Identification and Prognostic Significance of Novel IRF8 Transcripts in AML

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As a heterogeneous group of blood cancers, acute myeloid leukemia (AML) is subdivided according to cytogenetics, gene mutations, and deregulated gene expression for risk classification. Because of the molecular complexity of the disease, single or restricted numbers of biomarkers cannot accurately predict clinical outcomes for all AML patients. Identifying additional biomarkers could more accurately define patient prognosis when combined with known prognostic factors. Interferon regulatory factor 8 (IRF8) is a transcription factor important for blood cell development. Previous research by Dr. Derek Stirewalt (Clinical Research Division) and others described aberrant expression of *IRF8*in ageing hematopoietic stem cells and leukemic blast cells from adult AML patients; however, it has been unclear whether aberrant *IRF8* expression predicts patient prognosis. In a new study, lead author Era Pogosova-Agadjanyan and colleagues in the Stirewalt laboratory identify expression of alternative *IRF8* transcripts in leukemic cells and determine that high *IRF8* expression predicts increased disease relapse.

IRF8 controls the transcription of a number of genes necessary for hematopoiesis, particularly in the subset of white blood cells known as myeloid cells. Loss of IRF8 function promotes granulocyte maturation and blocks monocyte development, while constitutive expression of IRF8 has opposite effects on these processes. Studies have demonstrated that inactivation of *IRF8* in mice leads to myeloproliferative disease that progresses to an AML-like disease. Dr. Stirewalt and colleagues first described decreased *IRF8* expression in ageing healthy patient hematopoietic stem cells (Stirewalt *et al*., 2009). Furthermore, AML patient cells show decreased *IRF8*expression.

In the current study, Pogosova-Agadjanyan *et al*. identified three novel splice variants of *IRF8* with a cryptic initial exon originating in the first intron, while the wild type first exon was missing. The researchers assessed expression of wild type *IRF8* and the novel splice variants in 194 adult AML patient samples using optimized quantitative assays for the transcripts. The majority of AML patients had low levels of all *IRF8* transcripts, but a greater than 2-fold increase was observed in 12% of patients for wild type *IRF8* and 14% of patients for the splice variants. No significant association was found for any of the *IRF8* transcript variants with age, molecular biomarkers, or cytogenetics.

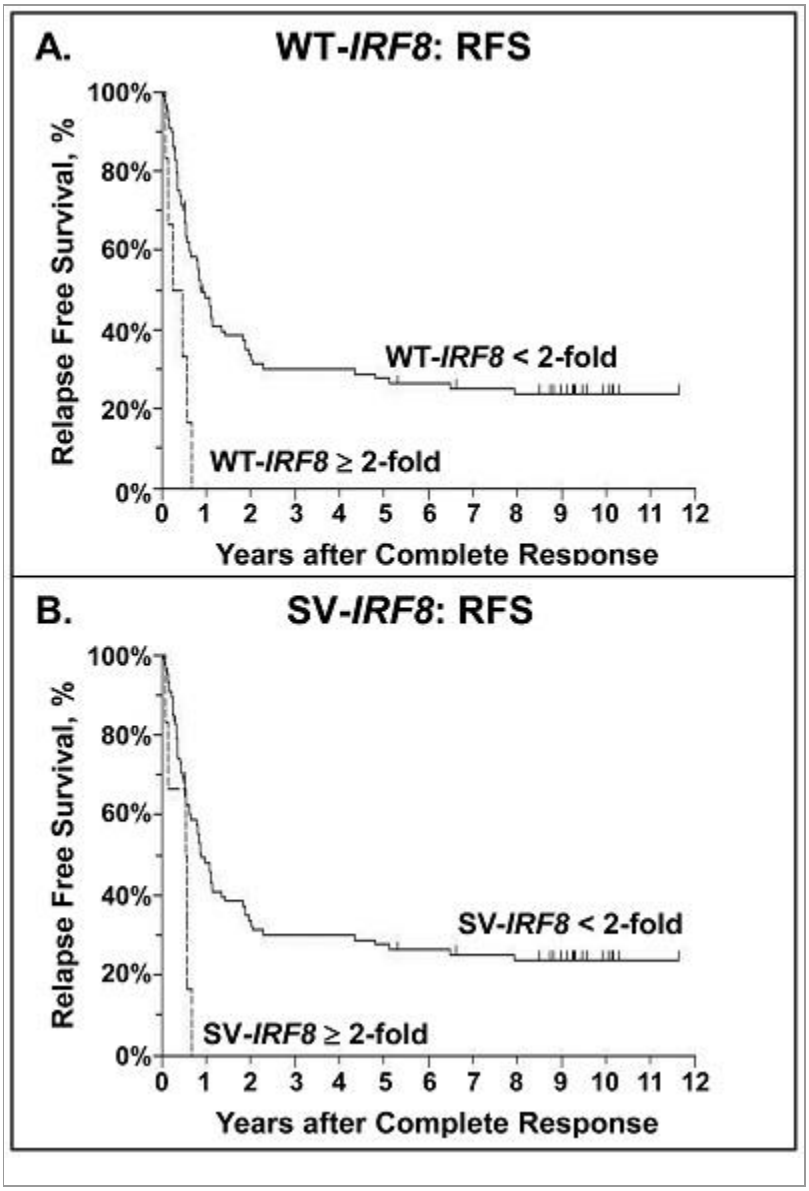
Furthermore, no significant association between*IRF8* expression levels and complete response or overall survival was detected taking into account other prognostic factors. However, relapse-free survival was significantly associated with increased expression of both wild type and splice variant *IRF8*as a single prognostic factor in univariate analysis (P = 0.01 and P = 0.026) and with other prognostic factors in multivariate analysis (P = 0.019 and P = 0.021).

Whether the alternative splice variants produce functional proteins remains to be determined. If produced, the novel proteins may disrupt normal IRF8 function during hematopoiesis and lead to disease, or may alternatively serve as surrogate biological markers for another biological state. Preliminary studies indicate that initiation of the alternative splice variants is governed by promoter hypermethylation; thus, these splice variants could serve as a biomarker for global hypermethylation in AML cells.

"This study showed that malignant cells from patients with AML frequently initiate transcription at aberrant regions within the genome, leading to the expression of alternative splice variants," according to Era Pogosova-Agadjanyan and Dr. Stirewalt. "Using RNA Sequencing and other methods, we have identified other genes that display similar alternative transcripts due to initiation of transcription at aberrant regions in the genome." These changes are being monitored in distinct populations of AML patient cells and the Stirewalt laboratory is pursuing the functional significance and prognostic impact of these changes.

Pogosova-Agadjanyan EL, Kopecky KJ, Ostronoff F, Appelbaum FR, Godwin J, Lee H, List AF, May JJ, Oehler VG, Petersdorf S, Pogosov GL, Radich JP,Willman CL, Meshinchi S, Stirewalt DL. 2013. The Prognostic Significance of IRF8 Transcripts in Adult Patients with Acute Myeloid Leukemia. *PLoS One* 8:e70812.

Also see: Stirewalt DL, Choi YE, Sharpless NE, Pogosova-Agadjanyan EL, Cronk MR, Yukawa M, Larson EB, Wood BL, Appelbaum FR, Radich JP, Heimfeld S. 2009. Decreased IRF8 expression found in aging hematopoietic progenitor/stem cells.*Leukemia* 23:391-3.



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Decreased relapse-free survival in AML patients with increased expression (greater than 2-fold) of both (A) wild type IRF8 (WT-IRF8, n = 94 patients) and (B) novel IRF8 splice variants (SV-IRF8, n = 92 patients). Kaplan-Meier estimates were calculated for patients who achieved complete remission status.