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An *In Vitro* Platform to Dissect Drug Responsiveness in Refractory Anemia with Ringed Sideroblasts (RARS)

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Background

Myelodysplastic syndromes, or MDS, are a heterogeneous group of clonal hematopoietic disorders characterized by dysplastic bone marrow morphology, variable cytopenias in the presence of a hypercellular marrow and an increased risk of transformation to acute myeloid leukemia (AML). International Prognostic Scoring System (IPSS) assess a patient's survival and likelihood to transition to AML. This system separated MDS patients into three groups based on the hazard of 25% patients developing leukemia into low, intermediate and high risk categories, with the intermediate risk further stratified into intermediate-1 (Int-1) and Int-2 categories. Overall, patients with low and Int-1 risk of transformation were collectively referred to lower risk (LR-MDS) group and those with Int-2 and high risk as higher risk (HR-MDS) disease.

Despite intensive investigation, The pathophysiology of LR-MDS remains unknown and therapeutic options are limited. In a phase-II trial, treatment with oral rigosertib, a small molecule inhibitor of PI3K and PLK pathways, was shown to have a 39% response rate (i.e., transfusion independence), in patients with LR-MDS (ASH 2013 Abstract 2745). Notably, rigosertib caused an increase in hemoglobin, a decrease in transfusion requirements, and, in some patients who were previously refractory, "re-sensitization" to erythropoietin (EPO) therapy. However, thus far, the mechanism of this responsiveness remains unknown. Whole exome sequencing revealed a broad spectrum of mutations in these patients, including mutations in SF3B1, SRSF2 and TET2. However, there was no correlation between mutational spectrum and responsiveness.

Methods

CD34+ cells were isolated from bone marrow (BM) mono nuclear cells derived from BM aspirates. Bone marrow stromal cells (BMSC) were isolated from bone biopsies of patients with MDS. Normal CD34+ cells and BMSC were obtained commercially. CD34+ cells were co-cultured with stromal cells plated a day before and stimulated with concentrations of EPO and rigosertib. Differentiation was assessed by staining cells with various markers of erythroid differentiation and analyzed by FACS. We show that co-culture of CD34+ stem cells from the bone marrow of patients with their own BMSC recapitulates key features of the MDS phenotype and rigosertib responsiveness.

This study is approved by the Institutional Review Board of Columbia University and informed consent was obtained from all the individuals participated in the study.

Results

Erythroid differentiation of EPO stimulated human CD34+ cells in an *in vitro* BMSC/CD34+ co-culture system

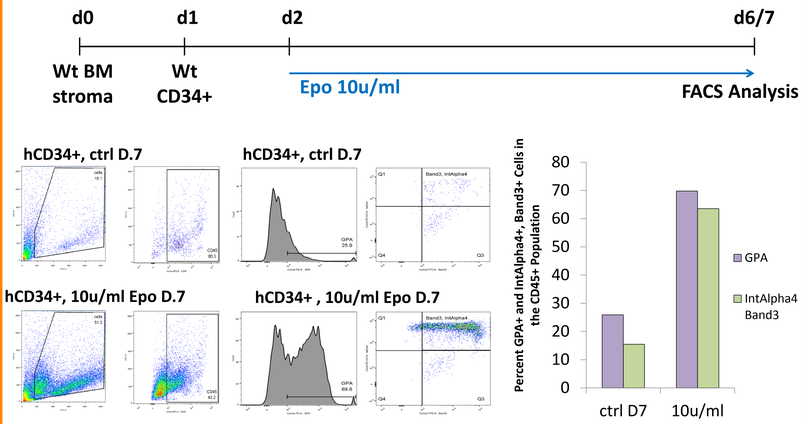


Figure 1. Normal CD34+ cells co-cultured with normal BMSC showed striking responsiveness to EPO stimulation, as evidenced by the increased production of CD45- low/GPA+/ band 3+/Integrin-Alpha4+ erythroid cells. Cells were analyzed for erythroid differentiation using GPA/Alpha 4/Band 3 antibodies and flow cytometry.

Rigosertib enhances erythroid differentiation of EPO stimulated CD34+ cells *in vitro* BMSC/CD34+ co-culture system

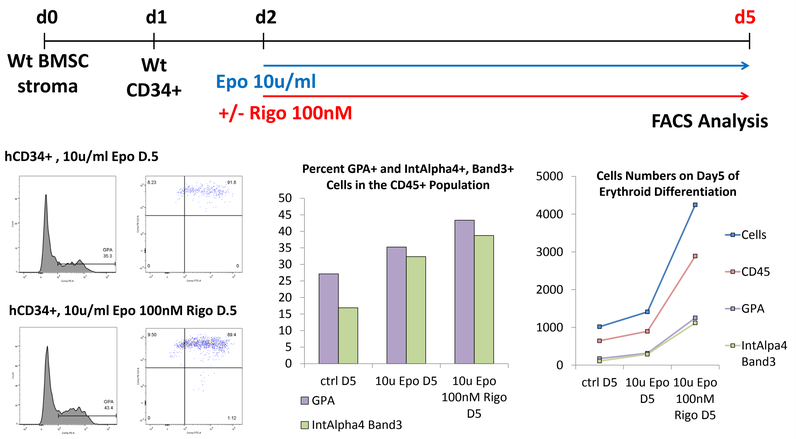
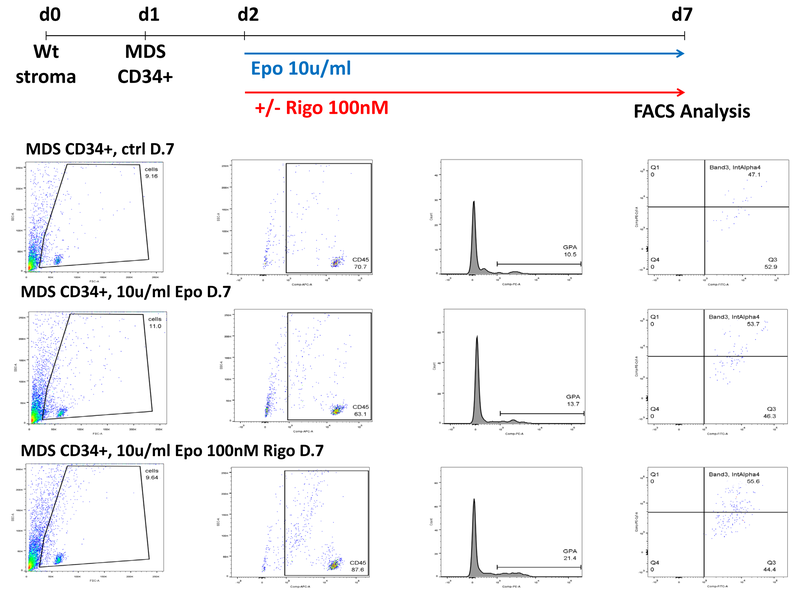


Figure 2. Normal CD34+ cells co-cultured with normal BMSC and stimulated with EPO alone or EPO and Rigosertib show increased response, as evidenced by the increased production of CD45- low/GPA+/ band 3+/Integrin-Alpha4+ erythroid cells. Cells were analyzed for erythroid differentiation using GPA/Alpha 4/Band 3 antibodies and flow cytometry.

Rigosertib enhances erythroid differentiation of EPO stimulated LR-MDS patient derived CD34+ in an *in vitro* BMSC/CD34+ co-culture system



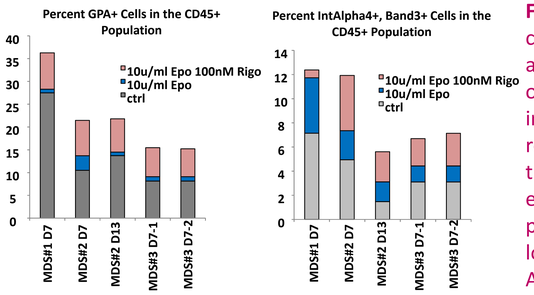


Figure 3. MDS CD34+ cells co-cultured with normal BMSC and stimulated with EPO alone or EPO and Rigosertib show increased response in patient responsive to Rigosertib treatment (MDS#2) as evidenced by the increased production of CD45-low/GPA+/ band 3+/Integrin-Alpha4+ erythroid cells.

Conclusions

We have created an in vitro platform to dissect the mechanism of rigosertib responsiveness in RARS patients. This is a novel co-culture-based system that recapitulates key features of the RARS phenotype. Without reliable animal models for this disease, this platform may offer a viable method to characterize drug responsiveness, dissect mechanisms, and offer patient-specific drug responsiveness information, since individual CD34+ cells are co-cultured with a patient's own BMSC. We wish to use this in vitro co- culture test to prospectively predict responsiveness to experimental drugs in patients, thereby focusing the drug on only selected LR-MDS patients.

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