Insights into the Mechanism of Action of Lenalidomide in Patients with Del(5q)

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Welcome to *Managing MDS*. I am Dr. Benjamin Ebert, and today, I will be providing highlights from my lecture at the 14th International Symposium on MDS in Valencia, Spain. I am going to tell you about the mechanism of action of deletion 5q MDS and mechanisms of response to lenalidomide. You may know thalidomide and its derivatives, lenalidomide and pomalidomide, have one of the most extraordinary histories in the history of drug development.

In particular, thalidomide was developed in the 1950s as a sedative. It was thought to be an extremely safe drug, partially because in mouse and rat studies the drug had absolutely no toxicity at all, and so it was used over-the-counter in many countries and was widely used as a hypnotic and antiemetic for women during pregnancy. As you know, it was subsequently discovered that thalidomide was causing birth defects in women who were exposed to thalidomide during pregnancy, but research on thalidomide continued. It was found to have some anticancer properties, but in particular, powerful anti-myeloma activity. Then derivatives of thalidomide, lenalidomide and pomalidomide, had even better activity in multiple myeloma. In particular, there was activity of lenalidomide in patients with MDS, specifically in patients with deletions of chromosome 5q, and lenalidomide was approved for MDS with deletions of chromosome 5q. Of note, all of this clinical development occurred in the absence of any understanding of how the drugs work. It was really just empiric clinical observations that thalidomide, lenalidomide, and pomalidomide had activity in multiple myeloma of the B-cell malignancies and deletion 5q MDS.

We and others have discovered that all of these drugs bind to a protein called CRBN, otherwise known as cereblon. CRBN is the substrate adaptor for ubiquitin ligase; it is actually called the CRL4-CRBN E3 ubiquitin ligase. What ubiquitin ligase does is, it is an enzyme that targets particular proteins for destruction by the cell. It does that by transferring ubiquitin groups serially, so that a protein becomes polyubiquitinated and that ubiquitinated protein gets destroyed by the proteasome. An E3 ligase, like the CRL4-CRBN ubiquitin ligase, recognizes particular proteins and then targets them for degradation by the proteasome by ubiquitination. The absolutely extraordinary aspect of the way lenalidomide and its other related molecules work, is that it binds to this ubiquitin ligase. Particularly, it binds to CRBN (which is a substrate adaptor which recognizes particular proteins for degradation) and it does not inhibit the activity of this enzyme. Most drugs inhibit activities of enzymes, lenalidomide does not. It binds to this protein CRBN and increases the affinity for particular substrates, and leads to the

polyubiquitination and degradation of those substrates. We first found that lenalidomide binds CRBN and induces degradation of IKZF-1 and IKZF-3, otherwise known as Ikaros and Aiolos. Those are transcription factors that are essential for the survival of multiple myeloma cells, and that explains the activity of these drugs in multiple myeloma. However, that did not explain why lenalidomide works in del 5q MDS.

What we subsequently found was that lenalidomide induces binding ubiquitination and degradation of another protein called casein kinase 1 alpha. The gene-encoding casein kinase 1 alpha is located in the common deleted region for del 5q MDS and is already expressed at heterozygous levels or haploinsufficient levels in del 5q MDS patients, and when casein kinase levels are further lowered by ubiquitination and degradation after exposure to lenalidomide, the cells are killed. This is because heterozygous loss of casein kinase 1 alpha is tolerated, homozygous loss is not tolerated by a cell. The cells that are already at haploinsufficient levels are sensitized to further degradation with casein kinase 1 alpha and elimination of those cells. That is a mechanism that has been proposed for years in cancer research, that heterozygously deleted genes could be targeted for the selective elimination of cancer cells. It turns out that lenalidomide is the first FDA-approved example of this mechanism of action working. We now understand a lot more about how lenalidomide works. We think this is the beginning of a larger class of drugs that will target ubiquitin ligases leading to ubiquitination and degradation of various proteins that are relevant to cancer and other diseases – and hopefully also relevant to MDS – but this is the first in this class of drugs to be identified.

Very briefly, I want to tell you another story that I described at the MDS International Symposium, and that is related to a manuscript that we published in the last few months in the *New England Journal of Medicine* on bone marrow transplantation for MDS. We looked at all of the American patients with MDS who underwent allogeneic stem cell transplantation over a 10-year period, who had samples banked in a central national registry called the CIBMTR and had samples available for us to sequence. In total, we sequenced over 1,500 cases of MDS, sequencing the pre-transplant sample and then analyzing the effect of the various mutations we identified on the clinical phenotype and overall survival of those patients. The dominant gene effect that we saw was that p53 mutant cases did very, very poorly – much worse than any other mutated case or other mutated gene. Most of that mortality was due to relapsed disease that occurred quite early, often within the first year posttransplant. Those patients had aggressive disease leading to mortality before the real immunologic benefit of the transplant, the graft versus MDS effect, could actually take place. That was very aggressive, however, there was about 10% of cases with p53 mutant MDS with long-term survival. It is possible that if we could keep those patients in remission for longer, then we would have much more long-term survival in those cases. We found a couple of other interesting genotype-phenotype associations.

One is that patients with RAS mutations also had high relapse, but those patients with RAS mutant MDS benefited from full myeloablative conditioning. The adverse prognosis

of having a RAS mutation was limited to the cases of MDS. Patients who underwent allogeneic stem cell transplantation who got a reduced intensity conditioning regimen, those who got full myeloablative conditioning, eliminated the adverse effects of the RAS mutation and their disease. Another gene that is really fascinating is the JAK2-mutated MDS cases. JAK2 is more commonly mutated in myeloproliferative neoplasm but is mutated in MDS as well. Those cases had a higher non-relapse-related mortality. Perhaps some aspect of cytokine signaling that gets in an inflammatory pathway, or changes to the microenvironment that occur in JAK2-mutated MDS, led to higher non-relapse-rated mortality unlike PVD3 in RAS and led to higher relapse-related mortality.

The final interesting thing that we found was that there were a substantial number of undiagnosed congenital bone marrow failure syndromes amongst the cases of MDS who underwent allo transplant, in patients under the age of 40. In particular, we found a number of undiagnosed Shwachman-Diamond Syndrome patients. They had homozygous or compound heterozygous SBDS mutations consistent with the genotype of Shwachman-Diamond Syndrome. All of the patients that we had with these homozygous SBDS mutations acquired p53 mutations and did very poorly. We think that it is useful in patients under the age of 40 to work them up for bone marrow failure syndromes. We found undiagnosed, not only Shwachman-Diamond Syndrome but telomeropathies and congenital GATA2 mutations. We think that there are undiagnosed cases of congenital bone marrow failure syndromes leading to MDS, particularly in cases of MDS under the age of 40, so being vigilant for that is warranted clinically. Thank you for listening. I wanted to tell you today about the mechanism of action of lenalidomide in del 5q MDS. Lenalidomide binds CRBN or cereblon, which is the substrate adaptor for ubiquitin ligase that leads to ubiquitination and degradation of protein called casein kinase 1 alpha, and degradation of that protein leads to elimination of del 5q cells through a p53 dependent mechanism. I also told you about a recent study that was published in the *New England Journal of Medicine* about allogeneic stem cell transplant for MDS; finding that p53 mutations confer a higher relapse-related mortality, RAS mutations confer higher relapse-related mortality, and that this effect can be eliminated by complete myeloablative conditioning. JAK2 mutations cause non-relapse-related mortality. In patients with MDS under the age of 40, there are a number of undiagnosed congenital bone marrow failure syndromes that would be useful to identify and diagnose in those patients. I appreciate your attention. Thank you for listening.