



Cellular immune profiling after sequential clofarabine and lenalidomide for high risk myelodysplastic syndromes and acute myeloid leukemia



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ABSTRACT

Patients with high risk myelodysplastic syndromes (MDS) and acute myelogenous leukemia (AML) are commonly older with multiple co-morbidities, rendering them unsuitable for intensive induction chemotherapy or transplantation. We report preliminary cellular immune profiling of four cases receiving sequential clofarabine and lenalidomide for high risk MDS and AML in a phase I study. Our results highlight the potential of immune profiling for monitoring immune-modifying agents in high risk MDS and AML.

1. Introduction

High risk MDS and AML are heterogeneous myeloid malignancies and usually have a very poor prognosis. Advanced age and comorbidities often make the patients unsuitable candidates for intensive chemotherapy or allogeneic stem cell transplantation (allo-SCT) [1]. New less toxic treatments that improve response rates and extend survival are needed for such patients. In this phase I study, we sought to determine safety and efficacy of sequential therapy with low-dose clofarabine and lenalidomide in high risk MDS and AML. Both clofarabine lenalidomide have partial efficacy as single agents for MDS and AML [2,3]. Although the immunomodulatory effects of lenalidomide are well described [4,5], little is known about the effect of clofarabine on cellular immunity. We hypothesized that the lymphocyte depleting effect of clofarabine would create a favorable immunological microenvironment for subsequent lenalidomide therapy promoting T cell and NK cell reconstitution. Here we report cellular immune profiles of the four cases receiving sequential clofarabine and lenalidomide for high risk MDS and AML.

2. Materials and methods

The study was designed as an open label, single institution phase I study at the National Heart, Lung, Blood Institute, National Institutes of

Health and was approved by Institutional Review Board (NCT01629082). All patients signed an informed consent prior to enrollment and the study was conducted in compliance with the Declaration of Helsinki. The eligibility criteria included patients with a diagnosis of high risk MDS, CMML, and AML who were not candidates for standard intensive chemotherapy or allo-SCT and had failed at least one prior therapy. Subjects received a single course of intravenous low-dose clofarabine 5 mg/m²/day for five days. At 28 days after induction therapy, oral lenalidomide therapy was initiated with dose escalation from 25 mg to 50 mg daily for 21 days of 28 days for up to 12 cycles. Response assessment was performed according to International Working Group (IWG) response criteria [6]. Detailed study design was described in [Supplementary material](#). Flow cytometric analysis was performed to characterize T cell and natural killer (NK) cell subsets, with functional markers of T cell exhaustion and activating and inhibitory NK cell receptors. Antibodies used in the panel are listed in [Supplementary Table 1](#). Relative changes in RNA expressions of various genes related to cancer immunology were evaluated by custom made 384 well PrimePCR™ Assay Panels for Real-Time PCR (Bio-Rad Laboratories, Hercules, CA, USA).

3. Results

Four subjects with relapsed or refractory high-risk MDS (IPSS risk

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Table 1
Clinical and Hematological Characteristics of study population.

Subject ID	UPN1	UPN2	UPN3	UPN4
Age (years), gender	79, male	70, male	60, female	64, male
Disease	RAEB-I IPSS intermediate	RAEB-II IPSS high risk	AML with multilineage dysplasia	AML with multilineage dysplasia
Cytogenetic abnormality	del(12)(p11.2p13), +8	-Y	normal	-7, inv (9)(p11q13),
Prior treatment	Azacitidine, Decitabine	Azacitidine, Revmimid (10 mg) growth factors	Azacitidine, hydroxyurea	Azacitidine, HIDAC, MEC, FLAG, Auto BMT
Response to clofarabine	Stable Disease	Stable Disease	Partial Response	Refractory Disease
Off treatment reason	Disease progression	Disease progression	DLT-liver	Disease progression

Abbreviations: RAEB, refractory anemia with excess blasts; AML, acute myeloid leukemia; DLT, dose limiting toxicity; HIDAC, High dose cytarabine; MEC, mitoxantrone, etoposide, cytarabine; FLAG, fludarabine, high dose cytarabine, G-CSF; IPSS, International Prognostic Scoring System; WHO, World Health Organization

score > intermediate 2) or AML were enrolled. Clinical and hematological characteristics are summarized in Table 1. Two subjects achieved stable disease with lenalidomide maintenance for 4–6 months, but were taken off study on days 162 and 206 respectively due to progression of disease. One subject had a partial response in the erythroid lineage after clofarabine induction. This subject was taken off study on day 69 due to asymptomatic transient grade 3 liver toxicity during lenalidomide therapy. One subject had disease progression during lenalidomide therapy and was taken off study on day 70. The clinical trial is now closed without dose escalation beyond the first cohort for reasons of poor accrual and lack of durable response.

In 3 subjects with partial response (PR) and stable disease (SD), Clofarabine reduced the count of all lineages of lymphocytes, T cells (CD4 and CD8), NK cells, and B cells. However, in the subject with refractory disease, CD4, CD8, and NK cells increased after clofarabine treatment (Fig. 1). T cell subset analysis demonstrated shift in memory subsets after clofarabine. Fractions of central memory (CM) T cells in CD4 cells decreased in responders (0.61 fold change) and increased in partial responder and non-responder (1.22 fold change). Fractions of effector memory (EM) decreased in all but one subject (stable responder). Fractions of naive cells in both CD4 and CD8 T cells increased in all subjects following clofarabine treatment. Before treatment, the exhaustion markers PD1, LAG3, and TIM3 in peripheral blood were moderately increased in both CD4 and CD8 T cells in comparison to healthy volunteers. After clofarabine, absolute numbers of PD1⁺ CD4 cells decreased in the subjects with stable disease (UPN1 and UPN2) and partial response (UPN3). Absolute numbers of PD1⁺ CD4 cells were increased in UPN4 with refractory disease. Similar changes were seen in the exhaustion markers LAG3 and TIM3. FoxP3⁺CD25⁺ regulatory T (Tregs) cells were largely unchanged but rose in UPN4 (Supplementary Figure 1). Thus the failure of lymphodepletion and rise of exhaustion markers and Tregs following clofarabine correlated with treatment failure.

In NK cells, CD57⁺ and LIR1⁺ populations fell among the subjects with stable disease and partial response, while NK cells in the subjects with refractory disease increased their CD56^{dim} population expressing

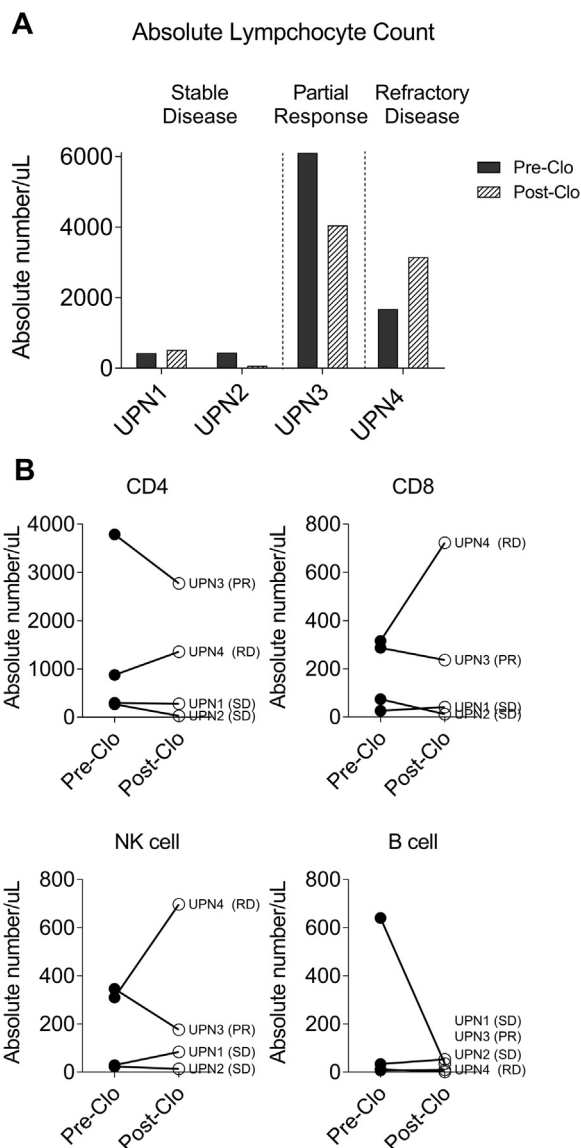


Fig. 1. Lympho-depleting effect of clofarabine. A. PBMC samples (n=4), were collected pre clofarabine(pre-clo) and 28–42 days post clofarabine(post clo). Analysis of post clofarabine samples showed persistently low (UPN1 and UPN2, stable disease) and decreased (UPN3, partial response) absolute lymphocyte counts. In contrast absolute lymphocyte count was increased in UPN4 (refractory disease). B. All lineages of lymphocytes, CD4 T cells, CD8 T cells, NK cells and B cells were decreased in subjects with stable disease (SD) and partial responder (PR), however CD4 T cells, CD8 T cells, and NK cells were increased in subject with resistant disease (RD).

CD57 and LIR1 (Fig. 2). These findings indicate restoration of NK cell repertoires after clofarabine and a switch to a less inhibitory NK profile with response or stable disease. The CD4 and NK cell immune profiles in two subjects (UPN1 and UPN2) demonstrated dynamic immune reconstitutions in the subsequent lenalidomide therapy (Fig. 3). Targeted RNA expression profiles in UPN 2 were analyzed and significant up-regulations of genes were observed in IL-15, IFN γ , CSF1, IL7R, and IL-2 after lenalidomide therapy (Supplementary Fig. 2). This finding indicates that lenalidomide induced expressions of genes related to cytotoxic T cell and NK cell activation.

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