Prognostic impact of immunophenotypic aberrancies of blasts in lower risk myelodysplastic syndrome

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ABSTRACT
Objective/background: Low risk myelodysplastic syndrome (MDS) is a marrow failure state eventually leading to transfusion dependence. Flow cytometry has previously been demonstrated as prognostic tool in MDS, however not thoroughly studied in lower risk MDS. In this study, we assessed whether assessment for immunophenotypic blast aberrancies by flow in low risk MDS patients has a prognostic role in these patients.

Methods: A total of 63 consecutive patients diagnosed with low/intermediate risk MDS were included. We recorded initial flow results, and collected time to transfusion dependence, and AML progression.

Results: On multivariate cox regression analysis, increasing IPSS-R score, an increase in the number of blast aberrancies on flow cytometry, and aberrant expression of CD7 on myeloid blasts increased likelihood of transfusion dependence.

Conclusion: Low risk MDS patients with increasingly aberrant blast phenotypes by flow may be at risk for earlier transfusion dependence.

1. Introduction
The myelodysplastic syndromes (MDS) represent a clonal hematopoietic stem cell disorder with variable clinical presentation and course [1]. Oftentimes, higher risk MDS shares features with Acute Myeloid Leukemia, while lower risk MDS is characterized by eventual development of transfusion dependence, frequently red blood cell dependence. Diagnosis and classification of MDS occurs through a multimodal approach, including the use of flow cytometry.

In addition to aiding diagnosis, flow cytometry has previously been shown to provide prognostic value [2]. In higher risk MDS, studies have shown that aberrant CD7 expression is associated with shorter survival [2]. Wells et al developed a flow cytometry scoring system (FCSS) to aid in the diagnosis and prognostication of MDS [3]. The FCSS has since been validated in predicting outcomes, including risk for transfusion dependence, and risk for progression to AML, independent of other scoring systems for MDS [4,5]. While FCSS provides prognostic information, it has not been adopted into standard workflows at many institutions, including ours.

At our institution, routine flow analysis at time of MDS diagnosis includes the assessment of immunophenotypic (IP) aberrancies in myeloblasts, and we have previously described neoplasia-specific blast aberrancies in this context [6]. Little has been published regarding the relevance of aberrances in low risk MDS cases. Therefore, in this study, we investigated the prognostic significance of IP aberrances in low or intermediate risk MDS, namely, whether the presence of aberrances is associated with development of red blood cell transfusion dependence.

2. Methods
This study was performed with Institutional Review Board approval from the Medical College of Wisconsin. We performed a retrospective chart review of patients who received an initial diagnosis of MDS at our institution between January 2010 and December 2017. IPSS-R score was calculated for all identified patients and those with a low or intermediate risk score (IPSS-R <4.5) were included for the study. Patients diagnosed with myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) and chronic myelomonocytic leukemia (CMML) were excluded from the study. Patients who were lost to follow up after initial BM biopsy were also excluded from the study.

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Patients were identified as red blood cell transfusion dependent if they had two or more episodes of packed red blood cell (PRBC) transfusions over the course of four weeks. In patients meeting these criteria, date of transfusion dependence was defined as the date of the second PRBC transfusion. Patients who underwent allogeneic hematopoietic stem cell transplantation prior to development of transfusion dependence were censored at the time of transplant.

Only patients who had initial diagnostic studies, including flow, at our institution were included. All flow reports were reviewed by a single pathologist for the purpose of this study. Flow cytometry (4- or 8-color) was performed on bone marrow aspirates for the following antigens: CD7, CD11b, CD13, CD14, CD15, CD19, CD20, CD33, CD34, CD36, CD38, CD45, CD56, CD64, CD117, and HLA-DR using a FACS Calibur/Canto. Blasts were identified using cluster analysis (Paint-A-Gate™ software, BD Biosciences, California), after exclusion of all other populations as cohesive, well-delineated clusters, with consistent light scatter and CD45 expression patterns across multiple tubes. Blast IP aberrancies were defined as $\geq 1/4$ log compared to normal controls. Neoplasia-specific blast IP aberrancies were defined as abnormalities seen exclusively in neoplastic non-acute myeloid diseases, and were adopted from a previous study to include: expression of CD7, CD11b, CD13, CD15, CD33, CD34, CD36, CD38, CD45, CD56, CD64, CD117, and HLA-DR.

### Results

Between January 2010 and December 2017, we identified 63 patients with a new diagnosis of low or intermediate risk MDS at our institution. The median age of patients was 66 years, and most were male, n=35 (56%). At diagnosis, mean hemoglobin was 10.0 g/dL (range: 5.2-15.3 g/dL), mean platelet count was 155.4 $\times 10^9$/L (range: 8960 $\times 10^9$/L), and mean ANC was 2.5 $\times 10^9$/L (range: 0.1-13.8 $\times 10^9$/L). The mean bone marrow blast percentage was 1.9 % (range: 0.0-9.2 %). Overall, 15 (24%) patients were categorized as IPSS-R very low risk, 29 (46%) patients were IPSS-R low risk, and 19 (30%) patients were IPSS-R intermediate risk. Table 1 summarizes demographics. Of note, eight patients initiated therapy with an erythrocyte stimulating agent or hypomethylating agent prior to development of transfusion dependence.

### Multivariate analysis of variables for transfusion independence

| Description              | Point Estimate | Lower 95% Wald Confidence Limit | Upper 95% Wald Confidence Limit | p-value | Overall p-value |
|--------------------------|----------------|---------------------------------|---------------------------------|---------|-----------------|
| IPSS-R                   | 1.976          | 1.130                           | 3.454                           | 0.0169  | 0.0169          |
| Blast % at diagnosis     | 1.000          | 0.998                           | 1.002                           | 0.9291  | 0.9291          |
| Number of aberrancies    | 1.453          | 1.060                           | 1.992                           | 0.0203  | 0.0203          |
| Aberrant CD7 expression  | 3.727          | 1.359                           | 10.218                          | 0.0106  | 0.0106          |

4. Discussion

Among lower risk MDS patients, our study demonstrates a potential prognostic role for simply assessing the number of IP aberrancies on blasts by flow cytometry. In patients with MDS with low or intermediate IPSS-R scores, the presence of blasts with three or more aberrancies at diagnosis was associated with more rapid development of transfusion dependence. In the absence of aberrancies, patients in this study rarely
developed transfusion dependence. Furthermore, like previous studies, aberrant CD7 expression was associated with poorer outcomes; this also indicated an earlier time to transfusion dependence.

As more centers routinely perform flow analysis during initial assessment for MDS, stratifying blasts into high (3+) IP aberrances or low IP aberrancies, is a simple assessment that may inform clinicians into how carefully to monitor their LR-MDS patients. Prospective studies are required to confirm these results, and future studies could also examine the relationship between high IP blast aberrancy burden and pathologic mutations present with MDS.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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