Effect of Measurable (“Minimal”) Residual Disease (MRD) Information on Prediction of Relapse and Survival in Adult Acute Myeloid Leukemia

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LETTER TO THE EDITOR

The likelihood of therapeutic resistance (i.e. failing to achieve complete remission [CR] or relapsing from CR) varies widely in adult acute myeloid leukemia (AML). Conceivably, accurate identification of patients who will have poor outcomes with standard therapies would enable their assignment to investigational treatments and facilitate interpretation of trial results. Yet, our previous studies indicated significant limitations in our ability to predict outcomes for individual patients even when many clinical and disease-related characteristics are jointly considered.1,2 Although inclusion of additional pre-treatment information might improve predictions, inclusion of post-treatment data, particularly measurable (“minimal”) residual disease (MRD) after the initial chemotherapy cycle(s), might also be useful. Here, we explore this possibility with data from S0106 (NCT00085709), a randomized SWOG trial testing the addition of gemtuzumab ozogamicin (GO) to “7+3” in patients aged 18-60 years with newly diagnosed de novo AML.3

CONFLICT OF INTEREST
The authors declare no competing financial interests.

Supplementary information is available at Leukemia’s website.
In S0106, submission of bone marrow specimens obtained at baseline and at time of CR was encouraged for centralized, prospective assessment of MRD by multiparameter flow cytometry (MFC), using an early generation, 3 tube, 10-color assay. MRD was identified by an experienced hematopathologist (B.L.W.) by visual inspection as a cell population showing deviation from normal antigen-expressing patterns seen in specific cell lineages at specific stages of maturation as compared with normal and regenerating marrow, an approach estimated to be applicable to ~90% of AML patients. The assay sensitivity varies with the type of phenotypic aberrancy and immunophenotypes of normal cells in background populations. However, the assay detects MRD when present in the large majority of cases down to a level of 0.1% and in progressively smaller subsets of patients below that level. When identified, the abnormal population was quantified as a percentage of the total CD45+ white blood cell events. Any measurable level of residual disease was considered positive (MRD\textsuperscript{pos}). Overall survival (OS) and relapse-free survival (RFS) were estimated using the Kaplan-Meier method. Risk of relapse (RR) was summarized using cumulative incidence estimates. We used Cox regression analyses to assess the association between the outcomes of interest and the covariates: age, gender, performance status, white blood cell (WBC) count, platelet count, bone marrow blast percentage, cytogenetic risk, FLT3-ITD and NPM1 mutational status, number of induction courses (1 vs. 2), and MRD (present vs. absent). We used the C-statistic to quantify a model’s ability to predict outcomes, with values of 0.6-0.7, 0.7-0.8, and 0.8-0.9 commonly considered as poor, fair, and good, respectively. The relative importance of predictors in the multivariable regression models was evaluated by the partial Wald Chi-squared statistic minus the predictor’s degrees of freedom. All analyses were performed using R (http://www.r-project.org). Institutional review boards of participating sites approved all protocols, and patients were treated according to the Declaration of Helsinki.

Four hundred sixteen of the 595 eligible patients (70%) treated on S0106 achieved CR, with CR rates unaffected by GO. Paired baseline/CR samples for MRD assessment were submitted for 174 patients (42% of those achieving CR). Four sample pairs were excluded for poor viability (n=2) or uninformative immunophenotype (n=2), leaving 170 patients for analysis. Of the 170, 148 (87%) were in CR after one cycle of induction therapy with the remaining 22 (13%) patients requiring two treatment cycles to achieve CR, consistent with the CR rates in the full study (p=0.88). There were also no significant differences in patient characteristics or in the outcomes of RFS, OS, and RR between patients who did and did not have MRD data available. One hundred thirty-two of the 170 patients (78%) were MRD\textsuperscript{neg}, whereas 38 (22%) were MRD\textsuperscript{pos} (Supplemental Table 1). Among MRD\textsuperscript{pos} patients, the median level of MRD was 0.205% (range: 0.002-10.4%), with 2 patients having levels above 5%. Patients who needed two cycles of induction therapy were more likely to be MRD\textsuperscript{pos} than those who obtained CR with the first chemotherapy cycle (41% vs. 20%, p=0.05). MRD\textsuperscript{pos} patients had worse post-remission outcomes than MRD\textsuperscript{neg} patients (Figure 1), with MRD status being significantly associated with OS (hazard ratio [HR]=2.32 [1.42-3.77], p<0.001), RFS (HR=2.28 [95% confidence interval: 1.45-3.60], p<0.001), and RR (HR=2.17 [1.27-3.70], p=0.005) on univariate analysis; estimates of cumulative incidence of relapse for various cytogenetic/molecular subgroups are shown in Supplemental Figure 1.

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We then assessed the ability of covariates (univariate and multivariable) to predict RFS and OS in individual patients (Table 1); similar statistical methods for competing risk outcomes such as RR are not available. In univariate analyses, MRD status, cytogenetic risk, NPM1/FLT3-ITD status, age, platelets, and bone marrow blast percentage were the strongest (but poor) individual predictors for RFS (C-statistics: 0.55-0.58). For OS, the strongest individual predictors were cytogenetic risk, age, NPM1/FLT3-ITD status, bone marrow blasts percentage, and MRD status (C-statistics: 0.56-0.59). Excluding MRD data, multivariable models yielded C-statistics of 0.65 and 0.69 for the prediction of RFS and OS (of note, in the 246 CR patients without MRD data, the C-statistics values for RFS and OS models were very similar [0.62 and 0.68]); addition of treatment arm as covariate did not change these findings. Inclusion of MRD data improved the models only minimally, yielding C-statistics of 0.66 and 0.70 for the prediction of RFS and OS, despite the fact that MRD was the most important predictor of both RFS and OS on multivariable analysis (Supplemental Figure 2).

In line with previous studies, this analysis from S0106 demonstrates that MRD after completion of induction chemotherapy is significantly associated with OS, RFS, and RR, and can robustly stratify cohorts of patients based on risk of AML recurrence and length of survival, even in cytogenetically/molecularly-defined disease subgroups. For individual patients, the MRD status was also the single most important predictor of OS and RFS in S0106. Still, the accuracy of multivariable models predicting these outcomes on an individual level is only minimally increased when MRD information is included and remains limited. This observation may caution against excessive reliance on MRD as a tool to dictate management in individual patients. A variety of reasons may contribute to this limitation. First, for S0106, we only had data on cytogenetics and FLT3-ITD and NPM1 mutational status but not detailed molecular profiling available. We previously found that results from genetic profiling increase the accuracy of multivariable models predicting therapeutic resistance or survival in younger adults with newly diagnosed AML, with the magnitude of improvement being roughly the same as that afforded by knowledge of the FLT3-ITD/NPM1 mutation status. Thus, perhaps, the accuracy of the models built for the S0106 cohort could be refined if additional molecular profiling data were available. Our group and others are also working on identifying novel potential molecular and clinical biomarkers that may continue to improve our ability to risk-stratify AML patients. Second, determination of MRD at the time of CR is, fundamentally, a measure of the leukemia’s sensitivity to induction chemotherapy. Still, a single assessment several weeks after treatment initiation may not fully capture response dynamics and may need to be supplemented with assessments at earlier (e.g. early disease clearance from the peripheral blood or bone marrow) and/or later times (e.g. after consolidation therapy). And third, there are significant limitations inherent to current MFC methods to detect MRD. Perhaps most importantly, while applicable to the vast majority of AML patients, MFC-based MRD assays do not have uniform sensitivity across all cases, and the sensitivity may not reach that of other methods, e.g. polymerase-chain reaction (PCR)-based techniques. There is thus a greater possibility of misclassification, particularly the classification of some MRD patients as MRD and not correctly identifying those as high-risk individuals, when MRD is assessed by MFC rather than PCR. For molecularly well-defined patient subsets, e.g.
those with NPM1 mutation, it is conceivable that PCR-based MRD data improve the accuracy of relapse and survival prediction to a larger extent than MFC-based MRD data.

Even with additional information and better MRD technologies, however, our ability to predict long-term outcomes in adult AML after achievement of CR may remain limited. RR, RFS, and OS are all affected by post-remission therapy, and survival estimates are impacted by non-relapse mortality. OS is additionally influenced by therapies used after disease recurrence. Indeed, in S0106, a survival plateau was noted at 3.5 years in the MRDpos cohort. Consequently, the accuracy of predictions generally increases as the period over which prediction is desired decreases. Consistent with this notion, our models improved if we attempted to predict shorter-term endpoints such as 6-month and 12-month RFS. For these two endpoints, C-statistics were 0.68 and 0.60 (univariate models) as well as 0.82 and 0.67 (multivariable models). Particularly for the shorter RFS prediction, the contribution of MRD data to the multivariable model's accuracy was more pronounced than for the RFS model built initially (C-statistics of 0.78 and 0.65 for 6- and 12-month RFS without MRD data). There were only 18 events in the 6-month RFS analysis, limiting the inference that can be drawn from the multivariable models; larger cohorts will be needed to test this idea further. If confirmed, our studies may form the basis for the development of relatively accurate shorter-term RFS prediction models, in which MRD data should be included.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Outcome of patients on S0106, stratified by post-remission MRD status
Estimates of the probability of OS (A) and RFS (B) as well as cumulative incidence of relapse (C) in patients who achieved CR with induction chemotherapy, shown separately for patients with and those without MRD at completion of CR achievement.
**TABLE 1**
C-statistics for univariate and multivariable Cox regression analyses

| Parameter                              | RFS  | OS   |
|----------------------------------------|------|------|
| **Univariate analyses**                |      |      |
| MRD status                             | 0.58 | 0.59 |
| Age                                    | 0.56 | 0.56 |
| Gender                                 | 0.50 | 0.50 |
| Performance status                     | 0.51 | 0.53 |
| White blood cell count                 | 0.52 | 0.53 |
| Platelet count                         | 0.56 | 0.55 |
| Bone marrow blast percentage           | 0.56 | 0.58 |
| Cytogenetic risk                       | 0.56 | 0.59 |
| \( NPM1/FLT3\)-ITD status              | 0.55 | 0.57 |
| Number of induction courses            | 0.54 | 0.55 |
| **Multivariable analyses**             |      |      |
| Basic covariates *                     | 0.61 | 0.63 |
| Basic covariates + cytogenetic risk    | 0.63 | 0.66 |
| Basic covariates + cytogenetic risk + \( NPM1/FLT3\)-ITD status | 0.65 | 0.69 |
| Basic covariates + cytogenetic risk + \( NPM1/FLT3\)-ITD status + MRD status | 0.66 | 0.70 |

*Age, gender, performance status, white blood cell count, platelet count, bone marrow blast percentage, number of induction courses*