The Genetics of Multiple Myeloma

By Brian Van Ness, PhD

ABSTRACT

What genetic abnormalities contribute to multiple myeloma? One intriguing and confounding issue in myeloma research is the heterogeneity of the genetic abnormalities that influence disease progression and therapeutic response. Moreover, because of the tumor-host interactions, alterations in gene expression occur within the normal cells of the bone marrow microenvironment, and contribute to complications and disease progression. While there are currently no known genetic alterations that are associated with every case of myeloma, there are some genetic abnormalities found in a relatively high percentage of cases. These abnormalities are mutations, deletions, translocations, and aberrant expression of various genes, such as oncogenes, tumor suppressor genes, growth factor receptor genes, genes responsible for adhesion, and apoptotic protecting genes. These abnormalities may affect growth and death pathways, as well as alter signal transduction pathways leading to activation of transcription factors. New directives by the National Cancer Institute that promote the use of new genetic technologies will greatly refine cancer classification and facilitate novel approaches to myeloma therapy.

Oncology Spectrums *2001;2(2):96-100*

Multiple myeloma is characterized by the accumulation of clonal plasma cells in the bone marrow. It accounts for 2% of all cancer deaths and 15% of all hematologic neoplasms.[1] It is a malignancy that is much more common in elderly adults (median age of 63) than in younger adults or in children. In the United States, it is estimated that 50,000 patients are being treated for multiple myeloma, with about 14,000 new cases and nearly 11,000 deaths from the disease each year.[1] Current treatments frequently include combination chemotherapy with the common use of alkylating agents (melphalan), antimitotic agents (vincristine), steroids (dexamethasone, prednisone), cyclophosphamides, and other DNA damaging agents (doxorubicin), as well as high-dose therapies with stem cell rescue. Despite active research and new therapeutic approaches that provide significant tumor reduction, relapse almost always occurs and improvements in prolonged survival have been limited; patients survive about 36-40 months from the time of diagnosis. Death commonly results from infections associated with a severely compromised immune system and therapeutic toxicities. Evidence presented by the Eastern Cooperative Oncology Group Myeloma Committee clearly demonstrates that immune dysfunction is a consequence of the disease, and the chemotoxicity can lead to immunosuppression failure to recover normal bone marrow function. Indeed, immune status is highly predictive of survival. Treatment of the malignancy must often be accompanied by palliative care for complications; the medical cost of treating secondary complications frequently exceeds that of treating the primary tumor

One could easily make the case that all disease has a genetic component, and there are certainly multiple points of altered gene regulation in myeloma (see Figure 1). Ultimately, deregulation or alteration of gene expression contributes to malignancy and associated complications. What is both intriguing and confounding in myeloma research is that no consistent genetic abnormality has been found in association with this malignancy. Indeed, it is well known that considerable genetic heterogeneity in the malignant plasma cell exists among patients.[2] However, the difficulty in defining and treating this disease is exacerbated by the genetic deregulation that occurs in the microenvironment of the bone marrow after insidious invasion by tumor cells. The bone marrow stromal cells that normally regulate hematopoiesis and immunologic function are reprogrammed by the tumor cells to alter gene expression, which in turn contributes to tumor growth and bone destruction. As a result, the complexity of this disease is magnified. Finally, the therapeutic approaches themselves undoubtedly alter gene function and response, and relapses are a consequence of genetic responses. Indeed, cumulative doses of DNA-damaging therapeutic agents (such as adriamycin and melphalan) have been shown to lead to second malignancies in patients who are otherwise considered to be long-term survivors of myeloma. Thus, a clearer definition of the genetic issues is critical to understanding the disease, predicting its progression, and developing effective new strategies for treatment.

TALKING POINTS	Physicians	Pharmacy	Formulary	Cancer Nurses

As genetic definition of cancer impacts therapeutic choices, physicians will be able to develop more personalized treatments.

New drug discovery, based on targets identified in the laboratory, will mean new formulations of cocktails that include specific biologic targets in the cancer cells or in the cells that promote tumor progression.

Consequences of personalized therapy will include new data bases in the management of the disease, and need for additional administrative supervision.

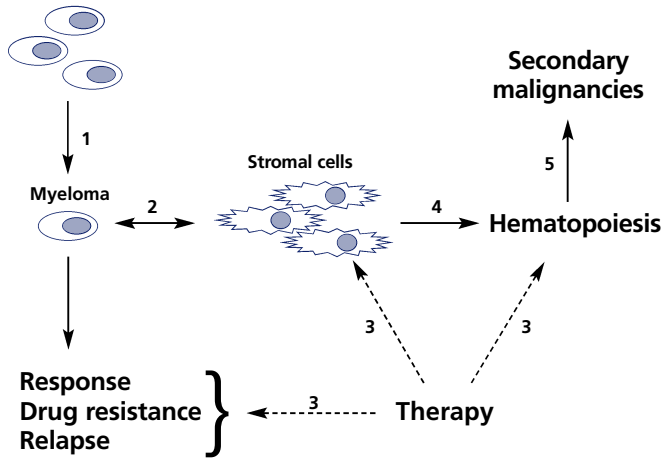
Delivery of new agents that interfere with specific tumor functions may provide quicker responses, with potentially less toxicity, thus improving quality of life.

Dr. Van Ness is a professor and head of the Department of Genetics, Cell Biology and Development at the University of Minnesota in Minneapolis, and serves as the program director for cancer genetics at the University of Minnesota's NCI-designated Comprehensive Cancer Center.

Despite the overwhelming challenges in the management of myeloma, recent advances in research technology have generated enormous excitement about redefining cancer and its treatment. Not surprisingly, the National Cancer Institute (NCI) has issued a Director's Challenge to provide a new genetic classification of cancer. The advances in the Human Genome Project and the rapid identification and isolation of all human genes are making possible the development of comprehensive genetic profiles of normal and abnormal cells. There is literally a data explosion. Given the nearly 100,000 genes of the human genome, which are the critical genes? The characterization of oncogenes and tumor suppressor genes that could be deregulated in myeloma is certainly of interest. However, associated with the complex intracellular signaling pathways that influence cell growth and death is a rapidly expanding list of relevant genes and their protein products. There are genes that regulate cell adhesion in the bone marrow; genes associated with growth factor receptors; intracellular signaling cascades that lead to the activation of transcription factors that regulate other genes; genes involved in cell proliferation; and genes that regulate programmed cell death or apoptosis. In any given cancer, there are clearly multiple genetic events, and even between patients with the same diagnosis of myeloma, there are differences in genetic abnormalities that contribute to differences in disease progression and response.[3]

In one current application of genetic testing in myeloma, immunoglobulin genes serve as unique clonal markers of the malignant plasma cell.[4] The expression of unique immunoglobulin gene sequences can be characterized and used in the molecular detection of the disease. Before malignant transformation, gene rearrangements and DNA mutations take place in the immunoglobulin loci as the early B cell matures to the plasma cell. As a result, the heavy chain locus of the immunoglobulin gene is a unique genetic marker of a clonal plasma cell. Thus, immunoglobulin gene rearrangement becomes a useful diagnostic marker of clonal B-cell tumors. The molecular detection of minimal residual disease has become more important with advances in myeloma treatment. Several techniques have been described to detect minimal residual disease including flow cytometry, fluorescent in situ hybridization, and polymerase chain reaction (PCR).[4-7]

FIGURE 1. GENETIC ALTERATIONS IN MYELOMA



Genetic alterations can influence multiple cells, cellular interactions, disease progression, and therapeutic response in myeloma. These genetic alterations can 1) contribute to transformation of the plasma cell, 2) alter the interactions of the myeloma cell with the environment, 3) alter the response to therapeutic agents, 4) disrupt normal hematopoiesis, and 5) lead to secondary transformations and malignancies.

Van Ness B. *Oncology Spectrums.* Vol 2. No 2. 2001.

Allele-specific oligonucleotide (ASO)-PCR is currently the most sensitive technique to detect minimal residual disease.[4,8-11] Briefly, DNA primers that are unique to the sequence of the tumor's immunoglobulin heavy chain gene are synthesized. These primers are used to amplify the junctional regions of the rearranged immunoglobulin heavy chain gene from the diagnostic bone marrow of the patient with myeloma by PCR. The ASO-PCR technique is highly specific, sensitive enough to detect one malignant cell in a background of 100,000 normal cells, and can be quantitative.[4] Thus, as therapeutic techniques improve, remission may be monitored by molecular techniques that are sensitive to very low levels of residual disease. The widespread application of ASO-PCR and similar techniques is prohibited by their requirements of labor intensive effort and high technical proficiency, so these techniques are currently not available in most hospital settings. Although ASO-PCR has not reached application as a standard test, it remains a useful tool in academic settings to monitor novel therapies that may achieve significant tumor reduction.

Defining the factors that control plasma cell growth and signaling may lead to identifying therapeutic targets. During normal B-cell development, interleukin-6 (IL-6) promotes terminal differentiation of mature B-cells into antibody-secreting plasma cells.[12] Although

IL-6 stimulates immunoglobulin secretion, it does not promote normal B-cell proliferation.[13] In contrast, IL-6 has been shown to be a potent growth factor for myeloma cells. Early work by Potter and Boyce[14] demonstrated that plasmacytomas developed in pristane-treated mice, and subsequent work showed that IL-6 served as a potent growth factor in tumor development.[15-17] Massive plasmacytosis has been shown to develop in transgenic mice carrying the human IL-6 gene fused to an immunoglobulin heavy chain transcriptional enhancer sequence.[18] Moreover, a human myeloma cell line transfected with an IL-6 expression vector was successfully transplanted into immunocompromised mice as a subcutaneous tumor.[19] The addition of IL-6 in cell culture media augments the clonal proliferation of myeloma cells, whereas anti-IL-6 or antisense IL-6 oligonucleotides inhibit the proliferation of myeloma cell lines.[20] Additionally, mutations that affect IL-6 signal transduction pathways are often associated with myeloma (see below). Thus, IL-6 and its associated signal pathways may be important in the disease progression. The source of IL-6 has been the subject of some controversy. Kawano and colleagues[16] have reported that fresh myeloma cells are capable of autocrine IL-6 production and these cells also express IL-6 receptors. In contrast, Klein and colleagues[21] have reported that the IL-6 found in high levels in the bone marrow of patients with myeloma is derived from adherent stromal cells in the marrow.

"Mutation or aberrant expression of genes such as c-myc and cyclin D1 that regulate cell cycle can lead to uncontrolled proliferation."

As emphasized above, there is considerable heterogeneity in genetic alterations associated with multiple myeloma. While there are currently no known genetic alterations that are associated with every case of myeloma, there are some genetic abnormalities found in a relatively high percentage of cases. These abnormalities are mutations, deletions, translocations, and aberrant expression of various genes, many of which affect growth and death pathways. Thus, the genetic alterations can affect many different properties of the myeloma cell. Mutations can affect signal transduction through constitutively activated signaling molecules such as *ras*.[22] Activating *ras* mutations occur in 40–50% of myelomas. Mutation or aberrant expression of genes such as c-myc and cyclin D1 that regulate cell cycle can lead to uncontrolled proliferation. Changes in expression of death-inducing or death-promoting genes (eg, the Bcl family of genes) can lead to cells that are more resistant to apoptosis. Moreover, changes in any of these pathways or control mechanisms can lead to altered responses to therapeutic agents and other outside stimuli. Results from our own laboratory have shown that myelomas harboring mutations in *ras* or p53 genes can have very different responses to therapeutic agents.[3] Thus, an understanding of the genetic alterations and their functional consequences will influence therapeutic design and response.

One of the obvious abnormalities seen in myeloma is the frequent appearance of gross chromosomal abnormalities, including aberrant chromosomal joining and translocations. The t(4;14)(p16;q32) translocation is found in approximately 15%–25% of myeloma cases. This translocation results in the deregulated expression of both fibroblast growth factor receptor 3 (FGFR3) and the MMSET (multiple myeloma SET domain) gene.[23] The role of the MMSET gene in myeloma development is still unknown. Stimulation of FGFR3 by a fibroblast growth factor activates signaling pathways that lead to cell growth. Numerous reports have suggested that deregulated expression of FGFR3 may lead to additional proliferative or anti-apoptotic signals provided by bone marrow stromal cells expressing fibroblast growth factor. More recently, deletions in chromosome 13 have been associated with poor prognosis,[24] although the specific gene or genes involved have not been defined. Generally, most myelomas have apparent chromosomal abnormalities that have not been functionally characterized.

Among the most exciting new research approaches is the use of gene microarray technology. It is now possible to examine the levels of expression of thousands of genes in a single analysis. Microscope slides or silicon chips containing microdots of 60,000 different human genes are available for assessing the complexity of gene expression with mRNA isolated from any cell population. Potential applications of this technology would answer such questions as: What is the genetic expression of a malignant plasma cell compared to that of a normal plasma cell? How is the gene expression associated with a highly progressive or metastatic disease different from that associated with a disease that responds well to treatment? What genetic changes occur when a patient in remission suddenly relapses and fails to respond to therapy that was initially effective? The next generation of the Human

Genome Project promises new excitement—"functional genomics" that defines not only the genes, but also the gene actions. The future development of genetic expression profiles will provide the new definitions promoted by the NCI Director's Challenge. Along with this comes "pharmacogenomics"—the use of genetic data to predict or design therapies. Gene arrays have recently been used to reclassify patients with diffuse large B-cell lymphoma on the basis of a gene expression profile of 18,000 genes.[25] The new classifications correlated closely with patient survival. Similar classifications based on gene expression profiles are currently being used in myeloma treatment to predict therapeutic response and patient survival. In addition, gene arrays can be used to follow genetic changes associated with disease progression. Cell banks are being set up with samples collected from patients at the time of diagnosis and throughout disease progression and treatment. Gene expression profiles from a single patient can be followed throughout disease progression and treatment response to determine which genes may be involved.

In December 2000, the NCI convened a panel of national experts, called the Progress Review Group, to develop new initiatives in funding for lymphoma, leukemia, and myeloma. A full report will be issued after a complete review of the information available; however, several themes are emerging that would facilitate novel approaches. A new definition of lymphoid disease that integrates molecular approaches—including new gene array technologies, protein analyses, and pharmacologic responses—must be developed. Part of the evaluation will require a very important analysis of tumor-host interactions, recognizing the critical importance of the environmental conditions that support the growth of the tumor cell. With these definitions, new hypotheses can be generated to delineate the roles of specific genetic abnormalities that contribute to tumor survival, progression, and response. This role delineation will require further development of laboratory models. Cell lines exist that can be manipulated in the laboratory and evaluated for gene expression and protein expression; these expressions may then be correlated with therapeutic response. With identification of novel pathways and gene expression patterns comes the opportunity to identify novel targets. In the past, cancer therapy has involved many chemotherapeutic agents with broad toxicities, including steroids and DNA-damaging agents. New technologies have already identified novel targets in specific growth pathways (such as *ras*) and signaling pathways (affecting tyrosine kinases), and very specific transcription factors (such as Stats) that regulate specific genes within the tumor. Clinical trials are underway with protein kinase inhibitors, proteosome inhibitors, and angiogenic inhibitors to increase the specificity of treatment and minimize the toxicity. The new paradigm will replace the 'maximal tolerated dose' with the 'minimal pharmacologic effective dose.'

"The new paradigm will replace the 'maximum tolerated dose' with the 'minimal pharmacologic effective dose.'"

Indeed, one important limitation of the high-dose chemotherapies being employed is the often lethal toxicity. Is there a genetic basis to this? Within our cells, we produce many toxic byproducts of metabolism, and evolution has provided families of genes involved in detoxification and even DNA repair. The expression of these genes likely affects the detoxification of therapeutic agents as well. In defining risk, it is important to recognize that within the population, genetic polymorphisms (differences in DNA sequences among individuals) exist that can result in altered detoxification activity. For most people, such differences in DNA sequences may never be revealed because even low levels of detoxification activity are sufficient to counter daily metabolic activities and environmental exposures. However, when stressed by highly toxic agents, such genetic polymorphisms may influence therapeutic recovery. A new genetic field that identifies the consequences or risks associated with genetic polymorphisms is emerging. Genetic tests to identify single nucleotide polymorphisms (SNP analysis) are being conducted and correlated to cancer incidence and therapeutic outcome. These analyses are just beginning, but their results will add to the genetic information that can provide risk assessment and prognostic indicators of response.

One of the critical issues to be resolved is how genetic information is handled so that patients may provide informed consent and receive confidentiality protection. The power of genetic screening to identify patients' cancer risk and predict their treatment response requires an important effort to educate both physicians and patients with respect to informed consent and confidentiality protection. As genetic profiles become available, could they be used to define risks of

interest to medical insurance companies or employers? There is clearly a need for national discussion and policies to regulate the general dissemination of this information without impeding the benefit of such information to the physician and patient.

What is the impact of applying new technologies to the definition and treatment of myeloma? The goals are to improve outcome, reduce hospital stays, reduce morbidity of both the disease and its therapy, and ultimately to elicit a durable response leading to a cure. Although myeloma is a relatively uncommon cancer, it and other lymphoid malignancies are better understood with respect to their cellular and molecular biologies than many of the more common cancers. Thus, the lymphoid malignancies have become an important paradigm of how biological, genetic, and biochemical understanding can lead to effective treatments. The technology is rapidly advancing and clinical care is likely to improve greatly from the knowledge that will be gained in the next 5 years.

"Although myeloma is a relatively uncommon cancer, it and other lymphoid malignancies are better understood with respect to their cellular and molecular biologies than many of the more common cancers."

REFERENCES

1. Parker S, Tong T, Bolden S, Wingo P. Cancer Statistics. *CA—A Cancer Journal for Clinicians*. 1996;46:5-27.
2. Van Ness B. Molecular genetics in multiple myeloma and related disorders. In: Wiernick P, Canellos G, Dutcher J, Kyle R, eds. *Neoplastic Diseases of the Blood*. Edinburgh, England. 1996;525-535.
3. Rowley M, Liu P, Van Ness B. Heterogeneity in therapeutic response of genetically altered myeloma cell lines to IL-6, dexamethasone, and melphalan. *Blood*. 2000;96:3175-3180.
4. Billadeau D, Blackstadt M, Greipp P, et al. Analysis of B-lymphoid malignancies using allele-specific polymerase chain reaction: a technique for sequential quantitation of residual disease. *Blood*. 1991;78:3021-3029.
5. Ward MS. The use of flow cytometry in the diagnosis and monitoring of malignant hematological disorders. *Pathology*. 1999;31:382-392.
6. Genevieve F, Zandecki M, Lai J-L, et al. Evaluation of minimal residual disease by interphase FISH in multiple myeloma: does complete remission exist? *Leukemia*. 1999;3:641-644.
7. Gerard CJ, Olsson K, Ramanathan R, et al. Improved quantitation of minimal residual disease in multiple myeloma using real-time polymerase chain reaction and plasmid-DNA complementary determining region III standards. *Cancer Res*. 1998;58:3957-3964.
8. Billadeau D, Quam L, Thomas W, et al. Detection and quantitation of malignant cells in the peripheral blood of multiple myeloma patients. *Blood*. 1992;80:1818-1824.
9. Martinelli G, Terragna C, Zamagni E, et al. Molecular remission after allogeneic or autologous transplantation of hematopoietic stem cells for multiple myeloma. *J Clin Oncol*. 2000;18:2273-2281.
10. Ladetto M, Donovan JW, Harig S, et al:. Real-time polymerase chain reaction of immunoglobulin rearrangements for quantitative evaluation of minimal residual disease in multiple myeloma. *Biol Blood Marrow Transplant*. 2000;6:241-253.
11. Cavo M, Terragna C, Martinelli G, et al. Molecular monitoring of minimal residual disease in patients in long-term complete remission after allogeneic stem cell transplantation for multiple myeloma. *Blood*. 2000;96:355-357.
12. Muraguchi A, Hirano T, Tang B, et al. The essential role of B cell stimulating factor 2 (BDF-2/IL-6) for the terminal differentiation of B cells. *J Exp Med*. 1988;167:332-344.
13. Alderson MR, Pike BL. Recombinant human IL-6 (B cell stimulatory factor 2) enhances immunoglobulin secretion by single murine hapten-specific B cells in the absence of cell division. *Int Immunol*. 1989;1:20-28.
14. Potter M, Boyce C. Induction of plasma cell neoplasms in strain BALB/c mice with mineral oil and mineral oil adjuvants. *Nature*. 1962;193:1086-1088.
15. Nordan RP, Potter M. A macrophage-derived factor required by plasmacytomas for survival and proliferation in vitro. *Science*. 1986;233:566-569.
16. Kawano M, Hirano T, Matsuda T, et al. Autocrine generation and requirement of BSF-2/IL-6 for human myelomas. *Nature*. 1988;332:83-85.
17. Zhang XG, Klein B, Bataille R:. Interleukin-6 is a potent myeloma cell growth factor in patients with aggressive multiple myeloma. *Blood*. 1989;74:11-13.
18. Suematsu S, Matsuda T, Aozasa K, et al. IgG1 plasmacytosis in interleukin-6 transgenic mice. *Proc Nat Acad Sci USA*. 1989;86:7547-7551.
19. Okuno Y, Takahashi T, Suzuki A, et al. Acquisition of growth autonomy and tumorgenicity by an interleukin-6 dependent human cell line transfected with interleukin-6 cDNA. *Exp Hematol*. 1992;20:395-400.
20. Schwab G, Siegall CB, Aarden LA, et al. Characterization of an interleukin-6 mediated autocrine loop in the human multiple myeloma cell line U266. *Blood*. 1991;77:587-593.
21. Klein B, Zhang XG, Jourdan M, et al. Paracrine rather than autocrine regulation of myeloma cell growth and differentiation by interleukin-6. *Blood*. 1989;73:517-526.
22. Liu P, Leong T, Quam L, Billadeau D, et al. Activating mutations of N- and K-*ras* in multiple myeloma show different clinical associations: Analysis of the Eastern Cooperative Oncology Group phase III trial. *Blood*. 1996;88:2699-2706.
23. Chesi M, Nardini E, Lim RS, et al. The t(4;14) translocation in myeloma disregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. *Blood*. 1998;92:3025-3034.
24. Shaughnessy J, Barlogie B. Chromosome 13 deletion in myeloma. Curr Topics Micro Immunol. 1999;246:199-203.
25. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000;403:503-511.