Reviewer's report

Title: The aberrant asynchronous replication -- characterizing PHA-stimulated lymphocytes of patients with hematological malignancies -- is erased following stem cell transplantation

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Reviewer: Jose Rueff

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The present paper by Nagler et al. follows a line of research by the same group and extends previous observations in patients with various cancer types.

Major Compulsory Revisions

1. The groups of patients now studied with various haematological malignancies encompass highly heterogeneous conditions, with different aetiological mechanisms and prognosis, requiring also different therapeutic approaches which in the whole can hardly be grouped together to study whatever genomic effect, might it be asynchronous DNA replication or persistent aneuploidies. On what basis can one compare non-Hodgkin Lymphoma with CML, for example? Or CML in blast crisis with CML in chronic phases. And was the non-Hodgkin Lymphoma a B-cells or T-cells lymphoma. Or still, what mutations in BCR/ABL, if any, were found in CML. The same reasoning pertaining to the cellular and molecular characterization of the various conditions could be applied to the other conditions studied. It is thus mandatory to describe in detailed terms what exactly had been studied to attempt any comparisons and possible meaningful conclusions.

2. The few patients studied in total and for each group, with considerable scattering of ages (3-80 years old range) also add extra factors to hamper solid conclusions from the study.

3. It is difficult to agree that asynchronous replication could serve as good biomarker of a successful treatment, might it be an allogenic transplant or otherwise chemotherapy. Firstly, because the study does not supply any clear cut data on any other treatment besides allogenic transplantation that the patients might have done. Secondly, because the patients’ cells studied after transplantation are indeed donors’ cells and thus any different epigenetic effect could only occur if a rejection of the allogenic transplant would take place (or a graft-vs.-host disease).

4. The conclusion that replication asynchrony could be used for long-term follow-up of the patients and as marker of therapeutic success goes beyond the data presented at this stage, unless the authors supply any data on long-term follow-up and the possible relevant value of DNA asynchrony as a marker of effect.
5. The authors make use of the impressive and somehow imprecise concept of ‘cancerous phenotype’ (page 20, line 3) which by all means needs clarification, notably when such a concept is used to explain epigenetic allelic asymmetry. Moreover, the authors do not supply any data on the still elusive mechanism that may lead to asynchrony of replication on peripheral blood cells of patients with a wealth of different types of cancer and this would represent an enormous advance on the understanding of the basis of their observations.

6. In what concerns persistence or recurrence of aneuploidy it is not clear if that effect might have been due to G-CSF treatment of the donors’ cells, and consequently not an effect of the malignancy. In any case, some more data (which is not presented, as mentioned in page 14, lines 11-14) should be supplied, even if that implies additional studies.

7. The Kolmogorov–Smirnov test should have been used in order to verify the normality of the continuous variables (e.g. age) and the Levene test to analyze the homogeneity of variances. The statistical analysis of the homogeneity of age distributions between cancer patients and controls should have been carried out using the Student’s t-test.

Minor Essential Revisions

8. The paper should supply data on the efficiency of hybridization (for example, by measuring hybridization levels in metaphase chromosomes for the four probes used in the study).

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.