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Deferasirox And ROS Production In Hematopoietic Stem Cells: A New Molecular Mechanism For Mielodysplastic Syndromes Treatment

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Deferasirox (DFX) is an iron chelator used to prevent and treat complications related to transfusional iron overload in myelodisplastic patients. Myelodysplastic syndromes (MDSs) are a group of clonal stem cell disorders characterized by ineffective hematopoiesis and increased risk of development of acute myeloid leukaemia (AML). Most MDS patients present anaemia and this is why they require red blood cell transfusion. Intriguingly, a robust percentage of patients treated with DFX recovers correct hematopoietic stem cell (HSC) differentiation whereas other chelators, like deferoxamine (DFO) did not. Consolidated evidence highlights the importance of redox signalling in the homeostasis of fundamental processes, particularly in controlling the balance between self-renewal and differentiation of stem cells. In this setting, reactive species of oxygen (ROS) would act as secondary messengers, modulating the expression of master transcription factors and regulatory proteins leading or (pre)conditioning stem cells towards differentiation. In the present study we investigated the effect of DFX and DFO on ROS production in hematopoietic stem/progenitor cells (HSPCs) in order to identify a molecular mechanism explaining the differential effect of iron chelators in rescuing altered hematopoiesis.

Human HSPCs were isolated upon informed consent from peripheral blood of G-CSF-treated healthy donors by immuno-selection against the specific markers CD133 and CD34. HSPCs were treated with 100 µM DFX or DFO for 24 hrs. To completely abrogate ROS production, cells were co-incubated with diphenil iodide (DPI) 100µM. Cell viability was determined by trypan blue staining. ROS levels were analyzed by laser scanning confocal microscopy (LSCM) and flow cytometry after the incubation at 37°C for 15 min with the specific probe dichlorodihhyrofluorescein-diacetate (H2DCFDA) 10µM. Sox17 gene expression was evaluated by Realtime PCR, BMI1 protein levels were assessed by western blotting. Data were presented as mean±s.d. and were compared by unpaired Student T-Test; a $p<0.05$ was considered significant.

DFX treatment of HSPCs resulted in a significant up-regulation of ROS levels (p=0.00002 *versus* CTRL) whereas no relevant change was observed following DFO treatment. Importantly, ROS abrogation by DPI reduced their DFX-induced production ($p=0.01$ *versus* DFX). Then, we evaluated the effect of drugs on sox17, a transcription factor up-regulated during lineage differentiation and on BMI1 expression, a regulatory protein maintaining immaturity of HSCs.

DFX treatment caused a significant increase of sox17 transcription levels (p=0.04 *versus* CTRL) and a strong reduction of BMI1 expression ($p=0.02$ *versus* CTRL). Interestingly, all these effects were reverted by DPI co-incubation. Conversely, DFO treatment influenced neither sox17 nor BMI1 expression.

Our results show that DFX treatment influences key factors able to restore the hematopoietic function of HSCs. This effect was seemingly independent on its iron-chelating property but it

was mediated by ROS production which represents therefore a further pharmacological target in MDS treatment.