Are next-generation sequencing results knocking on Heaven's door for transplantation planning in chronic myelomonocytic leukemia?

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Chronic myelomonocytic leukemia (CMML) is a heterogeneous malignant myeloid disorder included in the 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia in the category of myelodysplastic syndromes/myeloproliferative neoplasms. CMML patients show a diverse biological, clinical picture and heterogeneous prognosis, with short overall survival (OS) and increased risk of progression to acute myeloid leukemia. The diagnosis of CMML requires a monocytosis, defined as an absolute monocyte count above $1x10^9$/L that should represent >10% of the white blood count (WBC) differential, which persists for more than 3 months and for which other causes of reactive monocytosis have been excluded. The current World Health Organization classification includes three CMML groups, divided on the basis of blast counts, for better prognostication: CMML-0 [<2% peripheral blood (PB) and <5% bone marrow (BM) blasts]; CMML-1 (2-4% PB and/or 5-9% BM blasts); and CMML-2 (5-19% PB and/or 10-19% BM blasts). The classical French-American-British classification, still widely used, divides CMML into so-called "dysplastic" CMML (WBC ≤$13x10^9$/L) and "myeloproliferative" CMML (WBC >13 $x10^9$/L).

Cytogenetic abnormalities and somatic mutations are found in, respectively, 25-30% and up to 95% of CMML patients and both have a strong influence on OS. The validated CMML-specific cytogenetic risk classification recognizes three risk categories: low-risk (normal karyotype or loss of the Y chromosome as a single anomaly; ~78%), high-risk (trisomy 8 or abnormalities of chromosome 7, or complex karyotype; ~12%), and intermediate-risk (all other abnormalities; ~9%).[1] The most frequently mutated genes in CMML affect epigenetic regulation and DNA methylation (*ASXL1* and *TET2*), RNA splicing (*SRSF2*), and transcription (*RUNX1*) and signaling pathways (*RAS*).[2-4] Frameshift and nonsense *ASXL1* mutations confer an adverse prognosis,[2-4] aggravated when *EZH2* and *ASXL1* mutations co-occur.[5] *DNMT3A* and *TP53* mutations, although less common in CMML, have also been associated with poorer OS.[6,7] It is noteworthy that the number of mutations also influences patients' outcomes, as recently demonstrated in a study in which a shorter OS was observed in CMML patients with three or more concomitant mutations.[7]

Specific prognostic scoring systems for individual risk assessment are essential in order to provide risk-adapted treatment. The most commonly used are the CMML-specific prognostic scoring system (CPSS),[8] the MD Anderson Cancer Center prognostic score (MDAPS)[9] and the revised International Prognostic Scoring System (IPSS-R)[10] (the last only being applicable to "dysplastic" CMML). More recent scoring systems that also include somatic mutations are those by the *Groupe Français des Myélodysplasies* (GFM),[2] the Mayo Clinic[3], and the molecular CPSS.[4] All these molecular prognostic scoring systems consider *ASXL1* mutations; the molecular CPSS also takes into account mutations in *NRAS*, *SETBP1*, and *RUNX1*.

Allogeneic hematopoietic cell transplant (HCT) is the only potentially curative therapy for patients with CMML but the number of transplant-eligible patients is low because of these individuals' advanced age, comorbidities, and frailty. A recent multicenter retrospective study with 1,656 CMML patients of whom 89 received an allogeneic HCT demonstrated the benefit of HCT for patients with higher-risk disease as determined by the CPSS[11] and multiple retrospective studies have documented a 3-year OS rate of 30-40%.[12-15] For high-risk transplant-ineligible and/or lower-risk patients the most widely used therapies are hydroxyurea, hypomethylating agents, and best supportive care. Recent evidence suggests that hypomethylating agents might be superior to hydroxyurea.[11]

Many studies have evaluated the prognostic factors for transplantation outcomes in CMML patients, with contradictory results.[16] As would be expected, patients transplanted in complete remission[13] as well as those with <5% blasts at transplantation[14] had better outcomes in comparison to those with more advance disease at transplantation. The favorable effect of using hypomethylating agents before transplantation over intensive chemotherapy is debatable.[14-16] In a large study by the Center for International Blood and Marrow Transplantation Research (CIBMTR), the CPSS score at the time of HCT strongly influenced OS after transplantation.[15] Table 1 shows the predictive factors for increased relapse or reduced OS evidenced in major studies on allogenic HCT for CMML.

In this issue of *Haematologica*, Woo and colleagues, analyze long-term outcomes after allogeneic HCT in 129 patients with CMML from a single institution and evaluate clinical and molecular risk factors associated with outcomes.[17] Of note, this study is the first to evaluate the impact of somatic mutations determined by next-generation sequencing (NGS) on allogeneic HCT outcomes in a large and homogeneous series of patients from a single institution. In a subcohort of 52 patients in whom a NGS panel of 75 genes was used, 85% of patients had at least one mutation, congruent with previous reports on

Table 1. Predictive factors for overall survival after allogeneic hematopoietic cell transplantation in relevant series of patients with chronic myelomonocytic leukemia.

	EBMT {Symeonidis:2015[13]}	MDACC {Kongtim:2016[14]}	CIBMTR {Liu:2017[15]}	FHCRC {Woo:2019[17]}
Study period	1988-2009	1991-2013	2001-2012	1986-2017
N. of patients	513	83	209	129
Median age, years (range)	53 (18-75)	57 (18-78)	57 (23-74)	55 (7-74)
Disease at HCT (CMML/AML, %)	56 / 44	57 / 43	100 / 0	71 / 29
Factors predicting OS				
Complete remission at HCT	Favorable	Favorable	NA	No effect
Higher-risk categories by CPSS/MDAPS	NA	NA	Unfavorable/NA	Unfavorable/Unfavorable
Performance Status (KPS≥90)	NA	No effect	Favorable	NA
HCT-CI ≥4	NA	NA	NA	Unfavorable
High-risk CMML-specific cytogenetics	No effect	No effect	NA	Unfavorable
Graft source (peripheral blood)	No effect	NA	Favorable	No effect
Transplant from matched related donors	No effect	Favorable	NA	No effect
Prior HMA treatment	No effect	Favorable	No effect	No effect
Molecular profile	NA	NA	NA	Increased relapse: -Mutations in NRAS, ATRX, WT1 - ≥10 gene mutations - ≥4 mutations in epigenetic regulators
Conditioning intensity	No effect	No effect	NA	No
Development of chronic GvHD	No effect	Favorable	NA	NA
Age	No effect	No effect	NA	No effect
Year of transplant	No effect	No effect	NA	No effect

EBMT: European Society for Blood and Marrow Transplantation; MDACC: MD Anderson Cancer Center; CIBMTR: Center for International Blood and Marrow Transplantation Research; FHCRC: Fred Hutchinson Cancer Research Center; HCT: hematopoietic cell transplant; CMML: chronic myelomonocytic leukemia; AML: acute myeloid leukemia; OS: overall survival; CPSS: CMML-specific prognostic scoring system; MDAPS: MD Anderson prognostic scores; KPS: Karnofsky Performance Score; HCT-CI: Hematopoietic Cell Transplant-Comorbidity Index; PB: peripheral blood; HMA: hypomethylating agents, GvHD: graft-versus-host disease; NA: information not available.;

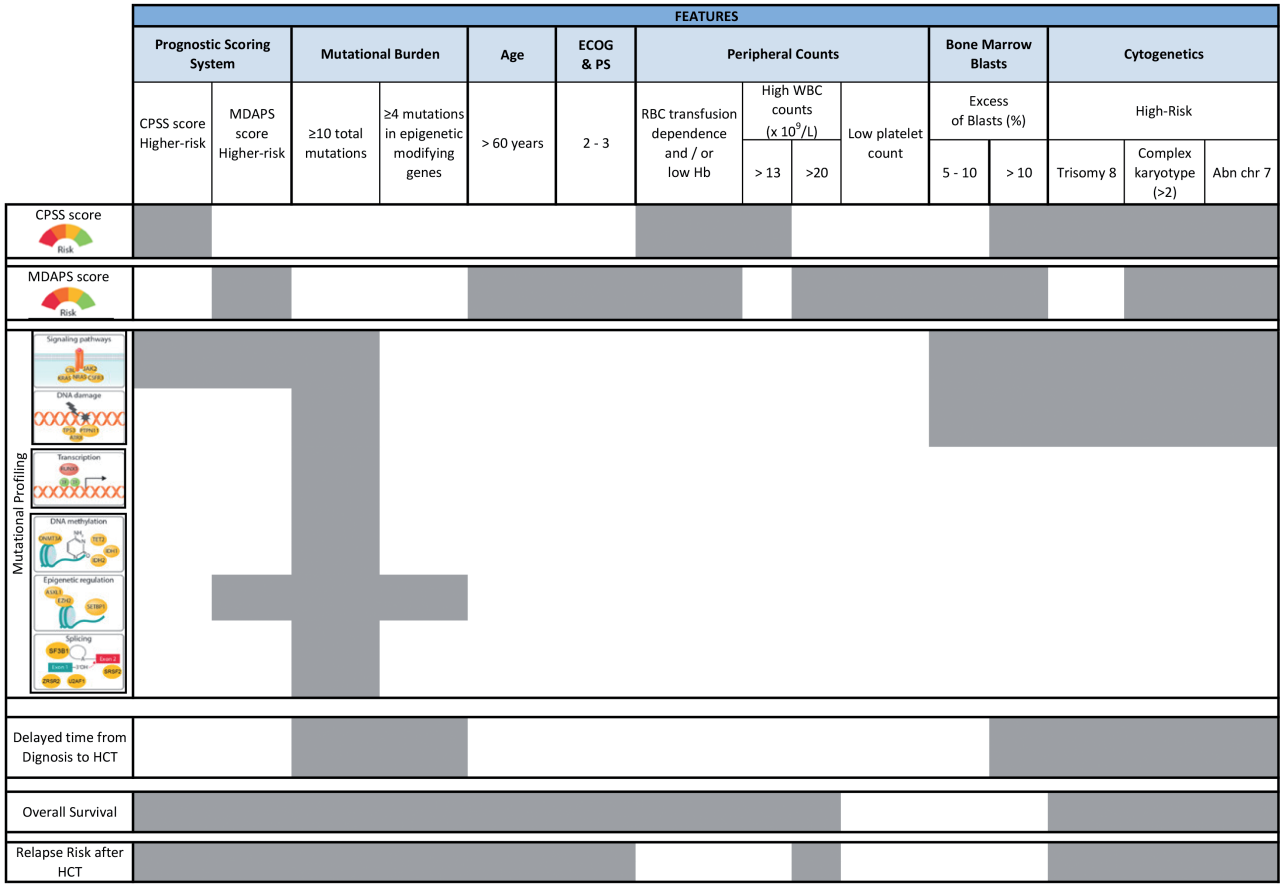
CMML. The most commonly mutated genes were *ASXL1* (52%), *TET2* (42%), and *SRSF2* (25%). Other frequently encountered mutations were evident for WT1 (27%), *RUNX1* (17%), *DNMT3A* (17%), *SMC1A* (17%), *EZH2* (12%), and *ATRX* (12%), highly likely because most patients had intermediate-2 or high-risk disease according to the CPSS.[17]

In the study by Woo *et al.*, mutations in *NRAS* were associated with an increased relapse risk whereas mutations in *ATRX* and *WT1*, conferred both a higher relapse risk and inferior OS. Moreover, this study showed that a high overall mutation burden (≥10 mutations) as well as the presence of four or more mutated epigenetic regulatory genes were linked to a higher risk of relapse. Unsupervised clustering revealed two higher-risk groups with specific associations between mutations and clinical features. The presence of a higher mutation burden was closely related to a longer period between diagnosis and transplantation but not with complex chromosomal abnormalities or an excess of blasts. The currently published recommendation of an international expert panel is to use the CPSS for considering a patient as a candidate for allogeneic HCT and to transplant those CMML patients belonging to the intermediate-2/high CPSS risk groups.[18] Whether the better outcomes observed with lower CPSS scores and the lower mutational burden observed in less advanced disease could argue in favor of transplanting patients with CMML earlier in the course of their disease (e.g., extending transplantation to patients with intermediate-1 CPSS risk score) is debatable and can only be properly answered by a carefully designed study.

As previously reported in other HCT series on CMML, in the study by Woo *et al.*, relapse risk was also significantly associated with adverse cytogenetics, higher-risk CPSS and MDAPS scores, and measurable residual disease by cytogenetics at transplantation. A higher mortality was seen in patients with high-risk cytogenetics and a high HCT Comorbidity Index. Of interest, neither disease status at transplantation (complete remission *vs.* non-complete remission) nor pre-transplant therapy (intensive chemotherapy or hypomethylating agents) nor conditioning intensity had a clear and independent impact on transplant outcomes. Additionally, the year of transplant did not affect the risk of relapse or OS, clearly indicating that newer transplant strategies are needed to improve those outcomes.[17]

In summary, this study has identified both clinical and novel molecular risk factors for outcomes after allogeneic HCT that add relevant information which could be taken into account when planning transplantation for CMML patients. Table 2 illustrates the clinical and molecular risk factors for allogeneic HCT outcome in CMML patients, among whom different groups of higher-risk patients amenable to transplantation can be detected at different

Table 2. Schematic representation of the relationships between clinical and hematologic characteristics, cytogenetics, somatic mutations, prognostic scoring systems and transplant outcomes in patients with chronic myelomonocytic leukemia.



ECOG: Eastern Cooperative Oncology Group; PS: Performance Status; CPSS: CMML-specific prognostic scoring system; MDAPS: MD Anderson prognostic score; RBC: red blood cells; Hb: hemoglobin; WBC: white blood cells; Abn: abnormalities; Chr: chromosome; HCT: hematopoietic cell transplant

time points of the course of the disease.

However, even though NGS studies could therefore be used to select a group of high-risk CMML patients for transplantation, our view is that it might still be premature to incorporate the results of NGS techniques into the decision-making process for CMML patients undergoing allogeneic HCT. The main reasons are that NGS techniques and results are not well standardized, their reproducibility is unproven, the characterization of allele variants as pathogenic is not homogenously defined, and the variant allele frequency threshold used to define the presence of mutations is widely variable (5% in the series by Woo *et al.*) Furthermore, the impact of co-occurring mutations on prognosis is still unclear.[19] Additionally, the value of molecular profiling for treatment decision-making in CMML patients is diminished by the limited number of treatment alternatives and because there is no single somatic mutation that favors the use of a particular treatment approach.[19]

In conclusion, molecular profiling will likely emerge in the near future as highly valuable for planning transplantation in CMML patients, adding up to other well-recognized patient and disease characteristics such as higher-risk CPSS and MDAPS categories and HCT-Cormorbidity Index. Prospective cooperative studies focused on NGS results before and after transplantation and involving large numbers of patients will be eventually required to improve the cure rate afforded by allogeneic HCT in CMML.

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Leukemia stem cell gene expression signatures contribute to acute myeloid leukemia risk stratification

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The majority of patients with acute myeloid leukemia (AML) will die of their disease. Nevertheless, the prognosis of AML varies widely. Some AML patients may be cured by chemotherapy alone, while others require approaches such as allogeneic stem cell transplantation to have the best chance of long-term survival. As physicians, we are often asked by our AML patients: "How likely is this treatment going to work, and how long do I have to live?"[1]

Prognostication in AML has evolved over time. Initially, models for prediction of response to therapy were based on patient's parameters such as age and performance status in combination with cell characteristics such as morphology and chromosomal karyotype. With technological advancements, our understanding of disease biology has evolved and factors including molecular mutations and minimal residual disease have been integrated into prognostication schemes. Recently, an international expert panel on behalf of the European LeukemiaNet (ELN) published a revised version of a widely utilized prognostication scheme that categorizes AML patients into three risk groups (Favorable, Intermediate, and Adverse) based on genetic abnormalities (incorporating chromosomal karyotype and specific molecular mutations).[2] These AML risk groups have profound clinical implications, particularly with regard to post-remission therapy for younger fit patients. In general, fit Favorable-risk AML patients who achieve a first complete remission after induction chemotherapy go on to consolidation chemotherapy with curative intent. However, even fit patients with Adverse- and Intermediate-risk AML are unlikely to be cured by chemotherapy alone, and therefore it is reasonable to consider allogeneic stem cell transplantation for Intermediate- and Adverse-risk patients upon achievement of first complete remission.

Why is AML so often resistant to chemotherapy? The biology of AML chemoresistance is complex. However, at a basic level, adverse-risk AML cells are more likely to evade conventional chemotherapeutics that target the cell cycle. It has therefore been hypothesized that one powerful driver of adverse prognosis in AML may be the properties of the leukemia stem cell (LSC), a type of cell that exhibits cell cycle quiescence, self-renewal, and chemoresistance.[3-6] Although AML LSC remain challenging to isolate, assessment of AML LSC gene expression signatures has been proposed as a method to further refine prognosis – with LSC-like AML phenotypes contributing to adverse risk.