Reviewer's report

Title: Phospho-specific flow cytometry identifies aberrant signaling in indolent B-cell lymphoma

Version: 1 Date: 19 June 2012

Reviewer: Stefania Gobessi

Reviewer's report:

In the study by Blix et al., the authors evaluate the basal and induced signaling status in lymphoma B-cells and T-cells present in the biopsies from SLL/CLL and MZL patients, by using the powerful technique of phospho-flow cytometry. In particular, due to the important role of microenvironmental stimuli (antigen encounter, cytokines and cell-cell contacts with other immune and stromal cells present in the lymphoid tissues) in the pathogenesis and maintenance of malignant B-cells, the authors examine the phosphorylation/activation status of several signaling molecules following the engagement of B-cell receptor (BCR), CD40 and cytokine receptors in lymphoma B-cells and in infiltrating T-cells.

In my opinion, this is an interesting paper that fully integrates the biochemical studies we and other groups performed in the recent past, in order to characterize the most important signaling pathways that govern the pathobiology of SLL/CLL and MZL, and to identify aberrant signaling molecules as potential targets for new therapeutic treatments.

Minor Essential Revisions:

1) In the paragraph “Background”, the authors describe the molecular mechanisms of BCR signal transduction. In the sentence “SYK is recruited and phosphorylated by ITAMs……” a conceptual error is present. ITAMs are conserved sequences of four amino acids present in the Ig-alpha/Ig-beta subunits of BCR complex that become phosphorylated following receptor engagement but they do not have kinase activity. Thus, they cannot phosphorylate SYK. The correct sentence could be “Syk is recruited to the tyrosine-phosphorylated immunoreceptor tyrosine-based activation motifs (ITAMs) in the Ig-alpha and Ig-beta chain of the BCR and becomes activated by sequential phosphorylation at conserved tyrosine residues”.

2) In the paragraph “Methods: Activation of Signaling and Fluorescent cell barcoding” the barcoding technique should be explained in more detail. This could facilitate the readers that are still “naïve” in the field of multiplex phospho-flow analysis.

3) I do not understand where and when anti-BCL2 antibody was used.

Discretionary Revisions:
1) Could be helpful if the authors provide a Table that describes available patients’ characteristics (e.g. SLL/CLL patients: gender, age, VH gene status, ZAP-70 expression…).

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**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