MULTI CENTRE CONTROLLED STUDY ON THE IMPACT OF STEM CELL DONATION EITHER AFTER MOBILISATION WITH GRANULOCYTE COLONY STIMULATING FACTOR OR BONE MARROW HARVEST ON UNRELATED BONE MARROW DONORS

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End-of-trial Report

Granulocyte–colony-stimulating factor (G-CSF) currently remains the standard mobilizing agent for peripheral blood stem cell (PBSC) donation. The G-CSF mobilization however, may be associated with some side effects such as bone pain which resolve rapidly after administration is discontinued. Concerns were raised that G-CSF might cause genomic instability and act as trigger for development of hematological malignancy. Lymphocytes of G-CSF mobilized donors were found to display epigenetic and genetic changes similar to those observed in leukemia patients. While these effects are transient, the possibility of more durable impact on chromosome integrity of G-CSF treated donors and hence increased leukemia risk has not been evaluated so far.

To assess possible genetic effect of short-term exposure to G-CSF, screening was carried out by fluorescence in situ hybridization (FISH) at interphase and metaphase cell levels for aberrations of chromosomes 7, 8 and 17, the most commonly affected in leukemia. Since our study involved a medicinal product it was conducted as a clinical trial with two arms:

(a) ***Retrospective arm:*** in order to evaluate possible *long-term* genetic effects we investigated 50 peripheral blood stem cell (PBSC) donors 3 to 5 years post peripheral blood stem cell donation after G-CSF mobilization in comparison with 50 Bone Marrow (BM) donors. It was thought important to screen the latter group as temporarily increased levels of endogenous G-CSF are a frequent finding in post bone marrow (BM) donation and may cause similar genetic damage. The BM donors were age and gender matched to the PBSC donors. In addition, a parallel study using the same analytical set-up as the *Retrospective arm* was conducted on a group of 50 healthy subjects who had no prior exposure to G-CSF and had neither donated BM or PBSCs. These subjects served as a negative control group.

The results from the *Retrospective arm* of our study showed lack of any type of abnormalities in both G-SCF treated and BM donors when compared with the control group and with published data. In particular, neither increased levels of single random changes or the presence of clonal chromosome aberrations (i.e. more than 3 cells carrying the same aberration) were observed when 200 interphase and 50 dividing cells from each sample were investigated. Instead, single event random

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chromosome changes were observed in both dividing and quiescent peripheral blood lymphocytes at the same low frequency in all three groups of individuals studied. These data conclusively demonstrate the absence of long term detrimental effect on the chromosome integrity caused by G-SCF administration and bone marrow harvesting alike, that could be linked to occurrence of hematological malignancy.

(b) *Prospective arm*: To assess the *short-term* effect we monitored 50 PBSC donors and collected peripheral blood samples at four different time points: 1) before GCSF admission; 2) on the day of donation together with 1ml apheresis product; 3) 90 days after and finally 4) 180 days after GCSF admission.

The laboratory analysis of the second part of the study, *the prospective arm* has just been completed. The recruitment of the donors started in Septembers 2011 and was successfully finalized in November 2012 with the last Day 180 sample being collected on 25 May 2013. Altogether 50 donors were enrolled, collection of a full set of 5 samples at the four time points as specified was obtained from only 45. The sample collection in the remaining 5 donors was incomplete, short of either day 90 or 180 sample. By end of June 2013, 240 samples in this arm had been collected and processed with chromosome preparations and cell pellets ready for FISH screening. Out of total 737 samples collected in the prospective arm some 40 remained to be analyzed at this point. In the following 8 weeks, in spite of the cessation of our research funding, altogether 125 FISH assays were performed and data from 36,000 metaphase and 140,000 interphase cells were successfully obtained. This marked the completion of the data collection from the prospective arm.

In conclusion, the prospective arm of this study clearly demonstrated lack of clonal chromosomal aberrations in the peripheral blood lymphocytes of healthy donors subjected to G-CSF stimulation at any time point of observation. These results are in agreement with the retrospective arm data (3-5 years post stem cell donation) and conclusively determine absence of both long and short-term effects of G-CSF administration as part of PBSC donation in healthy individuals. We envisage having a draft manuscript summarizing the data from both arms of the study ready for publication by December 2013.

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