CXCL8 level in extracellular milieu was observed to be significantly increased on treatment with RIGO alone (p value < 0.01) and sequential combinations (p value < 0.01) whereas Azacitidine did not impact. 90% and 83% inhibition in colony formation was observed in MDS-L cells treated with RIGO/AZA and RIGO respectively, whereas as Azacitidine formed 30% fewer colonies compared to controls.
Summary/Conclusion: RIGO/AZA combinations effects CXCL8 an RLR signaling responsive gene and may have an impact on hematopoiesis. Further studies are underway to determine the effects of RLR signaling pathways on hematopoiesis both *in vitro* and *in vivo* in the HMA clinical resistance setting to identify potential therapeutic targets to reverse bone marrow failure in pts with HMA resistance.

PB1979 FEATURES OF FUNCTIONING OF HEMATOPOIETIC BONE MARROW PROGENITOR CELLS IN MDS, WHICH IS TRANSFORMED INTO ACUTE MYELOBLASTIC LEUKEMIA

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Background: Myelodysplastic syndromes (MDS) are heterogeneous group of clonal origin diseases, characterized by ineffective hematopoiesis, pronounced refractory to therapy, peripheral blood cytopenia, hyperplasia or dysplasia of bone marrow (BM), eventual ability to transform into acute leukemia, predominantly with myeloid origin (AML). Numerous studies have shown that the trigger mechanisms of MDS are associated with occurrence of somatic genetic or chromosomal abnormalities in the stem cell of the myeloid lineage, as well as the activation and disruption of epigenetic mechanisms. Another characteristic of MDS is the reduced cluster and colony forming ability of hematopoietic progenitor cells in the CFU assay *in vitro*.
Aims: The purpose of the study was to investigate the morpho-functional features of hematopoietic progenitor cells in MDS transformed into AML.
Methods: There were 8 patients with MDS RAEB II aged 57 to 69 years, with a mean age of 61.7±3.5 years, 3 women and 4 men. The diagnosis was verified according to the WHO criteria for myeloid neoplasms and acute leukemias in 2008. All studies were conducted with respect for human rights and moral and ethical standards in accordance with the principles of the Helsinki Declaration of Human Rights. Patients were also subjected to an immunomodulator (lenolidomide) treatment. Transformation of MDS RAEB II into AML occurred in 2, 3 and 6 months for 2, 3 and 3 patients respectively. Immunophenotyping of BM cells was performed on a flow cytometer. In parallel, samples of patients BM were cultured for 14 days under conditions of absolute humidity, 37^{0}C in DMEM medium with 20% FBS, 1% of antibiotics (penicillin/streptomycin) and L-glutamine, and 50 ng/ml GM-CSF in semisolid agar. Hematopoietic progenitor cells isolated from rib fragments of healthy subjects during orthopedic surgery were used as controls.
Results: The analysis of the obtained data shows that in patients with MDS RAEB II during the diagnosis, in the sample of BM an extension of the erythroid lineage was evident, due to narrowing of the granulocyte series. Significantly pronounced morpho-phenotypic features of dysgranulocytopoiesis, dysmonocytopoiesis were observed. It was established that 15,24 to 18,43% of blast cells with the phenotype of CD45 (dim) +/- 117+34+4+(16+56)+38+33. At the time of transformation of MDS RAEB II into AML, increase in the percentage of blast cells from 35 to 50% was observed in BM, with the phenotype of CD45 -/+ 117+4+(16+56)+15+cMPO+71+HLA-DR+34 and loss of CD33-CD38 in three cases. At the level of the cell culture, proliferation and differentiation activity indicated complete inhibition of colony forming ability compared with the control (2.1 ± 0,2 and 61.5±3,7 at 1x10^5 of explanted cells, respectively). Presence of irregularly shaped clusters of fibroblast cells was evident. Among the formed cellular aggregates no colonies were found.
Summary/Conclusion: Thus, transformation of MDS RAEB II into acute myeloid leukemia is accompanied by changes in erythroid and granulocytic lineages of hematopoiesis, as well as marked dysgranulocytopoiesis and dysmonocytopoiesis . There is also a significant increase in the number of blast cells. The colony-forming ability of bone marrow progenitor cells is absent, and cluster formation is significantly reduced compared to control. These changes correspond clonal origin of MDS and connected with the inhibition of hematopoietic function at progenitor cells level, causing ineffective hematopoiesis and and severe cytopenias of granulocyte lineage.

Myelodysplastic syndromes - Clinical

PB1980 EARLY TRANSITION OF PERIPHERAL BLOOD WT1 MRNA LEVELS AFTER 5-AZACITIDINE PREDICTS HEMATOLOGICAL IMPROVEMENT IN PATIENTS WITH HIGHER RISK MDS

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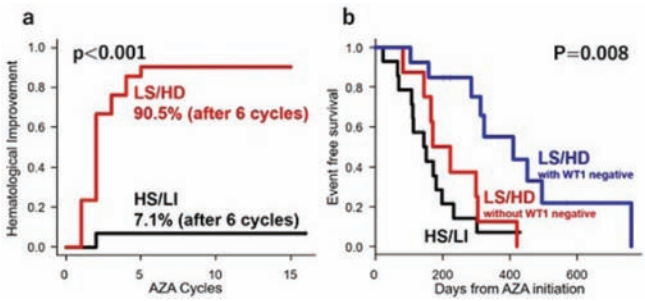
Background: Patients with higher risk myelodysplastic syndromes (MDS) treated with 5-azaciditine (AZA) have exhibited improved overall survival compared with conventional therapies, especially when hematological improvement (HI) was achieved. Increased levels of Wilms' tumor 1 (WT1) mRNA have been used as the marker of disease progression and poor prognosis in MDS patients. However, it remains to be clarified whether the dynamic changes of WT1 levels of peripheral blood (PB) correlates with response to AZA.
Aims: In this study, we evaluated whether WT1 levels in PB samples at baseline and during the therapy with AZA could predict hematologic response or not.
Methods: We retrospectively analyzed WT1 expression in PB samples (using Otsuka kit; Otsuka Pharmaceutical Co., Ltd.) of transplant-ineligible patients with higher risk MDS who received AZA (≥3 cycles) as an initial therapy between 2012-2019 in our institution. The patients who did not evaluated WT1 expression before and during AZA treatment were excluded in this study. According to the transition of WT1 levels from AZA initiation to the end of 3 cycles, we defined four categories as follows: low-stable (LS): patients maintaining low WT1 levels (< 1000 copies/μg); high-stable (HS): patients maintaining high WT1 levels (≥1000 copies/μg) without ≥1 log reduction levels of WT1; high-decreasing (HD): patients with high WT1 levels at baseline but showing ≥1 log reduction levels of WT1; low-increasing (LI): patients with low WT1 levels at initiation but increasing to high WT1 levels. HI was assessed according to IWG-2006 criteria. Event-free survival (EFS) was defined as the time from commencement of AZA therapy to the date of death from any cause, progression to acute myeloid leukemia, loss of HI, or cessation of AZA, whichever occurred first.
Results: Thirty-five patients were included in this study (median age at AZA initiation, 75 years, range, 61-86 years; 77.1% male). At the median follow-up of 335 days from AZA initiation, estimated overall survival (OS) rate and event-free survival (EFS) rate at 2 years were 36.7% and 9.1%. Median number of AZA cycles was 9 (range; 3-34). Median expression of WT1 at baseline was 1600 copies/μg (range; < 50-320000 copies/μg). Patients were classified as LS (n = 14, 40%), HS (n = 11, 31.4%), HD (n = 7, 20%) and LI (n = 3, 8.6%). Twenty patients (57.1%) achieved any HI. Cumulative incidence of any HI after 6 cycles of AZA was significantly higher in LS/HD cohort than in HS/LI cohort (90.5% vs 7.1%, p < 0.001, Fig. a). Thirteen patients achieved WT1 negative (< 50 copies/μg) during courses of AZA and all of whom were in LS/HD cohort (23.1% HD). These patients showed significantly better EFS (Fig. b).



Summary/Conclusion: The early transition of PB WT1 levels was a surrogate marker for HI in patients with higher risk MDS. Especially among the LS/HD patients, the achievement of WT1 negativity was correlated with better EFS.