Published in final edited form as:

Leuk Res. 2009 July ; 33(7): e81–e82. doi:10.1016/j.leukres.2008.11.030.

Assessment of *ATR*X Expression in Patients With Myelodysplastic Syndromes Treated with Decitabine

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Treatment with one of the DNA methyltransferase inhibitors (DNMTIs, “hypomethylating agents”), azacitidine or decitabine, is now considered standard care for patients with higher-risk myelodysplastic syndromes (MDS) (1) – especially since results were released from the AZA-001 trial (2), which demonstrated a 9-month survival advantage with azacitidine therapy compared to supportive care. However, many patients with MDS do not respond to treatment with DNMTIs as they are currently used, and adverse events are common. It is desirable to be able to predict *a priori* which patients are most likely to benefit from DNMTI therapy, in order to spare patients unlikely to respond from the risks and cost of treatment, but there are currently no well-established molecular methods for DNMTI response prediction.(3)

*ATR*X is an X-encoded chromatin-associated protein with an evolutionarily conserved Nterminal DNA methyltransferase (DNMT3-like) domain.(4) Germline mutations in *ATR*X cause a mental retardation-dysmorphology syndrome often associated with mild alpha thalassemia, which gave the gene its name (*ATR*-X syndrome: alpha thalassemia, retardation, X-linked) (5); in contrast, somatic point mutations in *ATR*X have been linked to acquired alpha thalassemia arising in the context of MDS, which may be present in as many as 8% of patients. (6-8) Germline mutations in *ATR*X are associated with widespread and diverse DNA methylation abnormalities across the genome.(9) Recent evidence suggests that the *ATR*X protein also has an important role in chromosome dynamics during mitosis, as *ATR*X-depleted cells exhibit defective sister chromatid cohesion and congression at the metaphase plate, as well as abnormal chromosome alignment. (10,11)

Circumstantial evidence for a role of *ATR*X in neoplasia is increasing. In an array-based gene expression profiling study of 42 children with acute myeloid leukemia (AML) associated with somatic FLT3 mutations, the expression ratio of the transcription factor *RUNX3* (formerly *AML2*) to *ATR*X predicted event-free survival (EFS).(12) In a subsequent study of 132 adults with de novo AML by another investigative group, low *ATR*X expression correlated with high-risk karyotype and poor clinical outcome.(13) Altered *ATR*X expression levels have also been reported in gene expression profiling experiments using prostate cancer primary cells (14), esophageal squamous cell carcinoma cell lines (15), chronic lymphocytic leukemia primary cells (Neil Kay, personal communication October 2007), and irradiated breast cancer cell lines (16).

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Given the known effects of ATRX on global DNA methylation, the suggestive but still puzzling findings with respect to ATRX in cancer generally and myeloid neoplasia in particular, and the hypothesized mechanism of action of decitabine as a DNMTI, we studied *ATRX* and *RUNX3* gene expression levels in MDS patients enrolled in the DACO-020 clinical trial, an open-label North American multicenter Phase 2 study of decitabine administered at a dose of 20 mg/m²/day IV daily for 5 days every 4 weeks.(17)

The clinical trial enrolled 99 consenting patients with International Prognostic Scoring System (IPSS) Intermediate-1 or higher risk MDS, who were treated at 28 different institutions. The study was approved by the Institutional Review Boards of participating institutions. RNA was isolated from patients' density centrifugation-separated peripheral blood mononuclear cells (PBMCs) prior to treatment. We confirmed RNA quality, generated cDNA, and performed real-time PCR (RT-PCR) with the Hs00230877_m1 *ATRX* and Hs00231709_m1 *RUNX3* multiplex primer-probe sets with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) controls. Additionally, we performed brilliant cresyl blue stains to look for hemoglobin H inclusions and sequenced *ATRX* in all patients with inclusions (previous investigations (8) have shown that *ATRX* is wild-type in MDS patients without hemoglobin H inclusions.).

We were able to extract RNA of sufficient quantity and quality for analysis from PBMCs of 80 of the 99 patients enrolled in the multicenter clinical trial (81%). Clinical response data were available from all patients, and cytogenetic data from 74 patients; 38 (51%) had an abnormal karyotype. *ATRX* and *RUNX3* expression or the ratio did not correlate with either the presence or complexity of a cytogenetic abnormality, and also did not predict clinical response to decitabine therapy ($p>0.20$ for all comparisons). Therefore, we were unable to confirm a correlation between *ATRX* or *RUNX3* expression levels and either cytogenetic patterns or treatment outcomes in MDS. Only 1 patient had any Hb H inclusions, and sequencing of *ATRX* in this patient did not reveal any mutations.

There are several possible reasons for failure to confirm the hypothesis. The cell population studied (PBMCs) may not have allowed a true measure of *ATRX/RUNX3* expression levels in the neoplastic clone. We were limited to using PBMCs because of small volumes of sample received from various sites, precluding separation of CD34+ cells, and a high rate of neutropenia among enrolled patients. It is also possible that *ATRX* expression has distinct effects in MDS compared to other neoplasms such as AML. These possibilities require further investigation, as the recurrent finding of *ATRX* expression changes in various neoplasms suggests a previously unrecognized role for the ATRX protein in cancer.

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