Early Fluorescence in situ Hybridization Assessment during Acute Myeloid Leukemia Induction Chemotherapy

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The standard of care for upfront remission induction therapy for “fit” adults with de novo acute myeloid leukemia (AML) is continuous-infusion cytarabine with concurrent anthracycline (i.e., “7+3” regimen) [1]. Fourteen days after the initiation of chemotherapy, bone marrow is re-evaluated to identify patients who may benefit from immediate receipt of an additional cycle of remission induction chemotherapy. Current NCCN guidelines specify that a second cycle of induction chemotherapy should be administered prior to count recovery if (1) more than 5% of the cells in the day-14 bone marrow sample are morphologically consistent with myeloblasts and (2) the clinical status of the patient permits safe administration of cytotoxic therapy [2].

Bone marrow cellularity on day 14 is often less than 10–20%, limiting the absolute number of cells available for analysis and potentially changing the accuracy of morphologic blast estimation. Morphologic evaluation of aplastic bone marrow aspirates also lacks the precision to differentiate physiologic recovering myeloblasts from leukemic blasts, indicative of persistent disease. Between

46 and 77% of the patients with >5% of bone marrow cells morphologically identified as myeloblasts on day-14 marrow examination who do not receive additional chemotherapy still go on to achieve first complete remission (CR) from 7+3 chemotherapy [3, 4]. Thus, the limited potential to morphologically differentiate between physiologic and aberrant myeloblast populations in the day-14 aspirate presents a prognostic dilemma [5].

Highly sensitive diagnostic methods for differentiating physiologic from leukemic myeloblasts are routinely applied to the diagnostic bone marrow examination. These include multiparameter flow cytometry (MFC), fluorescence in situ hybridization (FISH), real-time quantitative polymerase chain reaction, and next-generation sequencing [6], which have detection sensitivities of 10^{-4} , 0.3–5%, 10^{-4} to 10^{-5} , and 10^{-4} to 10^{-6} , respectively [7]. Most commonly utilized among these is MFC, which can capture a majority of leukemia immunophenotypes and has shown to predict good response at day 14 [8]. However, MFC is limited by the variability in instrumentation between institutions [9–11].

Table 1. Patient characteristics and bivariate analysis associated with achieving CR

Patient characteristics (<i>n</i> = 112)	Proportion of patients obtaining CR		
	%	crude odds ratio (95% CI)	<i>p</i> value
Age (median = 54 years)	n.a.	0.95 (0.92, 0.99)	0.02
Disease risk by ELN			
Favorable, 20 (18)	95	Referent	0.10
Intermediate, 44 (39)	80	0.21 (0.02, 1.74)	
Poor, 34 (30)	65	0.10 (0.01, 0.81)	
Unknown, 14 (13)	64	0.10 (0.01, 0.93)	
Received additional chemotherapy			0.05
No, 96 (86)	79	Referent	0.15
Yes, 16 (14)	56	0.34 (0.11, 1.02)	
Gender			<0.01
Female, 51 (46)	82	Referent	
Male, 61 (54)	70	0.51 (0.21, 1.27)	0.06
Day-14 bone marrow morphologic blasts			
<5%, 63 (56)	90	Referent	<0.01
≥5%, 49 (44)	57	0.14 (0.05, 0.39)	
FISH on day 14 (<i>n</i> = 50)			
Undetectable, 17 (34)	94	Referent	0.06
Detectable, 33 (66)	67	0.13 (0.02, 1.07)	

Values are *n* (%) unless otherwise indicated. CR, complete remission; ELN, European LeukemiaNet; FISH, fluorescence in situ hybridization.

FISH is readily available at most institutions and is frequently applied to the day-14 and remission bone marrow evaluations, if chromosomal abnormalities were identified on the diagnostic specimen. Conversion to a normal karyotype at the time of count recovery as measured by FISH correlates with positive long-term outcomes [12]. However, the prognostic implication of persistent FISH-detectable cytogenetic abnormalities in the day-14 marrow sample is unknown. We analyzed day-14 FISH results from 50 adult patients diagnosed with AML who had a diagnostic FISH abnormality treated at our institution and compared these results to treatment outcome.

Chart Selection and Data Collection

An Institutional Review Board-approved retrospective chart review was performed at the Medical College of Wisconsin to identify all adult patients diagnosed with AML from January 1, 2010, to March 15, 2015, who received standard-of-care induction chemotherapy defined as 7 days of continuous infusion of cytarabine (100–200 mg/m² per day) and 3 days of concurrent idarubicin (12

mg/m² per day) or daunorubicin (60 mg/m² per day). Demographic information (age, gender) as well as leukemia-specific information (European LeukemiaNet [ELN]) risk category [13], bone marrow cellularity, and morphologic myeloblast percentage from the time of diagnosis and the day-14 bone marrow biopsy were collected. Treatment information and response including administration of additional induction chemotherapy during aplasia and achievement of CR1 from the induction chemotherapy regimen were also recorded.

FISH Analysis

Dual-color, dual-fusion, deletion, break-apart, and locus-specific DNA probes that detect characteristic rearrangements and genetic abnormalities observed in AML (BCR-ABL1, PML-RARA, RUNX1T1-RUNX1, CBFB, monosomy 5/deletion 5q, and monosomy 7/deletion 7q) were applied to day-14 bone marrow aspirate specimens by FISH. Two-hundred unstimulated interphase cells were analyzed per probe, and the results were reported using the International System for Human Cytogenetic Nomenclature (ISCN 2009 and 2013).

Table 2. AML patient characteristics by day-14 FISH results

Patient characteristics	Undetectable FISH abnormalities on day-14 bone marrow evaluation (<i>n</i> = 17)	Detectable FISH abnormalities on day-14 bone marrow evaluation (<i>n</i> = 33)	<i>p</i> value
Median age (range, years)	54 (23–73)	56 (20–72)	0.69
Gender			0.47
Female	8 (47)	12 (36)	
Male	9 (53)	21 (64)	
Disease risk			0.01
Favorable	5 (29)	5 (15)	
Intermediate	9 (53)	5 (15)	
Poor	3 (18)	21 (64)	
Unknown	0	2 (6)	
<5% morphologic myeloblasts on mid-cycle bone marrow evaluation	14 (82)	12 (36)	0.002
Received additional chemotherapy	0	24 (73)	0.02
Complete remission	16 (94)	22 (67)	0.03

Values are *n* (%) unless otherwise indicated. AML, acute myeloid leukemia; FISH, fluorescence in situ hybridization.

Conventional Cytogenetics

Chromosome analysis was performed on unstimulated cultures from bone marrow aspirates. Twenty metaphase cells (when available) were analyzed by 2 ASCP-certified cytogenetic technologists, and abnormalities were described using the International System for Human Cytogenomic Nomenclature (ISCN 2016)

Statistical Analysis

First, the likelihood of achieving CR among different groups of patients was examined. A second analysis was performed on those patients who had cytogenetic abnormalities detected by FISH at the time of diagnosis. These patients were divided into 2 groups based on their day-14 bone marrow findings: those whose diagnostic cytogenetic abnormality was no longer detectable by FISH (i.e., FISH negative) and those who retained cells with the cytogenetic abnormality as detected by FISH.

Patient/disease-related factors were compared between the groups by using the Wilcoxon rank-sum test for continuous variables (age) and the χ^2 test for categorical variables. Bivariate analyses were performed to examine factors associated with achieving a CR. A statistical significance of $\alpha = 0.05$ was used throughout, and

SAS OnDemand, version 9.4 (SAS Institute, Cary, NC), was used for all analyses.

Patient characteristics and odds of achieving CR are described in Table 1 and are consistent with the published literature [1]. The relapse-free survival for those achieving CR with the first induction therapy was 58%, and the overall survival at the last noted clinical encounter for the entire cohort was 42%. Older age and $\geq 5\%$ myeloblasts detected by morphology on mid-cycle bone marrow evaluation were associated with lower odds of achievement of subsequent CR (OR = 0.952, 95% CI [0.915, 0.991]; OR = 0.140, 95% CI [0.051, 0.387], respectively). There were no significant differences in CR rates observed between ELN disease risk subgroups [13] or by gender.

Fifty patients had detectable FISH abnormalities in their leukemic blasts at diagnosis. Seventeen (34%) converted to FISH negative by the mid-cycle bone marrow evaluation, and 33 (64%) retained FISH-detectable abnormalities (Table 2). Patients whose mid-cycle bone marrow sample was FISH negative for AML-related abnormalities were more likely to (1) have no morphologically detectable myeloblasts (82 vs. 36%, $p = 0.002$) and (2) obtain a subsequent CR (94 vs. 67%, $p = 0.031$) compared with those in whom day-14 FISH was with persistent AML-related abnormalities. As expected, individuals with a favorable and intermediate risk of AML were more likely to have converted to FISH negativity on mid-cycle

evaluation compared to those with poor risk ($p = 0.005$) (Table 2).

Of the 12 patients with FISH-positive disease but <5% blasts morphologically on mid-cycle evaluation, 7 (58%) obtained a CR without additional chemotherapy, 3 received reinduction and obtained a CR, 1 received reinduction but had refractory disease, and 1 was considered having refractory disease/induction failure in the absence of further chemotherapy administration. Chromosomal abnormalities for the 7 who obtained a CR without additional chemotherapy are as follows: t(8;21); t(11;17); +8, idem, +i(5); +8, +9; der(16)inv(16)t(4;16); inv(16); +8, inv(16); and add(X), +8. The 3 who received additional chemotherapy did so based on subsequent biopsies performed during a period of prolonged count recovery. Conventional cytogenetic (CC) analysis was attempted on 29 of the day-14 biopsy samples. Of those, 7 were failures due to insufficient cell growth or metaphase chromosomes, 9 were karyotyped as normal, and 13 were positive. Three were positive by FISH and negative by CC; one was negative by FISH and positive by CC; 18 had concordant results (both positive or both negative); 4 were positive by FISH and failed to grow or generate sufficient metaphase chromosomes, and 3 were FISH negative and failed to grow or generate sufficient metaphase chromosomes.

This study demonstrates that conversion from FISH positive to FISH negative by the day-14 postinduction bone marrow evaluation strongly correlated with higher CR rates from standard AML induction chemotherapy in our patient population. However, the significance of the persistence of these chromosomal rearrangements at day 14 is less clear. While 40% of the patients with AML will

not have cytogenetic abnormalities that can be detected by standard FISH probes, the remaining 60% do [14]. We conclude that normalization of FISH-detectable abnormalities at the mid-cycle bone marrow evaluation is potentially prognostic and merits a prospective study to validate these findings in a larger multi-institutional cohort. Although less routinely available, application of additional genetic evaluation techniques such as next-generation sequencing for mid-point bone marrow evaluation may similarly correlate with treatment outcome and should be considered for prospective examination.

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Disclosure Statement

The authors declare that there are no conflicts of interest to disclose.

Author Contributions

R.S., K.C., and P. Hosking conceptualized this retrospective review, and R.S., K.C., C.B., and P. Hosking completed the data collection. R.S. and R.B. performed the statistical analysis. K.C., R.S., C.B., P. Hari, J.P., E.A., and L.M. participated in the clinical care of the patients, and R.S. and K.C. wrote the manuscript that was reviewed, edited, and agreed upon by all authors.

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