Relative Receptor Tyrosine Kinases and Anti-Apoptotic Transcripts Hold Potential for Predicting Inferior Outcome in Adult Acute Myeloid Leukemia: A Prospective Pilot Study

Surender Kumar Sharawat,[1] Sreenivas Vishnubhatla,[2] Radhika Bakhshi,[3] Vinod Raina,[1] Lalit Kumar,[1] Atul Sharma,[1] Sameer Bakhshi[1]

Abstract

Studies on simultaneous expression of proliferative (Fms-like tyrosine kinase 3 [*FLT-3*], *c-KIT* [v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog]) and anti-apoptotic genes (*BCL-2* [B-cell CLL/lymphoma 2]) in acute myeloid leukemia (AML) are not available in the literature. In a stepwise Cox regression model, high *FLT-3* and *c-KIT*/*BCL-2* ratio predicted overall survival (OS; hazard ratio [HR], 2.29). Our data are in concordance with global gene expression profiles of adult AML patients (Microarray Data Set: GSE1159). Interrelationship of proliferative and antiapoptotic markers are independent predictors of survival in AML.

Introduction: Acute myeloid leukemia is characterized by accumulation of immature cells because of imbalance between proliferation and apoptosis. In AML, simultaneous expression of proliferative (*FLT-3*, *c-KIT*) and antiapoptotic genes (*BCL-2*), are unknown. **Patients and Methods:** We prospectively assessed proliferative and antiapoptotic gene transcripts using Taqman probe chemistry in 48 adult AML patients. A stepwise Cox regression model was applied for independent prognostic factors. **Results:** Thirty-two of 48 (75%) patients achieved complete remission. At follow-up ranging from 0.5 to 57.3 months, event-free survival (EFS) was 26.9 ± 6.3% (range, 15.5%-39.6%) and OS 34.5 ± 7.46% (range, 20.5%-48.9%). High white blood cell count correlated with an inferior complete remission rate ($P = .021$). Cytogenetics and *FLT-3* internal tandem duplication did not predict EFS or OS. The transcripts of *FLT-3*, *c-KIT*, and *BCL-2* showed a significant linear association with each other in Pearson correlation (*FLT-3* vs. *c-KIT*: $R = 0.8234$; $P < .001$; *c-KIT* vs. *BCL-2*: $R = 0.3377$; $P = .01$; *FLT-3* vs. *BCL-2*: $R = 0.3815$; $P = .007$). In a validation cohort (Microarray Data Set GSE1159) of adult AML patients, the global gene expression profile depicted a similar interrelationship. Patients with a greater platelet count were associated with increased transcript levels of *BCL-2* ($P = .034$). In univariate analysis, a high transcript level of *FLT-3* and high transcript ratio of *FLT-3*/*BCL-2* and *FLT-3* and *c-KIT*/*BCL-2* significantly predicted OS ($P = .043$, .028, and .028, respectively). In a stepwise Cox regression model, high *FLT-3* and *c-KIT*/*BCL-2* ratio predicted OS (HR, 2.29). **Conclusion:** To our knowledge, this is the first study that evaluated proliferative and antiapoptotic transcripts simultaneously, and results have shown that it is the relative levels of these transcripts that determine outcome in AML patients rather than their expression in isolation.

Clinical Lymphoma, Myeloma & Leukemia, Vol. 14, No. 6, 501-8
Keywords: Acute Myeloid Leukemia, *BCL-2*, *c-KIT*, *FLT-3*, Real-time PCR

[1]Department of Medical Oncology, Dr. B.R.A. Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi, India
[2]Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, India
[3]Department of Biomedical Sciences, Shaheed Rajguru College of Applied Sciences, University of Delhi, New Delhi, India

Submitted: Feb 7, 2014; Revised: Mar 21, 2014; Accepted: Apr 3, 2014; Epub: Jun 11, 2014

Address for correspondence: Sameer Bakhshi, MD, Department of Medical Oncology, Dr B.R.A. Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi 110 029, India
Fax: 91-11-26588663; e-mail contact: sambakh@hotmail.com

2152-2650/$ - see frontmatter
http://dx.doi.org/10.1016/j.clml.2014.04.005

Introduction

Acute myeloid leukemia (AML) is characterized by a predominance of altered myeloblasts and loss of normal hematopoiesis mainly because of an imbalance between proliferation and apoptosis.[1] Proliferative markers, receptor tyrosine kinases (RTKs) contribute significantly for leukemogenesis.[2-5] The key proliferative RTKs for AML include the *c-KIT* receptor and its ligand stem cell factor, and Fms-like tyrosine kinase 3 (*FLT-3*) receptor and its ligand. Mutations of these receptors have been extensively evaluated for their prognostic significance in AML.[6-8] In AML, there are data to suggest that high *FLT-3* expression is associated with a poor prognosis but data on *c-KIT* expression are controversial with regard to outcome.[9-11]

Apoptosis and cell cycle regulation is the inherent mechanism to check uncontrolled growth of cells; alteration in these key regulatory pathways confers a proliferative advantage to the cells. In the apoptosis regulatory system, the balance of proapoptotic and antiapoptotic proteins in a cell affect the apoptotic state of that cell. *BCL-2* (B-cell CLL/lymphoma 2), an antiapoptotic gene, has been studied extensively in different cancers and leukemia to determine the apoptotic state of the oncogenic cell. Previously, *BCL-2* and relative *BAX* (BCL2-associated X protein)/*BCL-2* was evaluated in AML for its clinical, biological, and pathological contribution in leukemia development, and has been variably linked to outcome in AML.[12-14]

In the current study our primary objective was to investigate the cumulative effect of gene expression (*FLT-3*, *c-KIT*, and *BCL-2*, involved directly or indirectly in proliferation) on survival outcome of AML patients. Although they have been individually evaluated in AML patients, their simultaneous expression and how they vary with respect to each other and their relationship with outcome is not known. Logically one would expect that increased expression of all of these 3 proteins would indirectly lead to increased proliferation of myeloblasts. In the current study, we prospectively assessed proliferative and antiapoptotic gene transcripts, their interrelationship, and effect on outcome in adult AML patients.

Patients and Methods

Patient Selection, Treatment, and Sampling

Forty-eight consecutive patients newly diagnosed with AML (except acute promyelocytic leukemia) > 18 years (< 60 years) of age were enrolled from March 2008 until June 2010 prospectively. The study was approved by the institute ethics committee and informed consent was taken for evaluation of peripheral blood and/or bone marrow for the study. AML was classified into good-, intermediate-, and poor-risk cytogenetic groups.[15] A uniform treatment protocol was followed for all patients (daunorubicin 60 mg/m^2 for 3 days with cytarabine 100 mg/m^2/d over 24-hour infusion for 7 days); patients who were not in morphological complete remission (CR) after first induction received the ADE (cytarabine 100 mg/m^2 slow intravenous push twice a day for 10 days, daunorubicin 50 mg/m^2 daily for 3 days, and etoposide 100 mg/m^2 daily for 5 days) protocol.[16] Patients who were not in CR after 2 inductions were declared refractory. After achieving remission patients received 3 cycles of high-dose cytarabine at 18 mg/m^2 per cycle.

Five milliliters of peripheral blood (n = 31 patients; if peripheral blast count was > 30%) or otherwise 5 mL bone marrow (n = 17 patients) was collected using ethylenediaminetetraacetic acid (EDTA) as an anticoagulant in EDTA-coated sterile vacutainers (BD Vacutainer).

RNA Isolation and Reverse Transcription

Total RNA was isolated from 10 million Mono Nuclear Cells (MNCs) using the trizole method. RNA was evaluated for quality and quantity using spectrophotometry. cDNA was synthesized from 1-µg aliquots of total RNA in a 20-µL standard reaction mixture using a reverse transcription (RT) kit (Roche Applied Sciences, Mannheim, Germany) according to the manufacturer's instructions. The quality of cDNA was checked using β-actin as a housekeeping gene of 1100 base pairs. Good quality cDNA were processed further for absolute quantification of transcripts.

Absolute Quantification Using Taqman Probe-Based Real-Time Polymerase Chain Reaction

For absolute quantification of transcripts using TaqMan chemistry, probes for *FLT-3* and *BCL-2* were conjugated with Yakima yellow as a reporter dye at 5′ and BlackBerry Quencher (BBQ) at 3′, and *c-KIT* was conjugated with 6-carboxyfluorescein as a reporter

Table 1 Patient Characteristics at Baseline

Variable	AML Patients (n = 48)
Median Age, Years (Range)	35 (19-59)
Sex (Male:Female)	1.3:1
Median Hemoglobin (Range), g/dL	7.7 (2.0-12.2)
Median WBC Count (Range)/mm^3	20,200 (1000-685,000)
Median Platelet Count (Range)/mm^3	36,000 (10,000-266,000)
Cytogenetics (n = 35)	
Good Risk	5 (16.2%)
Intermediate Risk	26 (70.2%)
Poor Risk	4 (13.5%)
FAB AML subtype	
M0	1 (2.08%)
M1	5 (10.42%)
M2	29 (60.42%)
M4	6 (12.50%)
M5	5 (10.42%)
M6	2 (4.17%)
***FLT-3* ITD**	
Positive	8 (16.67%)
Negative	40 (83.33%)

Expression of *FLT-3*, *c-KIT*, and *BCL-2* (Transcript Copies per microgram of RNA; n = 48

	FLT-3	*c-KIT*	*BCL-2*
Median (Range)	20.2 × 10^6 (0.054 × 10^6-682.0 × 10^6)	0.36 × 10^6 (0.0034 × 10^6-16.3 × 10^6)	0.71 × 10^6 (0.0017 × 10^6-11.2 × 10^6)

Abbreviations: AML = acute myeloid leukemia; FAB = French-American-British; ITD = internal tandem duplication; WBC = white blood cell.