Gene Mutation Revelation Points to New Target for Myeloma Treatment, Studies Say

By Ken Garber

Multiple myeloma is a disease in the spotlight. Although it is relatively rare, accounting for about 2% of all cancers in the U.S., new treatments arrive almost every year. Bortezomib, thalidomide, and lenalidomide have all been approved since 2003, and new bone marrow transplant regimens have extended survival. The multiple myeloma field “has exploded,” said Louis Staudt, M.D., Ph.D., of the National Cancer Institute. But the disease is still considered incurable.

Now two papers in the August issue of Cancer Cell mark another advance in myeloma biology, as well as a promising new approach to treatment. Groups led by Staudt and Leif Bergsagel, M.D., of the Mayo Clinic in Scottsdale, Ariz., independently identified a variety of gene mutations and other genetic abnormalities in the NF-κB signaling pathway in myeloma. The Staudt group concluded that 82% of patients with myeloma have activated NF-κB. This result, Staudt said, “rockets it up the list of pathways you’d have to consider as targets in myeloma.” The Bergsagel report suggests that NF-κB pathway mutations can be used to select patients for bortezomib treatment. And myeloma patients may eventually benefit from direct NF-κB inhibitors now under development by many drug companies.

Two Paths, One Conclusion
That NF-κB is important in myeloma is no surprise. NF-κB is a prolific transcription factor that, in cancer, activates genes involved in inflammation, cell cycle control, and apoptosis. In 1996, a group led by Harvard University researcher Ken Anderson, M.D., showed that when myeloma cells bind to bone marrow stromal cells, the myeloma cells trigger the NF-κB–dependent transcription of cytokines that promote tumor survival. Other links to myeloma have surfaced since then. “Inhibiting NF-κB has the potential to inhibit the tumor, the tumor–host interaction, and the microenvironment,” Anderson said.

What’s new about the Cancer Cell reports is the identification of specific gene mutations and other genetic abnormalities in NF-κB signaling. The only other known case of an NF-κB mutation in myeloma was reported back in 1991, in one patient. The new studies “validate the importance of NF-κB in myeloma cell biology by showing mutations, with constitutive [independent] activation as a result,” Anderson said. These mutations probably do not initiate cancer but instead seem to allow myeloma cells to survive and grow independent of their bone marrow microenvironment—a major hallmark of cancer.

The Staudt and Bergsagel groups arrived at their NF-κB discoveries from different directions. Staudt began with a drug from Millennium Pharmaceuticals in Cambridge, Mass., targeting IkB kinase beta (IKKβ), which works upstream of NF-κB. Because the drug killed myeloma cells in culture, Staudt and NCI colleague Michael Kuehl, M.D., began looking for mutations in NF-κB–related genes that could be activating the pathway and sensitizing the cells to the drug.

Bergsagel’s group, on the other hand, collaborated with Agilent Technologies in Santa Clara, Calif., to undertake a broad search for copy number abnormalities in myeloma cell lines and patient samples, using a technique called array comparative genomic hybridization, which compares tumor DNA to normal DNA on the basis of the intensity of fluorescent labels. Finding an unusual number of two-copy deletions in NF-κB pathway genes, Bergsagel focused on NF-κB. Both groups now report many of the same mutations, derived from completely separate patient populations. (Bergsagel’s list of mutated genes is somewhat larger.)

Although both reports highlight the importance of NF-κB in myeloma, they have some major differences and draw some starkly different conclusions. One difference is how NF-κB activation is measured. The Staudt group, working with John Shaughnessy, Ph.D., of the University of Arkansas in Little Rock, developed an 11-gene expression signature for NF-κB activation, and they found high expression in the 82% of myeloma patient samples tested. Bergsagel used a low expression level for an NF-κB signaling protein called TRAF3 as a proxy for NF-κB pathway activation. (Bergsagel identified in that report TRAF3 as a tumor-suppressor gene in myeloma.) He concluded that 20% of myeloma patients have TRAF3 mutations and estimates that 40% have NF-κB activation via other mechanisms—60% in all.

“A lot of people do the analysis, and they get a global view, and they wait for the computer program to tell them what the answer is. I don’t think that works. I think you need to approach this global genomic data with a hypothesis.”
The two groups also disagree on whether the mutations affect mainly the “canonical” or “classical” pathway, which is important for inflammation, or the “non-canonical” or “alternative” NF-κB signaling pathway, which is involved in B-cell development. Bergsagel placed most of the mutations in the alternative pathway but acknowledged some effect on the classical pathway. Staudt, on the other hand, saw more effect of the genes on the classical pathway—an important distinction because there are drugs in development specific for the classical pathway but not the alternative pathway. For example, several companies, including Millennium, are developing drugs to block IKKβ in the classical pathway.

**Clinical Implications**

The most controversial finding is Bergsagel’s report that 90% of patients with low levels of TRAF3 respond to bortezomib, a drug that inhibits the proteasome, where cellular proteins are degraded. Bortezomib is thought to block NF-κB signaling, among many other effects. Bergsagel hopes that a predictive test for low TRAF3 levels can eventually be developed to guide bortezomib therapy. “With a 90% response rate, you would probably want to use a bortezomib regimen up front,” he said. Bortezomib is currently approved only for relapsed myeloma.

“The 90% result “is a very provocative finding, but it needs to be validated,” Shaughnessy said. Staudt points out that the 90% figure was derived from a clinical trial using bortezomib as a single agent, which is not how the drug is routinely used now. “The role of TRAF3 mutations [in] daily treatment of myeloma patients is less clear,” he said. Anderson agreed that validation is needed and warned against excluding people without TRAF3 mutations from bortezomib treatment, because many do respond to the drug. “We don’t know the full story of how proteasome inhibitors work, quite frankly,” he said.

Several groups are already planning to correlate TRAF3 expression with bortezomib response in ongoing bortezomib myeloma clinical trials “We may have the opportunity to quickly validate that finding,” Shaughnessy said.

Bortezomib, while often effective in myeloma, is not the ideal anti-NF-κB drug. More specific drugs are needed to fully capitalize on the new findings. Besides Millennium, biotech companies Nereus Pharmaceuticals and Reata Pharmaceuticals have reported developing IKK inhibitors. Meanwhile, such inhibitors “have already been developed by many major pharmaceutical companies and are going to go into clinical trials in one setting or another,” Staudt said. Millennium’s IKKβ inhibitor is now being tested in humans with rheumatoid arthritis.
arthritis, and Shaughnessy’s group plans to test it in mouse models of myeloma in preparation for a possible human trial. Because of the promiscuity of NF-κB signaling in human biology, these drugs will have side effects, but Staudt and others hope that myeloma cells will be especially dependent, even “addicted,” to NF-κB, creating a therapeutic window.

**A Model Disease**

The *Cancer Cell* reports have implications for other cancers besides myeloma. Many other groups are now using array comparative genomic hybridization to look genomewide for copy number abnormalities (and ultimately mutations) in a variety of tumor types. Bergsagel, who began with a general search but quickly focused on NF-κB signaling, argues for a hypothesis-driven approach. “A lot of people do the analysis, and they get a global view, and they wait for the computer program to tell them what the answer is,” he said. “I don’t think that works. I think you need to approach this global genomic data with a hypothesis.”

Because of the myeloma field’s success in generating new treatments, other cancer researchers are beginning to look to it as a model for translational research. The NF-κB work and other recent advances in myeloma treatment are due partly to the relative ease of obtaining cancer cells from patients as opposed to from solid tumors. But myeloma research has succeeded, Anderson argued, also because cancer cells are studied in the context of the tumor microenvironment, not in isolation. Bone marrow connective tissue from patients can be grown in laboratory dishes for 3–6 weeks before myeloma cells are added, allowing researchers to identify genes in the tumor microenvironment (like NF-κB) that confer a survival advantage or drug resistance to tumor cells. Mouse models of myeloma incorporating the human tumor microenvironment have also been developed.

“We identify new targets in the tumor or the microenvironment, [and] we can validate, using our model systems, targeted therapies,” Anderson said. “And then those that are chosen and validated can be rapidly translated to clinical trials.” NF-κB inhibitors could easily be the next in line.

© Oxford University Press 2007. DOI: 10.1093/jnci/djm164