The impact of category, cytopathology and cytogenetics on development and progression of clonal and malignant myeloid transformation in inherited bone marrow failure syndromes

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The impact of category, cytopathology and cytogenetics on development and progression of clonal and malignant myeloid transformation in inherited bone marrow failure syndromes

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Short running title: Myeloid transformation in inherited bone marrow failure syndromes

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ABSTRACT
Inherited bone marrow failure syndromes are a group of rare, heterogeneous genetic disorders with a risk of clonal and malignant myeloid transformation including clonal marrow cytogenetic abnormalities, myelodysplastic syndrome and acute myeloid leukemia. The clinical characteristics, risk classification, prognostic factors and outcome of inherited bone marrow failure syndromes-associated clonal and malignant myeloid transformation are largely unknown. The aims of this study were to determine the impact of category, cytopathology and cytogenetics, the three components of the Category Cytology Cytogenetics Classification of Pediatric myelodysplastic syndrome, on the outcome of inherited bone marrow failure syndromes-associated clonal and malignant myeloid transformation. We used data from the Canadian Inherited Marrow Failure Registry. Among 327 inherited bone marrow failure syndromes patients enrolled on the registry, the estimated risk of clonal and malignant myeloid transformation by the age of 18 years was 37%. The risk of clonal and malignant myeloid transformation varied by inherited bone marrow failure syndromes but was highest in Fanconi anemia and Shwachman-Diamond syndrome. Developing clonal and malignant myeloid transformation significantly impacted overall survival. Mortality varied based on cytopathological group. The largest group was refractory cytopenia. Clonal marrow cytogenetic abnormalities were identified in 87% of patients with clonal and malignant myeloid transformation and different cytogenetic groups had different impact on disease progression. We conclude that category, cytopathology and cytogenetics in cases of inherited bone marrow failure syndromes-associated clonal and malignant myeloid transformation have an important impact on outcome and that the classification of such cases should incorporate these factors.
INTRODUCTION

Inherited bone marrow failure syndromes (IBMFSs) are a group of rare genetic disorders with single or multi-lineage cytopenia resulting from impaired hematopoiesis and a variable degree of cancer predisposition. These disorders carry a risk of clonal and malignant myeloid transformation (CMMT), which includes isolated clonal marrow cytogenetic abnormalities (CMCA), myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).1-8 There are multiple reports of IBMFS cases with early CMMT changes that eventually progressed to either severe bone marrow failure, advanced MDS and/or AML.9,10

The precise definition of adult and pediatric de-novo CMMT is still in evolution, but it is widely accepted that it should be based on peripheral blood cell numbers and types, and on bone marrow blasts, cellularity, cytogenetics and presence of dysplasia. Unfortunately, there is no widely accepted definition of IBMFS-associated CMMT.11 For example, Hasle & Colleagues defined pediatric MDS based on peripheral blood counts, marrow morphologic dysplasia, CMCA and blasts.12 These are measurable criteria and potentially excellent tools to define MDS. However, the ability of these criteria to include all cases of IBMFS-associated MDS without falsely including cases without true transformation has never been tested.

Aside from challenges in defining IBMFS-associated CMMT, the classification and grading of this disorder presents another challenge, because some of the clinical and biological features that are used to characterize de-novo CMMT (e.g. prominent dysplasia and increased marrow cellularity) are less common in IBMFS-associated CMMT. In 2002 we developed the Category Cytology Cytogenetics (CCC) classification for pediatric MDS, which aimed to address aspects of not only de novo MDS, but also of therapy-related and IBMFS-associated MDS.11 In the present study we examined the three components of the CCC classification on a large cohort of patients with CMMT from one comprehensive population-based IBMFS registry and hypothesized that they have prognostic utility.

METHODS

The Canadian Inherited Marrow Failure Registry
The Canadian Inherited Marrow Failure Registry and its inclusion and exclusion criteria were published previously,\textsuperscript{13,14} and are summarized in the Supplementary Methods.

**Definition and diagnostic criteria**

CMMT was defined as having bone marrow failure and at least one of the following: 1) an isolated CMCA, 2) MDS or 3) AML.

CMCA was defined as the presence of at least two hematopoietic cells with the same cytogenetic abnormality detected by metaphase cytogenetics or the presence of positive cells by fluorescence in situ hybridization in a percentage that is higher than the reference value.

The diagnostic criteria of pediatric MDS proposed by Hasle and Colleagues were used. They include meeting at least two of the following criteria: 1) sustained unexplained cytopenia, 2) at least bilineage prominent morphologic myelodysplasia ($>$10\% of the cells in each lineage), 3) acquired clonal cytogenetic abnormality in hematopoietic cells, and 4) blasts of 5-29\%.\textsuperscript{12} Also, MDS cases should not have CMCA\textsuperscript{s} that are pathognomonic for AML, nor should they have very rapid leukemic blast cell growth as indicated in repeat bone marrow testing 2-3 weeks after diagnosis. Information on all cases is reviewed centrally, and the diagnosis of MDS may be modified based on these criteria. Clinical cases of MDS that do not meet all diagnostic criteria are carefully reviewed to determine if the existing diagnostic criteria need to be modified.

AML was defined as a documentation of $\geq$30\% myeloid leukemic blasts in the bone marrow or pathognomonic AML-type CMCA and very rapid leukemic blast cell growth as indicated in repeat bone marrow testing 2-3 weeks after diagnosis.

**Classification of cases with CMMT**

Grading or classification of CMMT was based on the CCC classification for pediatric MDS 2002.\textsuperscript{11} We made minor modifications to the system we published in 2002 (Table 1). First, we changed the term ‘cytology’ to ‘cytopathology’ to reflect changes found on bone marrow biopsy testing. Second, due to the natural progression of MDS to AML, we added the category of AML secondary to MDS or chronic bone marrow failure syndrome. Third, some degree of dysplasia is
a feature of the IBMFS bone marrow morphology and does not necessarily indicate malignancy,\(^1,^{15,16}\) while single lineage dysplasia is an inherent feature of certain inherited disorders. Therefore, we included only patients that had prominent dysplasia (>10% of cells) in each lineage and in at least two lineages to qualify for a diagnosis of refractory cytopenia with dysplasia (RCD). Fourth, emerging genomics data can be incorporated in the future to characterize and understand IBMFS-associated CMMT; therefore, we changed the heading ‘Cytogenetics’ to “Cytogenetics/Genetics”.

**Definition of disease progression**

For patients who underwent HSCT, the length of disease progression was calculated as the time from CMMT diagnosis to the day before the transplant preparatory therapy was started. There were no deaths that were unrelated to CMMT or transplant. HSCT was applied at the discretion of the treating physician. The indications for transplant included at least one of the following: severe cytopenia, progressive cytopenia approaching severely low counts and excess blasts.

Disease progression was defined as developing one or more of the following 3 criteria:

1. One of the following cytopathological changes:
   a) Refractory Cytopenia (RC) or Refractory Cytopenia with Ringed Sideroblasts (RCRS) to Refractory Cytopenia with Dysplasia (RCD) or Refractory Cytopenia with Excess Blasts (RCEB) or AML
   b) RCD to RCEB or AML
   c) RCEB to AML

2. One of the following cytogenetic changes: a) New cytogenetic abnormality in patients with normal cytogenetics at baseline, b) New cytogenetic abnormality in those with a single cytogenetic abnormality (such as del(20q) and trisomy 8, or i(7q) in Shwachman-Diamond Syndrome, SDS)

3. One of the following changes in the severity of bone marrow failure: a) Single-lineage severe cytopenia to bi-lineage or tri-lineage severe cytopenia, b) Bi-lineage-severe cytopenia to tri-lineage severe cytopenia. The various lineages were defined as severely reduced if i) the platelet count was less than \(20 \times 10^9/L\) or transfusion requirement, ii) the hemoglobin was less than 70g/L
or transfusion requirement and iii) the absolute neutrophil count was less than \(0.5 \times 10^9/L\) or granulocyte-colony stimulating factor therapy requirement.

**Data analysis**

Survival and risk were estimated by Kaplan-Meier analysis and Wilcoxon test was used to determine significant differences. P-values of <0.05 were considered statistically significant. Some analyses (e.g. risk of CMMT) were stopped at the age of 18 years, due to the possibility of referral bias of patients with CMMT who are older than 18 years and are not treated at pediatric centers. We did not include solid tumors in this study due to the small number of patients with this complication. We chose IBMFS categories that had more than 10 patients for our analyses. Also, we included only categories that had more than 3 patients with CMMT to assess the impact of category on progression and overall survival of patients with CMMT. This included the categories of FA, SDS and unclassified-IBMFS. We analyzed only cytopathological groups and cytogenetic groups with more than 3 patients to determine how cytopathology and cytogenetics affect progression and overall survival. The cytogenetic groups included were 1) complex cytogenetics (\(\geq 3\) cytogenetic abnormalities), 2) del(7), del(7q) and deletion or translocation at areas 7q32-34, 3) i(7q) and 4) normal cytogenetics (constitutional abnormalities without CMCAs were included). Statistical analyses were performed using XLSTAT and Microsoft Excel software.

**RESULTS**

**The IBMFSs have a High Risk of Developing CMMT**

As of August 31st, 2011, 327 patients were enrolled on the registry. Seven patients were excluded due to missing information for a total of 320 patients analyzed. The distribution of specific categories of IBMFSs is in Table 2. Forty-four patients met the criteria of MDS or had frank AML. An additional patient with the MDS/AML-predisposition syndrome (constitutional trisomy 8) had cytopenia and a hypercellular marrow without dysplasia, CMCA or excess blasts, and did not fit criteria for any other blood dyscrasia but MDS, and thus was also included. This gave a crude CMMT prevalence of 14.1% among IBMFS patients. Kaplan-Meier analysis showed a risk of 37% by the age of 18 years (Fig 1A). The peak incidence of CMMT was between the ages of 5-10 years (Supplementary Fig 1).
Forty-seven percent of patients were diagnosed with CMMT at the time of IBMFS diagnosis. The median age at diagnosis of IBMFS in those with CMMT was 61.5 months (range 0-376 months). The median age at diagnosis of CMMT was 104 months (range 8-756 months), compared to a median age of diagnosis of about 70 years in the general population of western countries. The incidence of MDS in the United States in persons aged 0-18 years is reported as 1 per 1,000,000 per year and the risk of developing AML before the age of 18 years is 8 per 1,000,000 per year.\textsuperscript{17-20} Given that the risk of developing CMMT in our study was 37% at the age of 18 years, the risk of CMMT (combined MDS and AML) during childhood among the IBMFSs is estimated to be 2284 fold higher than the risk in the general population.

Twenty-five of the patients with CMMT (55.6%) were males and 20 (44.4%) were females. Most patients (28/45, 62.2%) had physical malformations along with blood dyscrasia. Thirteen patients (28.9%) had physical anomalies involving three or more non-hematological systems (data not shown).

The 45 patients with CMMT were followed for a total of 394 person years with a median duration of 66 months per patient (1-423 months). Fifteen out of 45 patients (33.3%) progressed. Of the 30 patients who did not progress, 14 required HSCT due to significant cytopenia or advanced MDS at presentation with CMMT; the remaining 16 patients were followed for a total of 153 person years with a median duration of 75 months per patient (range 1-309 months). The predicted risk of progression within 10 years of diagnosis with CMMT was 60% (Fig 1B).

**Impact of CMMT on Survival**

Overall mortality among the 45 patients with CMMT was 33.3% (15/45), while overall mortality among the non-CMMT group was 5.8% (16/275). Kaplan-Meier analysis predicted 56% survival of the CMMT group compared to 92% in those without CMMT (\textit{p}=0.02) by 18 years of age (Fig 1C). The median survival after diagnosis of CMMT was 32 months (range 1-174 months). Causes of death in the CMMT group included: post-transplant complications in eight patients (four therapy related, two non-engraftment and two secondary graft failure), failure to achieve remission (1 patient), and CMMT-related complications (e.g. infections and bleeding
complications) (3 patients). The median age of death among patients with CMMT was 12.8 years (5.2-43.1 years) compared to 7.1 years (0.2-34 years) among those without CMMT.

The Impact of Category of IBMFS on CMMT Risk and Outcome

Among the whole group of patients on the registry, CMMT was most common in patients with FA (14 patients), followed by SDS (9 patients) and unclassified-IBMFSs (13 patients) (Table 2). Other categories included Kostmann/severe congenital neutropenia (K/SCN), dyskeratosis congenita, and congenital amegakaryocytic thrombocytopenia. There were two patients with constitutional mosaic trisomy 8, one with constitutional 4p- syndrome1 and one with constitutional supernumerary ring chromosome 1 among the patients with non-primary IBMFSs who had CMMT.

FA patients had the highest actuarial risk of 75% of developing CMMT by 18 years of age. This was followed by patients with dyskeratosis congenita, unclassified-IBMFS, K/SCN and SDS with about 25%, 24%, 24% and 20% risk by 18 years of age, respectively (Fig 1D-H). None of the patients with Diamond-Blackfan anemia (DBA) developed CMMT. When we compared the three categories with the largest numbers of patients, DBA, FA and SDS, the difference in risk of developing CMMT by 18 years of age was significant (p=0.005)(Fig 1I). This difference was still significant (p=0.02) when we compared DBA, FA, SDS, unclassified-IBMFS, dyskeratosis congenita and K/SCN (data not shown). The earliest age of onset of CMMT was 78 months in FA, 8 months in SDS, 201 months in dyskeratosis congenita, 57 months in K/SCN and 11 months in unclassified-IBMFS.

The risk of progression within 10 years of diagnosis with CMMT in FA, SDS, and unclassified-IBMFS was not significantly different between categories, and was estimated to be 41%, 72% and 43%, respectively (Fig 2A-E). The overall survival at 10 years from diagnosis with CMMT in FA, SDS and unclassified-IBMFS was also not significantly different between categories, and was estimated at 57%, 25%, and 91%, respectively (Fig 2F-J).

We did not find a correlation between IBMFS category and bone marrow failure severity, specific cytopathology, or cytogenetics at presentation with CMMT (data not shown).
**Cytopathology of IBMFS-Associated CMMT and Impact on Outcome**

The majority of patients with CMMT had RC (60%), followed by RCEB (18%), RCD (9%), leukemia (AML, 7% and B-precursor acute lymphoblastic leukemia, 2%) and RCRS (4%) (Table 3).

The risk of CMMT progression to more advanced CMMT tended to be higher in the RCEB group at 70% within 3 years of CMMT diagnosis (Fig 3A). The rate of progression of RC was 37% and 65% at 3 and 10 years from CMMT diagnosis, respectively (Fig 3B). Patients with RCRS and RCD had the lowest rates of progression (Fig 3C-D).

The overall survival after diagnosis of CMMT was significantly different between the various cytopathologic groups (Fig 3). Patients with RCEB and AML had the poorest overall survival (p<0.0001). Importantly, patients with RC did poorly with an overall survival of 37% at long-term follow-up of 10 years.

There was no correlation between cytopathology and bone marrow failure severity or specific cytogenetic abnormalities at presentation with CMMT (data not shown).

**Cytogenetics of IBMFS-Associated CMMT and Impact on Outcome**

CMCAs were present in 93% of the patients at presentation with CMMT (Table 4). One patient developed a CMCA at follow-up. Monosomy 7, complex cytogenetics and i(7q) were the most common CMCAs, and were seen in 36%, 21% and 11% of the patients with CMCAs, respectively. These abnormalities were analyzed for their prognostic values. Other cytogenetic groups were too small for statistical analysis.

Rates of progression between the cytogenetic groups were significantly different. Patients with monosomy 7 and complex cytogenetics had the highest risks of progression of 54% and 60% at 5 years from CMMT diagnosis, respectively (p=0.02; Fig 4A). There was no statistically significant difference in overall survival between the various cytogenetic groups (Fig 4B).
All patients with i(7q) were alive at last follow-up; however, this anomaly was associated with significant morbidity. Among the four patients with SDS and i(7q), one patient suffered from severe multilineage cytopenia and one from severe neutropenia at presentation. A third patient with i(7q) developed an additional CMCA consisting of trisomy 8, as well as severe neutropenia at follow-up. One of the two patients with del(20q) developed RCEB and subsequently AML 11 years after the first appearance of this CMCA in the bone marrow.

There was no correlation between cytogenetics and the severity of bone marrow failure or specific cytopathology at presentation with CMMT (data not shown).

**DISCUSSION**

The present study provides for the first time prognostic data related to the category, cytopathology and cytogenetics of IBMFS-associated CMMT on a large group of patients from one population-based registry. Our data showed an estimated 2284-fold higher risk of CMMT compared to the general population. Furthermore, the development of CMMT in patients with IBMFS signals a high likelihood of malignant progression and significantly increased mortality.

The risk of CMMT among the whole group of IBMFSs has never been studied. In our cohort of 320 patients, that included those diagnosed with the most common categories of IBMFSs, the estimated risk of CMMT by the age of 18 years was 37%. It is known that the cumulative risk of IBMFS-associated CMMT further increases in adulthood. However, similar to all other studies that have been conducted on IBMFSs, our registry may also suffer from referral bias of patients with CMMT who are older than 18 years of age and who are not treated at pediatric centers. Thus, we did not analyze certain factors (e.g. risk of CMMT) for patients older than 18 years of age.

Little population-based data is available about the differential risk of developing CMMT among patients with various categories of IBMFSs. Although the various IBMFSs share many clinical and morphological phenotypes, their respective IBMFS genes play roles in several different biochemical pathways. Therefore, it is reasonable to hypothesize that mutations in different IBMFS genes may have different impacts on the malignant potential and behavior of bone
marrow cells. Our data indicate a significantly different CMMT risk by the age of 18 years between DBA, FA and SDS. CMMT was most common among FA patients, who had a 75% risk by this time, which is much higher than that described in other studies,\(^5, 8, 22-24\) and is even higher than that reported in studies where patients with isolated clones where included.\(^22\) This may reflect our comprehensive registry inclusion criteria and meticulous collection of data on all bone marrow testing. SDS patients had a previously unpublished high risk of 30% of developing CMMT by the age of 18 years. This information provides the rationale for routine leukemia surveillance in children with FA and SDS.\(^25\) In contrast, no patient with DBA developed CMMT in our cohort, suggesting that cancer surveillance with annual bone marrow testing during childhood is not indicated in DBA. The need for surveillance for patients with DBA after the age of 18 years is yet unclear. The development of CMMT in children with K/SCN and dyskeratosis congenita is consistent with the literature and reinforces the need for surveillance in these populations.

Unclassified-IBMFSs manifest bone marrow failure and heterogeneous clinical and genetic characteristics. We found a substantially, and previously unreported, high risk of CMMT (24%) before 18 years of age in this population. This underlies the critical need to initiate cancer surveillance even when the precise syndrome and genetic group is unknown. Although the majority of patients with unclassified-IBMFS underwent extensive genetic testing that was negative, not all of them were tested for mutations in all known IBMFS genes. Therefore, some of the unclassified cases with CMMT in this group might have mutations in genes such as \(RUNX1\) and \(GATA2\). Importantly, patients who present with idiopathic MDS are often not comprehensively tested for mutations in IBMFSs. Hence, a proportion of the IBMFS patients who have neither a positive family history nor physical malformations and present with idiopathic MDS, would not be captured by our registry. The magnitude of this patient population is still to be determined.

Our results indicate for the first time that developing CMMT is progressive and significantly impacts survival, which indicates a major need to develop strategies for prevention and early detection. It is noteworthy that the high rate of progression was identified at a median follow-up of 66 months, and the prognosis may be even worse with longer follow-up. The impact of
underlying category of IBMFS on the outcome of CMMT is unknown. The results of our study indicate no differences in disease progression and survival between patients with the common IBMFSs after they develop CMMT. This might be due to small numbers of patients in each category. Alternatively, it may be that the long-term outcome is substantially affected regardless of the specific IBMFS category. Interestingly, the pattern of progression and mortality was different between FA and SDS patients (Fig 3). FA patients had initially high progression and mortality rates and then a plateau, whereas SDS patients had a persistent risk of progression and drop in survival over time. The early and fast drop in survival in FA patients is likely due to early intervention with HSCT due to concomitant signs of severe cytopenia. Indeed, 11 of 14 patients with FA and CMMT received HSCT and the majority survived. Only one patient with SDS and CMMT received HSCT. Therefore, the data likely reflects the natural history of SDS patients with CMMT, and although past studies suggested that progression of these patients from CMCAs to advanced MDS and AML might be slow,\textsuperscript{1, 26, 27} the data demonstrates that the risk of progression and mortality continue without a plateau from time of CMMT diagnosis. Although the number of SDS patients with CMMT is small, these results indicate a crucial need to further confirm these findings and to study whether early intervention with HSCT in certain SDS patients can improve outcome.

In our study, RC was the most common cytopathology in IBMFS-associated CMMT.\textsuperscript{12, 28} Patients with RC fared significantly better than those with RCEB with respect to progression and survival. The predicted risk of progression in those with RCEB was high early after diagnosis, in line with previous studies of MDS.\textsuperscript{29, 30} Surprisingly, the risk of progression in patients with RC persisted and continued to increase over time with no apparent plateau. Clearly, the impact of early intervention with HSCT in this group on overall survival should be studied.

In contrast to de novo MDS, where 30-50% of patients have a CMCA,\textsuperscript{31-33} CMCAs were seen in 93% of our patients with IBMFS-associated CMMT at the time of presentation. Based on the International Prognostic Scoring System for adult MDS, monosomy 7 and complex cytogenetics ($\geq$ 3 chromosomal abnormalities) are classified as poor prognostic features.\textsuperscript{34} Monosomy 7 occurred in one-third of our patients and was the most common CMCA, similar to children and adolescents with de novo MDS.\textsuperscript{7} The predicted risk of progression and estimated survival of
patients with monosomy 7 at 5 years post CMMT diagnosis were 54% and 85%, respectively. Over one third of the patients with monosomy 7 did not receive transplant and were alive at last follow-up. These results suggest a novel concept that HSCT may not be automatically necessary for all patients with monosomy 7, but only offered to those who have either significant or progressive cytopenia or excess blasts. Discovery of co-existing genetic alterations and definition of their prognostic significance will help refine the transplant indications in this group. Patients with complex cytogenetics had poor outcome in our study with a 60% predicted risk of progression as early as one year post CMMT diagnosis, and a 50% predicted overall survival at 5 years. This is similar to reports of poor outcome in patients with de novo MDS and complex cytogenetics.31, 34, 35

Isochromosome 7q and del(20q) can be seen for many years in marrows of stable SDS patients and can even become periodically undetectable.1, 36-38 Surprisingly, in the present study, three out of four patients with i(7q) and SDS had significant complications at presentation or at last follow-up. In addition, one of the two patients with del(20q) developed RCEB and AML 11 years after the first appearance of this CMCA. These data may suggest that newly appearing clones with i(7q) and del(20q) signal a slowly progressive bone marrow dyscrasia that eventually requires treatment. Larger cohorts with longer follow-up are needed to determine the true impact of these cytogenetic abnormalities on progression to more severe bone marrow failure and/or leukemia.

Two classifications of pediatric MDS have been proposed due to increasing awareness of the biological and clinical differences between pediatric and adult MDS. In addition to the 2002 CCC pediatric MDS classification, a World Health Organization (WHO) pediatric MDS classification was developed in 2003 and revised in 200812, 39-41. This classification focuses on MDS cytopathology and includes three classes: RC of childhood (RCC), RAEB (myeloblasts of 5-19%) and RAEB in transformation (RAEB-t, myeloblasts of 20-29%). RCRS was omitted from the pediatric WHO MDS classification due to its rarity in childhood. RCD was combined with RCC due to yet unclear differences from RCC. The pediatric WHO classification has several limitations. First, although 40% of children with MDS have an underlying IBMFS11, it is mainly based on experience from de-novo MDS and designed for this MDS category. The
application of this classification to IBMFSs has never been studied. Second, the omission of RCRS in the pediatric WHO classification did not take into account that despite its rarity, this cytopathology exists in children as evidenced by this series and other reports in the literature.\textsuperscript{42} Had we used the pediatric WHO MDS classification we could not have categorized both of our cases of RCRS. Third, the significance of RCD has not been systematically and thoroughly studied in IBMFSs. Although our numbers are small, there is a suggestion that this category carries a different risk of progression and survival than RC (Supplementary Figure 2 and 3). Hence, further studies are required before RCD is omitted as a separate entity from the pediatric MDS classifications. The pediatric CCC classification was designed to include all categories of MDS (de-novo, therapy-related and syndrome-associated). To our knowledge the present study tests for the first time the prognostic significance of a pediatric MDS classification in a large cohort of patients with IBMFS-associated MDS or CMMT.

One of the cases in the present series did not fit the MDS diagnostic criteria proposed by Hasle and Colleagues.\textsuperscript{12} However, the patient clearly had MDS, since he had an MDS/AML predisposition syndrome (constitutional trisomy 8), progressive cytopenia and a hypercellular marrow. He had no prominent dysplasia, no ringed sideroblasts, no excess blasts, no cytogenetic abnormalities and no indication of any other dietary, metabolic or infectious etiologies that could account for the blood dyscrasia. We propose to modify the diagnostic criteria of pediatric MDS (Supplementary Table 1) and to include cytopenia with hypercellular bone marrow that cannot be explained by causes other than MDS.

The data in the present study help define the impact of category, cytopathology and bone marrow cytogenetic abnormalities on the characteristics and prognosis of IBMFS-associated CMMT. These components of the CCC classification simplify the wide assortment of variables that constitute pediatric MDS and CMMT into one scheme, and hence can be used clinically to describe the disease at presentation and follow-up. Due to the rarity of the disorders, the numbers in certain categories, cytopathologies and cytogenetic groups were small; thus, enrolling further patients and longer follow-up will be important for replicating the data and defining additional cytogenetic and genetic variables as risk factors. Emerging genomics data can be incorporated and be used to further characterize and understand IBMFS-associated CMMT.
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AUTHORSHIP
Contribution: MC performed research, analyzed data, and wrote the manuscript. CIS performed research, analyzed data, and wrote the manuscript. RK is a study co-investigator, contributed vital data and edited the manuscript. CVF is a study co-investigator, contributed vital data and edited the manuscript. RY is a study co-investigator and contributed vital data. JW is a study co-investigator and contributed vital data. YP is a study co-investigator, contributed vital data and edited the manuscript. MS is a study co-investigator and contributed vital data. JHL is a study co-investigator and contributed vital data. BM is a study co-investigator and contributed vital data. SA is a study co-investigator and contributed vital data. MacS is a study co-investigator and contributed vital data. RS is a study co-investigator and contributed vital data. MB is a study co-investigator and contributed vital data. VB is a study co-investigator and contributed vital data. LJ is a study co-investigator and contributed vital data. LG is a study co-investigator and contributed vital data. LS analyzed data and edited manuscript. MarS analyzed data and edited the manuscript. PS analyzed data. BZ performed research and analyzed data. YD designed and supervised the research, analyzed data and wrote the paper.
REFERENCES


Table 1: The modified Category, Cytopathology, Cytogenetics Classification of Childhood MDS

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<td>2. RCRS</td>
<td>2. RCRS</td>
<td></td>
</tr>
<tr>
<td>3. RCD</td>
<td>3. RCD</td>
<td></td>
</tr>
<tr>
<td>4. RCEB</td>
<td>4. RCEB</td>
<td></td>
</tr>
<tr>
<td>5. AML secondary to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS or other chronic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bone marrow failure</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cytogenetics/Genetics</strong></td>
<td><strong>Cytogenetics</strong></td>
<td></td>
</tr>
<tr>
<td>1. Marrow cytogenetics/</td>
<td>1. Marrow cytogenetics</td>
<td></td>
</tr>
<tr>
<td>genetics group (to be</td>
<td>genetics abnormality (to be specified)</td>
<td></td>
</tr>
<tr>
<td>specified)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Normal marrow</td>
<td>2. Normal marrow</td>
<td></td>
</tr>
<tr>
<td>cytogenetics/ genetics</td>
<td>cytogenetics</td>
<td></td>
</tr>
<tr>
<td>group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Unknown marrow</td>
<td>3. Unknown marrow</td>
<td></td>
</tr>
<tr>
<td>cytogenetics/ genetics</td>
<td>cytogenetics</td>
<td></td>
</tr>
<tr>
<td>group</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. For a diagnosis of RC, IBMFS patients should have clear evidence of clonality (e.g. CMCA) in addition to cytopenia, but should not fulfill the diagnostic criteria for RCRS, RCD or ECEB as defined below. Cases with hypercellular/normocellular RC as defined in Supplementary Table 1 are also included.
2. RCRS is defined as having RC and \( \geq 15\% \) ringed sideroblasts in the bone marrow (around \( \geq 1/3 \) of the nuclear circumference). Patients should not fulfill the criteria for RCD and RCEB.
3. RCD in the modified system specifically requires having RC and prominent dysplasia (\( >10\% \) of cells) in at least two cell lineages, but no excess blasts
4. RCEB in both versions of the classification includes cases with 5–29% blasts, and can include in addition either RC, RCRS or RCD

Marrow cytogenetics/genetics abnormality refers to:
1. Acquired cytogenetics/genetics abnormality in the bone marrow that is not constitutional (e.g. constitutional +21 in Down syndrome, or +8 are not clonal marrow cytogenetic abnormalities)
2. The abnormality should appear in at least 2 cells

FISH analysis might be positive in cases where regular cytogenetics is negative. In such cases, the test type (FISH) has to be specified.

IBMFS, inherited bone marrow failure syndrome; FISH, fluorescent in situ hybridization; RC, Refractory single/multilineage cytopenia without obvious dysplasia; RCD, Refractory single/multilineage cytopenia with dysplasia; RCEB, Refractory single/multilineage cytopenia with excess blasts (5–30%); RCRS, Refractory single/multilineage cytopenia with ringed sideroblasts
Table 2: Categories of the IBMFS-associated clonal and malignant myeloid transformation cases

<table>
<thead>
<tr>
<th></th>
<th>Number of patients</th>
<th>Number of patients with CMMT</th>
<th>Median age at diagnosis of CMMT in months (range)</th>
<th>Median length of follow-up of patients with CMMT in months (range)</th>
<th>Median age of patients without CMMT at last follow-up in months (range)</th>
<th>Number of patients progressed to more advanced CMMT</th>
<th>Number of patients deceased</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBA</td>
<td>52</td>
<td>0 (0%)</td>
<td>NA</td>
<td>NA</td>
<td>118.5 (1-555)</td>
<td>NA</td>
<td>1 (No CMMT)</td>
</tr>
<tr>
<td>FA</td>
<td>41</td>
<td>14 (31%)</td>
<td>103 (78-489)</td>
<td>58 (8-127)</td>
<td>68 (4-219)</td>
<td>5</td>
<td>6 (CMMT)</td>
</tr>
<tr>
<td>SDS</td>
<td>40</td>
<td>9 (20%)</td>
<td>205 (8-503)</td>
<td>239 (59-407)</td>
<td>113 (11-618)</td>
<td>4</td>
<td>4 (CMMT)</td>
</tr>
<tr>
<td>K/SCN</td>
<td>13</td>
<td>3 (23%)</td>
<td>167 (57-756)</td>
<td>179 (65-423)</td>
<td>159 (15-372)</td>
<td>0</td>
<td>1 (CMMT)</td>
</tr>
<tr>
<td>DC</td>
<td>13</td>
<td>1 (8%)</td>
<td>201 (6-401)</td>
<td>32 (6-401)</td>
<td>64 (15-372)</td>
<td>1</td>
<td>3 (No CMMT)</td>
</tr>
<tr>
<td>CAMT</td>
<td>2</td>
<td>1 (50%)</td>
<td>80</td>
<td>98 (15-372)</td>
<td>20 (6-401)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>UC- IBMFS</td>
<td>85</td>
<td>13 (17%)</td>
<td>55 (11-200)</td>
<td>58 (11-174)</td>
<td>115 (1-662)</td>
<td>4</td>
<td>1 (CMMT)</td>
</tr>
<tr>
<td>Const +8*</td>
<td>3</td>
<td>2 (50%)</td>
<td>84.5 (45-124)</td>
<td>58 (1-662)</td>
<td>131 (1-662)</td>
<td>1</td>
<td>2 (CMMT)</td>
</tr>
</tbody>
</table>
Patients with non-primary IBMFSs have hypo-productive cytopenia; however this complication is not a major component of their syndrome and does not occur in the majority of patients. Therefore, patients with these disorders were not regularly enrolled on the Canadian Inherited Marrow Failure Registry, and epidemiological analysis could not be performed.

CAMT; congenital amegakaryocytic thrombocytopenia; CMMT, clonal and malignant myeloid transformation; Const, constitutional; DC, dyskeratosis congenita; DBA, Diamond-Blackfan anemia; FA, Fanconi anemia; K/SCN, Kostmann/severe congenital neutropenia; NA, not applicable; SDS, Shwachman-Diamond syndrome; UC-IBMFSs, unclassified inherited bone marrow failure syndromes.

<table>
<thead>
<tr>
<th>Const</th>
<th>1</th>
<th>1</th>
<th>55</th>
<th>78</th>
<th>0</th>
<th>0</th>
<th>1 (CMMT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4p-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Const</td>
<td>1</td>
<td>1</td>
<td>182</td>
<td>66</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>r(1)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other*</td>
<td>69</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>145</td>
<td>NA</td>
<td>1 (No CMMT)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2-690)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>320</td>
<td>45</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>15</td>
<td>15 (CMMT)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16 (No CMMT)</td>
</tr>
</tbody>
</table>

* Patients with non-primary IBMFSs have hypo-productive cytopenia; however this complication is not a major component of their syndrome and does not occur in the majority of patients. Therefore, patients with these disorders were not regularly enrolled on the Canadian Inherited Marrow Failure Registry, and epidemiological analysis could not be performed.
Table 3: Cytopathology of IBMFS-associated clonal and malignant myeloid transformation at presentation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of patients</th>
<th>Number progressed to more advanced CMMT (%)</th>
<th>Number of patients deceased (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory cytopenia</td>
<td>27</td>
<td>10 (37)</td>
<td>5 (19)</td>
</tr>
<tr>
<td>Refractory cytopenia with ringed sideroblasts</td>
<td>2</td>
<td>0 (0)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Refractory cytopenia with dysplasia</td>
<td>4</td>
<td>0 (0)</td>
<td>2 (50)</td>
</tr>
<tr>
<td>Refractory cytopenia with excess blasts</td>
<td>8</td>
<td>4 (50)</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>AML</td>
<td>3</td>
<td>1 (33)</td>
<td>2 (67)</td>
</tr>
<tr>
<td>ALL</td>
<td>1</td>
<td>NA</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>15 (33)</td>
<td>15 (33)</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CMMT, clonal and malignant myeloid transformation
Table 4: Cytogenetics of marrow samples at diagnosis of IBMFS-associated clonal and malignant myeloid transformation

<table>
<thead>
<tr>
<th></th>
<th>Number of patients</th>
<th>Number progressed to more advanced CMMT</th>
<th>Number of Patients Deceased</th>
</tr>
</thead>
<tbody>
<tr>
<td>-7</td>
<td>11</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Complex (≥ 3 cytogenetic abnormalities)</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>i(7)(q10)</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>-7, del(6)(q21)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-7 and del(7)(q22)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>-7, inv(2)(p11.2;q13)c</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>t(7;21)(q34;q22)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>t(14;16)(q11.1;p13.2)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>t(3;12)(q26.2;p13)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>del(2)(q33)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>del(16)(q22)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>del(20)(q11.2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>del(20)(q13.2)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>der(15)t(1;15)(q12;q11)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>der(4)t(4;8)(p16.3;p23.1)c</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>+i(l)(q10),+der(8)t(3;8)(q21;q23)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>der(18)t(18 ;21)(p11.2;q22),</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>der(5)t(2;5)(p13;p15)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>del(21)(q22) or r(21)(p11.1;q22),</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>+der(21)i(21)(q10) or del(21)(q22;q22)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>+8c, +8, t(3;21)(q21;q22)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>+X</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+X, +8</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-X and -21</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Count</td>
<td>NA</td>
<td>Total</td>
</tr>
<tr>
<td>----------------</td>
<td>-------</td>
<td>----</td>
<td>-------</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td>No clonal marrow cytogenetic abnormalities</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

CMMT, clonal and malignant myeloid transformation; NA, not applicable
FIGURE LEGENDS

Figure 1: Characteristics and Actuarial risk of clonal or malignant myeloid transformation (CMMT) among patients with IBMFSs. A. Actuarial risk of CMMT was estimated by Kaplan-Meier analysis. Censoring was performed at last follow-up or at the time of hematopoietic stem cell transplantation for patients who had undergone such a procedure. The figure represents risk with 95% confidence intervals. B. Risk of progression to more advanced transformational stage among patients with CMMT. The figure represents risk with 95% confidence intervals. C. Overall survival analysis of patients with and without CMMT until the age of 18 years. D-I. Actuarial risk of clonal or malignant myeloid transformation (CMMT) among the common categories of inherited bone marrow failure syndromes: Fanconi anemia (FA), Shwachman-Diamond syndrome (SDS), Dyskeratosis congenital (DC), Kostmann/severe congenital neutropenia (K/SCN), Unclassified-IBMFS (UC-IBMFS), Comparison between FA, SDS and DBA. The figures represent risk with 95% confidence intervals.

Figure 2: Actuarial risk of progression and survival of clonal or malignant myeloid transformation (CMMT) among the various categories of inherited bone marrow failure syndromes. A. Fanconi anemia (FA), B. Shwachman-Diamond syndrome (SDS), C. Kostmann/severe congenital neutropenia (K/SCN), D. Unclassified-IBMFS (UC-IBMFS). E. Comparative analysis between FA, SDS and UC-IBMFS. The figures represent risk with 95% confidence intervals. F. Fanconi anemia (FA), G. Shwachman-Diamond syndrome (SDS), C. Kostmann/severe congenital neutropenia (K/SCN), H. Unclassified-IBMFS (UC-IBMFS), I. Comparative analysis between FA, SDS, UC-IBMFS all groups with more than 3 patients with CMMT, J. Comparative analysis of three categories (without K/SCN). The figures represent Risk with 95% confidence intervals.

Figure 3 Impact of cytopathology on outcome of patients with clonal or malignant myeloid transformation (CMMT). Actuarial risk of progression after CMMT diagnosis among
the various classes of cytopathology at presentation: **A.** Refractory cytopenia (RC), **B.** Refractory cytopenia with excess blasts (RCEB), **C.** Refractory cytopenia with ringed sideroblasts (RCRS), **D.** Refractory cytopenia with dysplasia (RCD).

Comparative analysis between groups was not significant (p=0.18). Overall survival after CMMT diagnosis among the various classes of cytopathology at presentation: **E.** Refractory cytopenia (RC), **F.** Refractory cytopenia with excess blasts (RCEB), **G.** Refractory cytopenia with ringed sideroblasts (RCRS), **H.** Refractory cytopenia with dysplasia (RCD), **I.** Acute myeloid leukemia (AML). Comparative analysis showed significance (p<0.0001).

**Figure 4** Impact of cytogenetics on outcome of patients with clonal or malignant myeloid transformation (CMMT). **A.** Actuarial risk of progression of CMMT; **B.** Overall survival of patients with CMMT
Figure 1 (A-C)
Figure 1 D-I (Cont’d)
Probability of no Progression

A. RC
B. RCEB
C. RCRS
D. RCD

Time after diagnosis of CMMT (months)

P=0.18

Probability of Survival

E. RC
F. RCEB
G. RCRS
H. RCD
I. AML

P<0.001

Figure 3
Supplementary Methods

The impact of category, cytopathology and cytogenetics on development and progression of clonal and malignant myeloid transformation in inherited bone marrow failure syndromes

Michaela Cada, Catherin I. Segbefia, Robert Klaassen, Conrad V Fernandez, Rochelle A Yanofsky, John Wu, Yves Pastore, Mariana Silva, Jeffrey H Lipton, Jossee Brossard, Bruno Michon, Sharon Abish, MacGregor Steele, Roona Sinha, Mark Belltrutti, Vicky Breakey, Lawrence Jardine, Lisa Goodyear, Lillian Sung, Mary Shago, Joseph Bayene, Preeti Sharma, Bozana Zlateska, Yigal Dror

The Canadian Inherited Marrow Failure Registry and Inclusion and Exclusion Criteria
The Canadian Inherited Marrow Failure Registry is a multicenter collaborative study, established in 2001, which enrolls all consecutive patients with IBMFSs in Canada. The registry was approved by the Research Ethics Boards of all participating institutions, and includes all 16 pediatric tertiary care centers in Canada and one adult center. We estimate that these centers care for >95% of the eligible pediatric IBMFS population in Canada. This registry is population-based as >90% of the patients in this study are from centers that enroll >80% of the patients at their institutions.

Patients that fulfill specific diagnostic criteria for an IBMFS are recruited by hematologists at each centre. Briefly, patients are considered to have an IBMFS and are enrolled in the registry if they have chronic bone marrow failure in addition to having either a first-degree relative(s) with an IBMFS or associated physical malformations or are less than 1 year of age at presentation or have positive genetic testing. The diagnosis of the specific IBMFS is then established by the site co-investigator, and reviewed and modified at the central registry office at the Hospital for Sick Children, Toronto, according to previously described diagnostic guidelines based on published data. Patients are considered unclassified-IBMFSs if they do not fit the clinical, laboratory and genetic diagnostic criteria of known IBMFSs. The majority of these patients undergo extensive genetic testing, which was negative. Six of the 13 unclassified cases included herein were also tested by a next generation panel of 72 known IBMFS genes (including \textit{RUNX1} and \textit{GATA2}),
which were negative. Genetic testing is performed at the discretion of the referring physician. Patients registered between January 1, 2001 and August 31, 2011 were included in the current study.

Most patients on the registry have primary IBMFSs, i.e. inherited disorders that have hypoprolificytopenia as a major component, such as Fanconi anemia (FA). A small proportion of patients on the registry have inherited disorders, which typically do not have bone marrow failure as a component of their syndrome (non-primary IBMFSs), but have either bone marrow failure or CMMT at the time of their enrollment on the registry.

Patient information is collected on standardized forms and includes demographics, diagnosis, symptoms, family history, physical malformations, laboratory and genetic tests, imaging studies, treatment and outcomes. Actual reports stripped of identifiers are often included. Outcomes recorded include severe cytopenia(s), severe aplastic anemia, MDS, CMCAs, leukemia, solid tumors, hematopoietic stem cell transplantation (HSCT) and death. Follow-up information is collected on an annual basis. Patient information from all centers are reviewed at the central registry office for consistent and uniform definitions, eligibility and diagnoses. When necessary, information is clarified with the site research team and curated information is entered into a database at the central registry office.

**Exclusion Criteria**

Patients with the following groups of disorders are excluded from the registry: 1) acquired aplastic anemia, i.e. cases which do not fit the diagnostic criteria of an IBMFS, 2) de novo MDS or therapy related MDS, i.e. cases which do not have an underlying condition that fits the diagnostic criteria of an IBMFS, and 3) de novo leukemia or therapy related leukemia that is not associated with known IBMFSs or an antecedent bone marrow failure phase.

Supplementary Table

The impact of category, cytopathology and cytogenetics on development and progression of clonal and malignant myeloid transformation in inherited bone marrow failure syndromes

Michaela Cada, Catherin I. Segbefia, Robert Klaassen, Conrad V Fernandez, Rochelle A Yanofsky, John Wu, Yves Pastore, Mariana Silva, Jeffrey H Lipton, Jossee Brossard, Bruno Michon, Sharon Abish, MacGregor Steele, Roona Sinha, Mark Belltrutti, Vicky Breakey, Lawrence Jardine, Lisa Goodyear, Lillian Sung, Mary Shago, Joseph Bayene, Preeti Sharma, Bozana Zlateska, Yigal Dror
Supplementary Table 1: Diagnostic criteria for myelodysplastic syndrome

The patient should fulfill both criteria:

I. The patient has evidence of MDS by having either:
   a. Two of the following features:
      • Cytopenia. Levels of no more than one lineage may be concomitantly increased (e.g. combined anemia and thrombocytosis or combined anemia, thrombocytopenia and leukocytosis)
      • Dysplasia that fulfils the following criteria
         1. Prominent dysplasia in each affected lineage (>10% of the cells in the affected lineage)
         2. At least two lineages that manifest prominent dysplasia
      • Cytogenetic abnormality
         1. Non-constitutional, acquired clonal marrow cytogenetic abnormality that can be detected in at least 2 cells.
      • Blasts
         1. 5-29% in the bone marrow or peripheral blood
   Or
   b. Unexplained refractory cytopenia with marrow that is persistently cellular or hypercellular, when all other possible causes for such a combination were excluded including peripheral destruction of blood cells or hypersplenism. Supportive diagnostic features include an underlying MDS-predisposition syndrome, high fetal hemoglobin, high red blood cell mean corpuscular volume, patchy distribution of clusters (≥10 cells) of erythroid precursors in the bone marrow with increased numbers of proerythroblasts and increased numbers of mitoses.

II. The patient does not have signs of AML as evident by:
   • No classical AML cytogentetics: t(15;17)(PML/RARA), t(8;21)(Runx1;ETO), inv(16)(CBFB1/MYH11),t(9;11)(MLL/AF9)
   Doubling time in repeat BM in 2-3 weeks characteristic of MDS and not AML
Supplementary Figures

The impact of category, cytopathology and cytogenetics on development and progression of clonal and malignant myeloid transformation in inherited bone marrow failure syndromes

Michaela Cada, Catherin I. Segbefia, Robert Klaassen, Conrad V Fernandez, Rochelle A Yanofsky, John Wu, Yves Pastore, Mariana Silva, Jeffrey H Lipton, Jossee Brossard, Bruno Michon, Sharon Abish, MacGregor Steele, Roona Sinha, Mark Belltrutti, Vicky Breakey, Lawrence Jardine, Lisa Goodyear, Lillian Sung, Mary Shago, Joseph Bayene, Preeti Sharma, Bozana Zlateska, Yigal Dror

Supplementary Figure 1
Age at Presentation with Clonal and malignant myeloid transformation
Supplementary Figure 2
Differences in risk of CMMT progression based on differences in the CCC and WHO classifications of childhood MDS

*The Category, Cytopatlogy, Cytogenetics (CCC) Classification of Childhood MDS: Refractory Cytopenia (RC, myeloblasts < 5%), Refractory Cytopenia with Dysplasia (RCD, myeloblasts < 5%), Refractory Cytopenia with Ringed Sideroblasts (RCRS, myeloblasts < 5%) and Refractory Cytopenia with Excess Blasts (RCEB, myeloblasts of 5-29%).

**The World Health Organization (WHO) classification of pediatric MDS: Refractory Cytopenia of childhood (RCC, myeloblasts < 5%, this includes RC and RCD), Refractory Anemia with Excess Blasts (RAEB, 5-19% myeloblasts) and RAEB in transformation (RAEB-t, 20-29% myeloblasts). We combined RAEB and RAEB-t in our analysis due to relatively small numbers of patients in each category.
Supplementary Figure 3
Differences in overall survival after CMMT diagnosis based on differences in the CCC and WHO classifications of childhood MDS

*The Category, Cytopathology, Cytogenetics (CCC) Classification of Childhood MDS: Refractory Cytopenia (RC, myeloblasts < 5%), Refractory Cytopenia with Dysplasia (RCD, myeloblasts < 5%), Refractory Cytopenia with Ringed Sideroblasts (RCRS, myeloblasts < 5%) and Refractory Cytopenia with Excess Blasts (RCEB, myeloblasts of 5-29%).

**The World Health Organization (WHO) classification of pediatric MDS: Refractory Cytopenia of childhood (RCC, myeloblasts < 5%, this includes RC and RCD), Refractory Anemia with Excess Blasts (RAEB, 5-19% myeloblasts) and RAEB in transformation (RAEB-t, 20-29% myeloblasts). We combined RAEB and RAEB-t in our analysis due to relatively small numbers of patients in each category.