Multiple myeloma : new aspects of biology and treatment

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Abstract : Recently, considerable progress has been made in understanding of the biology and treatment of multiple myeloma. Molecular genetic abnormalities such as bcl-2,c-myc, ras, p53, and Rb genes have been identified in this disease and are related to a poor prognosis. Cytokine studies have revealed that interleukin-6 is a potent growth factor for myeloma cells and is also responsible for the progressive bone resorption together with interleukin-1 β and tumor necrosis factor. Myeloablative chemotherapy followed by allogeneic or autologous hematopoietic stem cell transplantation has increased the incidence of complete remission. However, relapses are still observed because of drug resistance of tumor cells. Immunotherapeutic approaches targeting to cell surface antigens and interleukin-6 signals are being developed to further eliminate myeloma cells. Translating new biological advances into treatment protocols is essential to improve the prognosis of multiple myeloma. J. Med. Invest. 44 : 127-136, 1998

Key Words **:** *multiple myeloma, biology, treatment, hematopoietic stem cell transplantation, immunotherapy*

Multiple myeloma (MM) is a disease characterized by the accumulation of neoplastic plasma cells mainly in the bone marrow. The clinical manifestations are heterogeneous, but common complications of MM include recurrent bacterial infections, anemia, osteolytic bone lesions, and renal insufficiency. The pathogenesis of these clinical features depends on aberrant phenotype and genotype of MM cells and monoclonal immunoglobulin which is produced by tumor cells. Recent studies have been devoted to the biology of MM, and new therapeutic strategies have demonstrated an increase of response rate and survival. In this review, we discuss the clinical and laboratory advances that have improved our understanding of the pathogenesis and treatment of MM.

EPIDEMIOLOGY

Although the incidence and mortality of MM have been increasing until recently, MM remains a relatively rare cancer (1). In the United States, MM accounts for 1.0% of all malignancies in whites and 2.0% in blacks. In most countries, men have higher rates than women. Incidence rates in northern Europe are similar to those in North American white populations, whereas the rates are slightly lower in the United Kingdom, eastern Europe, and South Africa. Substantially lower rates are observed in Asian populations. In Japan, the mortality ratio has been increasing to 2.2 per 100,000 populations (2).

The cause of MM remains unknown, however, potential risk factors include radiation and chemical products as carcinogens (1). The incidence of MM increases with age, more so in male, in US blacks, and in cases of immune defects (1, 3). Specific populations at increased risk include patients with monoclonal gammopathy of undetermined significance (MGUS) (4). Long term follow-up evaluation (for 30 years or more) of MGUS has shown that up to 16-40% will develop overt MM, with an annual actuarial risk rate of 0.8% (5). Epidemiological studies have shown similar risk factors for both MGUS and MM. Moreover, the occurrence of MGUS and MM in the same families points to the involvement of common genetic factors in both diseases (4). The strong link between MGUS and MM also supports the two-hit hypothesis of the oncogenesis of MM, which postulates a first oncogenic event that causes MGUS and a second that leads to MM (6). Substantial progress has been made in identifying some of the critical ordered progression from normal plasma cells to MGUS and MM (7).

CLINICAL FEATURES

Clinical presentations of MM are varied and depend on the morphological and immunological type of tumor cells, the extent (or stage) of the disease, and other complications. Monoclonal immunoglobulin is the most consistent biological marker of MM, but 1% of cases are nonsecretory MM. The class of monoclonal immunoglobulin is also related to the clinical characteristics (Table1). Osteolytic bone lesions, anemia, hypercalcemia, renal insufficiency, and recurrent bacterial infections are common clinical features and have prognostic value (8-10). They are associated with the presence of monoclonal immunoglobulin and/or light chain in the serum or urine and correlate directly with total mass of MM cells (11, 12). The pathogenesis of these clinical

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Table 1. Clinical characteristics of multiple myeloma according to the monoclonal immunoglobulin class

Ig class	IgG	IgA	IgD	IgE	BJP
Age (mean)	62	64	56	60	61
Male/Female	1 : 1	1 : 1	2.3 : 1	1.5 : 1	1 : 1
Organomegaly*	10%	10%	50%	30%	20%
Plasma cell leukemia	2%	2%	12%	20%	7%
Azotemia	30%	30%	70%	—	57%
k/λ ratio	2 : 1	2 : 1	1 : 9	2 : 1	1 : 1
Median survival (mo ; k/λ)	35/25	22/19	24/16	13/12	30/10

*Lymph node, liver, or spleen.

features also depends on cytokines produced by MM cells and stromal cells in the bone marrow microenvironment (13).

BIOLOGY

There is evidence that MM cells have an abnormal biology, especially an aberrant differentiation and survival pathway in the bone marrow. They have a marked immaturity culminating in the plasmablastic type and are usually $CD19^-$, $CD56^+$ (whereas normal plasma cells are $CD19^+$, $CD56^-$), and produce low amounts of immunoglobulin (14). The largest number of tumor cells is found in the bone marrow, indicating that there are important receptors on the surface of MM cells that bind to growth factors and matrix proteins in the bone marrow microenvironment. Bone marrow MM cells usually express adhesion receptors such as CD54 (ICAM-1), CD56 (N-CAM), CD40, CD44, CD49d (VLA-4), CD49 e (VLA-5), and CD138 (syndecan-1), but there is considerable heterogeneity within and between patients (15, 16).

Cytogenetic studies are difficult to perform because of the usually hypoproliferative features of MM with a low plasma cell labeling index of 1% or less at diagnosis (17). Chromosomal abnormalities are found in only about 30% of patients at diagnosis, although DNA aneuploidy is noted in up to 80% (18). The characteristic numerical abnormalities are monosomy 13, and trisomies of chromosome 3, 5, 7, 9, 11, 15, and 19. Nonrandom structural abnormalities most frequently involve chromosome 1 with no apparent locus specificity ; 14q32 (immunoglobulin heavy chain gene locus) in 20% to 40% ; 11q13 (bcl-1,cyclin D1 locus) in about 20% but mostly translocated to 14q32 ; 13q14 (Rb gene locus) interstitial deletion in 15% ; and 8q24 (c-myc locus) in about 10% with about half of these involved in a translocation (19, 20). Importantly, abnormalities of 11q or 13q, and 14q32 translocations correlate with the resistance to treatment and short survival characteristic of aggressive disease (21). Karyotypic instability is also detected in MGUS and these genetic abnormalities may prevent the differentiation and normal death of tumor cells (22).

The presence of somatic mutations of the immunoglobulin genes of MM cells with no intraclonal variations indicates that the putative MM cell precursors are already stimulated by antigens and are either memory B cells or migrating plasmablasts (23-25). Therefore, the transformation is thought to occur at the germinal center and MM clone will home to the bone marrow and proliferate and differentiate into mature plasma cells. During the maturation of B cells, the multistep process of chromosome change occurring on one abnormal clone might ultimately lead to clinical MM (26).

Although MM is thought to be a medullary disease, recent studies have shown that many of MM patients have circulating MM cells in the peripheral blood. The absolute number correlates with disease activity in overt MM, suggesting that these circulating MM cells could be responsible for tumor spreading (27).

ONCOGENESIS

Activation of oncogenes could support the further progression of tumor cells. Molecular genetic abnormalities have been reported to involve bcl-1, bcl-2, c-myc, N-ras, p53 and Rb genes, which collectively contribute to the remarkable resistance to drug-induced apoptosis and hence clinical resistance of MM (7). Translocations between 14q32 and its chromosome partners dysregulate oncogenes such as c-myc, bcl-1,and fibroblast growth factor receptor 3, and may be important part of tumorigenesis (28-31).

Mutations of ras oncogenes have been shown to occur in approximately one-third of patients most frequently involving the N-and K-ras genes (32). Recent studies have shown that activation of the N-ras gene can allow IL-6 independent growth of a previously IL-6 dependent cell line, and prevent apoptosis of cells in the absence of IL-6 (33). Ras genes have been found to be more frequently mutated in patients with more severe disease, and often mutations of these genes occur during progression of the disease. Thus, mutated ras which is constitutively activated may allow for the IL-6 independent growth which is often observed at the later stages of MM. K-ras mutations are associated with shorter survival (34).

Overexpression of bcl-2 in tumor cells plays a role in preventing apoptosis and drug resistance. Bcl-2 gene is frequently overexpressed in MM cells (35). Bcl-2 and other related proteins such as $Bcl\text{-}X_L$ have been shown to prevent cell death and maintain the tumor clone (36). Tumor suppressor gene p53 has many effects on cell growth and differentiation, and point mutations of p53 have been identified mainly in plasma cell leukemia (37, 38) (Fig 1). Another tumor suppressor gene, the retinoblastoma (Rb) gene is frequently mutated in MM patients and MM-derived cell lines (38, 39). The $p16^{INK4A}$ is an inhibitor of cyclin-dependent kinases (CDK) 4 and CDK6, and inactivation of p16 gene by hypermethylation occurs in MM, especially advanced disease states (7). Deletions of several different inhibitors of CDKs have been reported in MM cells (40). Cyclin D itself has been shown to be overexpressed in MM and this overexpression seems to

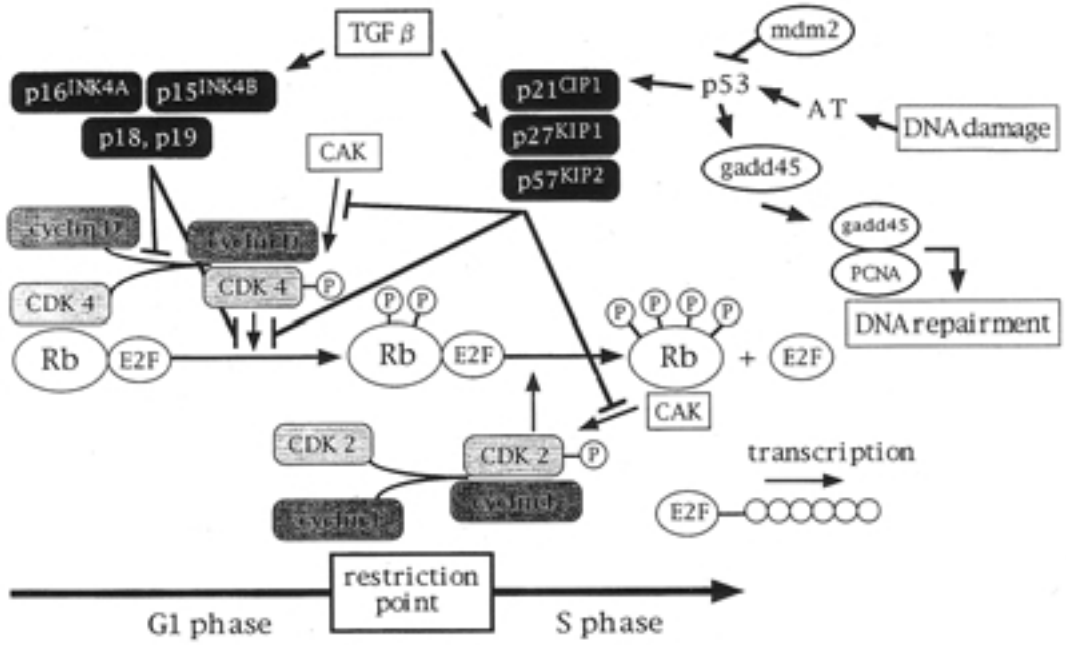

Fig.1. Tumor growth and suppressor proteins involved in cell-cycle regulation.

be a frequent feature of immature types of MM (30).

CYTOKINES

The survival advantage of MM is further enhanced by a number of pathophysiologically relevant cytokines (41). Specifically, interleukin (IL)-6 is a potent factor for the survival and growth of MM cells. The evidence that IL-6 is involved in the pathogenesis of MM was established by the experimental and clinical findings. MM cells produced IL-6 and expressed IL-6 receptor, and IL-6 could induce in vitro growth of MM cells (42, 43). In addition, anti-IL-6 antibodies inhibited the growth of MM cells in vitro and in vivo (44). IL-6 was initially found to be a growth factor for MM cells, but recently it was also shown to promote the survival of MM cells by preventing spontaneous or dexamethasone-induced apoptosis (45). IL-6 also activates osteoclasts close to MM cells and thus promotes bone resorption (46).

The proliferation and differentiation of MM cells are also regulated by a complex of growth factors and cell surface molecules expressed in the bone marrow microenvironment (Fig 2). Recent studies have shown that ligation of CD40 expressed on MM cells upregulates IL-6 secretion in MM cells and growth in an autocrine IL-6-mediated mechanism (47). MM cells can stimulate stromal cells, osteoblasts, and osteoclasts to release large amounts of IL-6. Transforming growth factor β1 produced by MM cells can trigger IL-6 secretion by stromal cells with related paracrine tumor cell growth (48). More recently, the presence of Kaposi's sarcoma-associated herpesvirus has been demonstrated in the bone marrow of MM patients (49, 50). Viral IL-6 produced by bone marrow dendric cells infected Kaposi's sarcoma-associated herpesvirus may be an alternative source for IL-6. The increased levels of IL-6 in the serum of MM patients can be explained by the overproduction of IL-6 mainly in the bone marrow.

IL-6 belongs to a family containing five other cytokines that use gp 130 as a transducer including oncostatin M, leukemia inhibitory factor, IL-11, ciliary neurotropic factor, and cardiotrophin-1. Accordingly, these six cytokines share some biological functions with IL-6 if the appropriate receptor α chain is expressed. Another potential mechanism contributing to the growth and expansion of MM is the agonistic effect of the soluble IL-6 receptor, which can amplify the response of MM cells to IL-6. MM cells shed the soluble form of IL-6 receptor α which is present in high amounts in the serum of MM patients, especially those with a poor prognosis (51). IL-6, soluble IL-6 receptor α, IL-1β and tumor necrosis factor seem to play a role in MM-associated bone disease, account for tumor burden-associated anemia, and possibly contribute to the myeloma kidney (41, 46).

Granulocyte colony-stimulating factor (G-CSF) and IL-6 induce activation of NF-IL-6, a transcription factor involved in the synthesis of IL-6. Thus, G-CSF is a potent growth factor for MM cells. Interferon α is another growth factor for MM cells in vitro, although growth inhibitory effects have been reported as well (52). IL-10 is a potent differentiation factor of B cells into immunoglobulin-secreting cells and this cytokine also stimulates the proliferation and long-term growth of some MM cells (53). This growth activity of IL-10 is not affected by anti-IL-6 and anti-IL-6 receptor antibodies. Insulin-like growth factor 1 and 2 have also been recognized as growth factors which stimulate the tumor cell growth and augment the IL-6 responsiveness of MM cells (54).

Binding of IL-6 to IL-6 receptor α induces the formation of a receptor complex composed of IL-6 receptor α and gp130, and mediates the phosphorylation of gp130. This

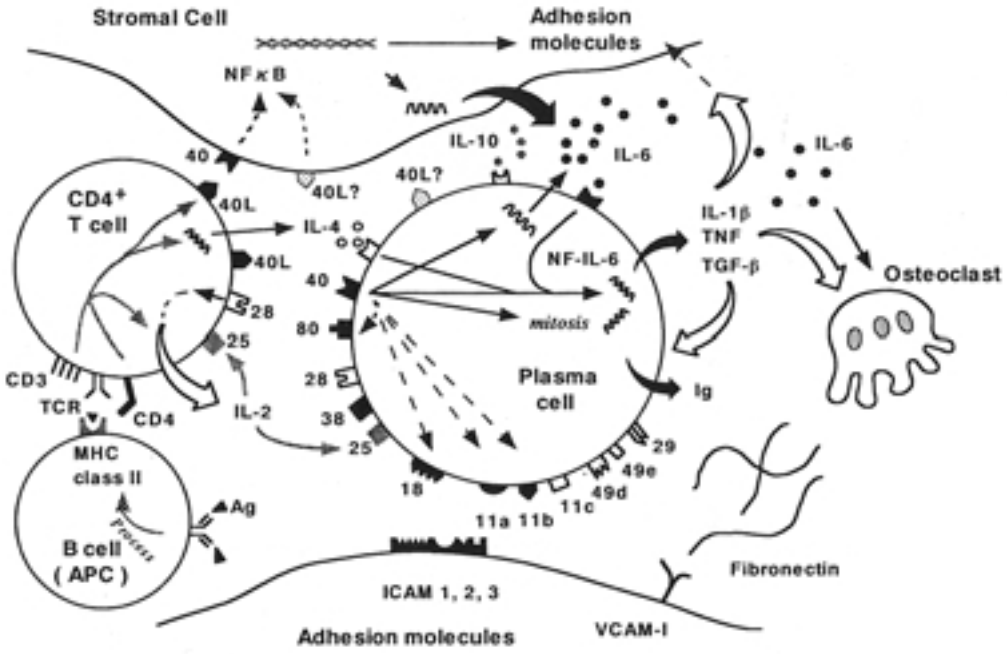

Fig.2. Interactions of myeloma cells with T cells and stromal cells for IL-6 secretion and adhesion to bone marrow microenvironment.

receptor complex further activates tyrosine kinases including members of the Janus kinase (JAK) and Src kinase families. Phosphorylation of STAT1 and/or STAT3 in MM cells occurs independently in the proliferative response to IL-6, but IL-6 induced activation of the Ras-MAP kinase cascade only in MM cells which proliferated in response to IL-6 (55). However, precise mechanism of IL-6 signaling cascades for the pathogenesis of MM remains unknown.

TREATMENT

Conventional chemotherapy

Among the anticancer drugs, alkylating agents have shown a significant antitumor activity against MM. The combination of melphalan or cyclophosphamide and prednisone is still the standard treatment for elderly patients (56). Combinations of other drugs, including vinca alkaloids, nitrosoureas and anthracyclines have been assessed, but all trials have failed to show a superiority than melphalan and prednisone (56). The regimen of vincristine, doxorubicin and dexamethasone (VAD) or a similar combination with high-dose methylprednisolone substituted for high-dose dexamethasone (VAMP) is effective for many patients (56). Its main advantage is the rapid induction of remission, but has not prolonged survival more than other regimens in a randomized clinical trial. Dexamethasone administration alone is as effective as melphalan and prednisone therapy for newly diagnosed patients, but the response rate is lower than VAD regimen (57).

The efficacy of drug-resistance modifiers, such as verapamil and cyclosporine, has been evaluated in patients with MM resistant to VAD (58, 59). However, because expression of p-glycoprotein is related to prior chemotherapy, they might have a limited value in MM patients (60).

High-dose chemotherapy

The lack of progress with conventional chemotherapy has heightened interest in high-dose therapy. McElwain et al have reported the efficacy of high-dose melphalan (140mg/m^2, given intravenously without stem cell support) even in refractory MM (61). High-dose melphalan therapy induced complete remission in 20-30% of patients, but therapy-related death associated with bone marrow aplasia occurred in 15-30% of patients (62, 63). The administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) or G-CSF reduces the duration of neutropenia (64). In addition, hematopoietic stem cell support enabled the use of melphalan in higher doses (200mg/m^2) or in combination with other cytotoxic agents and/or total body irradiation (65).

Allogeneic bone marrow transplantation has been performed in over 500 patients globally, although only approximately 7% of patients with MM are candidates for allogeneic bone marrow transplantation based on age, clinical condition, and donor availability. Allogeneic transplantation appears to be more effective than autologous transplantation with a higher remission rate up to 50% and a longer duration of disease free survival (66). This procedure even cures a small portion of patients, but transplant-related mortality is extremely high. Approximately 50% of patients died within one year of allogeneic transplantation. The late relapses are also observed and the relapse rate of patients in complete remission was about 50% at 5 years. The lack of plateau in the survival curves indicates that a single course of intensive therapy is not sufficient for curing MM. Development of novel conditioning regimens and means to enhance the graft-versus-myeloma effect are necessary to improve success rates (67).

Transplantation of autologous hematopoietic stem cells (obtained from bone marrow or blood) offers some advan-

Table 2. Autologous transplantation for multiple myeloma

Group (Reference)	No.	Age	Graft	Early death (%)	Complete response rate (%)	Event free survival	Overall survival
Anderson et al (68)	32	45	Purged ABMT	3	34	24mo	70% at 48mo
Fermand et al (69)	63	44	PBSCT	11	20	43mo	59mo
Cunningham et al (70)	53	52	ABMT	2	75	25mo	63% at 54mo
Harousseau et al (71)	81	55	ABMT	3	36	26% at 48mo	47% at 48mo
	51	49	PBSCT	6	37	47% at 48mo	49% at 48mo
Attal et al (72)	74	57	ABMT	3	30	39% at 60mo	68% at 60mo
Bensinger et al (73)	63	51	ABMT± PBSCT	11	40	21% at 36mo	43% at 36mo
Marit et al (74)	73	54	PBSCT	3	44	40% at 27mo	66% at 36mo
Vesole et al (75)	470	>50	PBSCT± allo-BMT	7	36	24mo	41mo

ABMT, autologous bone marrow transplantation ; PBSCT, peripheral blood stem cell transplantation ; allo-BMT, allogeneic bone marrow transplantation.

tages such as avoidance of the prolonged post-transplant immunosuppression and elimination of graft-versus-host disease. Specifically, the use of peripheral blood stem cells collected after high-dose cyclophosphamide and/or hematopoietic growth factors (such as GM-CSF or G-CSF) has shortened the duration of bone marrow aplasia, so that transplant-related mortality has markedly declined (Table2). Randomized study comparing conventional chemotherapy and high-dose chemotherapy followed by autologous bone marrow transplantation has shown that high-dose chemotherapy improved the response rate, event-free survival, and overall survival in MM patients (72). In addition, autologous stem cell transplantation may be useful as salvage therapy for patients refractory to standard chemotherapy and for patients in relapse with chemosensitive MM, but less so in patients with resistant relapse (76). Although higher rates of responses are obtained after high-dose chemotherapy, they do not lead to long-term remissions and cure. The result of clinical trials using peripheral blood stem cells does not increase the response rate but shortens the period of hematological toxicity and hospital stay as compared with autologous bone marrow transplantation (71). Likewise, the application of double transplants appears to improve the response rate in some patients, but has not resulted in an increase in survival (77).

To improve these problems, recent studies have focused on depleting the contamination of MM cells (78). The transplantation of purified $CD34^+$ progenitors is a promising alternative to the purging technique because 2-to 4-log reduction of tumor cells was obtained after positive selection (79, 80). However, it is not yet known if this will result in improved response rate and survival. Because polymerase-chain-reaction assays for clonally unique immunoglobulin gene mutations have detected MM cells in such purified $CD34^+$ cell fractions, an additional purging may be necessary to obtain grafts free of MM cells (24, 79-81).

Supportive therapy

The most common symptom is bone pain caused by pathological compression fractures. Bisphosphonates, which are potent inhibitors of bone resorption, have been widely used in MM, primarily for the treatment of hypercalcemia. Their ability to slow the progression of bone disease and improve bone density plays an important role in the management of MM (82-84). Specifically, pamidronate reduced the incidence of skeletal events, prevented hypercalcemia, alleviated bone pain, and improved the patient's quality of life (84).

The cause of anemia is multifactorial such as bone marrow infiltration of MM cells and renal failure. Clinical trials have had encouraging results with recombinant human erythropoietin for the treatment of anemia. These studies have shown that recombinant erythropoietin therapy is effective, especially in transfusion-dependent anemia patients (85, 86).

Immunotherapy

Several investigators have reported immunotherapeutic approaches targeting to cell surface antigens on myeloma cells, using anti-CD38 antibody, anti-CD54 antibody, or

soluble CD16 (87-91). However, because these molecules are also expressed on normal tissues including hematopoietic stem cells, these approaches may induce side effects related to crossreactivity in vivo. Recently, novel plasma cell-specific antigen, HM1.24, has been found and antitumor activity of anti-HM1.24 antibody against human myeloma xenografts has been reported (92, 93). Humanized anti-HM1.24 antibody might have a therapeutic potential for clinical use.

Based upon the fact that IL-6 is a major growth factor for myeloma cells functioning by an autocrine/paracrine fashion, immunotherapy targeting the IL-6-signaling system has also been reported. Clinical trials of murine anti-IL-6 monoclonal antibodies showed transient tumor cytostasis but did not result in tumor reduction (94). Trials with chimeric human-murine anti-IL-6 monoclonal antibodies, anti-IL-6 receptor antibodies and new IL-6 inhibitors are under way. IL-2, IL-4, interferon-γ and retinoic acid have been investigated in pilot studies (95).

The idiotype of the monoclonal immunoglobulin in an individual patient could be a tumor-specific antigen. Successful transfer of idiotype-specific immunity from a bone marrow donor has been reported after the donor was immunized with purified IgG of the patient (96). Other immunological approaches in MM currently under investigation include DNA vaccines and idiotype-reactive T-cell expansion (97, 98).

FUTURE DIRECTIONS

Conventional chemotherapy results in low complete response rates, and disease progression usually occurs within a couple of years. High-dose chemotherapy with hematopoietic stem cell transplantation has been shown to result in encouraging complete remission rates, however, there is no plateau of the survival curves in these trials. Thus, the development of maintenance chemotherapy or immunotherapy is necessary to eliminate minimal residual disease.

The molecular characterization of MM provides specific markers that may be useful in diagnosis and classifying tumors, as well as monitoring the minimal residual disease. In addition, further understanding of the molecular pathogenesis of MM may eventually permit the development of novel therapeutic strategies that target tumor-specific molecular lesions.

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