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Molecular Assessment of Pre-Transplant Chemotherapy in the Treatment of Juvenile Myelomonocytic Leukemia

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Abstract

Background: Despite the intensity of hematopoietic stem cell transplantation (HCT), relapse remains the most common cause of death in juvenile myelomonocytic leukemia (JMML). In contrast to other leukemias where therapy is used to reduce leukemic burden prior to transplant, many patients with JMML proceed directly to HCT with active disease. The objective of this study was to elucidate whether pre-HCT therapy has an effect on the molecular burden of disease and how this affects outcome post-HCT.

Procedure: Twenty-one patients with JMML who received pre-HCT therapy and were transplanted at UCSF were analyzed in this study. The mutant allele frequency of the driver mutation was assessed before and after pre-HCT therapy, using custom amplicon next-generation sequencing.

Results: Of the 21 patients, seven patients (33%) responded to therapy with a significant reduction in their mutant allele frequency and were classified as molecular responders. Six of these patients received moderate-intensity chemotherapy, one patient received only azacitidine. The 5-year progression-free survival after HCT of molecular responders was 100% vs. 61% for non-responders (p=0.12). Survival of molecular non-responders was not improved by use of high-intensity conditioning, but patients were salvaged if they experienced severe graft versus host disease. There were no baseline clinical characteristics that were associated with response to pre-HCT therapy.

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Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest statement: CCD declares consultancy with Jazz Pharmaceuticals. The other authors declare no conflict of interest.

Conclusions: Despite the myelodysplastic nature of JMML, patients treated with pre-HCT therapy can achieve molecular remissions. These patients experienced a trend towards improved outcomes post-HCT. Importantly, molecular testing can be helpful to distinguish between responders and non-responders and should become an integral part of clinical care.

Keywords

JMML; chemotherapy; molecular response; stem cell transplantation; outcome

Introduction:

Hematopoietic stem cell transplantation (HCT) is frequently used to treat both pediatric and adult high-risk myeloid malignancies[1–3]. In general, a low disease burden going into HCT is associated with superior long-term outcomes while transplanting patients with active disease is associated with poor survival[4–6].

Juvenile myelomonocytic leukemia (JMML) is a high-risk myelodysplastic / myeloproliferative overlap condition that affects young children. Patients usually present with clinical features both of peripheral myeloproliferation, including monocytosis and splenomegaly, as well as myelodysplasia[7–9]. Most patients with JMML will receive HCT yielding long-term survival in about 50% of patients[10,11]. The most common cause of death is relapse or transformation to acute myeloid leukemia (AML)[10–15]. While HCT is considered the standard of care for JMML, pre-HCT therapy is not routinely administered and the choice of therapy, when delivered, is largely at the discretion of the treating physicians. This is in contrast to AML, where all patients who eventually receive HCT are treated with intensive pre-HCT therapy with a goal of achieving a minimal residual disease (MRD) state prior to transplant. JMML is similar, however, to pure myelodysplastic syndromes that are frequently treated without pre-HCT therapy[16]. JMML has traditionally been suspected as being insensitive to traditional AML-type chemotherapy[17]. In a large transplantation study, where pre-HCT treatment was at the discretion of the providers, no difference in outcome was observed between patients who received AML-like chemotherapy and patients who received low-dose chemotherapy or no treatment prior to HCT[10]. The major limitations to that analysis include a possible selection bias by only treating patients using pre-HCT therapy for those with more aggressive disease and because a subset of these patients had already transformed to AML. In contrast, a benefit to pre-HCT treatment was observed in the largest cord-blood analysis conducted in JMML[18].

At the University of California San Francisco (UCSF) Benioff Children's Hospital, the institutional bias has been to treat JMML patients with moderately-intense, well tolerated myeloid-based chemotherapy to try and reduce disease burden prior to transplant. Data from a Children's Oncology Group transplantation study showed that pre-HCT chemotherapy (performed at the discretion of the provider) did yield molecular remissions, however, the number of patients was small and the study not powered to address this question[19].

Historically, monitoring disease burden in patients with JMML has been challenging as many patients present without an overt blast population by morphology or flow cytometry. Molecular monitoring of disease in AML has been shown to be superior to morphology and

flow cytometry in certain subsets including acute promyelocytic leukemia, *NPM1*-mutated AML, among other[20,21]. With the appreciation that 90–95% of patients have recurrent mutations in the Ras pathway, JMML would be ideal to study MRD using a molecular approach[22–24].

Here, we present a single-center study of 21 patients with JMML undergoing HCT in which we assessed their response to pre-HCT therapy using mutational monitoring. We asked the question whether pre-HCT chemotherapy is effective in reducing disease burden in JMML and whether this translates into improved outcome after HCT.

Methods:

Patients

From 1990 to 2018, 24 consecutive patients with a confirmed diagnosis of non-Noonan syndrome-associated JMML were transplanted at UCSF Benioff Children's Hospital and 21 of these patients had complete sample sets available to be retrospectively analyzed in this study. After transplant, all patients were routinely monitored for recurrence of disease via bone marrow analysis every three months for two years. Clinically validated, next-generation sequencing was additionally used for monitoring at those timepoints in the period since it became available. Acute graft versus host disease (GvHD) and chronic GvHD were diagnosed and graded according to standard criteria[25,26]. Patient specimens used for this study were all collected as part of the clinical care of the patients. The study was designed in accordance with the Declaration of Helsinki and reviewed and approved by the institutional review board of UCSF with a waiver of consent.

DNA extraction from patient samples

The mutational burden of the patients was retrospectively analyzed at the time of initial diagnosis and post-therapy at the last available timepoint before HCT (pre-HCT timepoint). If mononuclear cell DNA samples from routine clinical genetic analysis were not available for a patient, DNA was extracted from frozen cryopreserved mononuclear cells or 10μm slides of paraffin embedded bone marrow blocks. DNeasy blood and tissue kits were used or sections were scraped from slides, paraffin removed and DNA extracted using the AllPrep FFPE kit (Qiagen). Quantity and integrity of the extracted DNA was measured using Qubit (Thermo Fisher). Whenever possible, bone marrow samples were used for the pre-HCT assessment.

Next-generation sequencing

DNA samples were sequenced using a custom amplicon-based targeted sequencing approach. Libraries were prepared (Paragon Genomics) with a custom amplicon panel targeting 25 genes that are recurrently mutated in JMML (Supporting Information Table S1). The quality of the libraries was assessed on a Bioanalyzer (Agilent). Samples were then sequenced on a MiSeq platform (Illumina) at 760X mean coverage and a minimum mutant allele fraction (MAF) of 0.05 at diagnosis was required for reporting. Molecular remission was defined by a reduction of MAF of the driver mutation to <5% at the pre-HCT timepoint.

Statistical analysis

Median follow-up of this study was 4.07 years (range 0.75–23.76 years). Progression-free survival (PFS) was defined as time from transplantation to disease progression after HCT by a clinical or genetic diagnosis of JMML recurrence per the international working group definitions[27]. Overall survival (OS) was defined as time from initial diagnosis to death from any cause. Both were estimated using the Kaplan-Meier method. The significance level was set to p=0.05. Survival estimates were compared using the log-rank test. Statistically significant differences between molecular responders and non-responders to therapy were tested using Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. All calculations were performed using GraphPad Prism software (v8.0) and R (v3.4.1).

Results:

Patient characteristics

Clinical features of the 21 patients with full molecular datasets at diagnosis are summarized in Table 1. Of the 21 patients, 17 were male; the median age at diagnosis was 1 year, with a range from 4.6 months to 7.6 years. Of these, the majority (16/21 patients, 76%) received at least one course of a moderately-intense myeloid-based chemotherapy (i.e., $2g/m^2$ of cytarabine alone or in combination with fludarabine, $30mg/m^2$, or equivalent regimens). The other 5 patients were treated with either 6-mercaptopurine (6-MP) or azacitidine alone prior to HCT[28]. Splenectomy was performed in 5/21 (24%) patients prior to HCT.

Nineteen patients had mutations in canonical Ras-pathway genes. For the two remaining patients (UP2964 and UPN3065) no driver mutation was initially detected at the time of their clinical diagnosis using routine testing for JMML. However, both patients met the international criteria for JMML including hypersensitivity to the cytokine granulocyte macrophage - colony stimulating factor. Comprehensive molecular analyses at the time of this study including DNA and RNA-sequencing revealed these patients both harbored *FLT3* alterations (UPN2964 had a *FLT3* fusion and UPN3065 had a *FLT3* D835H mutation).

All patients received an allogeneic HCT (Supporting Information Table S2). Conditioning regimens varied in intensity and included between 1 and 3 alkylating agents. Total body irradiation (TBI) with 12 Gy was used as part of conditioning in 4/21 (19%) patients. An HLA-identical family donor was available in 7/21 (33%) cases, while 12/24 (57%) patients received a transplant from a matched unrelated donor and 2/21 (10%) were transplanted from a haploidentical donor. Five-year OS and PFS of the whole cohort were 77% (95% CI, 49%−91%) and 72% (95% CI, 44%−88%), respectively. Relapse after HCT occurred in 5/21 (28%; 95%CI, 4%−60%) patients at 1.9 – 31.8 (median 2.6) months post-HCT. Four of 21 (19%) patients received a second HCT, two from the same donor used during first HCT. Of these four patients, two died of progression, one died of treatment-related mortality (TRM). Only one patient remains alive in second remission.

Treatment-related mortality, occurrence of GvHD and veno-occlusive disease

Overall, therapy was well tolerated in our cohort. No fatal complications occurred from therapy prior to HCT. Veno-occlusive disease (VOD) occurred in 7/21 (33%) patients post-transplant, six of them grade 3–4[29]. There was no difference in the incidence of VOD depending on the type of pre-HCT therapy. Acute GvHD grade ≥2 occurred in 10/21 (48%) patients, 5/21 (24%) patients suffered from extensive chronic GvHD with only one patient developing cGvHD without prior aGvHD. However, one patient with chronic GvHD died after more than 5 years due to pneumococcal sepsis leading to a TRM of 5% of patients.

Response to therapy

Full sequencing datasets of the mutational burden both at diagnosis and at the last post-therapy/pre-HCT timepoint are shown in Table 2 (and Supporting Information Table S3). Of the 21 patients, one third (7/21) reached a reduction of MAF to below 5% at the pre-HCT timepoint (“molecular responders”) (Supporting information Figure S1). Moderately-intense chemotherapy yielded a molecular remission in 6/16 (38%) vs. 0/4 (0%) for 6-MP alone vs. 1/1 (100%) for azacitidine alone. Patient UPN2964, who was found to have a novel fusion involving *FLT3* (manuscript describing this case in press[30]), showed a response to therapy only after the addition of the FLT3-inhibitor sorafenib to chemotherapy.

Five-year PFS of molecular responders to pre-HCT therapy was 100% (95% CI, 100%). In contrast, molecular non-responders showed a 5-year PFS of 61% (95% CI, 30–82%) with 5/14 (36%) patients having a relapse (p=0.12; Figure 1). OS of molecular responders was also superior to the OS of non-responders (Supporting Information Figure S2).

Of note, the type of pre-HCT therapy itself (moderately-intense chemotherapy vs. low dose chemotherapy including azacitidine) had no effect on survival (p=0.42; Supporting Information Figure S3).

Comparison between molecular responders and non-responders to therapy

We next compared molecular responders and non-responders to evaluate potential factors influencing response. As previously described[23], the number of mutations at diagnosis impacted PFS and OS in our cohort (Supporting Information Figure S4). However, there was no difference in the number of mutations between molecular responders and non-responders. Furthermore, there were no statistically significant differences between the groups regarding baseline clinical characteristics including age, white blood cell count, platelet count and age-appropriate fetal hemoglobin levels at diagnosis (Table 3).

Prognostic impact of graft-versus-host disease in JMML

Equal percentages of molecular responders and non-responders developed significant GvHD (57% in both groups). While there was no impact on survival depending on the intensity of the conditioning regimen used for HCT or whether or not TBI was added, the presence of acute GvHD grade ≥2 and/or extensive chronic GvHD, had an impact on progression-free survival of the whole cohort. None of the patients who had aGvHD grade 2 or higher suffered a relapse, and only one patient who developed extensive cGvHD without prior aGvHD relapsed. Consequently, 5-year PFS for this group was 88% (95% CI, 39–98%),

compared to 53% (95% CI, 18–78%) for patients with no GvHD, aGvHD grade 1 or limited cGvHD (p=0.07; Figure 2A). Five-year OS was also significantly improved for patients developing aGvHD grade ≥2 and/or extensive cGvHD (p=0.02; Supporting Information Figure S5A). Even after 10 years, OS remained superior for patients with severe GvHD despite the fact that one patient died as a result of chronic immunosuppression (p=0.06; Supporting Information Figure S5B). Amongst molecular non-responders to pre-HCT therapy, 5-year PFS was 83% (7/8; 95%-CI, 27–97%) for those who developed GvHD, versus 33% (2/6; 95%-CI, 5–68%) for those who did not develop GvHD (p=0.046, Figure 2B).

Discussion:

In our cohort, we identified that approximately 40% of JMML patients responded to moderate-intensity pre-transplant therapy and achieved a molecular remission prior to HCT. The pre-HCT therapy was well tolerated and responding patients had an excellent outcome with 100% PFS after HCT. In contrast, patients who did not achieve a molecular response to pre-HCT therapy had poorer outcomes. Outcomes for the molecular non-responders were, however, significantly improved patients experienced acute (grade 2 or higher) and/or extensive chronic GvHD.

To better understand the factors that influence the likelihood of responding to pre-HCT therapy, we compared molecular responders and non-responders. Neither group differed in their baseline clinical characteristics. There were no differences in the number of secondary mutations, the type of driver mutation or cytogenetic abnormalities. Molecular Responders tended to be younger than non-responders, with age being an acknowledged risk factor for JMML. In summary, we were unable to identify a factor that could predict molecular response to pre-HCT therapy.

Similar to previous studies, we show that pre-HCT therapy does not yield improvements for every JMML patient[10,31–35]. However, by assessing for molecular responses, we were able to identify a subset of patients who experienced molecular remissions and went on to have an excellent PFS post HCT. We have thus demonstrated that the general dogma usually reserved for other malignancies[36,37] can also be applied to JMML: entering HCT in a state of molecular remission is associated with improved outcomes. Our hypothesis is that there is more time for the development of a graft-versus-leukemia effect for patients who enter HCT with a molecular response and low disease burden. Attempts to reduce the intensity of HCT conditioning for patients who have achieved a molecular remission may be warranted, as very intensive regimens may not be required to de-bulk disease in this setting.

Furthermore, our data indicate that GvHD can salvage patients with JMML who enter HCT with overt disease. This is in accordance with other studies demonstrating that chronic GvHD can protect JMML patients from relapse, and that donor lymphocyte infusions for patients with JMML were mostly effective when they led to the development of GvHD[14,38]. Even though no direct correlation between the occurrence of GvHD and improved survival was identified in other large studies[10,11], it has been suggested that reduced GvHD prophylaxis could be beneficial, especially in the setting of a second allogeneic

transplantation[10]. According to our data, patients with active disease at the time of transplant may also benefit from strategies to augment the graft-versus-leukemia effect during first transplantation.

In general, this study is limited by the small number of patients and thus many of these findings include trends towards, but do not reach, statistical significance. It is possible that molecular response to pre-HCT therapy is simply a biomarker of patients who would have done well after HCT regardless of disease remission. Larger, prospective trials of pre-HCT therapy are therefore warranted.

In summary, attempting to reduce the disease burden to a state of molecular remission before HCT is a reasonable strategy for newly-diagnosed JMML patients while evaluating patients for HCT. Moderately-intense myeloid-based chemotherapy was well tolerated, induced molecular remissions in about one third of patients in our study, and was associated with excellent outcomes. In addition, novel approaches to pre-HCT therapy like MEK-inhibitors and/or azacitidine should be further investigated in clinical trials to increase the number of patients going into HCT without active disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations key:

6-MP	6-Mercaptopurine
AML	Acute myeloid leukemia
GvHD	Graft versus host disease
HCT	Hematopoietic stem cell transplantation
JMML	Juvenile myelomonocytic leukemia
MAF	Mutant allele frequency
MRD	Minimal residual disease
OS	Overall survival
PFS	Progression-free survival
TBI	Total body irradiation

TRM	Treatment related mortality
VOD	Veno-occlusive disease

References:

1. Blume KG, Beutler E, Bross KJ, et al. Bone-Marrow Ablation and Allogeneic Marrow Transplantation in Acute Leukemia. New England Journal of Medicine. 1980;302(19):1041–1046. [PubMed: 6245359]
2. Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990;75(3):555–562. [PubMed: 2297567]
3. Brochstein JA, Kernan NA, Groshen S, et al. Allogeneic Bone Marrow Transplantation after Hyperfractionated Total-Body Irradiation and Cyclophosphamide in Children with Acute Leukemia. New England Journal of Medicine. 1987;317(26):1618–1624. [PubMed: 3317056]
4. Bader P, Kreyenberg H, Henze GH, et al. Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. J Clin Oncol. 2009;27(3):377–384. [PubMed: 19064980]
5. Knechtli CJC, Goulden NJ, Hancock JP, et al. Minimal Residual Disease Status Before Allogeneic Bone Marrow Transplantation Is an Important Determinant of Successful Outcome for Children and Adolescents With Acute Lymphoblastic Leukemia. Blood. 1998;92(11):4072–4079. [PubMed: 9834212]
6. Chen X, Xie H, Wood BL, et al. Relation of clinical response and minimal residual disease and their prognostic impact on outcome in acute myeloid leukemia. J Clin Oncol. 2015;33(11):1258–1264. [PubMed: 25732155]
7. Chang TY, Dvorak CC, Loh ML. Bedside to bench in juvenile myelomonocytic leukemia: insights into leukemogenesis from a rare pediatric leukemia. Blood. 2014;124(16):2487–2497. [PubMed: 25163700]
8. Loh ML. Recent advances in the pathogenesis and treatment of juvenile myelomonocytic leukaemia. Br J Haematol. 2011;152(6):677–687. [PubMed: 21623760]
9. Locatelli F, Niemeyer CM. How I treat juvenile myelomonocytic leukemia. Blood. 2015;125(7): 1083–1090. [PubMed: 25564399]
10. Locatelli F, Nollke P, Zecca M, et al. Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. Blood. 2005;105(1):410–419. [PubMed: 15353481]
11. Manabe A, Okamura J, Yumura-Yagi K, et al. Allogeneic hematopoietic stem cell transplantation for 27 children with juvenile myelomonocytic leukemia diagnosed based on the criteria of the International JMML Working Group. Leukemia. 2002;16(4):645–649. [PubMed: 11960345]
12. MacMillan ML, Davies SM, Orchard PJ, Ramsay NK, Wagner JE. Haemopoietic cell transplantation in children with juvenile myelomonocytic leukaemia. Br J Haematol. 1998;103(2): 552–558. [PubMed: 9827934]
13. Matthes-Martin S, Mann G, Peters C, et al. Allogeneic bone marrow transplantation for juvenile myelomonocytic leukaemia: a single centre experience and review of the literature. Bone Marrow Transplant. 2000;26(4):377–382. [PubMed: 10982283]
14. Smith FO, King R, Nelson G, et al. Unrelated donor bone marrow transplantation for children with juvenile myelomonocytic leukaemia. Br J Haematol. 2002;116(3):716–724. [PubMed: 11849238]
15. Yabe M, Ohtsuka Y, Watanabe K, et al. Transplantation for juvenile myelomonocytic leukemia: a retrospective study of 30 children treated with a regimen of busulfan, fludarabine, and melphalan. Int J Hematol. 2015;101(2):184–190. [PubMed: 25504334]
16. Locatelli F, Strahm B. How I treat myelodysplastic syndromes of childhood. Blood. 2018;131(13): 1406–1414. [PubMed: 29438960]
17. Niemeyer CM, Flotho C. Juvenile myelomonocytic leukemia: who's the driver at the wheel? Blood. 2019.

18. Locatelli F, Crotta A, Ruggeri A, et al. Analysis of risk factors influencing outcomes after cord blood transplantation in children with juvenile myelomonocytic leukemia: a EUROCORD, EBMT, EWOG-MDS, CIBMTR study. Blood. 2013;122(12):2135–2141. [PubMed: 23926304]
19. Dvorak CC, Satwani P, Stieglitz E, et al. Disease burden and conditioning regimens in ASCT1221, a randomized phase II trial in children with juvenile myelomonocytic leukemia: A Children's Oncology Group study. Pediatr Blood Cancer. 2018;65(7):e27034. [PubMed: 29528181]
20. Ivey A, Hills RK, Simpson MA, et al. Assessment of Minimal Residual Disease in Standard-Risk AML. N Engl J Med. 2016;374(5):422–433. [PubMed: 26789727]
21. Cicconi L, Fenaux P, Kantarjian H, Tallman M, Sanz MA, Lo-Coco F. Molecular remission as a therapeutic objective in acute promyelocytic leukemia. Leukemia. 2018;32(8):1671–1678. [PubMed: 30026570]
22. Caye A, Strullu M, Guidez F, et al. Juvenile myelomonocytic leukemia displays mutations in components of the RAS pathway and the PRC2 network. Nat Genet. 2015;47(11):1334–1340. [PubMed: 26457648]
23. Stieglitz E, Taylor-Weiner AN, Chang TY, et al. The genomic landscape of juvenile myelomonocytic leukemia. Nat Genet. 2015;47(11):1326–1333. [PubMed: 26457647]
24. Dvorak CC, Loh ML. Juvenile myelomonocytic leukemia: molecular pathogenesis informs current approaches to therapy and hematopoietic cell transplantation. Front Pediatr. 2014;2:25. [PubMed: 24734223]
25. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15(6):825–828. [PubMed: 7581076]
26. Sullivan KM, Shulman HM, Storb R, et al. Chronic graft-versus-host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. Blood. 1981;57(2):267–276. [PubMed: 7004534]
27. Niemeyer CM, Loh ML, Cseh A, et al. Criteria for evaluating response and outcome in clinical trials for children with juvenile myelomonocytic leukemia. Haematologica. 2015;100(1):17–22. [PubMed: 25552679]
28. Cseh A, Niemeyer CM, Yoshimi A, et al. Bridging to transplant with azacitidine in juvenile myelomonocytic leukemia: a retrospective analysis of the EWOG-MDS study group. Blood. 2015;125(14):2311–2313. [PubMed: 25838281]
29. Corbacioglu S, Carreras E, Ansari M, et al. Diagnosis and severity criteria for sinusoidal obstruction syndrome/veno-occlusive disease in pediatric patients: a new classification from the European society for blood and marrow transplantation. Bone Marrow Transplant. 2018;53(2): 138–145. [PubMed: 28759025]
30. Chao A, Meyer J, Lee A, et al. Fusion driven JMML: A novel CCDC88C-FLT3 fusion responsive to sorafenib identified by RNA sequencing. Leukemia. 2019.
31. Chan HS, Estrov Z, Weitzman SS, Freedman MH. The value of intensive combination chemotherapy for juvenile chronic myelogenous leukemia. J Clin Oncol. 1987;5(12):1960–1967. [PubMed: 2445935]
32. Diaz de Heredia C, Ortega JJ, Coll MT, Bastida P, Olive T. Results of intensive chemotherapy in children with juvenile chronic myelomonocytic leukemia: a pilot study. Med Pediatr Oncol. 1998;31(6):516–520. [PubMed: 9835905]
33. Hasle H, Kerndrup G, Yssing M, et al. Intensive chemotherapy in childhood myelodysplastic syndrome. A comparison with results in acute myeloid leukemia. Leukemia. 1996;10(8):1269–1273. [PubMed: 8709630]
34. Lutz P, Zix-Kieffer I, Souillet G, et al. Juvenile myelomonocytic leukemia: analyses of treatment results in the EORTC Children's Leukemia Cooperative Group (CLCG). Bone Marrow Transplant. 1996;18(6):1111–1116. [PubMed: 8971380]
35. Woods WG, Barnard DR, Alonzo TA, et al. Prospective study of 90 children requiring treatment for juvenile myelomonocytic leukemia or myelodysplastic syndrome: a report from the Children's Cancer Group. J Clin Oncol. 2002;20(2):434–440. [PubMed: 11786571]
36. Pulsipher MA, Carlson C, Langholz B, et al. IgH-V(D)J NGS-MRD measurement pre- and early post-allotransplant defines very low- and very high-risk ALL patients. Blood. 2015;125(22):3501–3508. [PubMed: 25862561]

37. van der Velden VH, van der Sluijs-Geling A, Gibson BE, et al. Clinical significance of flowcytometric minimal residual disease detection in pediatric acute myeloid leukemia patients treated according to the DCOG ANLL97/MRC AML12 protocol. Leukemia. 2010;24(9):1599–1606. [PubMed: 20668473]

38. Yoshimi A, Bader P, Matthes-Martin S, et al. Donor leukocyte infusion after hematopoietic stem cell transplantation in patients with juvenile myelomonocytic leukemia. Leukemia. 2005;19(6):971–977. [PubMed: 15800672]

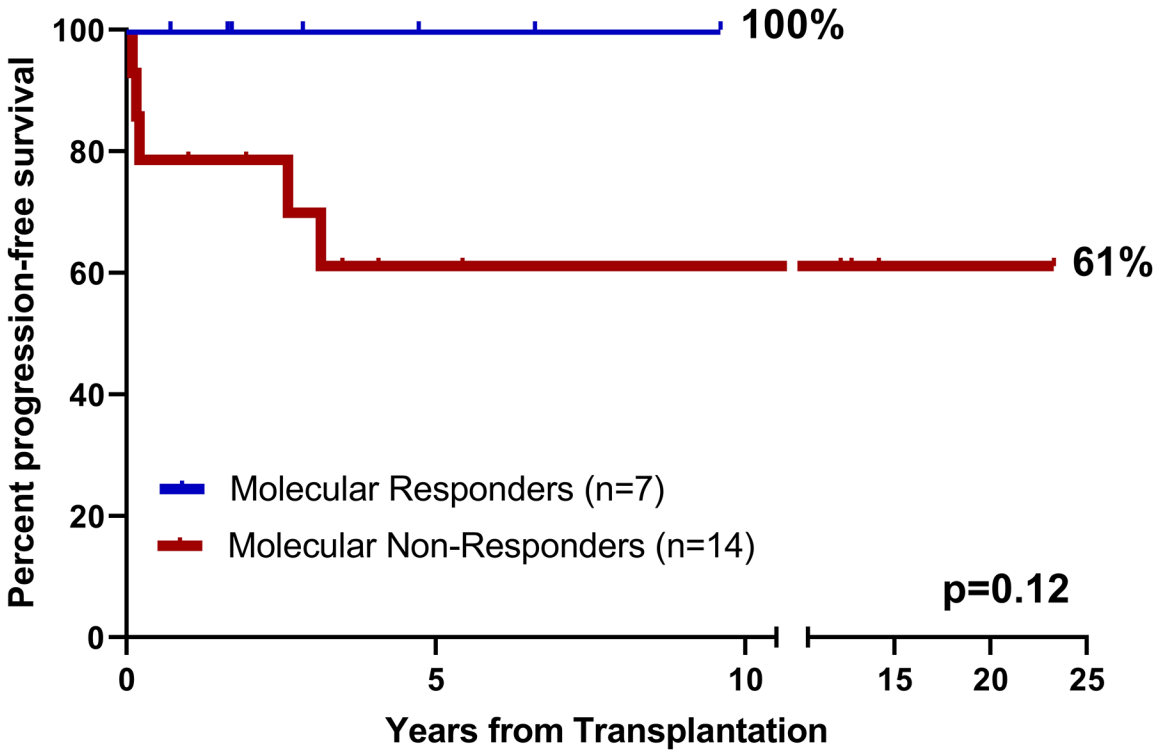

Figure 1:
Kaplan-Meier estimate of 5-year progression-free survival (PFS) by molecular response to therapy.

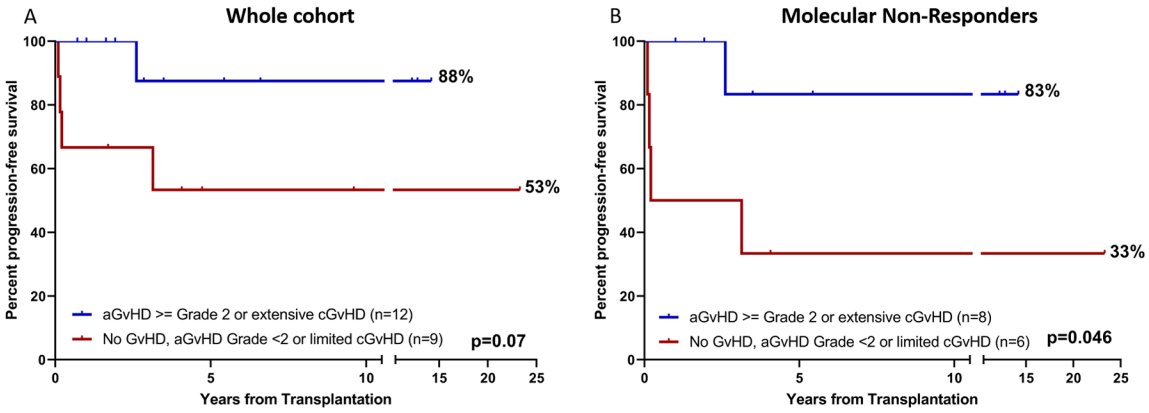

Figure 2:

Kaplan-Meier estimate of 5-year progression-free survival (PFS) by occurrence of graft versus host disease (GvHD). (A) For the whole patient cohort (n=21) and (B) for molecular non-responders (n=14).

TABLE 1:

Clinical patient characteristics.

Characteristics	Whole cohort (n=21)	Data n/a
Gender, Male n (%)	17 (81%)	
Median patient age at diagnosis, years (range)	1.0 (0.4–7.6)	
Median patient age at HCT, years (range)	1.9 (0.9–8.2)	
Median interval between diagnosis and HCT, months (range)	4.4 (2.0–12.7)	
Median WBC count at diagnosis, x10e9/L (range)	28 (4–181)	
Median monocyte count at diagnosis, x10e9/L (range)	3.5 (0–47)	n=2
Median platelet count at diagnosis, x10e9/L (range)	49 (5–223)	
HbF elevated for age, n (%)	11 (52%)	n=2
Karyotype		
Normal Karyotype, n (%)	11 (52%)	
Monosomy 7, n (%)	5 (24%)	
Other abnormalities, n (%)	7 (33%)	
Clinical evidence of NF1, n (%)	2 (10%)	n=4
Pre-Transplant Therapy		
Moderately-intense chemotherapy, n (%)	16 (76%)	
6-MP only, n (%)	4 (19%)	
Azacitidine only (4 cycles), n (%)	1 (5%)	

WBC, white blood cell count

HbF, fetal hemoglobin

NF1, Neurofibromatosis Type 1

6-MP, 6-mercaptopurine

TABLE 2:

Sequencing data of the driver mutations at diagnosis and pre-transplant time point.

				Diagnosis		Pre-HCT		
	UPN	**Number of Mutations***	**Type of Therapy**	**Driver Mutation**	**Allele Frequency**	**Driver Mutation**	**Allele Frequency**	**Disease burden reduction pre-HCT?**
Responders	UPN0647	1	Moderately intense chemo	NRAS p.Q61H	35%	NRAS p.Q61H	0%	yes
	UPN0830	2	Moderately intense chemo	PTPN11 p.E76K	47%	PTPN11 p.E76K	0%	yes
	UPN2610	1	Moderately intense chemo	PTPN11 p.E76K	47%	PTPN11 p.E76K	2%	yes
	UPN2964	1	Moderately intense chemo	FLT3-CCDC88C Fusion	133.5 (conc.)^	FLT3-CCDC88C Fusion	0.14 (conc.)^	yes
	UPN2892	1	Moderately intense chemo	NRAS p.Q61R	23%	NRAS p.Q61R	0%	yes
	UPN2860	1	Azacitidine only	KRAS p.G13D	8%	KRAS p.G13D	0%	yes
	UPN3020	3	Moderately intense chemo	RRAS2 p.Q72L	46%	RRAS2 p.Q72L	0%	yes
Non-Responders	UPN0868	1	6-MP only	PTPN11 p.E76K	39%	PTPN11 p.E76K	35%	no
	UPN0906	2	Moderately intense chemo	PTPN11 p.E76K	57%	PTPN11 p.E76K	50%	no
	UPN0969	2	Moderately intense chemo	NF1 p.T1972fs	89%	NF1 p.T1972fs	57%	no
	UPN1447	2	6-MP only	PTPN11 p.E76K	69%	PTPN11 p.E76K	14%	no
	UPN1711	4	6-MP only	NF1 p.N339fs	99%	NF1 p.N339fs	100%	no
	UPN1740	1	Moderately intense chemo	NRAS p.Q61K	43%	NRAS p.Q61K	11%	no
	UPN2629	1	6-MP only	KRAS p.G12V	17%	KRAS p.G12V	40%	no
	UPN2857	1	Moderately intense chemo	NRAS p.Q61K	37%	NRAS p.Q61K	46%	no
	UPN3064	2	Moderately intense chemo	PTPN11 p.D61Y	42%	PTPN11 p.D61Y	n/a	no#
	UPN3065	2	Moderately intense chemo	FLT3 p.D835H	38%	FLT3 p.D835H	42%	no
	UPN3066	1	Moderately intense chemo	NRAS p.G13D	n/a	NRAS p.G13D	42%	no
	UPN3067	1	Moderately intense chemo	PTPN11 p.E76K	46%	PTPN11 p.E76K	45%	no
	UPN2937	2	Moderately intense chemo	NRAS p.G13D	84%	NRAS p.G13D	89%	no
	UPN2983	1	Moderately intense chemo	KRAS p.G13D	41%	KRAS p.G13D	25%	no

UPN, universal patient number

*total number of mutations detected at initial diagnosis

^allele frequency of the *FLT3-CCDC88C*-fusion was reported as concentration of detected fusion alleles compared to wild-type *FLT3* alleles (detected through a custom droplet digital PCR assay)

response for patient UPN3064 was measured by persistence of monosomy 7 in cytogenetic analysis of a bone marrow aspirate at the pre-HCT timepoint

TABLE 3:

Comparison of responders and non-responders.

	Responders	Non-Responders	P-Value
Variable	n=7	n=14	
Age (y)			
Median	0.97	2.6	0.17
Range	0.39–3.7	0.44–7.2	
Gender, Male n (%)	6 (86%)	11 (79%)	1.0
Splenectomy, n (%)	1 (14%)	4 (29%)	0.62
WBC (x10^9/l)			
Median	27	31	0.6
Range	4.9–92	4.0–181	
Monocytes (x10^9/l)			
Median	2	3.7	0.38
Range	0–47	1.3–30	
Platelets (x10^9/l)			
Median	69	27	0.2
Range	6–124	5–223	
HbF elevated for age, n (%)	4 (57%)	7 (50%)	1.0
Driver Mutation, n (%)			
PTPN11	2 (29%)	5 (36%)	1.0
NRAS	2 (29%)	4 (29%)	1.0
KRAS	1 (14%)	2 (14%)	1.0
NF1	-	2 (14%)	-
FLT3	1 (14%)	1 (7%)	-
RRAS	1 (14%)	-	-
Number of secondary mutations			
Median	1	1	1.0
Range	1–3	1–4	
Abnormal Cytogenetics, n (%)	3 (43%)	7 (50%)	1.0
Monosomy 7, n (%)	2 (29%)	3 (21%)	1.0

WBC, white blood cell count

HbF, fetal hemoglobin