour cells, represent a paradigm shift in targeted therapy. After binding to the tumour cell, they exert their effects through multiple mechanisms. mAbs can cause direct cytotoxicity independent of the patients’ immune system both through activation of pro-apoptotic pathways after binding of the target antigen, or via the activity of conjugated toxins or radioisotopes against the malignant cell. The binding of the antibody can also flag the malignant cell to the patient’s immune system, capitalizing on endogenous immune mediated anti-oncogenic activity through mechanisms like antigen-dependent cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) (See Figure 5).

II. mAbs: AGENTS

Daratumumab

CD38 is a type II transmembrane glycoprotein highly expressed in multiple myeloma, making it a target for mAb-based immunotherapy. Daratumumab is a novel, high-affinity, humanized mAb that binds to CD38 and elicits signaling cascade and immune effector function engagement, leading to multiple direct and indirect mechanisms of action. This includes complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis, induction of apoptosis, as well as its ability to modulate vital cellular enzymatic activities associated with calcium mobilization and signaling.

While phase II trials have shown daratumumab’s impressive single agent activity, there are a number of studies exploring the efficacy of daratumumab in combination with other standard agents, including:

- A phase III randomized study comparing daratumumab + Len/dex vs. Len/dex in RR MM (NCT02076009)
- A phase III study examining the addition of daratumumab to bortezomib/Dexamethasone in patients with RR MM (NCT02136134)
- A phase III study comparing Dara/VMP to VMP in newly diagnosed, previously untreated patients (NCT02195479)

SAR650984

SAR650984 (SAR) is an investigational naked humanized IgG1 mAb that binds selectively to the human surface antigen CD38 highly expressed in MM cells and other hematological malignancies. Similar to daratumumab, SAR kills tumour cells using three different biological mechanisms: ADCC, CDC, and induction of apoptosis (pro-apoptosis). In an early phase I clinical trial, SAR has shown durable single agent activity in heavily pretreated relapsed/refractory MM patients (NCT01084252).
Elotuzumab

Elotuzumab is well-evaluated in MM and effectively acts against CS1, a glycoprotein highly specific to plasma cells. Single-agent results for this drug have been disappointing; however it has had excellent results in combination with lenalidomide and dexamethasone (upwards of 80% PR in relapsed patients). A phase III study is currently ongoing, looking at lenalidomide with dexamethasone +/- elotuzumab. The synergy is proposed to occur by an immune-mediated mechanism of elotuzumab modifying the plasma cells to be more prone to immune cell targeting, after lenalidomide prepares the NK and lymphoid cells for the immune synapse. Data to be reported at ASCO 2015 is eagerly awaited.

III. CLINICAL IMPLICATIONS OF MABS

• The impact of these agents, although not immediate, is greatly anticipated. Their ability to be used in combination with other approved agents makes this class of drugs extremely appealing. Such drugs will provide a new mechanism of action compared to currently available products.

• Administration of these agents may be a challenge due to infusion time and reaction. Strategies to address such challenges are currently being investigated.

'THEIR ABILITY TO BE USED IN COMBINATION WITH OTHER APPROVED AGENTS MAKES THIS CLASS OF DRUGS EXTREMELY APPEALING'
I. MECHANISM OF ACTION

Histone deacetylases are a group of enzymes with that contribute to epigenetic modification of the genome. The resultant repression of gene expression by deacetylation of the histone has downstream effects of inhibiting key pathways in cellular function. Histone deacetylases (HDAC) have recently emerged as a relevant clinical target in MM. Histone deacetylases (HDACs) and histone acetyl transferases regulate the acetylation of target proteins. Specifically, they remove acetyl groups from target proteins that regulate their activity. The mechanisms of action of deacetylase inhibitors (HDACi) is wide-ranging and in many respects is based on the epigenetic modifiers taken on by the tumour as it evolves away from “normal” tissue. It includes but is not restricted to: epigenetic inactivation of p53; targeting HDAC6, which inhibits aggresome formation, and acetylating HSP-90 which inactivates the chaperone system, both of which block the unfolded protein response.

To date, eighteen HDACs have been identified in humans and divided into 4 classes based on their homology to yeast HDAC (See Figure 6).

These classes differ in both their subcellular localization, with class I HDACs found in the nucleus and class II enzymes found in both the nucleus and cytoplasm, and their intracellular targets.

II. HDACi: AGENTS

Panobinostat

Panobinostat is among the most potent HDACi, with nanomolar HDAC inhibitory activity. It acts as a non-selective histone deacetylase inhibitor. Recently the FDA approved panobinostat in combination with bortezomib and dexamethasone for relapsed MM patients, based on the Panorama 1 trial.

In a randomized phase III clinical trial in patients with relapsed or relapsed and refractory MM (PANORAMA 1), the addition of panobinostat to bortezomib and dexamethasone led to a clinically relevant and statistically significant increase in progression-free survival (PFS) of approximately 4 months compared to placebo plus bortezomib and dexamethasone.

While there is very little single agent activity, it appears to work well in combination with other drugs such as bortezomib. More data on the use of this agent and its approval by Health Canada is anticipated.

III. CLINICAL IMPLICATIONS OF HDACi

- Although HDACis inhibit cell growth and induce apoptosis in multiple myeloma in vitro, the single agent clinical activity is limited. However, synergistic activity has been observed when used in combination with bortezomib. It remains unknown whether the addition of HDACi could reverse bortezomib resistance, but trials are currently being conducted to examine this, using panobinostat.

- There is also strong combination activity expected for HDACis with other proteasome inhibitors.
CONCLUDING REMARKS

The mechanisms of action of currently available and novel agents are imperative to understand, in order to anticipate both the effectiveness of single/combination regimens and their side effects. This would ultimately help to optimize use and timing of these agents. Additionally, understanding MoA is crucial in developing rational combinations of novel agents to broadly treat the clonal spectrum causing disease, thereby improving disease-free-survival and possibly even eliminating the malignancy. The sequencing of agents and rational combination therapy is an evolving field. Using combinations with different mechanisms of action will hopefully lead to synergy, resulting in more effective use of the drugs. Studies of such combinations will be important, as they may also reveal toxicities which may limit certain combinations. Practically speaking, the relevant combinations to most physicians will be ones that are potentially available. For the majority of patients in most provinces this means usually a triplet-based combination with a steroid (Dex or Pred), alkylator (cyclophosph or melphalan) and a novel agent (bortezomib, lenalidomide, pomalidomide or rarely thalidomide). Combinations of novel agents are not currently funded routinely, and newer drugs like the MABs are only available on study. More clinical trials and data are awaited on each of these agents to further clarify how they will be used in combination and sequentially.

‘MORE CLINICAL TRIALS AND DATA ARE AWAITED ON EACH OF THESE AGENTS TO FURTHER CLARIFY HOW THEY WILL BE USED IN COMBINATION AND SEQUENTIALLY’
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