Abstract

Background: Chemotherapeutic agents, like topotecan, induce DNA damage through the formation of single-strand breaks (SSBs) that are known to be repaired in cancer cells. Since there is limited information on the detection of SSBs in cancer patients treated with topotecan, we aimed to evaluate γH2AX as a marker for drug efficacy and assess the variation of results using the same phosphate backbone of DNA and leads to the generation of strand breaks. In this study we evaluated γH2AX as a marker for drug efficacy, using antibodies that recognize the phosphorylated S139 epitope.

Objective: To test the variation of results using the same procedure performed at two different laboratories.

Procedure: Cell line (SR and THP1) samples were treated with or without cyclophosphamide and sent to NCI and then stained at Apocell, and then scanned at NCI.

Results: The same samples were also stained at NCI and scanned at Apocell.

Conclusions: We have successfully developed an LSC-based assay to measure γH2AX within CD3+/CD14+ cells in blood samples collected from cancer patients treated with topotecan. Studies correlating the marker to outcome are ongoing. It would be valuable to rapidly stratify patients in clinical trials that involve therapies that induce DNA damage.

References


Table 1. Measurement of γH2AX in CD3+/CD14+ cells from different lymphocyte subpopulations

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<thead>
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<th>Donor</th>
<th>CD3+/CD14+ Cells</th>
<th>CD3+/CD14+ Cells</th>
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γH2AX Detection in Immunomagnetic Bead-Enriched CD3+ and CD14+ Cells

Objective: To determine if immunomagnetic bead isolation procedure could be used to isolate lymphocyte subsets from blood collected into CellSave tubes and if γH2AX expression could be measured from these isolated cells.

Procedure: PBMCs from healthy donors were isolated from blood collected into CellSave tubes and γH2AX induction in cancer patients treated with topotecan. Studies correlating the marker to outcome are ongoing.

Figure 1. Cross-Laboratory validation of the assay. Same pattern in γH2AX expression was observed by laboratories at NCI and at Apocell.

Figure 2. Immuneomagnetic bead capture of specific sub-populations of CD3+ T cells from 2 individuals (A and B).

Figure 3. Images of γH2AX staining, DAPI (nucleus) and merged image of both.