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Original Article

Human leukocyte antigen (HLA) alleles as predictive factors for benefit from lenalidomide in acute myeloid leukemia (AML)

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Abstract: Objectives: Lenalidomide is an active agent in acute myeloid leukemia (AML); response rates are about 15-30%. There are no well-defined predictive factors for benefit from lenalidomide in AML. One of the mechanisms of lenalidomide is natural killer (NK) cell activation; hence human leukocyte antigen (HLA) class I alleles (serving as killer immunoglobulin-like receptor ligands) could play a predictive role. We here evaluate the same when lenalidomide was used as a bridge to transplant. Methods: Consecutive AML patients started on lenalidomide as bridge to transplant between Aug-2013 to Aug-2018 were included in this single centre retrospective analysis. The starting dose and schedule of lenalidomide were at the discretion of the treating clinician. Lenalidomide was scheduled to be stopped about 2-4 weeks prior to planned transplant admission (or was stopped earlier if there was intolerance). For this study, event was defined as progression/relapse while on lenalidomide or within 4 weeks of stopping the drug. The primary endpoint was event free survival (EFS). Those who underwent transplant without an event were censored on the day of transplant. Toxicities and post-transplant outcomes were secondary endpoints. Results: Twelve patients (8 males, median age 29 years) were included. At start of lenalidomide, 7 had complete remission (CR)-1 (measurable residual disease or MRD by flow cytometry was positive in 3, negative in 3, and 1 unknown), 4 CR-2 (all MRD negative) and 1 active disease. In the whole cohort, median EFS was not reached with projected 3 year EFS being 80%. There was a significantly reduced risk of event with HLA A*24 (0% vs 75%, P=0.018) or with HLA B*40 (0% vs 60%, P=0.045). Only 1 patient needed discontinuation due to toxicities (cytopenias). Among patients who underwent transplant, grade II-IV acute graft versus host disease (GVHD) was seen in 83%. Conclusions: This is first study to show that HLA alleles may have a bearing on the effect of lenalidomide in AML and could serve as predictive biomarkers. These findings need to be confirmed in larger prospective studies.

Keywords: Lenalidomide, HLA alleles, acute myeloid leukemia

Introduction

Lenalidomide is an immunomodulatory drug and is effective in several hematological malignancies. Apart from multiple myeloma [1, 2] and myelodysplastic syndromes [3-5], it is also effective in follicular and marginal lymphomas [6] and even Hodgkin disease [7]. Apart from these diseases, lenalidomide has also shown to be effective in acute myeloid leukemia (AML), either alone [8-11] or in combination with other agents [10, 12-14]. A meta-analysis [15] evaluating the effectiveness of lenalidomide in AML found a complete remission (CR) rate of around 20%. Single agent lenalidomide has been shown to induce CR (including CR with incomplete count recovery) in about 30% of elderly patients with previously untreated AML [8, 9]. The CR rates are in relapsed refractory setting are lower with about 15% of patients achieving CR [8]. It has also been used as a maintenance therapy in high risk AML [16].

Although, there is a significant volume of data regarding the use of lenalidomide in AML, not much is known about the predictive factors

which can identify patients likely to benefit. One study identified that amongst patients treated with active disease with high dose lenalidomide, only those patients who had a baseline white blood cell count of $<10\times10^9$/L and baseline peripheral blood blast count <1000/microL were likely to attain a CR [9]. Because of lack of significant data on the predictive factors, a meta-analysis looking at the utility of lenalidomide in AML concluded that there is a need to better define the subgroup of patients with AML likely to benefit from lenalidomide.

Animal models and in-vitro experiments suggest that lenalidomide augments the killing of AML blasts by natural killer (NK) cells by reducing the expression of class I human leucocyte antigen (HLA) alleles on the surface of AML blasts [17]. HLA class I alleles serve as ligands for killer immunoglobulin-like receptors (KIRs) on the surface of NK cells. Given the recent identification of role of natural killer (NK) cells in AML [18-20], it seemed likely that HLA class I alleles could have an impact on the benefit from lenalidomide in patients with AML. Data from patients treated with conventional chemotherapy does suggest that expression of various activating or inhibitory receptors for NK cells on AML blasts affects the outcomes [21]. However, such data does not exist for treatment with lenalidomide.

At our centre, because of a waiting period of about 9-12 months for allogeneic transplant, we have often used lenalidomide as a bridge to transplant. Here we present our experience with lenalidomide as a bridge to transplant in AML, and evaluate whether HLA alleles can be predictive in that setting.

Patients and methods

Patients

This is a single centre retrospective analysis of all consecutive patients with AML started on single agent lenalidomide from August 2013 to August 2018 as a bridge to transplant. These patients were identified from a prospectively maintained database of transplant recipients, and a prospectively maintained database of patients on the transplant waitlist. All patients were included irrespective of the disease status at the time of start of lenalidomide. However, patients receiving lenalidomide as a part of combination therapy (eg along with azacytidine or cytarabine) were excluded. The study was approved by the institutional ethics committee (study number 900825). Given the nature of the study, the need for consent was waived off by the ethics committee.

Diagnosis and treatment of AML

All patients underwent bone marrow examination at baseline. The baseline studies included marrow morphology, flow cytometry, cytogenetics (both by fluorescent in situ hybridization and conventional karyotyping) and bone marrow biopsy. Molecular studies consisted of conventional polymerase chain reaction (PCR) for NPM, FLT3 and CEPBA in all patients. All patients received induction therapy with 1 course of daunorubicin and cytarabine (3+7) followed by 1-3 cycles of consolidation chemotherapy with high dose cytarabine. For those who relapsed, all of the above studies were repeated at relapse and the salvage regimen was usually CLAG (cladribine + cytarabine + G-CSF).

Lenalidomide therapy

Lenalidomide was started after recovery of counts from the prior chemotherapy (except when it was started with frank disease). The starting dose and schedule were at the discretion of the treating clinician. Lenalidomide was stopped 2-4 weeks before planned admission for transplant or if there were significant toxicities. Dose reduction and monitoring was at the discretion of the treating clinician.

Data collection

Data was collected using standardized data collection forms and included demographic parameters, flow cytometric parameters of AML, baseline cytogenetics and molecular parameters (and also cytogenetics and molecular parameters at relapse where applicable), disease status at time of starting (including minimal residual disease by flow cytometry), HLA alleles and KIR ligands and disease outcomes (relapse and death). For those who underwent transplant, data was also collected on the post-transplant outcomes (grade II-IV acute GVHD and survival outcomes).

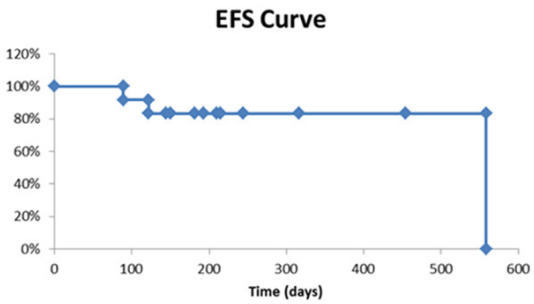

Figure 1. EFS of the whole cohort. EFS, event free survival.

HLA typing and KIR ligands

HLA typing was done by PCR based methods, specifically by sequence specific primer (SSP) and sequence specific oligonucleotide probe (SSOP). HLA-A, B, DR were typed for all. HLA-C and DQ were typed only for haploidentical transplants and matched unrelated donor (MUD) transplants. The low resolution typing was used for analysis. KIR ligands were obtained from online KIR ligand calculator (https://www.ebi.ac.uk/ipd/kir/ligand.html).

Objectives and statistics

The primary objective was to assess event free survival (EFS). EFS was calculated from the date of start of lenalidomide. For this study, event was defined as disease relapse/progression or death (from any cause) occurring while on lenalidomide or within 4 weeks of stopping it. For those who underwent transplant without an event, data was censored on the date of transplant. Secondary objectives included assessment of toxicities, and post-transplant outcomes (which included acute grade II-IV GVHD, and survival outcomes). Grading of acute GVHD was done using Glucksberg criteria [22]. *P* values were calculated with Fisher Exact test, and were 2 sided. A *P*-value of <0.05 was considered as statistically significant. Kaplan Meier curves were used to plot event free survival. Toxicities were graded as per standard criteria. All analysis was done using SPSS software (version 25).

Results

Baseline characteristics and lenalidomide details

Twelve patients were started on lenalidomide as a bridge to transplant in the above period, and all were included in the final analysis. The median age was 29 (range 17-49) years with 8 (66%) males. All patients had de novo AML. At the time of starting lenalidomide, 7 were in 1st complete remission (CR1), 4 in 2nd CR and 1 with frank disease. The minimal residual disease (MRD) status (by flow cytometry) at time of start was negative in 7, positive in 4 and unknown in 1. The starting dose of lenalidomide was 10 mg in 3, 15 mg in 6 and 25 mg in 3. Eight patients (66%) received lenalidomide on a continuous schedule. The median duration of lenalidomide was 217 days with range being 93-557+ days.

Event free survival

Five patients underwent transplant without an event. From the remaining 7 patients, 3 had an event (all disease relapse, among whom 1 underwent transplant), 1 stopped lenalidomide due to toxicity (hematological toxicity-cytopenias) and 3 were still continuing on lenalidomide at the time of analysis. Median EFS for the whole cohort was not reached, with projected 3 year EFS being 80% (**Figure 1**). Among patients who had an event, the median time to relapse/progression was 122 days. The details about the individual patients are summarized in **Table 1**.

Impact of HLA alleles on EFS

Among these 12 patients, 8 (66%) had HLA-A*24, and 7 (58%) had HLA-B*40. Of these, 6 patients had both the HLA alleles, 2 had only HLA-A*24 (but not HLA-B*40), and one had only HLA-B*40 (but not HLA-A*24). None of the 8 patients with HLA-A*24 had an event compared to 3 events among 4 patients (75%) without that allele (P=0.018). Likewise, presence of HLA-B*40 was also associated with reduced risk of event (0% vs 60%, P=0.045). The Kaplan-Meier curves for EFS according to the presence or absence of these alleles are shown in **Figure 2A** and **2B**. KIR ligands (grouped according to Bw4 or Bw6 and C1 or C2) were not predictive for event (P=NS for all comparisons).

Toxicities

The drug was well tolerated in this setting. All patients except 1 tolerated the drug well. In the remaining 11 patients no dose reductions were necessary. One patient who did not tolerate

Table 1. Details of individual patients

Sr No	Age	Gender	Baseline cytogenetics	Molecular profile (at baseline or at relapse)	Disease status at start	Flow MRD at start	Len dose (in mg)	Len Schedule	Event	Transplant
1	30	Female	Normal	FLT3 ITD and NPM positive	CR1	Negative	15	Continuous	No	Yes
2	27	Male	T (8:21)	Not available	CR2	Negative	15	Continuous	No	No
3	17	Male	Normal	Not available	CR1	Positive	15	Continuous	No	No
4	21	Male	Not available	Negative	CR2	Negative	15	Continuous	Yes	Yes
5	36	Male	Negative	Negative	CR1	Positive	10	Continuous	Yes	No
6	21	Male	T (8;21)	Negative	CR1	Negative	15	Continuous	No	No
7	19	Female	Del 19p	Monoallelic CEBPA	CR2	Negative	10	Continuous	No	No
8	49	Male	Negative	Negative	CR1	Positive	25	Not known	Yes	No
9	46	Female	T (4;12)	NPM positive	CR2	Negative	15	Continuous	No	Yes
10	19	Female	T (10;11)	Not available	CR1	Negative	10	Intermittant	No	Yes
11	45	Male	Trisomy 10	Not available	CR1	Not known	25	Intermittant	No	Yes
12	32	Male	Hyperdiploidy	Not available	1st relapse	Positive	25	Intermittant	No	Yes

Abbreviations: MRD: measurable residual disease, len: lenalidomide, CR1: first complete remission, CR2: 2nd complete remission.

lenalidomide had cytopenias even after reducing the dose to 5 mg/day.

Post-transplant outcomes

Overall, 6 patients underwent transplant-5 without an event, and one after disease relapse. In these patients, the median time of stopping lenalidomide prior to transplant was 28 days with range being 18-49 days. Of these 6 patients, 5 (83%) developed acute graft versus host disease. At the time of last follow up, 2 (33%) are alive and disease free, and 4 have died. The causes of death include transplant related complications in 3 and disease relapse in 1.

Discussion

Thalidomide and its analogous (lenalidomide, pomalidomide) have been extensively used in hematological oncology for a variety of indications. These drugs have several mechanisms of action-including anti-angiogenic, immunomodulatory and pro-apoptotic properties [17]. These also inhibit the cancer cell proliferation, and enhance the function of natural killer (NK) cells. The dominant mechanism in individual diseases is not very well known.

As far as AML is concerned, NK cell mediated killing seems to play an important role as shown by the impact of NK cell alloreactivity on transplant outcomes [23]. In an elegant series of in-vitro experiments and mouse models using blasts obtained from patients, Le-Roy and group have extensively investigated the effect of lenalidomide and pomalidomide on AML blasts and NK cells. Their series of experiments show that lenalidomide (and also pomalidomide) can directly kill the AML blasts, as well as enhance the killing by NK cells. They go on to show that the enhancement of NK cell killing of AML blasts widely varies from patient to patient. They also show that, this enhancement of killing does not differ if the source of NK cell is changed. These findings suggest that the enhancement of NK cell mediated killing of AML blasts does not depend on the source of NK cells, but depends on the AML blasts. In yet a step further, they show that lenalidomide exposure leads to significant down-regulation of HLA class I molecules on the AML blasts. The mean reduction in the expression of HLA class I molecules was 40%. To understand the functional important of this down-regulation, they further studied the impact of blocking antibodies to class I HLA alleles, and found a similar effect to that of exposure to pomalidomide. These findings indicate that down-regulation of HLA class I molecules is certainly one of the mechanisms of enhanced NK cell alloreactivity against AML blasts.

We hypothesize that the degree of down-regulation of HLA class I alleles depends on the alleles themselves, such that some alleles are likely more down-regulated compared to others. This hypothesis can explain the widely varying enhancement of NK cell killing of AML blasts observed in Le-Roy's series of experiments. It can also explain why the enhancement of NK

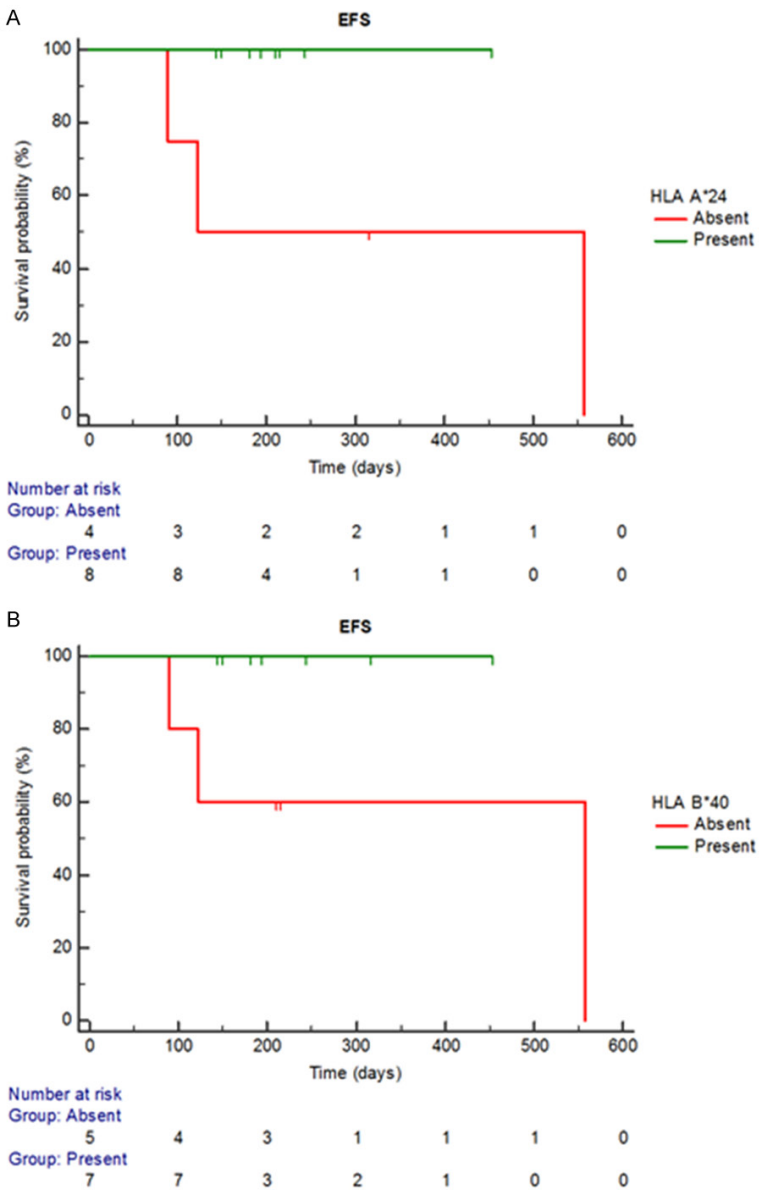

Figure 2. (A) (upper curve)-EFS according to presence or absence of HLA-A*24 and (B) (lower curve)-EFS according to presence or absence of HLA-B*40. (EFS: Event free survival).

cell mediated killing does not depend on the source of NK cells. Based on our findings, we feel that the alleles HLA-A*24 and HLA-B*40 have higher degree of down regulation compared to other alleles. Obviously, this needs to be confirmed by in-vitro or in-vivo experiments.

Our findings suggest that in AML, the benefits of lenalidomide possibly depend on patient's own HLA and might be predicted by presence or absence of particular HLA class I alleles. The limitations of our study include the retrospective nature of the study, small sample size and lack of a translational component. Hence, the findings need to be validated in prospective clinical studies with additional translational component to confirm our hypothesis. Whether the same findings hold true in other diseases like MDS or Hodgkin lymphoma also need to be studied.

In conclusion, HLA class I alleles potentially serve as predictive biomarkers for benefit or otherwise from lenalidomide therapy. To our knowledge, this is the first study to suggest the possibility of HLA alleles as potential biomarkers for lenalidomide. If confirmed in further studies, these findings could be valuable in choosing patients, both in clinical practice and in clinical trials.

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Disclosure of conflict of interest

None.

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