Epigenetic Therapies Move Into New Territory, but How Exactly Do They Work?

By Rabiya S. Tuma

Although several drugs aimed at reversing epigenetic modifications—nonsequence changes in DNA—are already the standard of care for some malignancies, the field scaled a major hurdle this spring when investigators showed for the first time that an epigenetic therapy improved overall survival in a randomized phase III trial.

An international team, known as the International Vidaza High-Risk MDS Survival Study Group, showed that patients with myelodysplastic syndrome who received azacitidine (Vidaza) survived longer than patients who received other standard treatments.

Those data move epigenetics and agents that target epigenetic modifications onto more secure footing in the cancer research community. But even as new therapies are in development, major questions remain with respect to exactly how the agents are working.

“I think the mechanism of action in cell culture models is pretty good, and in xenograft models,” said Peter A. Jones, Ph.D., director of the University of Southern California Norris Comprehensive Cancer Center in Los Angeles, who is credited with the discovery of azacitidine. “So the question is whether or not the same mechanism of action applies in humans—and the answer is still clearly we don’t know.”

It is generally accepted that genetic mutations cause cancer. Increasingly, however, researchers have found that epigenetic modifications also contribute to tumor formation and progression. Epigenetic modifications, such as DNA methylation and histone acetylation, enable a cell to switch its gene expression pattern in a long-lasting manner without altering the sequence of individual genes. And unlike mutations, epigenetic changes often affect large portions of the genome at once. For example, investigators have found that the genomes of many cancers are hypermethylated relative to normal cells, and that extra methylation appears to silence important regulatory genes, including tumor suppressor genes.

**Decrease in Methylation?**

With that information in hand, scientists hypothesized that drugs that reduce the amount of methylation, including azacitidine and decitabine (Dacogen), would reactivate the tumor suppressor genes and shut down tumor growth. In practice, things haven’t been that simple. Patients who respond to these agents do show a decrease in DNA methylation. However, the converse is not true: Not everyone who has a decrease in DNA methylation has tumor shrinkage. So demethylation may not be sufficient to induce response.

“People in the field very much wanted this simplicity of reactivation of tumor suppressor genes, said Jean-Pierre Issa, M.D., a leukemia specialist at the University of Texas M. D. Anderson Cancer Center in Houston, who has been involved with the development of azacitidine and decitabine and received research funding from Eisai Inc., maker of decitabine. “With decitabine, I am convinced that the core mechanism involves DNA methylation. What I don’t know is what happens after demethylation. Is it a change in tumor suppressor genes, or is it a change in microRNAs? Or is it affecting some part of the genome we don’t understand yet? Or does it induce an immune response?”

With hypomethylating agents, including azacitidine and decitabine, the demethylation tends to be transient in patients with myelodysplastic syndrome, according to Guillermo Garcia-Manero, M.D., also from M. D. Anderson. The transient nature of the epigenetic changes may be why most responders eventually relapse on these drugs. But a more basic question, he said, is why the hypomethylation has an antileukemia effect and why it appears in the first place. Perhaps the drug causes demethylation of the tumor cells’ DNA, as predicted by the initial hypothesis, or maybe the tumor cells are being killed off and replaced by more normal cells, which naturally have less methylation than the tumor cells.

**Or Some Other Mechanism?**

“I think the data we have more and more indicates that the concept of tumor suppressor reactivation may not really apply for most of the situations where we use these drugs,” Garcia-Manero said. “To be honest, we have not been able to demonstrate a relationship between hypomethylation or histone deacetylation induction and response. So it is possible that these drugs work through another mechanism that is not just the target of DNA methylation.” He noted that the drugs have a promiscuous mechanism of action. They induce DNA hypomethylation and change histone profiles, but they also induce DNA damage and autophagy and production of reactive oxygen species. “So the question is, which one of these mechanisms actually is the key?”

To conclude that these agents might be working by a mechanism other than an epigenetic one is further than some experts are willing to go. Stephen B. Baylin, M.D., deputy director of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University in Baltimore, who specializes in epigenetic regulation of cancer, agreed that the question is still unanswered.

Baylin noted that although these agents do have many effects on cells, the dose of the drugs has been reduced so far that the mechanism remaining is likely to be epigenetically based, rather than DNA damage, for example. He also pointed out that the time course of response in patients—often as
long as 4 months before a response appears—is unlike responses to cytotoxic chemotherapy, which occur relatively quickly. “No one really knows what is happening during that period or when it is the best time to look for biomarkers of demethylation and gene reexpression,” he said. “The hypothesis is that that is the definitive mechanism, but I don’t know that we have assayed properly for it.”

Baylin hopes to rectify that situation, however. He and USC’s Jones received a “Dream Team” award from Stand Up To Cancer, the charitable organization that the Entertainment Industry Foundation recently established. They will use some of the grant to do the critical experiments to test the hypothesis, such as analyzing DNA samples from myelodysplastic syndrome patients who participated in the international phase III trial. Baylin hopes that by looking at the whole epigenome in a large, randomized patient population, the team will be able to identify signatures that correlate response to azacytidine therapy.

In decitabine-treated patients, Issa’s team at M. D. Anderson has already found some epigenetic changes that correlate with response. For example, the team sees that p15, a tumor suppressor gene that is often silenced by DNA methylation in hematologic cancers, is reactivated and expressed in patients who respond to therapy, but not in those who do not respond. His group also has unpublished data showing that a microRNA that regulates cell division, miR124A, behaves similarly, with demethylation and expression occurring in responsive patients but not in those who do not respond. “What we don’t know really in all of these experiments is if that is why the patients respond. All of these studies are correlative,” Issa said. “And probably there are dozens of genes involved, so it is naïve to think that there is a single target for epigenetic therapy.”

When asked why he and Garcia-Manero may have different views of the likely mechanism of action, Issa pointed out that they focus on different agents: He focuses on decitabine, whereas Garcia-Manero focuses on azacytidine. And although both agents are DNA-demethylating agents, they may act differently. (Azacytidine, for example, incorporates into both RNA and DNA, whereas decitabine incorporates into DNA only.) In fact, though both agents are approved for clinical use, hematologists are unsure whether the agents work equally well. An Eisai-sponsored head-to-head randomized trial is expected to start later this year in patients with myelodysplastic syndrome and acute myeloid leukemia.

New Strategies, Targets
Despite the lack of clarity about how they work, clinical responses to the drugs have triggered interest in DNA-demethylating agents and histone deacetylase (HDAC) inhibitors, which target another form of epigenetic gene silencing. Academic and industry researchers are working to develop DNA-demethylating agents that are more stable in aqueous solution than decitabine and azacytidine, and there is “huge industry interest in HDAC inhibitors,” Jones said. Also, researchers want to follow up on cell culture data that suggest that using a DNA methylation agent followed by an HDAC inhibitor improves gene reactivation compared with either agent alone. “We have known for many years that if you use the two in combination you get a highly synergistic effect [in cells],” Jones said. “We used to think in a very compartmentalized way about DNA methylation and histone modification, but it is becoming very apparent that these epigenetic processes communicate with each other.”

Baylin and Jones plan to test the serial administration of the agents in patients as part of their Dream Team work, but Issa, who is also working with that team, isn’t as convinced that the strategy is the right one. In trials that have tested a DNA demethylation agent followed by an HDAC inhibitor, the results have been disappointing, he said. “I don’t mean that there were no responses, but they don’t seem to be necessarily better than single-agent demethylation agents.”

In addition to testing combinations of existing agents, researchers are working on inhibiting a new epigenetic target called histone methyltransferases. Unlike the DNA methyltransferases, which attach a methyl group to DNA during DNA replication, histone methyltransferases modify histone proteins and do not require that the cell be undergoing division. Previously, researchers found that high levels in tumors of one histone methyltransferase, called EZH2, are associated with a poor prognosis, suggesting that targeting the protein may have antitumor effects. Also, laboratory experiments show that EZH2 blockade stops tumor growth. “It provides an entirely new area of therapeutic targeting that could potentially be combined with DNA methylation inhibitors,” Issa said.

Investigators are also testing agents in new disease settings. Baylin’s group has preliminary evidence that the agents can work in solid tumors when used at the same dosing as in leukemias. In a phase I/II trial, his team is seeing “robust” and durable responses in patients with advanced non–small-cell lung cancer, he said. Because the trial is ongoing, he declined to provide specific data but said that he wants to expand the line of work to test whether using a DNA demethylation agent followed by an HDAC inhibitor in this setting would improve outcomes. He also plans to test the epigenetic therapies in breast and colon cancer patients. “We are very excited about this, and we really want to extend it,” Baylin said.

As in leukemia trials, the responses Baylin and his group are seeing appear to take time. With that in mind, success may depend on using unusual trial designs that require patience. Instead of looking for a response after just one or two cycles, as with cytotoxic agents, he thinks patients in the epigenetic therapy trials may need to be treated for several months before they are deemed responders or nonresponders. Baylin also plans to design the trials to look for predictors of response. He acknowledges, though, that there is a lot of work to be done. “There is much to understand at the basic level, and there is much to understand clinically.”

In Issa’s mind, though, the Dream Team award finally recognizes epigenetics’ role in cancer. “I think award of the Dream Team is belated recognition by the field, and the leaders of the field . . . and that recognition comes in part thanks to the success of epigenetic drugs.”