Criteria for evaluating response and outcome in clinical trials for children with juvenile myelomonocytic leukemia

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ABSTRACT

Juvenile myelomonocytic leukemia is a rare myeloproliferative disease in young children. While hematopoietic stem cell transplantation remains the only curative therapeutic option for most patients, children with juvenile myelomonocytic leukemia increasingly receive novel agents in phase I-II clinical trials as pre-transplant therapy or therapy for relapse after transplantation. However, response criteria or definitions of outcome for standardized evaluation of treatment effect in patients with juvenile myelomonocytic leukemia are currently lacking. Here we propose criteria to evaluate the response to the non-transplant therapy and definitions of remission status after hematopoietic stem cell transplantation. For the evaluation of non-transplant therapy, we defined 6 clinical variables (white blood cell count, platelet count, hematopoietic precursors and blasts in peripheral blood, bone marrow blast percentage, spleen size and extramedullary disease) and 3 genetic variables (cytogenetic, molecular and chimerism response) which serve to describe the heterogeneous picture of response to therapy in each individual case. It is hoped that these criteria will facilitate the comparison of results between clinical trials in juvenile myelomonocytic leukemia.

Introduction

Juvenile myelomonocytic leukemia (JMML) is a clonal disease in young children.[1,2] Patients with JMML present with leukocytosis, monocytosis and splenomegaly, features similar to those observed in the myeloproliferative subtype of chronic myelomonocytic leukemia (CMML) in adults.[3] Other clinical signs of JMML include thrombocytopenia, leukemic skin infiltration, elevation of fetal hemoglobin (HbF), and hypersensitivity of hematopoietic progenitors to granulocyte-macrophage colony-stimulating factor (GM-CSF).[4] Approximately 90% of patients with JMML harbor largely mutually exclusive mutations in *PTPN11, NF1, NRAS, KRAS,* or *CBL* in their leukemic cells resulting in hyperactivation of the RAS-MAPK pathway.[5-12]

Hematopoietic stem cell transplantation (HSCT) is still the only curative therapy for the vast majority of JMML patients.[13-15] However, with advances in understanding the underlying molecular mechanisms in JMML, the potential for the introduction of novel therapeutic agents has been recognized for some time. Several molecules, such as isotretinoin, zoledronic acid, and farnesyl transferase inhibitor R115777 have been evaluated in pre-HSCT windows or compassionately used.[16-18] Recently, azacitidine, a DNA-hypomethylating agent, was reported to induce hematologic and molecular remissions in some children with JMML,[19,20] and is currently being tested in clinical trials in Europe. Additional efforts are underway to employ therapeutic inhibition of the MEK/ERK and PI3K pathways.[21-23]

In order to evaluate the efficacy of any conventional or novel interventions for JMML, standardized criteria to define responses and relapse are urgently required. With the goal of defining widely accepted criteria of response to therapy, international experts from the European Working Groups of Myelodysplastic Syndromes in Childhood (EWOG-MDS), the Children's Oncology Group (COG) from the US, and from the Japanese Society of Pediatric Hematology/Oncology met to find an agreement at the JMML International Symposium, New Orleans, USA, (6 December 2013). The agreed criteria are presented in this manuscript.

Proposal of response criteria in clinical trials of therapy in JMML

Previous efforts for standardized response criteria for non-HSCT therapy in JMML

In adults, standardized response criteria proposed by an International Working Group of MDS have been widely used in clinical trials for MDS as well as CMML.[24,25] These criteria are, however, not applicable to JMML and myeloproliferative CMML because they focus on reduction of blast percentage and improvement of blood counts, and are not designed to evaluate myeloproliferative features such as organomegaly.

Bergstraesser *et al*. defined response criteria in JMML considering white blood cell (WBC) count, platelet count, as well as liver and spleen size.[26] These authors retrospectively evaluated the efficacy of 129 treatment courses other than HSCT administered to 63 children with JMML and reported a significant correlation between WBC count or spleen size and the efficacy of non-HSCT therapy. This finding was later applied to response criteria proposed by Chan *et al*. describing complete response (WBC $<20\times10^9$/L and normalization of spleen size) and partial response ($<50\%$ of initial WBC but total still greater than 20×10^9/L and 25% decrease in spleen size from initial size) based solely on these two criteria, WBC count and spleen size.[27] Due to the rarity of JMML there is no published prospective clinical trial that has actually applied these criteria, and there are evident limitations when only these two variables are used. The definitions are only applicable in patients with leukocytosis (WBC $\geq20\times10^9$/L) and splenomegaly (≥2 cm below the costal margin). However, among 497 JMML patients currently registered in the EWOG-MDS studies, 30% had a WBC count less than 20×10^9/L, and 12% a spleen size of less than 2 cm below the costal margin at diagnosis (*EWOG-MDS, unpublished data, 2014*). In addition, the criteria are not applicable to patients relapsing post HSCT who have reappearance of cytogenetic or molecular abnormalities after HSCT but who do not yet show the full clinical picture of relapse. Response criteria thus need to be applicable in many different clinical situations for a broad range of patients. Therefore, clinical variables other than WBC count and spleen size are needed, and cytogenetic and molecular variables are also necessary to describe disease status.

The concept behind the proposed response criteria

As outlined above, response measurements in JMML need to be applicable to the highly heterogeneous clinical features at presentation and to individual response patterns to different therapeutic agents. Therefore, 6 clinical vari-

ables and 3 genetic variables were selected (Table 1). For each of these variables (v), complete response (vCR), partial response (vPR) and progressive disease (vPD) are defined. This makes the evaluation of heterogeneous effects of each intervention possible. Because we recognize that each patient can present with different clinical, cytogenetic and molecular features, the number of evaluable variables at the start of therapy differs among patients. Based on the cumulative response of these 9 variables, the clinical and genetic remission status can be described (Tables 2).

Variables to evaluate response

In addition to WBC count and spleen size, clinical variables include presence of myeloid/erythroid precursors and blasts in peripheral blood, platelet count, percentage of blasts in bone marrow, and presence of extramedullary disease (Table 1).[4] Two additional hallmarks of JMML, the level of hemoglobin F (HbF) corrected for age and the presence of monocytosis, have intentionally been excluded as response criteria for various reasons. High HbF levels at diagnosis are known to predict outcome but are dependent on karyotype.[4] Moreover, so far there has been no report on serial HbF levels during the natural course of JMML or following HSCT. Therefore, further studies to evaluate HbF during the clinical course of JMML patients are necessary. Monocytosis more than 1.0×10^9/L is one of the diagnostic criteria of JMML,[4] but the absolute monocyte count generally correlates with the WBC. Since the usefulness of the WBC count has been previously confirmed, we chose the WBC count, but not the monocyte count, as a variable to be evaluated.[26] Moreover, monocytosis can be non-specific since it is observed in various conditions, such as infections or an early sign of bone marrow recovery after HSCT, and leukocytosis in JMML is manifested not only by monocytosis but also by circulating immature myeloid cells in the peripheral blood.

Since the spleen size is one of the variables evaluating treatment efficacy in JMML,[26] reliable methods to measure the size of this variably shaped organ are required. Size determination by palpation with measurement of the length between the spleen tip and the left costal margin is clinically appropriate[26,28-30] but may not be sufficient for clinical trials since its results are not verifiable. Measurements by computed tomography are to be avoided in children with JMML because of radiation issues, and magnetic resonance imaging generally requires deep sedation or anesthesia. For these reasons, we currently recommend evaluation of spleen size by ultrasound with the linear measurement of the splenic length, defined as the maximum distance between the dome and the tip of spleen in the right lateral decubitas position.[31]

The three genetic variables selected for evaluating response were cytogenetics, molecular alterations, and chimerism. Cytogenetic and molecular data must have been collected at diagnosis while chimerism is only applicable after HSCT. Abnormal karyotypes are observed in approximately 35% of JMML patients, with monosomy 7 being the most common aberration (25%).[4] Oncogenic molecular alterations in *PTPN11, NF-1, NRAS, KRAS* or *CBL*, noted in approximately 90% of patients are increasingly important tools for diagnosis and follow up of JMML.[5-12] We anticipate that additional somatic mutations will be discovered in JMML. Indeed, recently *SETBP1* and *JAK3* mutations were reported as a result of an exome sequencing project.[32] However, until these markers are fur-

ther validated, we would suggest that these mutations, which might indicate sub-clones, fall under the category of "acquired molecular abnormalities". Analysis of donor chimerism has been a standard measurement to follow JMML patients given allogeneic HSCT. While discussion of the various methods used to determine donor chimerism is beyond the scope of this consensus report, there was broad agreement that unsorted cell donor chimerism should be a common measurement in clinical

trials. Most JMML patients with persistent mixed chimerism experience clinical relapse of JMML,[33] and are thus candidates for early intervention with innovative therapies prior to development of a full clinical relapse.

Definitions of response to therapy other than HSCT in JMML

Based on the response of each applicable variable listed in Table 1, the clinical and genetic remission status can be

Table 1. Variables for evaluation of response to therapy in JMML.

Variables for response		Definition of response			Definition of disease progression or relapse (applicable to all patients)
		Assessment of CR and PR is feasible if the following are present before therapy	Requirement for CR for each variable (vCR)	Requirement for PR for each variable (vPR)	Requirement for PD for each variable (vPD)
Clinical variables	1) WBC count	>20×10⁹/L	3.0-15.0×10⁹/L	Decreased by ≥50% over pretreatment but still >15×10⁹/L	Increase by ≥50% and ≥20×10⁹/L
	2) Myeloid and erythroid precursors and blasts in PB*	≥ 5%	0-1%	Decreased by ≥50% over pre-treatment but still ≥ 2%	Increase from the baseline: < 5%: ≥ 50% increase and ≥ 5% ≥ 5%: ≥ 50% increase of total % of myeloid and erythroid precursors and blasts
	3) Platelet count	<100×10⁹/L	≥100×10⁹/L	For patients starting with ≥ 20×10⁹/L platelets: absolute increase of ≥ 30×10⁹/L For patients starting with < 20 ×10⁹/L platelets: increase by ≥ 100% and > 20×10⁹/L	Development of transfusion dependency or if patients have the baseline of the platelet count of ≥30×10⁹/L, decrease by ≥50% and <100×10⁹/L
	4) BM blasts	≥5%	<5%	Decreased by ≥50% over pre-treatment but still ≥5%	Increase from baseline; < 5%: ≥ 50% increase and ≥ 5% ≥ 5%: ≥ 50% increase of BM blasts
	5) Spleen size a) Clinical evaluation or	≥2 cm under the costal margin	No splenomegaly	50% decrease by cm under the costal margin	Increase by ≥100% if baseline <4cm from under the costal margin ≥50% if baseline 5-10 cm >30% if baseline >10 cm
	b) Sonography	Length of spleen ≥ 150% of upper limit of normal range	No splenomegaly	>25% decrease by length, but still splenomegaly	Increase by ≥25% of length
	6) Extramedullary disease#	Extramedullary leukemic infiltration	No evidence of extramedullary leukemic infiltration in any organ	–	Worsening or new lesions of extramedullary leukemic infiltration
	7) Cytogenetic response	Somatic cytogenetic abnormality detected	Normal karyotype	–	Reappearance or additional acquirement of cytogenetic abnormalities
	8) Molecular response	Somatic genetic anomalies detected **	Absence of somatic genetic anomalies	–	Reappearance or additional acquirement of JMML-specific somatic gene abnormalities
	9) Chimerism response (only for patients after HSCT)	>15% autologous cells after allo-HSCT	Complete donor chimerism	–	50% increase and >5% increase of autologous cells and >5%

*CR: complete response; PR: partial response; PD: progressive disease; WBC: white blood cell; PB: peripheral blood; BM: bone marrow. *Myeloid precursors include promyelocytes, myelocytes and metamyelocytes. The myeloid and erythroid precursors and blasts in PB are given as percentage of the total nucleated cells in PB (WBC including erythroblasts). **In NF-1, PTPN11, NRAS, KRAS, or CBL, thus the mutations are thought to be initiating. In patients with germ-line NF-1, PTPN11 or CBL mutation, only acquired mutations can be evaluated for response and relapse after therapy. The germ-line mutation remains even if patients achieved complete molecular response. #Extramedullary disease includes infiltration of skin, lung, and, very rarely, cranial nerves or central nervous system.*

defined for each patient (Table 2). In patients who achieve genetic complete remission (gCR), JMML cells are considered to have been eradicated, irrespective of clinical remission status. Patients with gCR may have persistent splenomegaly or leukocytosis from causes other than JMML, such as infections. However, it is unlikely that such a patient has clinical signs of progressive disease of JMML in the presence of gCR. In such a patient, any new clonal abnormalities, other possible errors of genetic examinations, or other disorders which give rise to JMML-like clinical features, should be excluded.

Response criteria for clinical trials of HSCT

There is a consensus that criteria for remission after HSCT are somewhat different from those stated above for remission after non-HSCT. The remission criteria in HSCT recipients include the results of chimerism analyses (Table 3). Appraisal of methodological consideration of chimerism studies will change over time and is beyond the scope of this consensus document. Patients undergoing HSCT who achieve neutrophil engraftment and complete donor chimerism with disappearance of acquired cytogenetic and molecular abnormalities are considered to have a complete remission of JMML. In these individuals, complete remission is defined irrespective of the spleen size or WBC counts, since post HSCT, patients often have persistent splenomegaly and leukocytosis without active JMML due to infections, graft-*versus*-host disease or other hepatic complications. For the very few cases with mixed chimerism after HSCT and no diagnostic cytogenetic or molecular marker, definition of complete remission requires the resolution of all clinical features indicative of JMML (Table 3).

Table 2. Definition of response following therapy other than HSCT in JMML.

Clinical remission status: variable 1-6 in Table 1		Genetic remission status: variable 7-9 in Table 1	
Clinical complete remission (cCR)	Patient fulfills the criteria of CR of all applicable clinical variables 1-6 of Table 1. The response variables must be maintained for at least 4 weeks.	Genetic complete remission (gCR)	Defined if the patient shows a normal karyotype and absence of acquired mutations in *PTPN11*, *NF-1*, *NRAS*, *KRAS*, or *CBL*.
Clinical partial remission (cPR)	Defined if the patient does not fulfill the criteria of cCR, but vPR was achieved in at least one of clinical variables (1-6) and none of clinical variables showed vPD.	–	–
Clinical stable disease (cSD)	Defined if the patient does not fulfill the criteria of cCR and cPR, but none of the variables showed vPD.	Genetic stable disease (gSD)	Defined if the patient does not fulfill the criteria of gCR, but none of the genetic variables (7-9) showed vPD.
Clinical progressive disease (cPD)	Defined if any of the variables 1-6 showed vPD.	Genetic progressive disease (gPD)	Defined if any of the variables 7-9 showed vPD.
Clinical relapse (cRel)	Defined if any of the variables 1-6 showed vPD after the achievement of cCR or cPR.	Genetic relapse (gRel)	Reappearance of an abnormal karyotype and/or mutation of genes related with JMML if previously undetected, and/or (only for patients after HSCT) increase in recipient chimerism with at least 10% of autologous cells and >50% increase above the baseline.

**In patients with germ-line NF-1, PTPN11 or CBL mutation, the germ-line mutation remains even if patients achieved a genetic complete remission.*

Table 3. Definition of complete remission and relapse after hematopoietic stem cell transplantation in children with JMML.

Complete remission is defined in the presence of neutrophil engraftment and:
1. full donor chimerism of unsorted cells from PB or BM and
2. disappearance of acquired cytogenetic and molecular abnormalities in patients with such a previously identified abnormality

For patients without cytogenetic or acquired molecular abnormalities at diagnosis, who do not achieve full donor chimerism (as defined above), all of the following features of clinical remission must be achieved for definition of complete remission:
a. absence of splenomegaly on exam and imaging, if splenomegaly was present at diagnosis
b. absolute leukocyte count $< 15 \times 10^9$/L
c. blasts in BM of <5%,
d. myeloid/erythroid precursors including blasts in PB ≤1%

Relapse is defined if one of the following criteria is fulfilled:
1. clinical JMML features and mixed chimerism >5%
2. blasts in BM ≥5%, total blasts and myeloid/erythroid precursors in PB ≥5%*
3. cytogenetic relapse: if applicable, reappearance of clonal cytogenetic abnormality
4. molecular relapse: if applicable, reappearance of acquired genetic anomalies

*PB: peripheral blood; BM: bone marrow. *Exclude other causes of appearance of blasts and myeloid/erythroid precursors in PB such as the regenerating phase after engraftment, severe infections and effect of granulocyte-colony-stimulating factor.*

Conclusion

In this paper we propose response and relapse criteria for patients who are diagnosed and treated for JMML, recognizing the complexities of disease presentation and therapeutic interventions. The usefulness and suitability of these criteria need to be proven in prospective clinical trials. It is likely that the proposed response criteria will require modifications in the future based on the accumulated experiences and advances of molecular biology in JMML. Because the goal of therapy of children with JMML is a cure, it is also important to be aware that responses need to be translated into an increase in long-term survival in well-controlled clinical trials.

Authorship and Disclosures
Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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