

## *Quantitative Determination of Apoptosis on Leukemic Cells by Infrared Spectroscopy*

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Quantitation of apoptotic cell death in vivo has become an important question for patients with acute leukemia. We describe herein a new analytical method, based on infrared (IR) spectroscopy, to estimate the percentage of apoptotic leukemic cells in two different cell lines (CEM and K562) induced with etoposide (VP-16). As the percentage of apoptosis increases, the dominant protein of the treated cells shifts from  $\beta$ -sheet to unordered coil, the overall lipid content increases and the amount of detectable DNA decreases. These changes can be directly related to the percentage of apoptosis as determined by two standard reference methods: flow cytometry and DNA ladder formation. The correlation between the significant IR spectral changes that in the DNA bands and the percentage of apoptotic leukemic cells in the two cell lines was optimal up to 24 h after etoposide treatment ( $r=0.99$  for CEM cells and  $r=0.96$  for K562 cells). Furthermore, IR spectroscopy is able to detect apoptotic changes in these cells already after 4 h treatment with VP-16, compared to flow cytometry which needs 6 h to observe significant changes. Our study suggests that IR spectroscopy may have potential clinical utility for the early, fast and reagent free assessment of chemotherapeutic efficacy in patients with leukemia.