Analyzing transformation of myelodysplastic syndrome to secondary acute myeloid leukemia using a large patient database

Ofir Shukron,[1] Vladimir Vainstein,[1,2] Andrea Kündgen,[3] Ulrich Germing,[3] and Zvia Agur[1]

One-third of patients with myelodysplastic syndrome (MDS) progress to secondary acute myeloid leukemia (sAML), with its concomitant poor prognosis. Recently, multiple mutations have been identified in association with MDS-to-sAMLtransition, but it is still unclear whether all these mutations are necessary for transformation. If multiple independent mutations are required for the transformation, sAML risk should increase with time from MDS diagnosis. In contrast, if a single critical biological event determines sAML transformation; its risk should be constant in time elapsing from MDS diagnosis. To elucidate this question, we studied a database of 1079 patients with MDS. We classified patients according to the International Prognostic Scoring System (IPSS), using either the French-American-British (FAB) or the World Health Organization (WHO) criteria, and statistically analyzed the resulting transformation risk curves of each group. The risk of transformation after MDS diagnosis remained constant in time within three out of four risk groups, and in all four risk groups, when patients were classified according to FAB or to the WHO-determined criteria, respectively. Further subdivision by blast percentage or cytogenetics had no influence on this result. Our analysis suggests that a single random biological event leads to transformation to sAML, thus calling for the exclusion of time since MDS diagnosis from the clinical decision-making process. Am. J. Hematol. 00:000–000, 2012. © 2012 Wiley Periodicals, Inc.

Introduction

Myelodysplastic syndromes (MDS), one of the most prevalent hematological disorders, constitute a heterogeneous class of stem cell malignancies, characterized by ineffective hematopoiesis in one or more bone marrow (BM) lineages [1]. About one-third of patients with MDS progress to MDS-related acute myeloid leukemia (secondary AML, sAML), whereas the remaining two-thirds can succumb to progressive BM failure, which leads to bleeding, recurrent infections, and severe anemia [2]. The prognosis of patients who undergo transformation from MDS into sAML is generally grave; most patients are resistant to currently available treatment options and the long-term survival rate among treated patients is $<10\%$.

MDS is genetically heterogeneous, showing various multiple cytogenetic abnormalities, whose causative role in disease progression is still not completely understood [3]. Recently reported fluorescence in situ hybridization (FISH) analysis demonstrated that cytogenetic evolution is not always associated with progression [2,4] and a study of gene mutations in a large cohort of patients with MDS identified five genes which correlate with worse prognosis [5]. Notwithstanding the large phenotypic heterogeneity in MDS, it is still unclear whether the onset of sAML is triggered by a single mutation or epigenetic event, or by an accumulation of multiple events. Some authors argue that one genetic event is sufficient for causing sAML [6–8] whereas others believe that several genetic events are required for the transformation [9,10]. The latest report on deep sequencing of the genome in patients with MDS before and after transformation to sAML [11] demonstrates complex clonal evolution with acquisition of multiple additional mutations, co-existing with the MDS founding clone. This phenomenon may represent general genetic instability without necessary causative relation. Indeed, recent reports of mutation analysis in *de novo* AML suggest that the vast majority of the detected genetic alterations do not play any important causative role in leukemogenesis [12].

If sAML transformation depends on the accumulation of multiple mutations, then the risk of developing sAML should increase over time. In contrast, if sAML depends on a single decisive mutation event, then the risk of transformation is expected to remain constant following MDS diagnosis [14]. Physicians must take into account this risk pattern when making critical treatment decisions, such as whether and when to suggest hematopoietic stem cell transplantation (HSCT), as is done in other high risk premalignant conditions [15]. Currently, HSCT, although highly toxic, is the only curative treatment that can prevent MDS from deteriorating to sAML [16], but studies, examining an optimal HSCT timing for patients with MDS, vary in their conclusions [17,18]. A statistical model, assuming constant risk of transformation, proposes delaying transplantation to improve survival [17], whereas early transplantation has been recommended based on the observations that patients with MDS who underwent HSCT relatively late, had shorter overall survival [18]. Therefore, elucidating the pattern of the sAML transformation risk since MDS diagnosis may have an important impact on the decisions about HSCT timing.

Epidemiological studies assessing the risk of AML show that the risk of all types of AML increases with age [19]. Surveillance Epidemiology and End Results Program, U.S.A (SEER) estimates the AML age-specific risk for patients examined between 1975 and 2007 (all races, both sexes), as 1.4–2 cases per 100,000, per year, when patients' age is <65, and 10 times higher for older patients (www.seer.cancer.gov). In contrast, a surveillance study of a small cohort of patients with MDS, showed that the risk of developing sAML declines with time after MDS diagno-

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sis. This decline in risk is said to be due to the tendency of patients with MDS to succumb to various age-related diseases before sAML transformation [4]. It appears, then, that the observed pattern of sAML transformation risk over time is still unclear, and cannot be associated with a specific pattern of biological events leading to sAML.

Analysis of age-dependent incidence data can be a simple way for estimating the risk of sAML transformation over time. Since the biological mechanism, leading patients with MDS to sAML transformation is not fully understood, this analysis seems to be the only presently available way of deducing whether the transformation results from a single event or from multiple events.

Statistical analysis of cancer incidence combined with mathematical modeling of the underlying dynamics has been employed to decipher the number of genetic events required for malignant transformation in cancer [20,21]. Such analysis is yet to be performed for MDS–sAML transformation.

In this study, we statistically analyzed a large database of patients with MDS from the Düsseldorf University Hospital registry, to evaluate sAML risk as a function of time following MDS diagnosis. By calculating the cumulative sAML risk curve and studying its qualitative behavior over time, we deduce the dependence of sAML transformation risk on time from MDS diagnosis. The time-dependence pattern, we discern, enables to assert whether a single event, for example, genetic mutation, or multiple events, lead to sAML transformation.

Methods

Patients. We retrospectively analyzed data from the Düsseldorf MDS registry, Heinrich-Heine University Hospital, Germany. Patients were registered and followed between the years 1982 and 2003. All patients had given written informed consent before any study procedure and the protocol was approved by the local ethics committee.

Patients in the database were grouped according to the IPSS criteria [22] into Low, Intermediate-1 (Int-1), Intermediate-2 (Int-2), and High risk groups; grouping is based on the number of cytopenias, BM blast percentage, and clonal cytogenetic changes. The cutoff for sAML definition used in IPSS is 30% according to FAB classification.

We studied the records of 1079 patients, excluding 50 patients, due to missing or contradicting data records. Ten additional patients had undergone HSCT and were also excluded. Among the remaining 1019 patients, 98 were secondary MDS. One hundred forty-three patients had received red blood cell transfusions during the follow-up period. Median age at MDS diagnosis was 66 years (range: 13–99). The patient group included 609 males and 410 females (ratio, 1.5:1).

Over the follow-up period, 311 (30.5%) patients were diagnosed with sAML. Median time to sAML (TTA, defined from the MDS diagnosis till transformation to sAML) was 17 months (range: 0–410). Median survival time with sAML was 4.2 months (range: 0–186). Median follow-up time was 22 months (range: 1–410). Three hundred forty-eight patients were still alive at the end of follow-up (year 2003). Of these patients, 29 had developed sAML.

Statistical analysis. We used the Kaplan–Meier product-limit estimator method [23] to calculate empirical cumulative hazard functions (CHFs) of the MDS-sAML transformation time. Censored patients were defined as those who died before sAML diagnosis or did not develop sAML during follow-up.

Using a log-rank test [24], we performed a univariate analysis to examine the effects of age at MDS diagnosis (higher or lower than median age), gender, and IPSS risk category on TTA. To examine the simultaneous effect of these factors on sAML transformation time, we carried out multivariate analysis using the Cox proportional hazard model [25].

To examine the temporal dependence of the TTA on sAML risk, we plotted the empirical Kaplan–Meier CHF of the TTA for each IPSS group, using a parametric form. We assumed that each biological event in the sequence leading from MDS to sAML is a Poisson process, having a constant occurrence rate. On the basis of this assumption it can be shown that if transformation to sAML is caused by a single random event, the resulting transformation risk curve will be constant, representing the uniform likelihood of this biological event at any given time [14]. The corresponding CHF is then a linearly increasing function. In contrast, a patient's transformation to sAML, being a multistage process, will result in a monotonically increasing risk function of sAML transformation over time, which, accordingly, has a CHF that increases at a higher-than-linear rate. The pattern of risk is then observed as the likelihood of undergoing the required number of successive or independent biological events, a likelihood that increases over time following the MDS diagnosis.

A CHF was initially calculated for each IPSS groups, incorporating all patients. A deviation from the trends expected of the CHF suggests heterogeneity among patients in these groups. To prevent a bias due to heterogeneity, patients with unusually long TTAs were excluded from the analysis. We excluded patients whose TTA or censoring time exceeded twice the inter-quartile range above the third quartile of their risk group's TTA (box plot criterion). Using the log-rank test, we compared characteristics of the excluded patients to those of the remaining patients in their respective risk groups to determine the nature and extent of the differences and to justify their exclusion.

We considered several nested families of parametric distribution (e.g., log-normal, generalized gamma, F, etc.) for describing the empirical CHF of the TTA. We graphically examined the goodness of fit of the various distributions, using the Cox-Snell residual plot. We chose to use the Weibull distribution in our analysis, owing to its simplicity, flexibility, and the good agreement with data obtained with the Cox-Snell residual plot (data not shown). Additionally, abundant theoretical and empirical evidence robustly support the a priori choice of this distribution [14]. The Weibull functions for the sAML transformation risk, $h(t)$, and for the CHF, $H(t)$, are given by the parametric forms:

$$H(t) = \lambda t$$
$$h(t) = \lambda\gamma t^{\gamma-1}$$

where $\lambda > 0$ is the scale parameter, and $\gamma > 0$ is the shape parameter.

We note that the Weibull is a nested distribution, the exponential being a particular case for $\gamma = 1$. The parameters of the Weibull CHF were estimated using the maximum likelihood estimator (MLE) method. We used log-likelihood ratio statistics for hypothesis examination of the values of the γ MLE shape-parameters [26].

To distinguish between an increasing-risk CHF and a constant-risk CHF, we used the calculated MLE parameters and examined the following null hypothesis:

$H_0 : \gamma = 1$: The underlying distribution is exponential (implying constant transformation risk) against the alternative.

$H_1 : \gamma \neq 1$: The underlying distribution is pure Weibull (implying non-constant transformation risk). In all statistical tests, results with a P value <0.05 were considered as statistically significant.

To comply with the new WHO definition of sAML cutoff point, namely 20% BM blast cell percentage [27], we reiterated our database analysis after excluding patients having BM blast cell percentage above the new cutoff point.

In our analysis we avoided the use of frailty or mixture models and adhered to the simplest models available to explain the TTA distribution. Although mixture models provide parametric descriptions of survival times in the presence of heterogeneity, use of these models would be hard to interpret. Such complications stem from a priori assumptions about frailty distribution. In addition, we used a simple objective exclusion criterion. Other, more sophisticated, exclusion or outlier detection techniques seemed too cumbersome.

Further analyses. Each IPSS Risk group was further subdivided according to blast percentage of its constituent members, and according to the IPSS cytogenetic score. Analysis of the CHF and hypothesis examination was performed on each subgroup similar to the analyses described earlier.

To eliminate a possible effect of the, rather, arbitrary IPSS cutoff point, we reiterated all the previous analyses after reclassifying the patients according to the newly updated definition of MDS, so that patients whose BM blast cell percentage exceeded 20% were considered as sAML patients [27]. The total number of patients remaining after this exclusion was 263, 396, 227, and 51 in Low to High risk groups, respectively. Among these, the number of patients who developed sAML was 35, 85, 104, and 30, respectively.

Results

Univariate analysis

A univariate analysis was carried out for evaluating the influence of individual prognostic factors on the TTA data in the studied patient group (609 men and 410 women; see Methods section). Three prognostic factors were examined:

TABLE I. Patient Characteristics

Analysis	Univariate						Multivariate
Variable Group	No. of Patients (% of total)	sAML evolution (% of group)	Age^{m}	TTA^{M} [mo]	sAML survival [mo]	Overall survival [mo]	TTA Hazard ratio (*P* value)
Gender							
Male	609 (59.7)	186 (30.5)	66	15.3	4	20	1.0.8 (0.27)
female	410 (40.3)	125 (30.5)	66	22.6	3.8	27.5	
IPSS							
Low	263 (25.8)	35 (13.3)	66.3	50.3	3.7	43.1	2.7 ($<10^{-5}$)
Int-1	396 (38.8)	85 (21.5)	66.2	21.7	3.3	24.9	
Int-2	227 (22.3)	104 (45.8)	66.5	8	5.5	13.5	
High	133 (13.1)	87 (65.4)	65	5	4.6	9	
Age							1 (0.4)
<66	506 (49.6)	160 (31.6)	58.7	19.1	5.5	20	
>66	513 (50.4)	151 (29.5)	72.4	15.2	3.4	19.5	

The univariate analysis of the effect of individual prognostic variables on the TTA. The prognostic variables examined (light gray boxes) were tested for influence using the log-rank test. Prognostic variables that were found significantly different in the log-rank test (*P* value < 0.05) are shaded dark gray. Hazard ratio found in the multivariate analysis is shown only for the effect on TTA, the values appearing in the rightmost column are the exponential of the coefficient of regression found in the Cox proportional hazard model when employed using the entire database.

m, median; M, mean; mo, months.

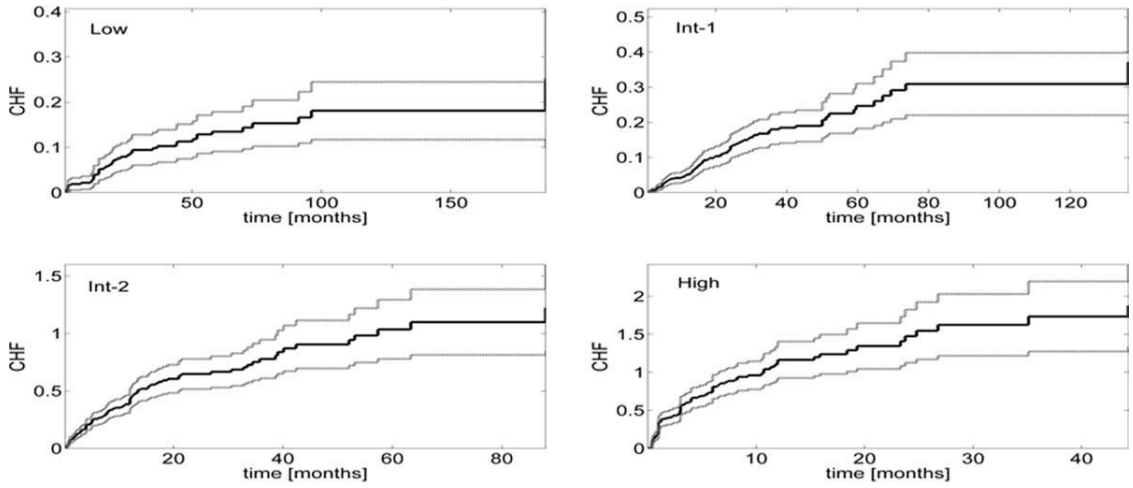

Figure 1. Empirical cumulative sAML transformation risk in each IPSS group decreases over time. Panel (A) Low risk group, (B) Int-1 risk group, (C) Int-2 risk group, and (D) High risk group. Empirical cumulative sAML transformation risk in each IPSS group (dark solid line); error bounds (dotted light gray).

age (higher or lower than the median 66 years), IPSS, and gender. Patients' characteristics and the influence of each prognostic factor on the TTA were summarized (Table I). IPSS was found to be the only prognostic factor with a statistically significant effect on TTA (*P* value <0.0005) [22]. This justifies the separate estimation of the CHF of each IPSS group, without incorporating age and gender data. IPSS groups Low, Int-1, Int-2, and High contained 263, 396, 227, and 133 patients, respectively. Age distributions were similar across the various risk groups (data not shown).

Decrease in sAML risk over time may be caused by intra-group heterogeneity

Calculating the empirical CHFs of each IPSS group shows that in all IPSS groups, the risk of transformation to sAML decreases with the time elapsed from MDS diagnosis at the late stages of follow-up (Fig. 1). This result does not reflect a multi-event leukemogenesis, expected to yield increasing transformation risk, nor does it reflect a single-event hypothesis, for which constant transformation risk is expected (see Methods).

A close inspection of the empirical CHF curves reveals that a few patients in each IPSS group had unusually long TTAs, disparate from the bulk of data of their respective risk groups. The inclusion of these patients in the IPSS group led to risk-group heterogeneity, which could obscure the underlying representative CHF, and was the plausible cause of the unexplained decrease in sAML transformation risk (see Discussion). Therefore, we excluded these patients from the dataset.

First, we identified candidates for exclusion according to the objective box-plot exclusion criterion. This criterion yielded TTA cutoff points of 108.7, 67.7, 36.4, and 21.1 months for the four Low to High risk groups, respectively. The numbers of patients having TTAs surpassing the cutoff points were 40, 53, 34, and 16, in Low to High risk groups, respectively. We statistically compared the data of the excluded patients with the data of the other patients in their respective IPSS groups, to determine the nature and extent of the difference (Table II). It was found that for IPSS groups Low, Int-1, and High risk, the excluded patients were significantly younger and their hemoglobin levels were significantly higher than the other patients in their IPSS groups (*P* values < 0.0005 and *P* values < 0.05, respectively). Moreover, in each IPSS group, the percentage of excluded patients receiving red blood cell transfusion was lower than that of the rest of the group. These statistics indicate better prognosis for the excluded patients.

When we excluded patients with unusually long TTAs from the analysis, the decreasing deviations of the empirical CHFs were weakened in Low to Int-2 groups, and the curves tended to be linearly increasing, implying a constant sAML transformation risk (Fig. 2). Note that a slight

TABLE II. Comparison of Candidates for Exclusion and their Respective Risk Group

Variable Group	Age[b]	% BM blasts[1]	PLT[a]	HB[a]	sAML surv. [mo]	Karyo[a]	%Ind[a]	Mean TTA* [mo]
Low								
Group	67.3	2	285	10.1	3.5	0	2	–
Excluded	59	1.9	264.2	11	13.3	0	0	186 (173)
Int-1								
Group	67.5	4.1	143.4	9.7	3.2	0	10	–
Excluded	57	3.4	162.9	10.4	4.6	0	4	93 (119)
Int-2								
Group	66.6	10.9	108.7	9.2	5.3	0.5	20	–
Excluded	65.8	11	141	10.4	6.7	0.25	16	52 (76)
High								
Group	66	21.6	77.9	8.8	4.3	1	43	–
Excluded	59.4	20.3	61.7	9.7	11	0.5	62	29 (108)

A summary of the differences in prognostic variables between long TTA patients (Excluded) and the remaining patients in their risk groups (Group). Variables, compared using the log-rank test, are shaded in light gray. Variables that differ significantly (P value < 0.05) between the groups are shaded in dark gray.

PLT, platelets; HB, hemoglobin; Karyo, karyotypes (scored by IPSS method, 0–Low, 0.5–intermediate, 1–High); Ind, induction; mo, months.

[a] Mean/

[b] Median.

* TTA for uncensored patients, time of death for censored-patient data.

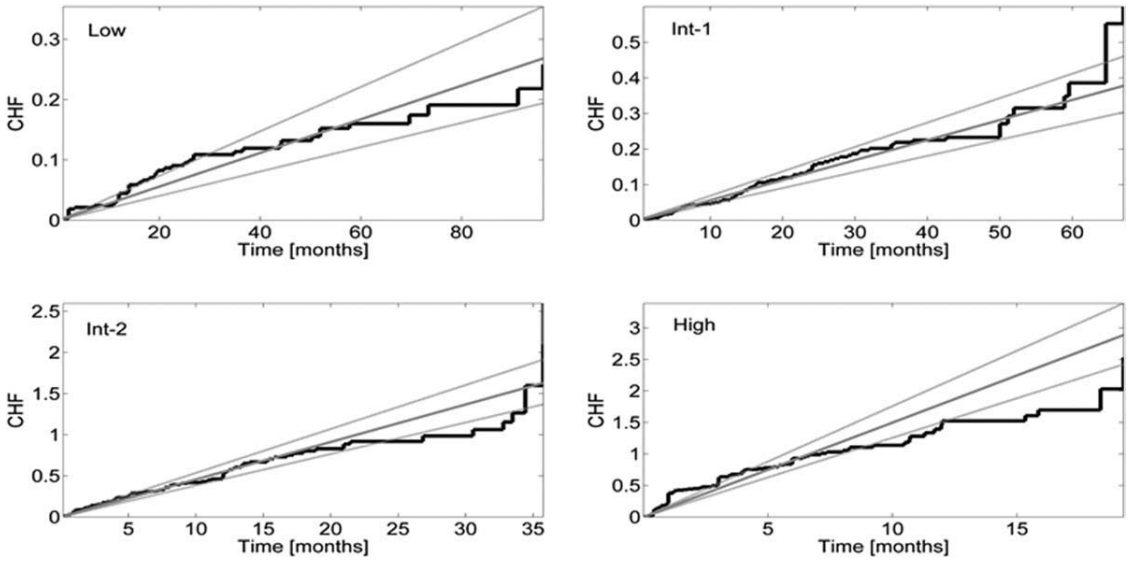

Figure 2. CHF following exclusion of patients with unusually long TTA suggest a constant age-dependent sAML transformation risk. The parametric exponential CHF (thin black line) is plotted with its given error bounds (dotted gray) showing the adequacy of the single event model (exponential) in describing the TTA distribution in risk groups (A) Low, (B) Int-1, and (C) Int-2. (D) In High risk group, a Weibull distribution with decreasing risk appears favorable according to the log-rank ratio statistic test. However, the apparent decrease in sAML risk over time stands in contrast to both hypotheses of multistage and single event leukemogenesis (see further details in Results and in Discussion).

decrease of the transformation risk over time was still seen in the High risk group.

The risk of sAML transformation is constant over time

The observed linearity in the CHF curves, following exclusion of outlier data, led us to hypothesize that MDS duration from diagnosis bears no prognostic relevance to sAML transformation risk. This hypothesis, if true, implies that a single genetic or epigenetic event, occurring at a random point in time, is likely to be responsible for the sAML transformation (see Methods for explanation). To examine this hypothesis, we described the empirical CHF by a parametric Weibull distribution and statistically tested the value of its shape parameter, γ, in each IPSS group. This value indicates whether the risk of transformation as a function of time is increasing, decreasing, or constant ($\gamma = 1$). Results show that in three out of four IPSS groups, the risk of transformation is constant. In the IPSS High group, the transformation risk still slightly decreases with time at the late stages of the follow-up (Table III).

These findings show that for patients in the IPSS Low, Int-1, and Int-2 risk groups, the risk of sAML transformation remains constant after MDS diagnosis, indicating a single genetic or epigenetic event underlying the transformation to sAML. For the IPSS High group, the constant transformation risk hypothesis was rejected, and a Weibull model with decreasing risk over time was favored ($\gamma < 1$). The parametric Weibull CHFs with constant risk ($\gamma = 1$) are presented, superimposed on the empirical CHFs (Fig. 2).

To verify that our results do not depend on the IPSS BM cutoff point defining sAML, which may be somewhat arbitrary, we reiterated our analysis after classifying patients according to the newly updated definition of sAML [27]. Results of the univariate analysis of the corrected patient groups are consistent with those of the uncensored data (Table I) and the null hypothesis was not rejected in any IPSS group (P values <0.05), suggesting that the model with constant sAML transformation risk is preferable over the alternative with increasing risk, for describing the TTA distribution (Weibull distribution parameter $\gamma = 0.82$, 1.13, 1.06, and 1.05 for IPSS groups Low to High, respectively). This result could also be inferred from the clear linear trends seen for the CHF (Fig. 3). We conclude that a

TABLE III. Test for Constant sAML Risk

Group Model parameters	Low	Int-1	Int-2	High
Weibull				
λ [CI]	0.002 [9.3×10^{-4}, 3.8×10^{-3}]	0.0069 [5.1×10^{-3}, 0.5×10^{-3}]	0.042 [0.034,0.052]	0.14 [0.12,0.18]
γ [CI]	0.82 [0.64, 1.07]	1.13 [0.96, 1.32]	0.91 [0.8, 1.03]	0.78 [0.68, 0.89]
Exponential				
λ [CI]	0.0028 [0.002, 0.0036]	0.0062 [0.0045, 0.0068]	0.045 [0.038, 0.053]	0.15 [0.12, 0.17]
sAML transformation risk	**Constant**	**Constant**	**Constant**	Decreasing

The maximum likelihood estimators of Weibull and exponential model parameters are given alongside the results of the log-likelihood ratio statistics test for adequacy of each model to describe the TTA distribution.

CI, confidence interval.

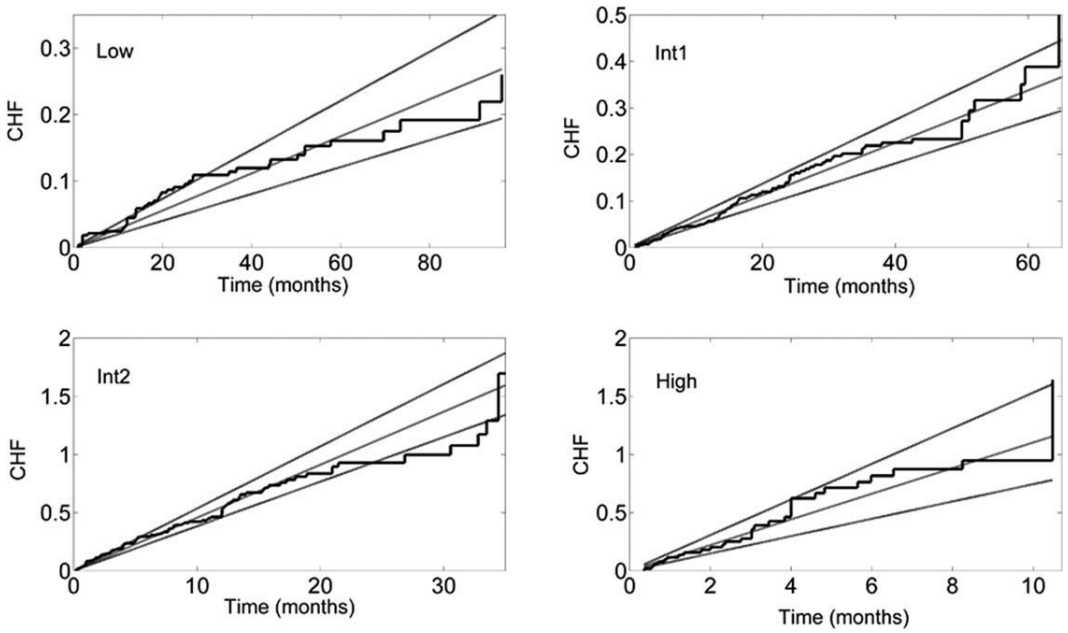

Figure 3. Cumulative risk of sAML according to WHO criteria (2008). Cumulative risk of sAML for the patients in the clinical database, excluding those with more than 20 blast cell percentage, is shown to increase linearly with time following MDS diagnosis in each risk group. The maximum likelihood estimator for the risk (λ) parameter of the exponential distribution, calculated based on patients' TTA (thick black line), shows the agreement between the observed empirical TTA and an exponential distribution (gray line; confidence interval is shown in dashed gray lines). In each box, the *x*-axis represents time post MDS diagnosis, *y*-axis is the cumulative hazard value.

similar mechanism of transformation exists in the IPSS High group, as in Low Int-2 groups, suggesting that a single biological event drives sAML transformation in the High group as well.

A large heterogeneity exists in MDS. Even within the IPSS groups, patients vary in blast percentage and cytogenetic abnormalities at diagnosis. Therefore, we checked whether or not further subdivision of the patients within each IPSS group according to these two parameters can alter our conclusions (see Methods). Our results show that the risk of transformation was mostly similar within each IPSS group (λs of the exponential distributions); the risk group High could not be evaluated, as it contained only two blast subgroups (Fig. 4). Moreover, the risk of transformation from MDS diagnosis is constant in all blast subgroups of all risk groups (Fig. 4).

The number of cytogenetic subgroups was: 1, 3, 3, and 2 in risk groups Low to High, respectively. All patients in risk group Low had Good cytogenetic scores, whereas no patients had Good cytogenetic score in risk group High. In risk groups Int-1 and Int-2 all subgroups were populated (not shown).

These results show that the exponential distribution was favored over the Weibull in all risk groups except in the Intermediate cytogenetic score subgroup, Int-1. One should conclude, then, that a constant risk of sAML transformation characterizes the majority of cytogenetic and blast subgroups, consistently with our main conclusion, namely, a single transformation event from MDS to sAML.

Discussion

Recent genome sequencing shows that MDS-to-sAML transformation is characterized by multiple cycles of mutation and clonal selection [11]. However, the causal relationships between these dynamic processes and MDS transformation to sAML is not clear. One possibility is that multiple mutations are essential for transition to sAML, so that early medical intervention can reduce its risk. Alternatively, the multiple mutations that characterize sAML may result from a permissive mutation-selection process following a single gene-driven transformation event. In the latter case the risk of sAML is expected to be constant in time.

In this study we analyzed a large population-based database of patients with MDS, showing that the risk of transformation of MDS to sAML is constant in time elapsed from MDS diagnosis. The most probable molecular mechanism underlying this risk pattern is a single genetic or epigenetic event occurring at a random time after MDS diagnosis in any single patient. This conclusion is primarily based on our finding that the risk of developing sAML is constant over time in three out of four IPSS groups and in all four risk groups when patients were classified according to the

WHO-determined blast percentage cutoff point for sAML. Other plausible molecular scenarios, such as a sequence of several obligatory random events, would have resulted in a significant increase in the transformation risk [14,28].

One may speculate that the event driving the transformation from MDS to sAML is a disruption of the apoptotic mechanism in BM blasts. Experimental data from studies examining the pathophysiological differences between MDS and sAML patients show compelling evidence that the apoptotic rate of immature BM blasts in sAML patients is significantly lower than that in patients with MDS, whereas proliferation rates remain similar. This discrepancy exists despite the fact that some anti-apoptotic genes can be activated, and some pro-apoptotic genes can be down-regulated in the early stages of MDS [9,29–32]. Probably, these mutations are still insufficient to actually turn off apoptotic mechanisms and additional derangements are required.

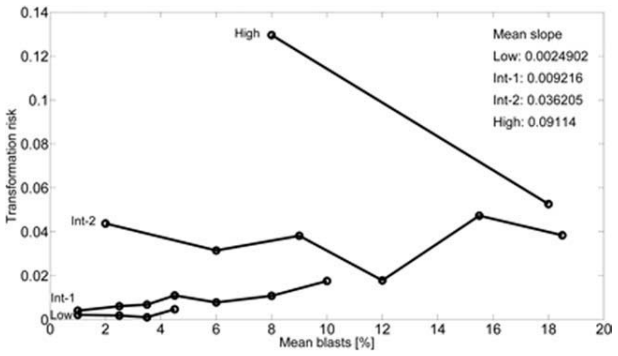

Figure 4. Exponential risk parameter of each blast cluster in each risk group as a function of mean blast percentage of each blast cluster. A near constant trend appears in all risk-groups (except for High, in which there exist only two clusters), suggesting that in each risk-group the patients' blast percentage at diagnosis is not a predictor for the TTA.

Our findings imply that even though many genetic alterations take place during sAML evolution, only one random biological event (genetic or epigenetic) is crucial, leading to abrupt or gradual patient's deterioration to sAML [33,34]. The majority of patients with MDS show abrupt type of progression [13].

Patients with MDS exhibit large heterogeneity in biological characteristics and disease severity at diagnosis [4]. This may be due to the relatively high prevalence of the disease in the population [35], the variability in the pathophysiological definition of its onset [32,36], and the various molecular and cytogenetic changes that are associated with it. Although the IPSS classification criteria deal with some of the heterogeneity, the stratification of patients with MDS into only four groups may bias the risk estimation, as several patients may present physical characteristics of one risk group, yet their prognosis may well fit another. In our analysis, the CHF for each IPSS group, incorporating all the patients, initially showed a decrease in sAML transformation rate over time (Fig. 1). This decrease is not expected to occur in either single- or multistage leukemogenesis, but can be explained by within-group heterogeneity caused by either early dying out of higher risk patients or by misclassification of a small, lower risk, subgroup. Therefore, we excluded from the statistical analysis of the CHF, patients with unusually long TTAs (lower risk in comparison to their IPSS groups), by using a one-sided objective exclusion criterion. The exclusion of these outliers, who constituted about 15% of their groups (1, 3, 9, and 6 patients for Low to High IPSS groups, respectively; Table II), had a prominent effect on the shapes of the CHFs, as was evident from the change in the qualitative behavior of the CHF curves—from decreasing transformation rate to constant transformation rate, in IPSS groups Low, Int-1, and Int-2.

In the IPSS High group, even after exclusion of the long surviving outliers, the sAML transformation risk remained

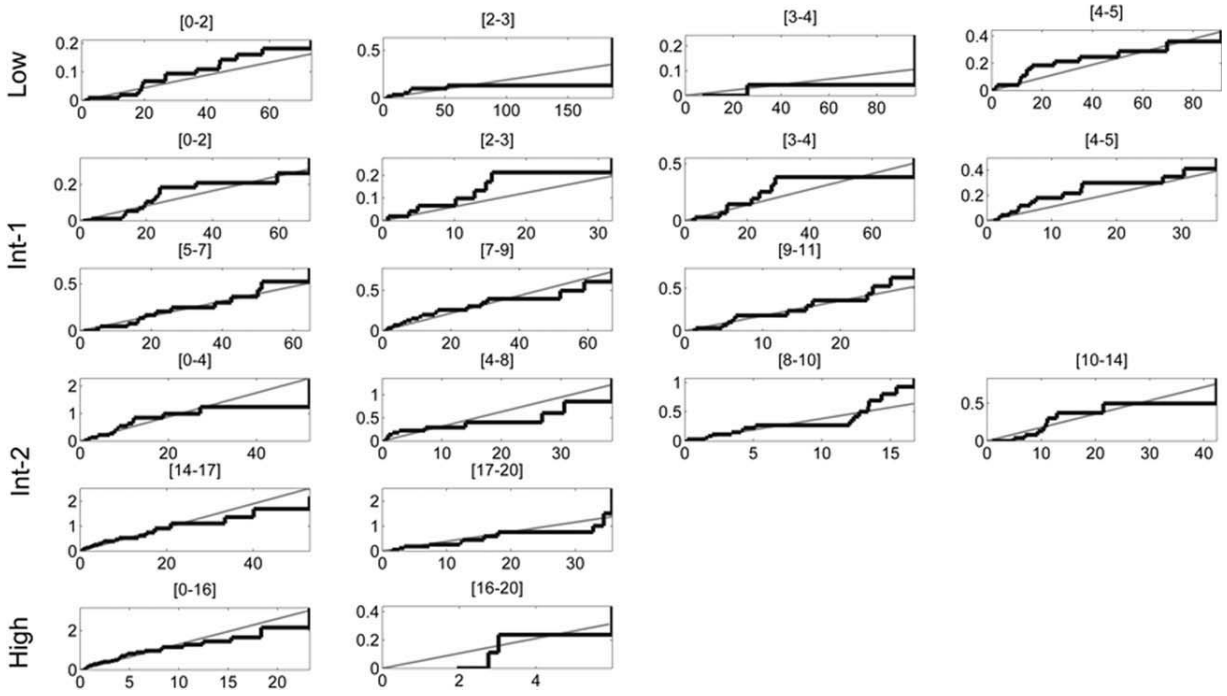

Figure 5. Empirical CHF calculated for the different blast subgroups of each risk group. The increasing risk model (see Design and Methods) and a constant risk model were evaluated using the empirical Kaplan–Meier CHF curve (thick black line) for each subgroup (subgroup's blast percentage limits appears in square brackets above each box). Results show that in all blast clusters of all risk groups, the constant risk model (gray line) was favored over the non-constant risk model, in line with the single event model. In each box, the *x*-axis represents TTA; the *y*-axis is the cumulative hazard value.

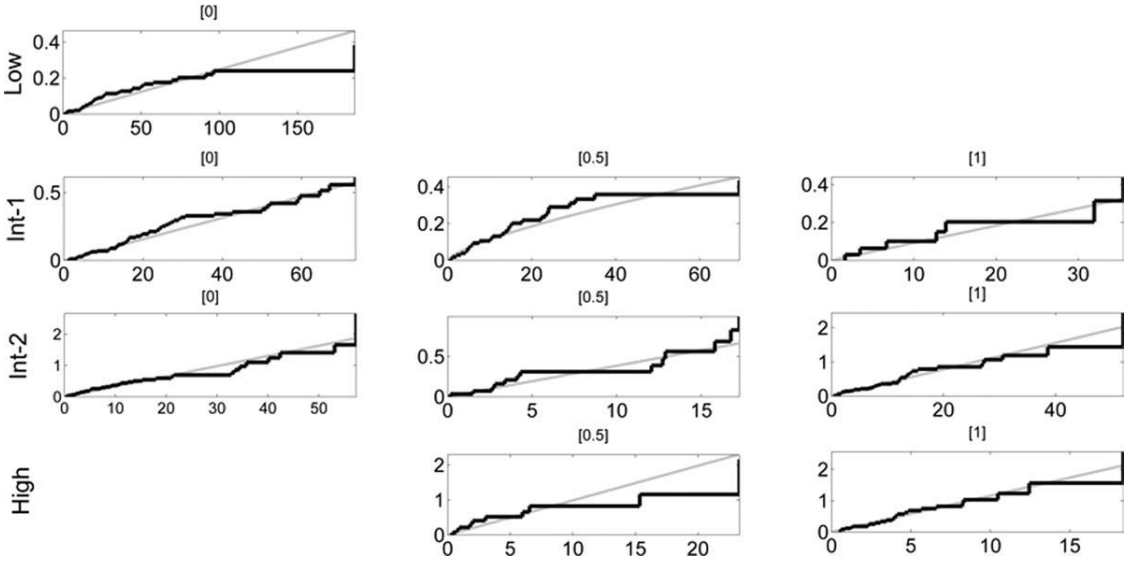

Figure 6. Testing the exponential TTA model for describing the empirical CHF of each risk group clustered according to cytogenetic changes score. Results show that in nearly all clusters the exponential CHF (thin gray line) is favored over the Weibull. The only exception being the Intermediate (0.5) cluster of Int-1, for which the Weibull with decreasing sAML risk was favored. This shows that homogenization of the risk groups according to karyotypes preserves the constant risk of transformation, in agreement with the single event model of sAML transformation. Empirical CHF, thick black lines; score of cytogenetic changes, square brackets above each box; 0, Good; 0.5, Intermediate; 1, Poor.

somewhat decreasing over time (Fig. 2). This result can be attributed neither to multiple transformation events, which are expected to yield an increasing sAML transformation risk, nor to a single sAML transformation event, which is expected to yield a constant sAML transformation risk. We believe that this decline may be caused by the residual heterogeneity of the IPSS High group, including patients who should already be classified as having sAML. We checked the effect of eliminating from the IPSS High group all the patients whose BM blast percentage exceeds 20%, suggested by the new MDS classifications as a threshold for definition of sAML [27]. Our results show that following this exclusion, sAML risk is constant in the IPSS High group (compare Figs. 2 and 3, lower right panels). This result supports the generality to all IPSS groups of our conclusion that the transformation from MDS to sAML is constant in time. Note that the analyzed patients were followed-up until 2003, that is, before wide acceptance of hypomethylating therapeutic agents. Therefore, we disregard the possibility that changes in the standard of care contributed to patient heterogeneity.

Comparison between the prognostic variables of the patients excluded from the different IPSS group and those of the other patients in their respective risk groups further support our exclusion procedure. This comparison showed that the candidates for exclusion were significantly younger, presented higher hemoglobin levels at MDS diagnosis, and fewer of them received red blood cell transfusions. In addition, although not statistically significant in all risk groups, candidates for exclusion had lower mean blast percentages at MDS diagnosis. These factors positively correlate with improved prognosis and lower risk [37], and thus give biological support to the exclusion of these patients from the analysis (Table II).

Improvement in the IPSS scoring method has been suggested by Germing et al., who proposed adding LDH as a prognostic variable in addition to the IPSS group [38], and Garcia-Manero et al. [39], who proposed refinement of criteria for classifying lower-risk groups. Here, we propose that patients with MDS stratification may be improved using additional recommended risk factors, such as those described earlier. This will render risk groups more homogeneous and the analysis of MDS deterioration to AML more precise. In general, our results suggest that it would be beneficial to use a scoring system that stratifies patients into more than four groups (such as the World Health Organization's classification system [27]) or takes into account time-varying prognostic factors for treated patients, as does the system formulated by Malcovati et al. (WPSS) [33]. Our analysis could then be repeated for the newly defined risk groups. Currently, sufficiently long follow-up of large cohorts of patients with MDS with specifications of all these new prognostic factors as well as defined mutational status of the aforementioned genes is unavailable.

Our finding of a constant risk of sAML transformation in each IPSS group may have important implications. It suggests that the likelihood of a newly diagnosed MDS patient developing sAML is equal to that of a patient from the same IPSS group who has had MDS for some time. Our results further show that the risk of sAML transformation differs between the IPSS groups, possibly because the mean BM blast population size at MDS diagnosis, and the number of patients with high risk cytogenetics, increase with IPSS risk category (Table II) [22]. If, as we find most probable, sAML transformation is driven by a single mutation in BM blasts, then the transformation risks differ among IPSS groups because these group vary in the multiplicative effect of blast population size and the genetic susceptibility to mutate (as reflected by cytogenetics).

Further subdivision of the patients within each of the IPSS risk groups according either to blast percentage or cytogenetic score did not change our main conclusion: the transformation rate remained constant over time within 11 out of 15 subgroups according to blast percentage subdivision, and 7 out of 8 of the resultant cytogenetic score resultant subgroups (Figs. 5 and 6).

In view of evidence that risk of cancer incidence increases with time in chronic myeloid leukemia (CML) [21], and pre-malignant conditions in colon cancer [15], it is of clinical importance to point out the difference between these diseases and sAML. In contrast to CML and colon cancer, the risk of sAML transformation in patients with MDS remains constant over time within each risk group.

This establishes a scientific basis to the current clinical practice that tends to ignore the time elapsing from MDS diagnosis, when patients are considered for HSCT.

In conclusion, the pathophysiological implication of our results is that only one obligatory stochastic, genetic, or epigenetic event, is most probably responsible for transforming patients with MDS to sAML. Our finding that the risk of sAML transformation remains constant after MDS diagnosis lays the scientific basis for physicians' disregard of the time elapsed following MDS diagnosis, when prioritizing patients for therapy, including HSCT. On the basis of circumstantial experimental evidence we speculate that an aberration in the apoptotic mechanism of BM blasts turns patients with MDS to sAML. Intensive research is warranted, focusing on the apoptosis-driving pathways as the plausible genetic aberration responsible for sAML transformation and as a possible target for therapeutic intervention.

Author Contributions

O.S. performed the statistical analysis, analyzed and interpreted data, and wrote the manuscript. V.V. designed the research, analyzed and interpreted data, and wrote the manuscript. U.G. and A.K. provided all patients' information and wrote the manuscript. Z.A. supervised the research, analyzed and interpreted data, and wrote the manuscript.

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References:

1. Tefferi A, Vardiman JW. Myelodysplastic syndromes. N Engl J Med 2009;361:1872–1885.
2. Steensma DP, Bennett JM. The myelodysplastic syndromes: Diagnosis and treatment. Mayo Clin Proc 2006;81:104–130.
3. Haase D. Cytogenetic features in myelodysplastic syndromes. Ann Hematol 2008;87:515–526.
4. Sperr WR, Wimazal F, Kundi M, et al. Survival analysis and AML development in patients with de novo myelodysplastic syndromes: Comparison of six different prognostic scoring systems. Ann Hematol 2001;80:272–277.
5. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 2011;364:2496–2506.
6. Harada Y, Harada H. Molecular mechanisms that produce secondary MDS/AML by RUNX1/AML1 point mutations. J Cell Biochem 2011;112:425–432.
7. Shih LY, Huang CF, Wang PN, et al. Acquisition of FLT3 or N-ras mutations is frequently associated with progression of myelodysplastic syndrome to acute myeloid leukemia. Leukemia 2004;18:466–475.
8. Watanabe-Okochi N, Kitaura J, Ono R, et al. AML1 mutations induced MDS and MDS/AML in a mouse BMT model. Blood 2008;111:4297–4308.
9. Parker JE, Mufti GJ, Rasool F, et al. The role of apoptosis, proliferation, and the Bcl-2-related proteins in the myelodysplastic syndromes and acute myeloid leukemia secondary to MDS. Blood 2000;96:3932–3938.
10. Raskind WH, Steinmann L, Najfeld V. Clonal development of myeloproliferative disorders: Clues to hematopoietic differentiation and multistep pathogenesis of cancer. Leukemia 1998;12:108–116.
11. Walter MJ, Shen D, Ding L, et al. Clonal architecture of secondary acute myeloid leukemia. N Engl J Med 2012;366:1090–1098.
12. Link CD.Genomics of AML: Clinical Applications, 53rd ASH Annual Meeting and Exsposition. St. Louis, MO: Washington University; 2011.
13. Tricot G, Boogaerts MA, De Wolf-Peeters C, et al. The myelodysplastic syndromes: Different evolution patterns based on sequential morphological and cytogenetic investigations. Br J Haematol 1985;59:659–670.
14. Armitage P. Multistage models of carcinogenesis. Environ Health Perspect 1985;63:195–201.
15. Campbell FC, Maxwell RJ. Rationale for cancer prevention strategies in high-risk ulcerative colitis. Surgeon 2009;7:96–9100.
16. Scott B, Deeg HJ. Hemopoietic cell transplantation as curative therapy of myelodysplastic syndromes and myeloproliferative disorders. Best Pract Res Clin Haematol 2006;19:519–533.
17. Cutler CS, Lee SJ, Greenberg P, et al. A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: Delayed transplantation for low-risk myelodysplasia is associated with improved outcome. Blood 2004;104:579–585.
18. Runde V, de Witte T, Arnold R, et al. Bone marrow transplantation from HLA-identical siblings as first-line treatment in patients with myelodysplastic syndromes: Early transplantation is associated with improved outcome. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant 1998;21:255–261.
19. Leone G, Mele L, Pulsoni A, et al. The incidence of secondary leukemias. Haematologica 1999;84:937–945.
20. Knudson AG, Jr. Mutation and cancer: Statistical study of retinoblastoma. Proc Natl Acad Sci USA 1971;68:820–823.
21. Michor F, Iwasa Y, Nowak MA. The age incidence of chronic myeloid leukemia can be explained by a one-mutation model. Proc Natl Acad Sci USA 2006;103:14931–14934.
22. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89:2079–2088.
23. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–481.
24. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966;50:163–170.
25. Cox DR. Regression models and life-tables. J Roy Stat Soc Ser B (Methodological) 1972;34:187–220.
26. Lee ET, Wang JW. Statistical Methods for Survival Data Analysis. New York: John Wiley; 2003. xxii, 513 p. p.
27. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002;100:2292–2302.
28. Moolgavkar SH, Luebeck EG. Multistage carcinogenesis: Population-based model for colon cancer. J Natl Cancer Inst 1992;84:610–618.
29. Economopoulou C, Pappa V, Papageorgiou S, et al. Cell cycle and apoptosis regulatory gene expression in the bone marrow of patients with de novo myelodysplastic syndromes (MDS). Ann Hematol 2010;89:349–358.
30. Lin CW, Manshouri T, Jilani I, et al. Proliferation and apoptosis in acute and chronic leukemias and myelodysplastic syndrome. Leuk Res 2002;26:551–559.
31. Mundle S, Iftikhar A, Shetty V, et al. Novel in situ double labeling for simultaneous detection of proliferation and apoptosis. J Histochem Cytochem 1994;42:1533–1537.
32. Albitar M, Manshouri T, Shen Y, et al. Myelodysplastic syndrome is not merely "preleukemia." Blood 2002;100:791–798.
33. Malcovati L, Germing U, Kuendgen A, et al. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 2007;25:3503–3510.
34. Zohren F, Strupp C, Kündgen A, et al. characteristics of disease progression in 3144 patients with myelodysplastic syndrome. Haematologica 2008;93:361.
35. Goldberg SL, Chen E, Corral M, et al. Incidence and clinical complications of myelodysplastic syndromes among United States Medicare beneficiaries. J Clin Oncol 2010;28:2847–2852.
36. Bernasconi P. Molecular pathways in myelodysplastic syndromes and acute myeloid leukemia: Relationships and distinctions: A review. Br J Haematol 2008;142:695–708.
37. Kao JM, McMillan A, Greenberg PL. International MDS risk analysis workshop (IMRAW/IPSS reanalyzed): Impact of cytopenias on clinical outcomes in myelodysplastic syndromes. Am J Hematol 2008;83:765–770.
38. Germing U, Hildebrandt B, Pfeilstocker M, et al. Refinement of the international prognostic scoring system (IPSS) by including LDH as an additional prognostic variable to improve risk assessment in patients with primary myelodysplastic syndromes (MDS). Leukemia 2005;19:2223–2231.
39. Garcia-Manero G, Shan J, Faderl S, et al. A prognostic score for patients with lower risk myelodysplastic syndrome. Leukemia 2008;22:538–543.