

# Multiple myeloma and persistence of drug resistance in the age of novel drugs (Review)

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**Abstract.** Multiple myeloma (MM) is a mature B cell neoplasm that results in multi-organ failure. The median age of onset, diverse clinical manifestations, heterogeneous survival rate, clonal evolution, intrinsic and acquired drug resistance have impact on the therapeutic management of the disease. Specifically, the emergence of multidrug resistance (MDR) during the course of treatment contributes significantly to treatment failure. The introduction of the immunomodulatory agents and proteasome inhibitors has seen an increase in overall patient survival, however, for the majority of patients, relapse remains inevitable with evidence that these agents, like the conventional chemotherapeutics are also subject to the development of MDR. Clinical management of patients with MM is currently compromised by lack of a suitable procedure to monitor the development of clinical drug resistance in individual patients. The current MM prognostic measures fail to pick the clonotypic tumor cells overexpressing drug efflux pumps, and invasive biopsy is insufficient in detecting sporadic tumors in the skeletal system. This review summarizes the challenges associated with treating the complex disease spectrum of myeloma, with an emphasis on the role of deleterious multidrug resistant clones orchestrating relapse.

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## 1. Introduction

Multiple myeloma (MM) is the second most prevalent hematological malignancy worldwide, with a median onset of 60 years of age (1-6). MM is currently incurable, albeit clinically manageable and typically manifests with an accumulation of terminally differentiated monoclonal plasma cells (PCs) in the bone marrow (3). It is distinguished from solitary plasmacytoma by the presence of aberrant PCs at numerous skeletal sites (7,8).

MM can be 'secretory' or 'non-secretory' depending on the serum/urine levels of secreted monoclonal immunoglobulin. 'Secretory MM' is characterized by the presence of abnormal levels of monoclonal proteins (M-protein) or paraproteins in circulation and urine. 'Non-secretory' MM accounts for 1% of all MM cases and lacks the hallmark of increased serum or urine M-protein or paraprotein. Consequently, the diagnosis of non-secretory MM depends rather on an increase in tumor burden and evidence of end organ damage (9,10). The complex spectrum of physiological impairment attributed to MM include lytic bone lesions, osteoporosis, compression fractures, bone pain and ultimately patient immobility. The abundance of malignant monoclonal PCs also severely compromises patient immunity and hematopoiesis (11).

The inclusion of immunomodulatory drugs (IMiDs) as part of high dose chemotherapy together with systemic and cytogenetic prognostic markers have improved patient survival in MM. Thalidomide, and its derivatives are currently approved for use across all phases of MM therapy. These drugs possess immunomodulatory, anti-angiogenic, anti-inflammatory and anti-proliferative capacity (12). Over the past few decades, a

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**Abbreviations:** MM, multiple myeloma; BM, bone marrow; PCs, plasma cells; M proteins, monoclonal proteins; IMiDs, immunomodulatory agents; ABC transporters, adenosine triphosphate-binding cluster transporters

**Key words:** multiple myeloma, plasma cells, combination therapy, multidrug resistant clones, ABC transporters, relapse, personalized management

30-40% complete response rate and an increase in median survival of 4-5 years have been achieved with these drugs in combination with auto-transplants in younger *de novo* patients (13).

Most MM patients respond successfully to initial induction therapy, however, all the patients eventually relapse, forcing a review of the treatment regimen (14). A significant contributor to treatment failure leading to clinical relapse is the emergence of multi-drug resistance (MDR) (15). MDR is the phenomenon whereby the cancer cells become resistant to a wide variety of structurally and functionally unrelated drugs following exposure to a single chemotherapeutic agent (16-18). Existing measures for assessing the clinical state of MM patients include serum markers [immunoglobulins,  $\beta_2$ -microglobulin ( $B_2M$ ), free light chain assays, creatinine, C-reactive protein (CRP) and thymidine kinase] followed by confirmation with invasive bone marrow biopsy. However, these do not offer a direct measurement of the presence or the evolution of proteins responsible for drug resistance on malignant PCs. MM is characterized by the presence of multiple clones with differing degrees of drug sensitivity at the time of diagnosis. Consequently, despite complex chemotherapeutic regimes (19), therapeutic response is unpredictable and extremely variable with MM patients. Furthermore, bone marrow biopsy cannot assess the patchy tumor infiltrates in multiple sites associated with MM and provides an indirect measure of tumor burden distributed throughout the skeletal system. This impacts the quality of life for the patient and translates to highly heterogeneous patient survival rates ranging from a few weeks to more than 10 years (20).

Aside from the significant physical and emotional costs associated with the emergence of MDR and subsequent relapse, there are also significant financial costs incurred with the management of MDR. The drugs used at relapse are typically novel, costly and with associated side effects. The estimated cost of an effective melphalan, prednisone and velcade regimen approximates \$119,102 (US), while a novel superior regimen utilizing melphalan and prednisone combined with lenalidomide maintenance can reach as high as \$248,358 (US) (21). Consequently, MM remains one of the most costly cancers to treat when total treatment costs are considered (21-24).

Here, we review the factors limiting the successful treatment outcome in the complex multiple myeloma clinical setting. We focus on the persistent issue of drug resistant clones in MM and the major role played by ATP-binding cassette (ABC) transporters along with other resistance mechanisms in relapse in the era of novel therapeutics.

## 2. Normal plasma cell characteristics

MM is a hematological malignancy characterized by the accumulation of aberrant PCs in the bone marrow (25). PCs are terminally differentiated activated B cells retained in the G1 phase of the cell cycle (26). PCs express surface markers that are reflective of their elaborate maturation and differentiation process. PCs typically can be distinguished from naïve B cells by the lack of CD10, CD19 and CD20 expression on their surface (27). Two specific surface antigens on PCs are CD38 and CD138 (28-31). CD38 is an ectoenzyme important

in signal transduction, cell adhesion and calcium signaling, and is expressed across all PC developmental stages (28). CD138 is a *trans* membrane proteoglycan that facilitates cell binding, cell signaling, cell-cell and cell-extracellular matrix interactions (32). Amongst the typical markers expressed on PCs, CD45 is considered an early PC marker (plasma blasts) (27). According to the maturation stages, PCs are grouped into plasma blasts (CD138<sup>-</sup> CD45<sup>++</sup>), early PC's (CD138<sup>+</sup> CD45<sup>+</sup>) and mature PC's (CD138<sup>++</sup> CD45<sup>-</sup> or weak CD45 expression) based on the antigen expression on their surface (5).

PCs are prime mediators of the adaptive immune response (5,26). The development of a normal B cell starts in the bone marrow (BM) and matures following migration into the peripheral lymphatic organs. The maturation process is aided by antigen exposure, dendritic co-stimulatory signals and somatic mutations that ultimately result in high affinity antibody production (3). B cells with high affinity antigen receptors further differentiate into memory cells and plasma blasts. Eventually, highly efficient PCs that survive these processes (long-lived PCs) migrate back to the bone marrow and localize in 'niches', which aid in the further differentiation and longevity of the immune response (3,33-35).

## 3. Pre-malignant plasma cell characteristics - monoclonal gammopathy of undetermined significance

Monoclonal Gammopathy of Undetermined Significance (MGUS) is a benign condition that can precede malignant transformation to MM (36). Clinically, MGUS is characterized by excessive PC growth whilst retaining a stable M-protein profile (37). Serum M-protein levels of <3g/dl, small amounts of monoclonal light chains in urine, the absence of end organ damage, absence of lytic bone lesions, anemia and hypocalcaemia define the pre-malignant condition MGUS (38). The rate of transition from MGUS to MM is ~1%/year (36,38).

## 4. Malignant plasma cell characteristics

The exact cause of malignant transformation of PCs remains unknown. However, ras mutations are absent in pre-malignant MGUS and are observed in MM (39). It has been suggested that the myeloma clone arises from a pre-switched B cell (40), preconditioned as a result of prior exposure to certain triggers (i.e. viruses, chemicals and radiation). Other reasons proposed are an incompetent immune system, age and a family history of lymphato-hematopoietic cancer (36).

In malignant cells, the genotype is aberrant with frequent chromosomal deletions or hyperdiploidy (chromosomes 3,5,7,9,11,15,19 and 21) that results in abnormal functions of cell cycle regulatory genes (*cyclin D1*, *D2* and *D3*) (41). Malignant PCs also present with aberrant phenotypes at diagnosis. Surface markers such as CD56, CD117 and CD20 are found in decreasing order of expression on aberrant PCs. Isolated strong CD56 expression is common in MM and can be used to distinguish MM from MGUS, while CD56<sup>-</sup> phenotype is said to be associated with a high risk subtype with chromosomal abnormality [t(11;14)] in terms of survival (42-44). Malignant PCs also display an increased expression of various adhesion molecules compared to non-malignant PCs. Fibronectin receptor, very late antigen 4 (VLA-4), the lympho-

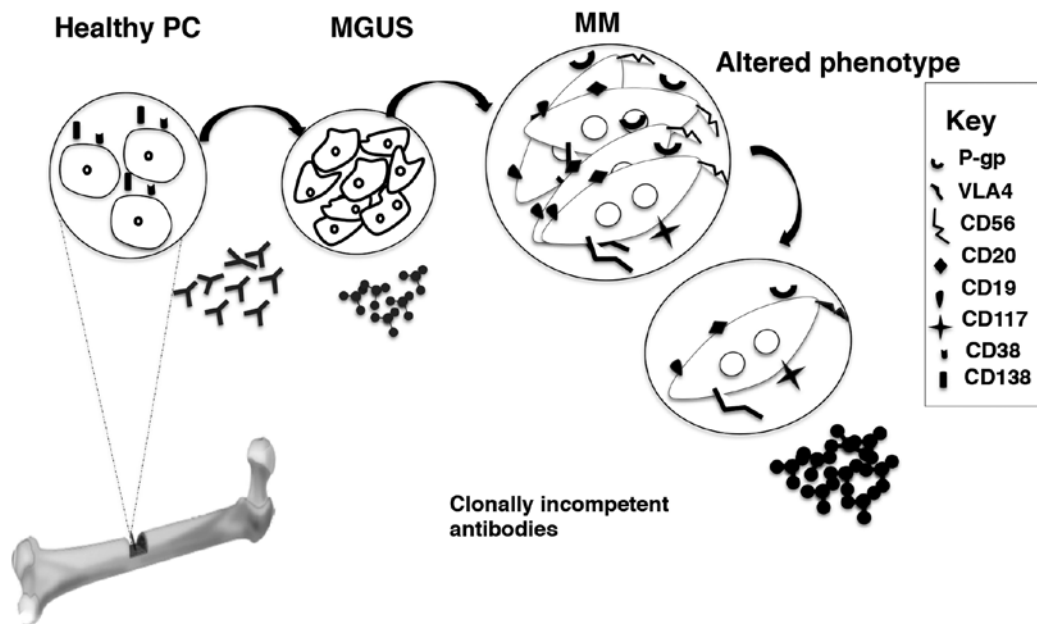


Figure 1. Malignant transformation of PCs: terminally differentiated healthy PCs reside in the bone marrow and typically express CD138 and CD38. Healthy PCs are major components of humoral response. The pre-malignant condition monoclonal gammopathy of undetermined significance (MGUS) is characterized by the presence serum M-protein and monoclonal light chains in serum and/or urine, however, patients remain well. In the malignant phase, PCs have aneuploidy, altered surface expression and patients experience high serum/urine levels of M or paraproteins along with other classic MM manifestations. In relapse, the MM initiating cells or 'side population' cells have immature B cell phenotype than the mature PCs. Major signaling pathways are aberrant resulting in the clonotypic MM cells. Clonotypic MM cells are highly proliferative and over express various MDR pumps (P-gp, MRP1 and BCRP) and early B cell marker on their surface and transporters such as vault proteins (LRP).

cyte homing receptor CD44 and neural cell adhesion molecule (N-CAM and CD56) are abundantly expressed on malignant PCs (45). In contrast, VLA-5, the laminin receptor VLA-6, and the vitronectin receptor CD51 are weakly expressed (45). In advanced MM, mature PCs escape the bone marrow niche and are found in circulation (46). Interestingly, only 50% of the circulating population expresses CD138, which is widely considered to be an exclusive mature PC marker of hematopoietic origin (27,47). Consequently, circulating PCs are also classified according to the presence or absence of CD138 apart from the maturation based classification of normal PCs mentioned above (27). The circulating CD138<sup>+</sup> PCs are thought to be plasma blasts as they express CD45, CD20, CD19 and human leukocyte antigen (HLA)-class II and more actively proliferating (5,47,48) (Fig. 1).

## 5. Multiple myeloma

**Diagnosis.** MM is diagnosed when M-protein or paraprotein exceeds 3 g/dl in serum or urine (49), when there are 10-15% aberrant PCs in the bone marrow and by the presence of skeletal lesions (5,37,50). An abnormal ratio of serum  $\kappa$  and  $\lambda$  free light chains above the normal range of  $\kappa/\lambda$  of 0.26-1.65 provides an alternative criterion if the M-protein status is not conclusive (9,10,50). In the case of 'non-secretory myeloma',  $\geq 10\%$  baseline clonal bone marrow PC provides the main criterion of diagnosis with renal and skeletal manifestations of MM (9,50,51).

Other clinical manifestations alone or in combination are also considered at diagnosis. These include elevated calcium levels or hypercalcemia  $>11.5$  mg/dl/ $>2.65$  mmol/l indicating

defective bone physiology, renal insufficiency signified by creatinine  $>2$  mg/dl/ $177$   $\mu$ mol/l or more, anemic hemoglobin levels of  $<10$  g/dl or  $2$  g/dl  $<$  normal levels or hemoglobin  $<12.5$  mmol/l or  $1.25$  mmol/l  $<$  normal levels, and bone lesions or pain (9). International uniform response criteria by International Myeloma Working Group recommends that amyloidosis and/or systemic light chain deposition disease (LCDD) should be correspondingly categorized as 'myeloma with documented amyloidosis' or 'myeloma with documented LCDD', requiring confirmation through bone marrow biopsy to ascertain the existence of  $\geq 30\%$  PCs and/or myeloma-related bone disease. Following diagnosis, MM patients are usually placed on induction therapy with conventional or novel agents followed by autologous stem cell transplant depending on eligibility of each patient (52). Response to treatment is subsequently evaluated through regular monitoring of serum and urine M-protein levels by immune fixation and confirmed by periodic bone marrow aspiration (53).

**Staging criteria.** MM is a highly heterogeneous disease with respect to survival and clinical manifestations (54), hence it is difficult to accommodate every criterion in one staging system (55). In 2005, the International Myeloma Working Group established the International Staging System (ISS) for MM (Table I) (54). Until 2005, MM staging predominantly relied on the Durie-Salmon Staging (DSS) system, which was established in 1975 (56). DSS correlates various biochemical factors with tumor burden for staging of malignancy. This makes it difficult to achieve consensus across various laboratories (57). The advantage of ISS is that it is a statistical model, which emphasizes the duration of survival based on

the measure of two parameters, B<sub>2</sub>M and serum albumin (55). ISS uses B<sub>2</sub>M as a measure of the rate of myeloma growth with serum albumin indicative of tumor burden (54,55,58). Since its launch, ISS has been validated, is statistically easier to assess and is more robust compared to the DSS system (55,59).

**Genotype and multiple myeloma.** Myeloma, unlike other hematological malignancies, is uniquely characterized by intricate cytogenetic and molecular genetic abnormalities resonant of epithelial tumors (60). A *de novo* patient usually presents hyperdiploid with multiple trisomies or hypodiploid with one of several types of immunoglobulin heavy chain (IgH) translocations (61). The importance of cytogenetic markers and gene profiling on therapeutic decision making is becoming increasingly evident in MM (62).

Chromosomal abnormalities associated with immunoglobulin heavy chain translocations result in abnormal gene regulation in MM (63). Cell cycle regulatory genes are impaired in MM and the dysregulation of *cyclin D1*, *D2* or *D3* is considered to be an initial oncogenic pathway in MM and MGUS (64). 25% of IgH translocations in MM directly affect *cyclin D1* (11q13), *cyclin D2* t(4;14), *cyclin D3* (6p21) or musculoaponeurotic fibrosarcoma (*MAF*) oncogene (c-*MAF*, 16q23 or *MAF* oncogene homolog B (MAFB), 20q11 (41,64). The recurrent translocations associated with MM are t(4;14)(p16;q32), and t(14;16)(q32;q23) which are correlated with a negative prognosis (61). Myeloma patients frequently present with chromosomal deletions of 13q14 and 17p13 (63). Several other genetic components such as tumor suppressor genes (p53, phosphatase and tension homolog-PTEN), retinoblastoma protein-Rb protein) and transcription factor, myelocytomatosis viral oncogene homolog (c-*myc*) also show abnormalities in MM, however, the exact origin of these genetic and epigenetic changes in the course of MM pathogenesis is not known yet (39).

Recently, the role of short non-coding RNAs (19-25 bp) in MM has been examined (65). A small number of microRNAs (miRNAs) are implicated in MM pathogenesis (65). Pichiorri *et al* demonstrated distinct miRNA profiles for malignant PCs (MGUS and MM) compared to those of normal PCs. In MGUS, *miR-21* and *miR-106b~25* clusters with oncogenic function are upregulated with *miR-21* blocking apoptosis (66). *miR-106b~25* has been shown to regulate pro-apoptotic genes and play a role in pathogenesis (67). It is believed that *miR-21* and *miR-106b~25* potentially initiate the lymphoproliferative transformation of PCs by hindering apoptosis, promoting survival of malignant cells and predisposing to secondary genetic abnormalities, leading to malignancy (65). Compared to the normal PCs, *miR-32*, *miR-17~92*, *miR-21*, *miR-106~2*, *miR-181a* and *miR-181b* are upregulated in MM. *miR-15a* and *miR-16-1* are implicated in regulating tumor proliferation in MM that are located in 13q14.3 which coincides with a frequent deletion in MGUS and MM cohort (65,68,69).

### Disease presentation

**Systemic monoclonal protein (M-protein or paraprotein).** Monoclonal protein (M-protein or paraprotein) production, is a salient feature of secretory MM (70). Based on immunoglobulin heavy chain structure, MM is classified into IgG, IgD and IgE subtypes of which IgG MM is most common (11).

Paraproteinemia and an associated hyperviscosity syndrome, arising from elevated systemic M-protein levels are typically associated with MM (11,71). Approximately 25% of MM patients present with paraproteinuria resulting in renal insufficiency, while ~50% have renal failure (11,37,50,72) resulting from direct damage and blockage to the kidney (73). Other MM associated renal complications include, myeloma cast nephropathy, amyloidosis, fibrillary glomerulonephritis, immunotactoid glomerular nephritis and light chain deposition disease (72).

**Immune incapacity.** MM patients are immunocompromised due to the defective hematopoiesis and the aberrant PCs producing clonally incompetent M-proteins. This is in addition to the gradual reduction in immune competence coinciding with late middle age (40). Yaccoby *et al* proposed limited mobility in the aged population resulting in reduced exposure to antigens as the potential reason for the reduced differentiation rate of the memory B-lymphocytes to PCs (74,75).

The manipulative tumor cells strategically elude the immune watch and facilitate tumor survival. One such mechanism is the phenomenon of 'trogocytosis' in which the surface antigen exchange occurs in lymphocytes creating unique cell phenotypes with specific function (76). The immune synapse facilitates unique cell types to maintain intracellular signaling in T cell subsets and aid in tumor-induced immune suppression (77). The phenomenon of trogocytosis is more common in MM compared to other mature B cell malignancies and T cells are more proficient in acquiring antigens from malignant PCs (78). Impaired immune system in MM patients also leads to recurrent infections with a life-changing impact on patients and care givers (79).

**Microenvironment-dependent disease manifestations.** One of the characteristic features of MM is the tendency of aberrant PCs to be confined to the bone marrow. Malignant PCs favor a microenvironment analogous to normal long-lived PCs (3,74,75) and tend to migrate to peripheral blood only in the terminal stage of the disease (3,45,74,75). These malignant PCs evolve 'autocrine growth supporting loops' at this terminal stage which facilitate microenvironment independent survival (35). The adhesion of MM cell with bone marrow stromal cell orchestrates homing via adhesion to the endothelium, invasion through the sub-endothelial membrane, and chemotactic migration within the bone marrow stroma (35,45) (Fig. 2).

Aberrant PC interaction with bone marrow stromal cells (BMSCs) and extra cellular matrix (ECM), subsequently alter the normal microenvironment to tumor advantage (80,81). Cytokines such as interleukin 6 (IL6), vascular endothelial growth factor (VEGF), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), insulin-like growth factor 1 (IGF1) support the growth of MM cells (82,83). Along with IL6 and IGF1, IL21 promote the tumor survival while VEGF plays a role in MM cell migration with stromal cell derived factor-1 $\alpha$  (SDF-1 $\alpha$ ) (84-87). The initial binding between MM cells and bone marrow stromal cells is mediated via adhesion receptor integrins (integrin  $\alpha 4\beta 1$ , VLA4), through their ligands [vascular cell adhesion molecule 1 (VCAM1)] (88,89). The binding, further, upregulates cytokine and/or chemokine release from

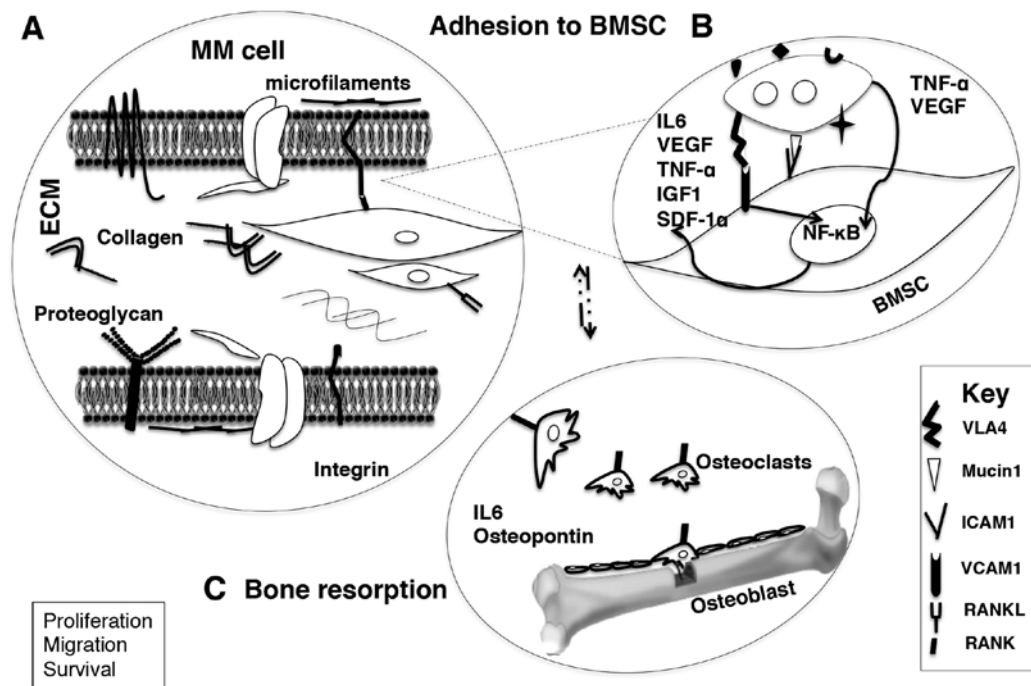


Figure 2. Microenvironment-dependent factors in MM: (A) Aberrant PCs homing to the microenvironment is mediated by integrin mediated adhesion to extracellular matrix (ECM) and bone marrow stromal cells (BMSCs). (B) The growth, survival and migration of an aberrant PC is cytokine mediated and facilitated by the adhesion of aberrant PC to the BMSC. In BMSCs, this contact triggers the cell signaling pathway and nuclear factor- $\kappa$ B and subsequent secretion of various adhesion molecules/cytokines on both cells. (C) Adhesion to the BMSC is also involved in the bone resorption. MM cells stimulate RANKL expression on BMSCs and reduce osteoprotegerin expression to promote osteoclastogenesis. Adhesion of MM cells to osteoclasts follows with the over production of osteopontin and IL6, supplementing MM cell growth and survival.

stromal cells to the microenvironment (Fig. 2A). In addition, the transcription factor nuclear factor- $\kappa$ B (NF- $\kappa$ B) plays a significant role in the initiation of various cell-signaling pathways in MM cell and BMSC following the adhesion (84,90). The adhesion of MM cell to stroma triggers NF- $\kappa$ B and mitogen-activated-protein kinase (MAPK) signaling cascade in BMSC, which in turn results in a change in phenotype of MM, and BMSC with co-expression of adhesion molecules. Subsequently, cytokines secreted from MM cells trigger inflammatory cytokine production and NF- $\kappa$ B activation in BMSC (IL6, TNF- $\alpha$  and VEGF). The inflammatory cytokines from BMSCs trigger signaling pathways in MM cells (MAPK, phosphatidylinositol 3 kinase/protein kinase B (PI3/AKT), Janus kinase/signal transducer and activation of transcription 3 (JAK/STAT3) pathways which enhance proliferation, cell cycle modulation and tumor survival via activation of antiapoptotic signals (91-93) (Fig. 2B).

Osteolytic lesions, compromise mobility, can result in spinal cord compression and moderate to severe nerve damage in MM. In fact, morbidity and mortality in MM is mostly associated with osteolytic lesions (80,81). Abe *et al* (81) demonstrated that peripheral blood mononuclear cell-derived osteoclasts enhance MM cell survival and growth in primary MM, as well as MM cell lines than stromal cells (75,80,81). Receptor activator of nuclear factor  $\kappa$ B (RANK) on the surface of osteoclasts and the ligand (RANKL) expressed on the BMSC activate the osteoclasts while osteoprotegerin on BMSCs a decoy ligand of RANK prevents RANK-RANKL communication (89). Manipulative MM cells stimulate RANKL expression on BMSCs simultaneously reduce osteoprotegerin expression which accordingly promotes osteoclastogenesis.

Consequent adhesion of MM cells to osteoclasts enhances the production of osteopontin and IL6, which augments MM cell growth and survival (88,89) (Fig. 2C).

## 6. Therapy

Treatment of MM typically involves combination chemotherapy including cyclophosphamide or melphalan, a steroid (dexamethasone or prednisolone), a novel agent [e.g. proteasome inhibitor, immunomodulatory drug (IMiDs)] and may be followed by autologous stem cell transplant depending on the age at diagnosis (2). Treatment of progressive MM consists of induction, maintenance and supportive regimens (50). In patients below 65 years of age, autologous stem cell transplant (ASCT) is considered (13). In many cases a single autologous stem cell transplant can result in progression-free survival in comparison with chemotherapy alone (94).

The IMiDs and the proteasome inhibitors (e.g. bortezomib and carfilzomib) have provided significant improvements in survival and quality of life in MM (95). IMiDs are structural and functional analogs of thalidomide that have potent immunomodulatory properties, anti-myeloma activity and better tolerability profiles (96). Thalidomide was the first immunomodulatory agent approved for use in MM. It is highly active against MM, however, is limited by considerable toxicity, particularly in older patients (97). Lenalidomide, an analog of thalidomide, possesses more potent activity with less toxicity and consequently is preferred for use across phases of MM treatment (98).

Thalidomide monotherapy when used for induction therapy produces a low response rate of ~35% (99,100). In the context

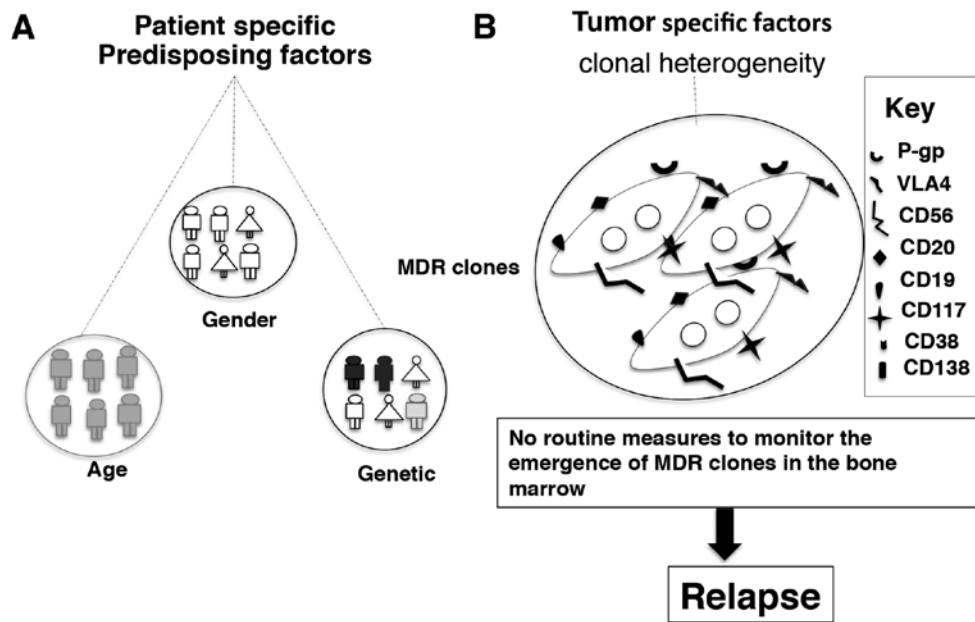


Figure 3. Patient and tumor specific factors impacting on predisposition and treatment in MM: (A) The demographic (age, gender and genetics) variables cause relative pre-disposition of MM in patients. The incidence of MM increases with age and MM manifestations mimic ageing symptoms. MM is more common in males than females. African Americans are more predisposed for MM in comparison with Caucasians or Asians. (B) Myeloma is incurable despite most patients responding to initial high-dose induction therapy and the introduction of novel class of drugs (proteasome inhibitors, IMiDs). The eventual relapse is a result of vigorous changes in MM biology and the development of drug resistance during the course of treatment. Currently, the emergence of MDR<sup>+</sup> MM cells is overlooked in the clinical setting.

of relapsed disease, thalidomide monotherapy results in a median event-free survival of 6-12 months and median overall survival of 14 months (101). Thalidomide's combination with dexamethasone improves the rate to 60-75% and is associated with a high incidence of grade 3-4 toxicity (102-104). For relapsed MM, the addition of an alkylating agent (cyclophosphamide or melphalan) further increases the response rate to 75-80% (105,106). In comparison with the response rates achieved using novel agents such as bortezomib or lenalidomide, thalidomide monotherapy is not superlative. In addition, combination of thalidomide with cytotoxic agents such as doxorubicin or cyclophosphamide, improves the response rate and quality of response further. Consequently, a three-combination regimen is more commonly used when thalidomide induction is considered (104). However, for consolidation/maintenance therapy, the impact of thalidomide on therapeutic outcome remains unclear. Results obtained from the British Myeloma Research Council Myeloma IX study demonstrates that thalidomide is associated with shorter post-relapse survival suggesting that thalidomide maintenance may induce drug resistance compromising duration of response and survival especially in patients with high risk genotype [t(4;14), t(14;16), t(14;20), 1q21amp, del(17p)] (107,108).

Other novel agents like thalidomide derivatives (lenalidomide) and proteasome inhibitor (bortezomib) combination chemotherapy increases the overall response rate to 90% or above (109-112).

A complete remission or complete response (CR) in MM is clinically defined as negative serum and urine immunofixation, no plasmacytoma and  $\leq 5\%$  PCs in bone marrow for at least 2 months (113), whereas partial response is stated by  $>50\%$  reduction of serum M-protein and  $>90\%$  of Bence Jones protein (113,114).

**Defining clinical relapse.** The malignant PCs enter a static phase with typically lower levels of proliferative markers such as thymidine kinase, high sensitive CRP marking the remission status of MM patient after successful induction therapy (115-117). However, MM cells eventually overcome this passive phase and become aggressive within a short space of time (118). This complex process is said to include loss of immune regulation, clonal evolution, cytokine deviance, oncogene stimulation and/or tumor suppressor gene anomaly (118). The mechanisms underlying initiation, a prolonged asymptomatic stage, progression and aggressive transformation of PCs are not yet clear (118). The failure of the current chemotherapeutic regimen to eliminate the malignant clone in MM is considered to be one of the major causes of consecutive relapse (118). Relapse from a complete response is clinically defined by the reappearance of the serum or urine M-protein (paraproteinemia),  $\geq 5\%$  bone marrow PCs, new lytic bone lesions and/or soft tissue plasmacytomas, an increase in the size of residual bone lesions and/or the development of hypercalcaemia (corrected serum calcium  $>11.5$  mg/dl) not attributed to another cause (114,119).

## 7. Patient-related predisposing factors complicating diagnosis and treatment in MM

**Patient age and gender.** The incidence and risk of developing MM increases with age, with predominantly 80% of affected patients being above the age of 60 (1,5,120). The classic disease manifestations in MM such as anemia, bone pain and associated fracture and renal involvement imitate the complications associated with ageing process (36). Consequently, patients discount the warning signals, which results in delayed diagnosis, which severely compromises the accessible therapeutic

decisions for the elderly patients. Myeloma is more common in men than women for reasons yet unknown (5) (Fig. 3A).

**Ethnicity.** The incidence MM is lowest among those of Asian descent, is intermediate in Caucasians and is highest in African Americans (25,121,122). Various independent studies have suggested that there may be a greater genetic predisposition to MGUS in Africans and African Americans than in Caucasians (123). Although the reason for this genetic pre-disposition is not known, a small number of studies have revealed that the variation in the prevalence of immunoglobulin subtypes and the overexpression of either  $\kappa$  or  $\lambda$  free light chain ratios in different races may contribute to the differential cytogenetic susceptibility between races (123). The presence of a rare deletion of 193 bp in the long arm of the pseudogene [poly(ADP-ribose) polymerase-allele B] of chromosome 13 (negative prognosis in MM) is more frequent in African Americans than Caucasians (69). Although the etiology of MM remains unknown, a family history of hematological disorders, either alone or combined with exposure to certain viruses, radiation and chemicals, is a proposed risk factor (36) (Fig. 3A).

## 8. Tumor and treatment-associated factors complicating treatment

**Clonal evolution.** Numerous studies have confirmed the presence of tumor-initiating cells (stem cells) in the bone marrow and their role in disease relapse (48,124). The primary bone marrow contain a small population of clonotypic B cells with an immature phenotype (CD138<sup>-</sup>) known as 'side population' or MM initiating cells with stem-cell characteristics besides the malignant 'main population' (48). These cells contain more quiescent cells than 'main population' cells in cell cycle analysis. The MM stem cells or 'side population' (SP) cells are enriched source of cancer stem cells and characteristically show low staining of Hoechst 33342 dye, have high clonogenic potential and possess self renewal capacity (48,125). The SP cells contain hypermutated Ig genes, overexpress members of the ABC transporter family such as permeability-glycoprotein (P-gp), multi drug resistance-related protein 1 (MRP1) and breast cancer related protein (BCRP) much like the stem cells (126). The self-renewal capacity of the clonotypic MM cells is mainly attributed to the abnormal signaling pathways found in MM such as Hedgehog, Notch and Wnt signaling pathways (126).

The overexpression of drug efflux pumps is known to compromise the treatment outcome in MM (124). As mentioned, the side population has high expression of MDR proteins. The inability of chemotherapeutics to eradicate MM clones is a major limitation in MM management and a major cause of relapse (127). The detrimental MM clone is persistent during the remission phase and possess high proliferating potential once activated (118). The presence of drug efflux pumps further adds to the deleterious potential of the aforementioned MM clone and cause inevitable relapse (124) (Fig. 3B).

**Multidrug resistance.** Primary or acquired drug resistance is a major obstacle in MM therapy. In the past, conventional chemotherapeutic treatment of MM, was primarily focused

on alkylator and corticosteroid based regimens (VAD regimen-vincristine, adriamycin or doxorubicin, dexamethasone) (128). The current therapeutic regimen includes IMiDs, proteasome inhibitors to improve outcome in MM patients. However, overexpression of MDR genes, topoisomerases and glutathione transferases mediate drug resistance in MM and many cancers (129). Cell adhesion mediated drug resistance (CAM-DR) and overexpression of anti-apoptotic proteins are typical resistance mechanisms also contributing to relapse in MM (130,131).

**Topoisomerase II.** Topoisomerase II (topo II) is a 170-173 kDa homodimeric protein involved in DNA replication, recombination and gene transcription (132,133). Topo II is an ideal drug target and anthracyclins (doxorubicin), anthracenediones (mitoxantrone) and intercalating agents (acridines) are the main topoisomerase inhibitors used in MM therapy. These drugs interact with topo II to form a temporary complex, which prevents chromosome segregation and DNA synthesis (129). Point mutations in essential domains of the malignant PCs modify the drug target topo II by epigenetic changes such as hypermethylation at the CpG. Island of promoter region affecting the gene expression (129). Structural changes to topo II ( $\alpha$  to  $\beta$ ) also contribute to drug resistance to topo II inhibitors used in MM therapy (129). The sub-cellular localization of topo II is also crucial in determining the drug effectiveness and is governed by the adhesion molecule-mediated resistance mechanism in MM (134). Turner *et al* demonstrated that tumor density plays a role in topo II resistance in such a way that in high density MM tumors, majority of the topo II is transported away from the DNA to the cytoplasm and the drugs fail to form cleavable complexes resulting in poor therapeutic outcome (135).

**Glutathione transferases.** Glutathione ( $\gamma$ -glutamylcysteinylglycine) is a tripeptide thiol present throughout the mammalian organ system specifically in the liver and kidney. Physiologically, glutathione plays a critical role in clearance of xenobiotics, harmful radiations and free radicals (129,136). Glutathione transferases (GST) are a family of detoxification enzymes catalyzing the non-covalent or covalent conjugation of glutathione with the diverse detrimental electrophilic compounds. GSTs also sequester toxic compounds and protect the cells from the oxidative stress through inherent organic peroxidase activity. The cytosolic and microsomal GST forms in humans are differentiated as GST- $\pi$ , - $\alpha$  and - $\mu$  of which GST- $\pi$  form is the most common enzyme. The conjugation with glutathione makes the toxic compounds water soluble facilitating an easy expulsion from the cells. In the malignant status, this effective detoxification mechanism also becomes unfavorable. Active GSTs are either increased in the cell or the expression levels of the isozymes are altered to protect the tumor by catalyzing the toxic chemotherapeutics (136,137). Alkylating agents, melphalan and cyclophosphamide used in myeloma therapy are inactivated by GST catalysis resulting in poor therapeutic outcome (129). In addition, high percentage of co-expression of GST- $\pi$  (82%) with P-gp which is another class of MDR protein (72%) in MM relapse is reported by Petrini *et al* (138). This implicates co-operation of two distinct MDR pathways in coordinating poor therapeutic response.

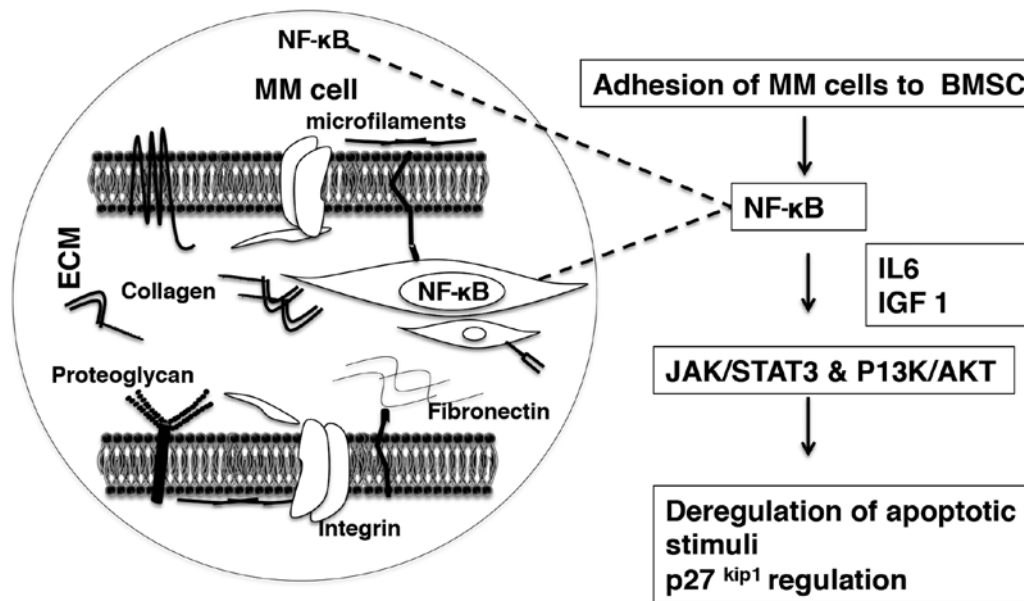


Figure 4. Microenvironment-mediated drug resistance in MM. The adhesion of MM cells to fibronectin in the extracellular matrix (ECM) enhances MM cell survival and growth. The adhesion triggers the deregulation of apoptotic stimuli and facilitates MM growth and survival through NF-κB pathway activation. The alteration of drug target (topo II), cytokine (IL6 and IGF1) mediated upregulation of cell signaling (JAK/STAT3 and PI3K/AKT) cascades also play a major role in initial drug resistance in MM.

**Microenvironment-mediated drug resistance.** Apart from microenvironment-mediated pathogenesis mentioned above, components of the bone marrow microenvironment contribute to treatment unresponsiveness in MM (139,140). The microenvironment related resistance mechanisms could be classified as integrin mediated adhesion to ECM (fibronectin) disrupting the apoptotic stimuli through cytokine-mediated upregulation of cell signaling and caspase mediated apoptotic cascade in MM cell. Microenvironment-dependent drug resistance in MM is considered as a bonus mechanism in MM cells by which the drug resistant cells are selected early on during initial therapy and they later acquire more explicit drug resistance during the course of chemotherapy (141).

**CAM-DR.** CAM-DR is induced following the interaction of malignant PCs to the ECM (141). Aberrant PCs express a variety of cell adhesion molecules, which function as cell-to-cell and cell-ECM through counter receptors. Fibronectin mediated adhesion has been shown to increase the tolerance of MM cell line (RPMI-8226) to chemotherapeutic agents and the induction of drug resistance in MM cells by suppressing apoptosis (142). Integrin molecules such as the VLA4, VLA5 and their respective receptors govern this resistance mechanism. The integrin molecules act as extrinsic factors eliciting intracellular response through focal adhesion points that stimulate signaling pathways and cytoskeletal modification (141). Damiano *et al* (142) demonstrated that the initial integrin mediated adhesion to fibronectin enhances MM cell survival and protects against apoptotic stimuli from doxorubicin and melphalan aiding tumor survival (141). The mechanism of CAM-DR can also be through blocking a specific element of the caspase mediated apoptotic pathway as shown by Shain and Dalton (141) in MM cell line RPMI-8226. The study showed direct inhibition of mitoxanthrone-induced caspase-3 and -7 cleavage. Once adhered to fibronectin, the cancer cells

use the microenvironment in a number of ways to develop *de novo* drug resistance such as overexpression of cell cycle regulatory protein (p27<sup>Kip1</sup>), alterations to drug target and by facilitating integrin mediated cell signaling and cytoskeletal reorganization (Fig. 4).

**Cytokine-mediated drug resistance.** The MM cell-BM microenvironment cytokines regulate apoptosis and MM cell survival through their participation in PI3K/AKT and JAK/STAT3 signaling pathways (84). Novel and conventional chemotherapeutics in MM target the caspase-mediated apoptosis pathways. Caspase-8/3 mediated death receptor pathway (IMiDs, melphalan) and caspase-9/3 mediated mitochondrial intrinsic pathway (dexamethasone) follow subsequent poly-(ADP-ribose) polymerase (PARP) cleavage resulting in apoptotic death of MM cells (92,143-145). The proteasome inhibitor class (bortezomib) targets both caspase-8/3 and caspase-9/3 pathways (146). The IL6 mediated activation of JAK/STAT3 signaling cascade results in upregulation of myeloid cell leukemia sequence 1 (MCL1) and B cell lymphoma/leukemia family (Bcl-XL) leading to dexamethasone resistance (147). PI3K/AKT signaling and NF-κB activation in MM cells are coordinated by IL6 and IGF1 by inducing inhibitors of drug-induced apoptosis resulting in treatment unresponsiveness and eventual survival of the tumor (148,149) (Fig. 5).

In conclusion, the MM cell-ECM interactions are a foundation for the *de novo* resistance to chemotherapeutics and thus pave the path for more mutative transformations or acquisition of classical MDR mechanisms during the course of treatment (141,142,150) (Figs. 4 and 5).

**Drug efflux.** Cancer cells often develop cross-resistance (to a large variety of chemically and pharmacologically unrelated drugs leading to the phenomenon of multiple (or multi-drug)



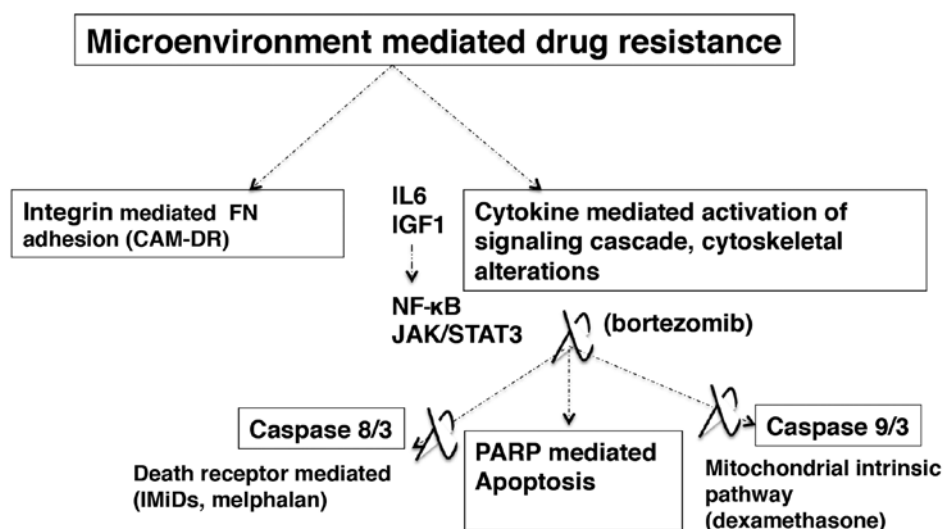


Figure 5. Microenvironment-mediated drug resistance pathways in MM: cell adhesion-mediated drug resistance and cytokine-mediated cell signaling cascade activation contribute to treatment failure in both conventional and novel therapies. Fibronectin-mediated adhesion to the ECM components trigger the cell cycle regulatory proteins (p27<sup>kip1</sup>) limit therapeutic success with doxorubicin. Death receptor-mediated apoptotic (caspase-8/3-IMiDs, melphalan) and mitochondrial intrinsic pathways (caspase-9/3, dexamethasone) and proteasome inhibitor targets both these pathways. Cytokine (IL6 and IGF1) activate NF-κB and JAK/STAT3 pathways, disrupting the apoptotic death of tumor cells.

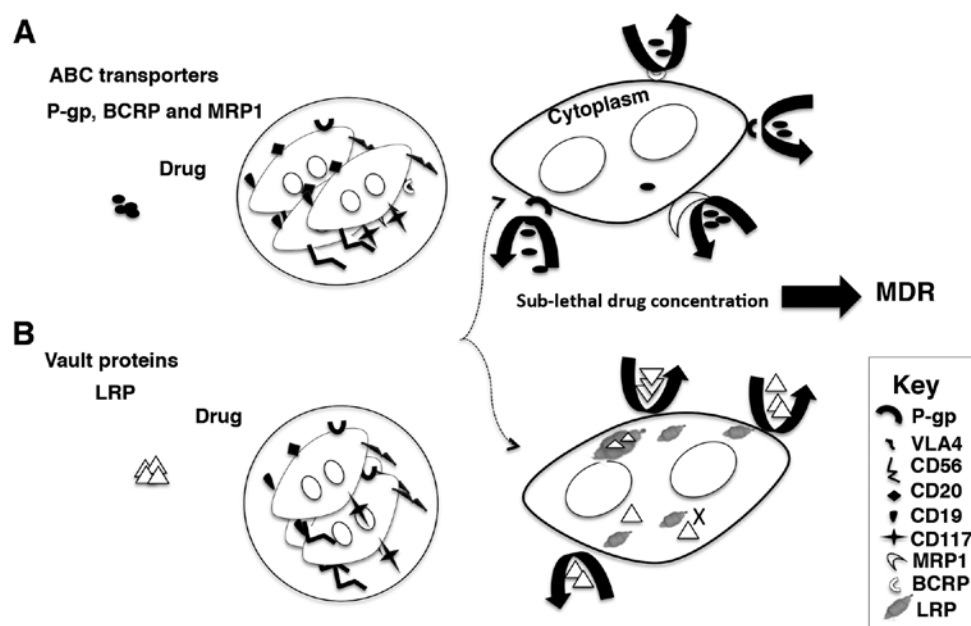


Figure 6. MDR proteins contributing to MM relapse: (A) The MDR<sup>+</sup> MM clones characteristically overexpress the ABC transporters and the vault proteins similar to the stem cell populations. ABC transporters maintain intracellular sub-lethal concentration of the drug resulting in relapse. (B) Vault proteins (LRP), efflux out or sequester its substrate drugs and prevent the entry to the nucleus, which result in treatment failure and MM relapse. In addition, the transporters cause high systemic drug concentration causing serious side effects in patients with renal insufficiency.

resistance (MDR) (17). MDR is mainly attributed to the overexpression of ATP-dependent efflux transporters belonging to the ABC superfamily (17) (Fig. 6). The overexpression of the ATP binding cassette (ABC) transporters on the plasma membranes of malignant PCs contribute to MDR in MM. P-gp, MRP1 lung resistance protein (LRP) and BCRP are all members of the ABC superfamily of membrane transporters and mediate MDR in MM through their drug efflux capacity (130).

In MM therapy, maximal response rates and improved survival is achieved through combination thalidomide therapy.

Combination chemotherapy is however compromised by the overexpression of the multidrug transporters (P-gp, MRP1, BCRP and LRP) on malignant cells, which maintain intracellular drug accumulation deficits in resistant cells. Although there is no current evidence to suggest that thalidomide itself is a substrate of these drug efflux pumps, the drugs used in combination as part of the recommended regimens are themselves substrates of one or more of these efflux transporters (Table II) (151-166). This contributes to compromised therapeutic effects, reduced rates of response and overall survival of the tumor.

Table I. Current International Staging System (ISS) in multiple myeloma.

ISS stage	Criteria	Median survival in months
I	Serum $\beta_2$ -microglobulin <3.5 mg/l Serum albumin $\geq$ 3.5 g/dl	62
II	Neither stage I nor stage III Or (i) Serum $\beta_2$ -microglobulin <3.5 mg/l and serum albumin <3.5 g/dl (ii) Serum $\beta_2$ -microglobulin 3.5 to <5.5 mg/l irrespective of serum albumin level	44
III	Serum $\beta_2$ -microglobulin $\geq$ 5.5 mg/l	29

Table II. Chemotherapeutics used in MM are substrates of MDR proteins.

Drugs	P-gp	MRP1	BCRP	LRP	References
Melphalan	+	+	-	+	(152-154)
Lenalidomide	+	-	-	a	(155-157)
Bortezomib	+	+	-	a	(158,159)
Thalidomide	-	-	a	a	(160)
Prednisone/prednisolone	+	-	a	a	(161,162)
Doxorubicine	+	+	+	+	(163,165,166)
Idarubicin	+	+	-	a	(164,167)

<sup>a</sup>Insufficient published data to comment.

*P-gp*. The *ABCB1* gene located on the long arm of chromosome 7 encodes 170 kDa P-gp (17). It is a cell surface protein distributed throughout the human body and is typically found in pharmacological interfaces protecting the cell from carcinogen or xenobiotic influx (17). It has been established that an excessive amount of *ABCB1* leads to MDR in many cancers (17). P-gp mediated drug efflux sustains a sub-lethal amount of drug concentration in the intracellular environment enabling the cancerous cell to evade the toxic chemotherapeutic insult resulting in the eventual survival (17).

The main components of conventional induction regimen in MM, the vinca-alkaloid (melphalan), anthracyclines (doxorubicin, daunorubicin) are common substrates of ABC transporters such as P-gp or MRP1 (Table II). Chemotherapeutic resistance in MM patients is frequently associated with the overexpression of P-gp (167). At least 5% of cases of untreated MM presents with P-gp which can compromise the induction therapy outcome significantly (168). In addition, the circulating B cells or the 'side population' in MM express P-gp comprising the resident MDR clone, which leads to MM relapse (169). Nuessler *et al* also reported that 33% of patients at relapse or progressive MM are positive for functional P-gp (170).

Several *in vitro* and *in vivo* pharmacogenomic and pharmacogenetic studies have revealed genetic polymorphisms of the ABC transport proteins as clinical MDR facilitators in MM. These polymorphisms show diverse function and manifestation across different ethnicities and patient cohorts (171-173). Fifty single nucleotide polymorphisms (SNPs) and 3 insertion/deletion

polymorphisms have been identified for P-gp. Of these, three (rs1045642, rs2032582 and rs1128503) were found to have potential therapeutic impact in MM (171,172), though, only rs1045642 (C<sub>3435</sub>T) showed correlation to the overall survival in MM. Minimal linkage disequilibrium was shown for the other two SNPs (171). It is believed that rs1045642 may alter the substrate specificity and influence therapy in MM (173). However, the statistical comparison within the MM patient group of Northern Irish ethnicity showed that although rs1045642 had an influence on overall survival, it was insignificant in statistical comparisons between healthy controls and MM patients (173). Another study involving 115 post-transplant MM patients investigating C<sub>3435</sub>T polymorphism reported that C/T and T/T genotypes showed a longer overall survival than C/C genotype under dexamethasone, adriamycin (doxorubicin) and vincristine (VAD) treatment regimen (174).

*MRP1*. MRP1 is a 190 kDa protein coded by the *ABCC1* gene and located on the plasma membrane of both normal and malignant cells (175). Specifically, in a cell, MRP1 transports multiple organic anions (some glutathione conjugates) protecting against oxidative stress and is reliant on the intercellular glutathione levels for anthracycline transport (176-178). MRP1 gene overexpression results in clinical MDR in patients treated with natural agents such as anthracyclines and vinca-alkaloids (175,179). Abbaszadegan *et al* reported frequent detectable MRP1 mRNA in MM (100%) (167). MRP<sup>ve</sup> cells have been shown to accumulate lower amounts

of drug relative to P-gp, potentially due to their dependence on glutathione metabolism (130,179). The presence of the polymorphism, MRP1/R723Q (p.Arg723Gln) results in changes in the physico-chemical properties (size and polarity) of the protein, and this structural change significantly increase time to progression, progression-free survival and overall survival in a group of MM patients treated with velcade and pegylated liposomal doxorubicin (178). It is postulated that the variance in capacity of MRP1/R723Q isoform in trafficking and expression may be the cause of the antitumor effect of anthracyclins in this study (178). The MRP1 expression and its prognostic significance in MM or in cancer in general is comparatively less studied than P-gp (180).

**BCRP.** Breast cancer resistance protein is another ABC transporter family member with a molecular weight of 72 kDa typically expressed at pharmacological barriers (181,182). Structurally, BCRP encoded by the *ABCG2* gene, consists of a single nucleotide binding domain (NBD) and one *trans* membrane domain (TMD). Consequently, BCRP requires at least two NBDs to function as a drug efflux pump and usually exists as an oligomer (183). BCRP was initially described in MCF-7/AdVrp human MDR breast cancer cell line that did not express P-gp or MRP1 (184,185). In MM, BCRP shows impaired function and is not associated with drug resistance in *de novo* patients (184). However, BCRP is closely associated with the compounding problem of clonogenic potential of MM cells leading to relapse. The 'MM stem cells' or 'side population' (Hoechst 33342 low staining) have higher BCRP mRNA levels and functional activity compared to the rest of the MM cells (main population) (186). Functional BCRP expression in MM is inversely proportional to promoter methylation in *ABCG2* gene in such a way that unmethylated promoter site results in moderate or high BCRP (*ABCG2*) expression (187). Numerous polymorphisms for BCRP have been reported in literature (V12M, Q141K, F208S, S248P, F431L, S441N and F489L) however they have not been linked to MM yet (188).

**Major vault protein (LRP).** LRP is a 110-kDa protein expressed in the kidneys, adrenal glands, heart, lungs, muscles, thyroid, prostate, bone marrow and testis. Most vaults are complex ribonucleoprotein particles comprising two large molecular weight proteins and a small RNA in addition to the 110 kDa LRP. They are mostly present in cytoplasm, with a small fraction present in the nuclear membrane and nuclear pore complex (189). They are assumed to translocate substances across the nucleus and cytoplasm and are said to be involved in MDR (130,190). Raaijmakers *et al* reported the prevalence of LRP in untreated MM patients (153). This study established the relevance of LRP as an independent predictor in comparison with current markers (PC labeling index, serum B<sub>2</sub>M or lactate dehydrogenase level) for therapeutic response and survival in MM patients treated with melphalan (melphalan and prednisone) (153). Thus, screening for LRP prior to treatment to identify the positive population is recommended in therapeutic design in *de novo* MM to circumvent LRP mediated drug resistance (153). There are currently more than 100 polymorphisms identified for LRP (191). LRP expression rather than polymorphic state have been correlated with therapeutic response (192-195).

**Circumvention of MDR.** In the past few decades, substantial research has focused on the development and trial of agents, which can reverse the drug efflux capacity of ABC transporters, in particular P-gp in cancer (15,130,196,197). Indeed, the pharmacological inhibition of P-gp activity has been a major focus in many MM clinical studies (198). In an attempt to circumvent acquired MDR, several inhibitors have been used to improve treatment outcome of patients with MM (16,199-201). The cyclosporin A reversal effect has been evident in phase II studies with MM and acute myeloid leukemia, although phase III clinical trials failed to give the expected response in progression-free survival and overall survival (197). Since the initial successful clinical trials, verapamil and cyclosporin were combined with vincristine, adriamycin and dexamethasone (VAD) in MM, however, these have had disappointing results mainly due to lack of improved efficacy or dose related toxicity (15,196,197).

In conclusion, management of MM relies on combination therapy and different drug resistance mechanisms, topo II $\alpha$  and GST- $\pi$ -dependent resistance, specifically the drug efflux pathways pose a significant challenge in MM clinical setting. Conservative regime in MM, are mostly substrates of ABC-transporters, topo II $\alpha$  and GST- $\pi$ -dependent resistance mechanisms (170). Recent studies have reported that the novel agents are also substrates of ABC transporters specifically P-gp (154,157).

## 9. Discussion

Herein, we explored the relevant innate and acquired challenges associated with the therapeutic management of MM including the role of MDR in therapeutic failure. Many cases of MM with late middle age onset fail to be accurately diagnosed early as recurrent infections, tiredness and bone/joint pain is often associated with normal ageing-related complications.

MM is currently an incurable and chronic disease, with 'non-secretory myeloma' exclusively dependent on frequent bone marrow aspiration for the assessment of molecular, cytogenetic markers including aberrant PC population, and categorizing complete response. Secretory myeloma is partially dependent on bone marrow aspiration for the confirmation of the clinical status (9). This is largely because the malignancy is restricted to the bone marrow and is rarely seen in peripheral blood (202). Current risk stratification in MM is also primarily dependent on cytogenetic markers and is assessed using invasive bone marrow biopsy. Nevertheless, the BM biopsy does not provide a sensitive assessment of genetic abnormalities in multiple tumor sites throughout the skeletal system of MM patients. Therefore, even invasive biopsy is not comprehensive in risk profiling patients with MM.

The current ISS, although, presents with distinct advantages over its predecessors, the precise indication of the higher ISS stage (stage III) is inconclusive in terms of whether it suggests tumor burden/aggressiveness or the level of end-organ damage or both (55). There are several reliable systemic markers present for prognosis, like B<sub>2</sub>M, M-protein, however these markers are insufficient in gauging the transition from the indolent phase of MM to an aggressive disease state (118). In the case of 'non-secretory myeloma', diagnosis and prognosis are further limited as it lacks the typical hallmark of the disease.

T cells proficiently acquire antigens from MM cells over any other cell type and create novel cell types through trogocytosis (76,77). It is not understood clearly if the novel T regulatory cell types provide new ligands for receptors and regulate signaling pathways, however, this mechanism enables the malignant PCs to effectively evade the immune system recognition and thereby stimulate tumor growth (203).

We have very little understanding regarding the intricate cycle of dormant and malignant phase of PCs in MM or in other words how PCs escape the plateau phase in the remission status and become aggressive again in relapse. This phenomenon underlines the fact that even aggressive therapy is not successful in eliminating the neoplastic origin of MM (118). As discussed, the MM stem cell population (SP cells) and circulating CD138<sup>+</sup> PCs are said to have an aggressive proliferative and dissemination capacity (5,27). In addition, they characteristically have self-renewal potential and overexpress the ABC transporters on their surface (124). The persevering MM clone with MDR phenotype potentially lead to treatment failure and currently this aspect is not routinely monitored in the clinical setting. Another complicating aspect of MM is the high heterogeneity in survival amongst patients. MDR phenotype, genetics of MM, including specific IgH translocations and individual immune profiles are potential players with a role in the disparity in survival amongst MM patients. Present systemic markers do not assist greatly in risk stratification, thus, it is more reliant on the cytogenetic markers in this aspect. Therefore, inclusion of more systemic markers, alone or in combination that would aid in early detection, tailor an individualized approach to optimize a prognostic surveillance at diagnosis and after primary surgery is highly recommended in MM (204-207).

The derivatives of thalidomide (IMiDs) have improved overall survival and have increased the cost of treatment significantly. However, in a phase 1 clinical trial conducted in 2011, involving 21 patients with refractory myeloma who were treated with lenalidomide and temsirolimus (mTOR pathway inhibitor-CCI-779), a high concentration of the drug was detected in the blood causing toxicity. The patients experienced unusual side effects such as electrolyte imbalance, rashes, fatigue, and neutropenia. Further investigation of the pharmacokinetic profiles of CCI-779 and lenalidomide suggested a drug-drug interaction, hinting that the disposition of CCI-779 is arguably mediated by CYP3A4/5 and P-gp (208-210). The clinical trial assessed toxicity or adverse effects and response to treatment by serum and urine M-protein quantification every four weeks. There was only limited documented clinical evidence suggesting lenalidomide and P-gp interaction and this possibility was investigated through *in vitro* studies to determine whether lenalidomide can be transported by P-gp. The *in vitro* studies proved that lenalidomide is actively transported by P-gp and this effect was reversed by CCI-779 and verapamil. In addition, *ABCB1* silencing RNA or short interfering RNA (siRNA) knockdown studies *in vitro* also showed more lenalidomide uptake, supporting lenalidomide and P-gp drug-drug interaction (154).

In light of emerging studies that these novel drugs that have been incorporated to MM therapeutic management are substrates of ABC transporters, the situation warrants a re-evaluation of the manipulative power of MM cells (154). It

is evident that opting for more aggressive chemotherapy has brought some promise of prolonging remission and survival in MM. However, this recent study serves as a reminder of aggressive chemotherapy pitfalls of side effects, toxicity and eventual development of MDR phenotype in patients (211). More importantly out of the innate MM complications contributing to treatment failure, MDR is an element that can be modulated and targeted, which, therefore invites specific attention.

The role of polymorphisms in *ABCB1* and *ABCC1* in both the predisposition to disease and the therapeutic outcome of MM have in recent years been studied extensively. The three most common MDR1 SNPs include 2677G>T/A in exon 21 (RefSNP ID: rs2032582), 3435C>T in exon 26 (RefSNP ID: rs1045642) and 1236C>T (RefSNP ID: rs1128503) in exon 12. The 2677G>T/A polymorphism translated into an amino acid exchange from Ala to Ser or Thr at codon 893, affecting the intracellular region of P-gp between *trans* membrane 10 and 11 (212). Both 3435C>T and 1236C>T are synonymous SNPs. The 3435C>T mutation results in a change from cytosine to thymine that translates to isoleucine. It is found in the second ATP binding domain, located between the Q-loop and the second signature motif on the intracellular side of the protein. This SNP is associated with altered MDR1 expression (213). 1236C>T affects the intracellular region of P-gp between the first A-loop and Walker A motif (212) and translates into a glycine residue. These three polymorphisms are and comprise the most common MDR1 haplotype (212). microRNAs, *-miR-15a*, *miR-16-1*, and *miR-17-92* are also shown to play a role in the heterogeneity in the clinical outcome of MM (65,68).

In terms of the therapeutic outcome, Buda *et al* investigated the prognostic role of MDR1 in the outcome of 115 MM patients treated with DAV (dexamethasone, doxorubicin and vincristine) followed by autologous stem cell transplant. This study showed that the C<sub>3435</sub>T polymorphism was prognostic with patients with the C/T and T/T genotypes demonstrating a longer overall survival compared to those with C/C genotype. The same polymorphism was again found to be associated with a longer time to progression and progression-free survival in relapsed and/or refractory MM patients treated with pegylated liposomal doxorubicin in combination with bortezomib (178). The T allele in SNP G2677T/A is likewise associated with a better response to DAV (214) and a better overall survival in MM (215).

The single-nucleotide polymorphism in MRP1 (rs4148356, R723Q) has also been shown to impact on the clinical outcomes of MM patients (178). The MRP1 mutation Arg723Gln has an effect on the protein expression and trafficking, significantly reducing MRP1-mediated resistance to a wide spectrum of drugs. The presence of R723Q results in extended time to progression, progression-free survival and overall survival in MM patients. This has been ascribed to the differential ability of the isoform in trafficking glutathione and/or regulating its expression (178). It is currently unknown whether polymorphisms of BCRP play a role in MM treatment outcome (216).

The integrin mediated (CAM-DR) drug resistance mechanism is considered to enable MM cells to survive the initial drug toxicity, which in the course of therapy aids in selective expression of classical drug resistance pathways such as ABC-transporter overexpression in MM cells (65,68,142).

Current measures of therapeutic response rely on invasive bone marrow biopsy, immunofixation, serum protein electrophoresis, quantitation, measurement of free light chain and CT/MRI scans (217). A full blood count, biochemistry screen, B<sub>2</sub>M and light chain assays are other prominent systemic markers along with radiology used for staging, diagnosis and monitoring in MM (1,58). None of the above markers, however, provide a direct assessment of the emergence of MDR or detect the expression and evolution of resistance markers, polymorphic variants of resistance markers or nucleic acid signatures, which may contribute to disease progression and individual therapeutic responsiveness.

## 10. Conclusion

Cancer biology in general is an intricate process, especially in MM, where individual immunological and tumor profiles change dynamically during the course of treatment. Despite our knowledge of the MM landscape, the intrinsic challenge of heterogeneity provides a significant complication in the management of MM, necessitating individualized analysis of MM pathogenesis and routine monitoring of evolution of drug resistance.

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