Activity of Lenalidomide in MDS Tied to Degradation of CSNK1A1

Written by Maria Vinall

Lenalidomide is a highly effective treatment for multiple myeloma (MM) and myelodysplastic syndrome (MDS) with deletion of chromosome 5q (del[5q]). In MM, the activity of lenalidomide is thought to be the result of activation of the cereblon (CRBN)-CRL4 E3 ubiquitin ligase to ubiquitinate the transcription factors IKZF1 and IKZF3 [Krönke J et al. *Science.* 2014]. Emma C. Fink an MD/PhD student at Brigham and Women's Hospital, Boston, Massachusetts, USA, reported the results of a study [Fink EC et al. ASH 2014 (abstr 4)] indicating that in MDS with del(5q), lenalidomide induces the ubiquitination of casein kinase 1A1 (CSNK1A1) by CRBN-CRL4 and its subsequent degradation by the proteasome. Haploinsufficiency for CSNK1A1 sensitizes del(5q) to lenalidomide treatment and results in p53-dependent killing.

The investigators used global proteomics profiling in the myeloid cell line KG-1 to identify CSNK1A1 as a novel and direct target of lenalidomide. CSNK1A1 is haploinsufficient in del(5q) MDS and is a negative regulator of p53 and β-catenin [Schneider RK et al. *Cancer Cell.* 2014]. Lenalidomide treatment increased ubiquitination and decreased protein levels of CSNK1A1.

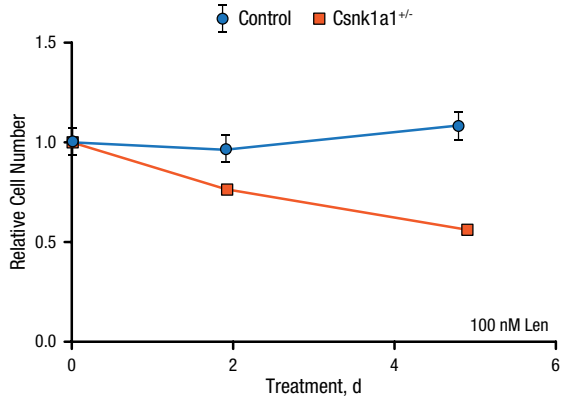
Additional analyses showed that lenalidomide treatment resulted in a dose-dependent decrease in CSNK1A1 protein levels in multiple human cell lines without altering CSNK1A1 mRNA levels. In addition, the investigators found that CSNK1A1 associates with CRBN-CRL4 only in the presence of lenalidomide, suggesting that lenalidomide induces the recruitment of CSNK1A1 to CRBN-CRL4. When mixed in vitro, CSNK1A1 is ubiquitinated by CRBN-CRL4. Ubiquitinated CSNK1A1 is then subsequently degraded by the proteasome leading to low protein levels.

The investigators then analyzed the effects of CSNK1A1 haploinsufficiency on lenalidomide sensitivity in a genetically defined Csnk1a1 conditional knockout mouse model. They found that mouse cells do not respond to lenalidomide. Expression of human CRBN rendered the cells sensitive to lenalidomide, suggesting that sequence differences between mouse and human CRBN, the direct binding partner of lenalidomide, explained this species-specific response. A single amino acid change in mouse CRBN (isoleucine to valine at position 391; I391V) was identified that restored lenalidomide response in mouse CRBN.

After identifying a method to make mouse cells sensitive to lenalidomide, the investigators returned to the Csnk1a1 conditional knockout mouse model. Bone marrow cells from control mice or mice heterozygous for Csnk1a1 were transduced with the mouse CRBN I391V allele then treated with lenalidomide. Unlike controls, which did not respond to lenalidomide, Csnk1a1$^{+/-}$ cells depleted almost 50% over 5 days of lenalidomide treatment. These results demonstrate that hematopoietic Csnk1a1$^{+/-}$ cells are more sensitive to lenalidomide than wild type cells with 2 copies of the gene (Figure 1).

Lenalidomide treatment led to the induction of the p53 target p21 in Csnk1a1$^{+/-}$ cells, suggesting that the activation of the p53 pathway is involved in lenalidomide's downstream mechanism. Deletion of a single allele of p53 completely rescued the lenalidomide sensitivity of Csnk1a1$^{+/-}$ cells, implying a p53 killing mechanism. Further, CSNK1A1 overexpression reduces lenalidomide sensitivity in del(5q) patient samples (Figure 2).

Figure 1. Csnk1a1$^{+/-}$ Cells Are More Sensitive to Lenalidomide



Csnk1a1, casein kinase 1A1; Len, lenalidomide.
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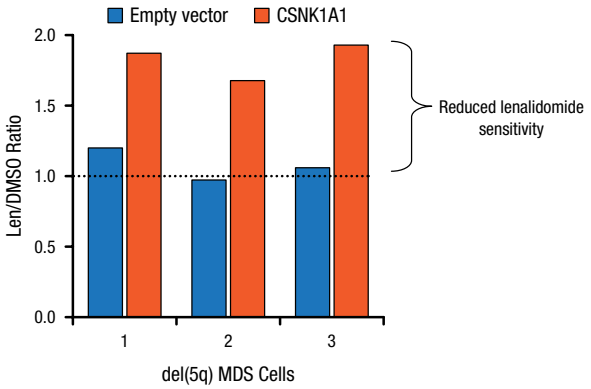
Figure 2. Effect of Overexpression of CSNK1A1 on Lenalidomide Sensitivity of del(5q)



CSNK1A1, casein kinase 1A1; del(5q), deletion of chromosome 5q; DMSO, dimethyl sulfoxide; len, lenalidomide; MDS, myelodysplastic syndrome.
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To conclude, Dr Fink noted that another study [Macbeth et al. ASH 2014 (abstr 3606)] showed similar results regarding lenalidomide inducing ubiquitination of CSNK1A1 by the CRBN-CRL4 and its subsequent degradation.

Th17-Prone CD146$^+$CCR5$^+$ T-Cell Population Is an Early Marker of Intestinal GVHD

Written by Maria Vinall

Acute graft-vs-host disease (GVHD) is a major complication of allogeneic hematopoietic stem cell transplantation (HSCT), primarily affecting the skin, liver, and gastrointestinal (GI) tract. GVHD limits the role of transplantation in other clinical settings, such as the treatment of severe autoimmune disorders. T lymphocytes in the peripheral blood play a central role in immunity and in the process whereby newly transplanted donor cells attack the transplant recipient's body.

In a late-breaking clinical trial [Li W et al. *Blood*. 2014], Wei Li, MD, PhD, Indiana University, Indianapolis, Indiana, USA, reported that early quantification of a novel Th17-prone CD146$^+$CCR5$^+$ inducible T-cell costimulator (ICOS)–induced population may identify patients at risk for GI GVHD development and subsequent mortality.

Peripheral blood cells from 214 HSCT patients (71 GI GVHD, 48 no GVHD, 33 non-GVHD enteritis, 22 skin-first GVHD, 40 isolated-skin GVHD) were analyzed using proteomics 14 days prior to the onset of GVHD symptoms. Biomarkers that increased 1.5-fold were identified in the plasma from GI GVHD patients and compared with HSCT patients without GVHD at matched time points. Two proteins were identified: CD146, a cell adhesion and trafficking molecule expressed on a subset of CD4$^+$ T cells and endothelial cells, and the chemokine (C-C motif) ligand 14, which binds to the chemokine receptor CCR5 on T cells.

CD146$^+$CCR5$^+$ T-cell frequency was significantly increased in patients with GI GVD compared with patients without GVHD ($P < .0001$), non-GVHD enteritis ($P < .0001$), or isolated-skin GVHD ($P = .007$) but not with skin-first and then GI GVHD ($P = .28$).

CD146$^+$CCR5$^+$ T cells were not correlated with GI histologic severity and increased prior to GVHD clinical onset. CD146$^+$CCR5$^+$ T cells were Th17 prone in that Th17 cells express more CD146 than Th1 cells. The activation marker ICOS, known to be critical for the development of human Th17 cells, was also critical for the expression of CD146$^+$CCR5$^+$ on T cells.

Th17 cells migrated more efficiently through endothelial-cell monolayers than their Th1 counterparts, suggesting that endothelium may play an important role in recruiting pathogenic T cells. This was supported by evidence showing that CD146 Th17 transmigration is reduced by CD146 shRNA knockdown on T cells. However, knockdown of CD146 on endothelial cells does not reduce T-cell transmigration.

Finally, to evaluate the in vivo function of CD146 T cells, donor human T cells knockdown with CD146shRNA were transmigrated into a xenogeneic GVHD mouse model. Mice did not lose weight, had similar human T-cell engraftment (hCD4), had fewer splenic CD146$^+$CCR5$^+$T cells, and expressed less interferon gamma 53 days after transplant, providing proof that CD146 promotes infiltration of pathogenic T cells into GVHD target organs.

The CD146$^+$CCR5$^+$ cell population is a biomarker of GI GVHD. Early quantification of this population of cells may predict the development of GI GVHD and offer more specific prevention and therapeutic strategies, thereby reducing mortality.

Sorafenib as Effective Treatment for Newly Diagnosed AML in Younger Patients

Written by Maria Vinall

Christoph Röllig, MD, Universitätsklinikum Dresden, Dresden, Germany, presented the results of the Study Evaluating Sorafenib Added to Standard Primary Therapy in Patients With Newly Diagnosed Acute Myeloid Leukemia Less Than 60 Years of Age [SORAML; Röllig C et al. ASH 2014 (abstr 6)]. Although overall survival (OS) was not different compared with placebo, sorafenib significantly improved event-free survival (EFS) and relapse-free survival (RFS) with a cost of higher incidence of infections and bleeding events in younger patients with acute myeloid leukemia (AML).

AML is the most common form of leukemia in adults [National Cancer Institute. http://www.cancer.gov/cancertopics/pdq/treatment/adultAML/Patient/page1. Accessed December 23, 2014]. Survival rate for this disease continues to remain unsatisfactory, particularly among patients aged > 60 years. The significant genetic diversity and abnormality in AML even within a tumor of a single individual make it difficult to treat [Cancer Genome Atlas Research Network. *N Engl J Med*. 2013]. Kinase mutations