Published in final edited form as:

Leuk Lymphoma. 2013 November ; 54(11): 2565–2567. doi:10.3109/10428194.2013.782610.

HMGA1 overexpression correlates with relapse in childhood B-lineage acute lymphoblastic leukemia

Sujayita Roy^{1,2}, Francescopaolo Di Cello^{1,2}, Jeanne Kowalski^{3,4,*}, Alexandra C. Hristov^{5,*}, Hua-Ling Tsai^{3,4}, Deepa Bhojwani⁶, Julia A. Meyer⁷, William L. Carroll⁷, Amy Belton^{1,2}, and Linda M. S. Resar^{1,2,4,8}

¹Hematology Division, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

²Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

³Department of Biostatistics, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁴Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁵Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁸Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁶Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN, USA

⁷New York University Langone Medical Center and Cancer Institute, New York, NY, USA

Despite dramatic improvements in treatment and survival, acute lymphoblastic leukemia (ALL) remains the leading cause of death in children with cancer [1]. ALL is the most common pediatric cancer, accounting for 25% of all cancers occurring in children under the age of 15 years [1]. The majority of cases are a B-lineage cell subtype of ALL (B-ALL). Although most children with ALL are cured with current chemotherapeutic regimens, approximately 15% will relapse and 8–9% will ultimately succumb to their disease [1]. Risk assessment is a critical component in the selection of appropriate therapy for individual patients. Unfortunately, current criteria used to stratify patients into groups at increasing risk for treatment failure or relapse are limited, and rely primarily on clinical characteristics (age, white blood cell count, immunophenotype, presence/absence of central nervous system disease or testicular disease) and underlying genetic lesions. Good risk genetic lesions include *ETV6-RUNX1* fusion or hyperdiploidy (50 or more chromosomes) with favorable chromosomal trisomies (4 + 10), while poor risk genetic lesions include *MLL* rearrangements (*MLL*-R), hypodiploidy, intrachromosome 21 (iAMP21) or Philadelphia

Correspondence: Linda Resar, The Johns Hopkins University School of Medicine, Ross Research Building, Room 1025, 720 Rutland Ave., Baltimore, MD 21205, USA. Tel: 410-614-0712. Fax: 410-955-0185. lresar@jhmi.edu.

*Current affiliation of Jeanne Kowalski is Department of Biostatistics and Bioinformatics, Emory University, Atlanta, GA, USA. Current affiliation of Alexandra Hristov is Departments of Dermatology and Pathology, University of Michigan Health Sciences Center, Ann Arbor, MI, USA.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahelthcare.com/lal.

chromosome positive (Ph+) ALL [1]. In addition, early treatment response is included in risk stratification and determined by measurement of minimal residual disease (MRD) at specific time points early in therapy. The 5-year overall survival in ALL has increased from only 10% in the 1960s to about 80% in the 1980s, and very recent studies indicate that 92% of children with ALL will be cured with current therapies [1,2]. Nonetheless, many patients are over-treated, whereas a significant number of children who were initially classified as favorable risk suffer a relapse. Thus, elucidating the molecular underpinnings of refractory disease in ALL is needed to identify patients who are likely to relapse and require more intensive chemotherapeutic regimens. Moreover, identifying key molecular pathways could also uncover novel therapeutic targets.

We previously discovered that the high mobility group A1 (*HMGA1*) oncogene induces leukemic transformation in cultured cells and causes aggressive T-cell acute lymphoid leukemia in transgenic mice [2–7]. The *HMGA1* gene encodes the HMGA1a and HMGA1b chromatin remodeling proteins (formerly HMGI and HMGY, respectively), which differ by an internal 11 amino acids that result from alternatively spliced mRNA [2]. *HMGA1* is overexpressed in diverse, high-grade or refractory malignancies, including hematologic malignancy and solid tumors [2–9]. Indeed, HMGA1 proteins are the most abundant, non-histone, chromatin binding proteins found in cancer cells [2]. *HMGA1* is also highly expressed during embryogenesis and enriched in embryonic stem cells, hematopoietic stem cells and leukemic stem cells [10–12]. A recent landmark study showed that HMGA1 is required for the cellular reprogramming of somatic cells to induced pluripotent stem cells by the four Yamanaka factors [11]. The HMGA1 proteins modulate gene expression by altering chromatin structure and facilitating DNA-transcription factor interactions as part of an enhanceosome. Blocking *HMGA1* expression or function interferes with multiple cancer phenotypes, including proliferation, anchorage-independent cell growth, migration, invasion, xenograft tumorigenesis and tumor progression in murine models [2,3,9]. Inhibiting *HMGA1* expression also blocks cancer stem cell phenotypes, such as three-dimensional sphere formation and limiting dilution tumorigenesis [9]. The transcriptional networks regulated by HMGA1 are emerging, and include genes involved in hematopoiesis, stem cell function, signal transduction, cellular motility and inflammation [2,6,9,11]. A prior study in T-ALL also found that HMGA1 regulates homeobox genes in a subset of T-ALL [13]. In addition, a recent report indicates that *HMGA1* cooperates with loss of function of the *CDKN2A* (*INK4A/ARF*) tumor suppressor locus in mouse models of leukemia [7]. In diverse solid tumors, *HMGA1* expression portends a poor prognosis [2,9]. Together, these studies suggest that *HMGA1* could serve as a marker for poor outcomes in hematologic malignancy, such as childhood B-ALL.

To determine whether *HMGA1* expression correlates with relapse in pediatric ALL, we interrogated gene expression profiles generated from leukemic blasts from children with relapsed B-ALL enrolled on contemporary Children's Oncology Group (COG) protocols [14]. Paired samples were available from patients at diagnosis and relapse. Gene expression profiles were generated (Affymetrix U133A/U133Plus 2.0 arrays) with RNA extracted from leukemic blasts. To assess *HMGA1* gene expression, the probe 206074_s_at was chosen because it targets the 3' region and is predicted to correlate with full length *HMGA1* gene expression. Among the 86 patients, 53 had early relapses (< 36 months) and 33 patients had

late relapses (> 36 months). Expression data were analyzed by the robust microarray (RMA) approach across all samples with log₂ scale [6,7,14]. *HMGA1* expression levels at diagnosis and relapse were compared using the paired *t*-test. In leukemic blasts from patients with early relapses, we found a significant increase in the mean expression of *HMGA1* at relapse [0.36; 95% confidence interval (0.17, 0.54); *p* = 0.0003; Figure 1(A)]. Similarly, there was a significant increase in the mean expression of *HMGA1* in blasts at relapse from patients who relapsed late [0.50, 95% confidence interval (0.11, 0.89); *p* = 0.01; Figure 1(A)]. There were no significant differences in *HMGA1* expression levels between early and late relapses at either diagnosis or relapse. Together, these data suggest that higher expression of *HMGA1* correlates with relapsed disease.

To independently validate these data in a separate cohort of patients, we assessed *HMGA1* gene expression from RNA isolated from leukemia blasts using quantitative reverse transcription-polymerase chain reaction (RT-PCR) as we previously described [4–6]. We found that *HMGA1* mRNA levels were higher in patients at relapse as compared to diagnosis [Figure 1(B)]. As shown, the mean expression at relapse was 1.87 ± 0.58 as compared to 1.41 ± 0.042 (*p* = 0.041 by paired *t*-test.) These results further implicate high expression of *HMGA1* as a potential driver of refractory disease.

Next, we consulted published databases using OncomineTM (Compendia Bioscience, Ann Arbor, MI) to determine whether overexpression of *HMGA1* was a consistent finding in other pediatric B-ALL studies [15–17]. We discovered that *HMGA1* is among the top 11% of genes as compared to B-cell progenitors in a prior study of pediatric B-ALL [*p* = 0.048; [16]; Figure 1(C)], and in the top 21% of genes overexpressed as compared to normal bone marrow in another pediatric B-ALL study [*p* = 0.000068; Figure 1(D)] [15]. This latter group also noted extremely high *HMGA1* expression in B-ALL samples with a translocation in *cMYC*t(8;14), although only two samples had this molecular lesion [15]. We previously discovered that *HMGA1* is a *cMYC* transcriptional target [3], which is consistent with high expression of *HMGA1* in the setting of translocations associated with deregulated *cMYC*. Significant overexpression of *HMGA1* was not observed in a third database with samples from multiple institutions ([17]; not shown). Although these databases did not link expression levels to outcome, they indicate that *HMGA1* is enriched in a subset of pediatric B-ALL blasts.

Here, we report for the first time that high expression of *HMGA1* correlates with relapse in leukemic blasts from children with B-ALL. Our recent study demonstrating a central role for *HMGA1* in embryonic stem cells and cellular reprogramming to an induced pluripotent stem cell [11] suggests that *HMGA1* could drive refractory disease by inducing stem-like transcriptional networks. Indeed, prior functional studies in murine tumor models of solid tumors indicate that *HMGA1* is required for cancer stem cell properties, including limiting dilution tumorigenesis and growth like stem cells as three-dimensional spheres [9]. Further studies will be needed to determine whether *HMGA1* is required for leukemic stem cell properties. Of note, *HMGA1* was identified in a leukemic stem cell signature in a murine model of acute myeloid leukemia [12]. In preliminary studies, we found enrichment of *HMGA1* or *HMGA2* in leukemic stem cells from primary acute myeloid leukemia samples (Resar, unpublished data). Although further studies are needed, these findings suggest that

HMGA1 plays an important role in refractory status in ALL and could serve as a potential biomarker or rational therapeutic target.

References

1. Hunger SP, Loh ML, Whitlock JA, et al. Children's Oncology Group's 2012 blueprint for research: acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2012 Dec 19. [Epub ahead of print]. doi: 10.1002/pbc.24420
2. Resar LM. The high mobility group A1 gene: Transforming inflammatory signals into cancer? *Cancer Res*. 2010; 70:436–439. [PubMed: 20068164]
3. Wood LJ, Mukherjee M, Dolde CE, et al. HMG-I/Y, a new c-myc target gene and potential oncogene. *Mol Cell Biol*. 2000; 20:5490–5502. [PubMed: 10891489]
4. Xu Y, Sumter TF, Bhattacharya R, et al. The HMG-I oncogene causes highly penetrant, aggressive lymphoid malignancy in transgenic mice and is overexpressed in human leukemia. *Cancer Res*. 2004; 64:3371–3375. [PubMed: 15150086]
5. Hillion J, Dhara S, Sumter TF, et al. The high-mobility group A1a/signal transducer and activator of transcription-3 axis: an achilles heel for hematopoietic malignancies? *Cancer Res*. 2008; 68:10121–10127. [PubMed: 19074878]
6. Schuldenfrei A, Belton A, Kowalski J, et al. HMGA1 drives stem cell, inflammatory pathway, and cell cycle progression genes during lymphoid tumorigenesis. *BMC Genomics*. 2011; 12:549. [PubMed: 22053823]
7. Di Cello F, Dhara S, Hristov AC, et al. Inactivation of the Cdkn2a locus cooperates with HMGA1 to drive T-cell leukemogenesis. *Leuk Lymphoma*. 2013 Jan 11. [Epub ahead of print]. doi: 10.3109/10428194.2013.764422
8. Nelson DM, Joseph B, Hillion J, et al. Flavopiridol induces BCL-2 expression and represses oncogenic transcription factors in leukemic blasts from adults with refractory acute myeloid leukemia. *Leuk Lymphoma*. 2011; 52:1999–2006. [PubMed: 21728742]
9. Belton A, Gabrovsky A, Bae YK, et al. HMGA1 induces intestinal polyposis in transgenic mice and drives tumor progression and stem cell properties in colon cancer cells. *PLoS One*. 2012; 7:e30034. [PubMed: 22276142]
10. Karp JE, Smith BD, Resar LS, et al. Phase 1 and pharmacokinetic study of bolus-infusion flavopiridol followed by cytosine arabinoside and mitoxantrone for acute leukemias. *Blood*. 2011; 117:3302–3310. [PubMed: 21239698]
11. Shah SN, Kerr C, Cope L, et al. HMGA1 reprograms somatic cells into pluripotent stem cells by inducing stem cell transcriptional networks. *PLoS One*. 2012; 7:e48533. [PubMed: 23166588]
12. Somervaille TC, Matheny CJ, Spencer GJ, et al. Hierarchical maintenance of MLL myeloid leukemia stem cells employs a transcriptional program shared with embryonic rather than adult stem cells. *Cell Stem Cell*. 2009; 4:129–140. [PubMed: 19200802]
13. Nagel S, Scherr M, Kel A, et al. Activation of TLX3 and NKX2-5 in t(5;14)(q35;q32) T-cell acute lymphoblastic leukemia by remote 3'-BCL11B enhancers and coregulation by PU.1 and HMGA1. *Cancer Res*. 2007; 67:1461–1471. [PubMed: 17308084]
14. Hogan LE, Meyer JA, Yang J, et al. Integrated genomic analysis of relapsed childhood acute lymphoblastic leukemia reveals therapeutic strategies. *Blood*. 2011; 118:5218–5226. [PubMed: 21921043]
15. Andersson A, Olofsson T, Lindgren D, et al. Molecular signatures in childhood acute leukemia and their correlations to expression patterns in normal hematopoietic subpopulations. *Proc Natl Acad Sci USA*. 2005; 102:19069–19074. [PubMed: 16354839]
16. Maia S, Haining WN, Ansén S, Xia Z, et al. Gene expression profiling identifies BAX-delta as a novel tumor antigen in acute lymphoblastic leukemia. *Cancer Res*. 2005; 65:10050–10058. [PubMed: 16267031]
17. Haferlach T, Kohlmann A, Wiecek L, et al. Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: report from the International Microarray Innovations in Leukemia Study Group. *J Clin Oncol*. 2010; 28:2529–2537. [PubMed: 20406941]

Figure 1.

HMGA1 expression is higher at relapse compared to diagnosis in B-ALL blasts. (A) *HMGA1* expression (by gene expression profile analysis) from leukemic blasts from children with B-ALL at diagnosis and early relapse (< 36 months; $n = 53$) or late relapse (>

36 months; $n = 33$). (B) *HMGA1* expression (by quantitative RT-PCR) from leukemic blasts from children with B-ALL at diagnosis and relapse ($n = 10$). (C) B-ALL blasts ($n = 19$) expressed about 2.5-fold higher *HMGA1* as compared to immature B cells from normal bone marrow (assigned a value of 1.0; $p = 0.048$ by Student's t -test.) Bars show mean $\pm$ standard deviation. Graph shows *HMGA1* expression as assessed by probe 206074_s_at. (D) B-ALL blasts ($n = 86$) expressed about 2.5-fold higher *HMGA1* as compared to blasts from normal bone marrow (assigned a value of 1.0; $p = 6.87 \times 10^{-6}$ by Student's t -test.) Bars show mean $\pm$ standard deviation. Graph shows *HMGA1* expression as assessed by IMAGE probe 782811(2); fold change for IMAGE probe 783811(1) was similar.