Name of Sponsor: IRCCS OSPEDALE SAN RAFFAELE - Prof. Fabio Ciceri	**Statement on discontinuation of the study**	
Investigational Product: Plerixafor	**EudraCT No.:** 2011-000973-30	
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Report Date: 17-SEP-2021	**Trial No. / Doc. No.:** AMD-THAL

Title of Trial:	PLERIXAFOR MOBILIZED STEM CELLS AS SOURCE FOR GENE THERAPY OF BETA-THALASSEMIA AMD-THAL
Trial Sites:	NA
Publications:	https://doi.org/10.1182/blood.V122.21.2901.2901
Clinical Phase:	II
Statement on discontinuation of the study:	This study aimed to explore the safety and efficacy of stem cell mobilization using the mobilizing agent Plerixafor (AMD3100, Mozobil®) in six adult patients with transfusion-dependent beta-thalassemia. However, the study was interrupted with the enrollment of only 4 patients due to lack of candidates. Although statistically significant conclusions cannot be drawn, the efficacy and safety data obtained from the 4 patients have been published (https://doi.org/10.1182/blood.V122.21.2901.2901)

420. The Challenge of HSCs Procurement for Gene Therapy of beta-Thalassemia: Exploring Plerixafor as Mobilization Agent

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Successful gene therapy of inherited blood diseases relies on transplantation and engraftment of a significant dose of autologous genetically engineered hematopoietic stem cells (HSCs). Gene therapy trials in young pediatric patients are performed by transplanting CD34+ cells from steady state BM, while in adults G-CSF mobilized peripheral blood cells are the preferred targets. In the context of gene therapy for thalassemia, the choice of HSC source is crucial since intrinsic characteristics of patients (splenomegaly and thrombophilia) dictate caution in the use of G-CSF and prompt investigation of new agents. A phase II clinical protocol exploring the use of Plerixafor as a single mobilizing agent in adult patients affected by transfusion dependent beta-thalassemia was approved (EudraCT 2011-000973-30) and started in 2012. Plerixafor selectively and reversibly antagonizes the binding of SDF-1 to its receptor CXCR4 with subsequent egress of HSCs to the peripheral blood. Aims of our trial were to explore the ability of Plerixafor in inducing safe and effective stem cells mobilization, to characterize mobilized stem/progenitor cells in the BM and peripheral blood and to achieve gene transfer effi ciency in mobilized CD34+ cells at a level comparable to that obtained using steady state BM cells. Four subjects were enrolled and treated by subcutaneously administration of Plerixafor at the single dose of 0.24 mg/kg followed by leukoapheresis. Mobilization of CD34+ cells occurred very rapidly with a peak between 7 to 9 hrs. Only one patient received a second dose (0.40 mg/kg) at 24 hrs after the fi rst one and underwent a second leukoapheretic procedure. Three out of four patients achieved the minimal target cell dose (2 x 10[6] cells/kg) and no severe adverse events occurred. Purifi ed CD34+ cells

from leukoaphereses were analyzed for their biological and functional properties, subpopulations composition and expression profile. In vivo reconstitution potential and lymphomyeloid differentiation were tested following transplantation in NSG mice. We also compared Plerixafor-mobilized peripheral blood cells with CD34+ cells derived from BM pre- and post-Plerixafor treatment. Cells were transduced with GLOBE lentiviral vector, carrying the beta-globin gene, to assess gene transfer effi ciency and transgene expression. The results indicate that cells mobilized by Plerixafor have a primitive phenotype with a high in vivo hematopoietic reconstitution potential and are effi ciently transduced, thus representing a suitable source of target cells for gene therapy.