The effect of Ara-C treatment on hematopoietic stem cell expansion and leukemogenesis in a mouse model of *CEBPA* mutant acute myeloid leukemia

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Acute myeloid leukemia (AML) is the most common acute leukemia in adults. Acquired mutations in the *CEBPA* gene are found in 11-12% of all AML cases and include N- and C-terminal mutations that are frequently found within the same patient on separate alleles. We have recently demonstrated that combining N- and C-mutations in mice resulted in loss of hematopoietic stem cells (HSC) quiescence and expansion of premalignant pool of cells, associated with accelerated AML. Therefore, pharmacologic targeting of the pre-leukemic HSCs has emerged as another critical step to combat tumor progression particularly relevant to prevent tumor relapse. Increased cycling of mutant pre-leukemic HSCs suggests that they could be susceptible to the action of anti-proliferative agents used in chemotherapy. We have addressed the impact of cytosine arabinoside (Ara-C) treatment on proliferating mutant HSCs survival, as well as long-term tumor development in a mouse model of *CEBPA* mutant AML. We evaluated whether 1) Ara-C treatment leads to a selective mutant HSCs apoptosis and a consequent drop in mutant HCS number; 2) reduction in mutant HSC number affects tumor development.
Cohorts of mice treated or not with Ara-C for 1 week were monitored overtime for: i) percentage of Mac1+ cells, ii) white and red blood cells counts in the peripheral blood and mice survival. Here found that Ara-C efficiently and selectively induced apoptosis in mutant HSCs and downregulated their frequency and total number. However it did not lead to their complete elimination. Interestingly, we found that mice treated with Ara-C at early stages of AML progression showed a greater accumulation of Mac-1+ cells and a reduction in the frequency of B and T cells in the peripheral blood at 4 and 6 months after treatment, as compared to untreated mice. Moreover mice treated with Ara-C showed a statistically significant increase in the number of white blood cells, a reduction in the number of red blood cells, hemoglobin and hematocrit at 6 months following the Ara-C treatment, suggesting an earlier onset of leukemic blasts accumulation and leukemia-associated anemia in these mice. These data demonstrate that Ara-C treatment induces preleukemic HSC apoptosis, but does not lead to complete mutant cell clearance, revealing that Ara-C resistant mutant HSC population exists and initiates leukemia. Moreover, our results demonstrate that Ara-C-mediated HSC reduction does not lead to delay in leukemia progression. To the contrary, several parameters show negative long-term effects of Ara-C treatment on AML progression. Caution therefore has to be taken in evaluating of the presence of residual mutant HSC in patients' bone marrow after chemotherapy. This study points to a critical role of therapy-resistant HSCs in leukemia progression or relapse and warrants further studies on their better characterization.