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Development of a bioassay to monitor circulating plasma Ki-67

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Proliferation a key feature of tumor progression is measured via immuno-histochemical staining for the nuclear antigen Ki-67 (also known as MKI67) [1]. The complete primary structure of the protein was published in 1993. Two protein isoforms are derived by alternative splicing of an mRNA precursor which is transcribed from a single gene. Both isoforms 320 and 359 kD in size respectively contain a large central region with 16 repetitive elements (Ki-67 repeats). The genomic sequence of Ki-67 protein spans almost 30 kb [2].

During all active phases of the cell cycle, (G1, S, G2, and mitosis), Ki-67 protein is present but is absent from resting cells (G0). It is an ideal biomarker therefore to assess the growth fraction of a given cell population [3]. Although the functional significance of Ki-67 remains to be fully elucidated, it has valuable prognostic utility in assessing solid tumors and hematological malignancies [4,5]. Presently bioassays for Ki-67 require tumor material. Detection in plasma would be advantageous as this would circumvent the need for costly and invasive biopsy procedures.

In this issue Bruey et al., describe an electrochemical based enzyme immunoassay which detects Ki-67 in plasma [6]. This is the first report of an assay which monitors circulating levels of this protein. The Ki-67 assay employs electrochemiluminescence (ECL)-based technology from Meso Scale Discovery (MSD), Gaithersburg, MD. This approach couples enzyme-linked immunosorbent assays (ELISAs) with ECL detection of protein binding on patterned arrays. In their study they explored the relationship between circulating Ki-67 (cKi-67) in acute lymphoblastic leukemia (ALL), a hematologic disease characterized by a poor long-term survival rate in adults.

Quantification of Ki-67 levels has become an important aspect in monitoring the progress of human neoplasms, notably prostate and breast carcinomas. In breast cancer elevated Ki-67 expression indicates poor disease prognosis and a favorable clinical response to chemotherapy [7–10]. Additionally high levels of proliferation and elevated levels of Ki-67 have been reported in patients with ALL, Burkitt's lymphoma and non-Hodgkin's lymphoma [11–14].

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Non-Hodgkin's lymphomas (NHL) are malignant tumors of the lymphoid system. Several studies to date have investigated the clinical utility of Ki-67 as marker for predicting survival in NHL (12–14). The Ki-67-associated proliferative index, clinical outcome, and statistical correlations were independently assessed by Miller *et al.*, on thin tissue sections from previously untreated patients with advanced stages of intermediate- and high-grade NHL histologies (12). A proliferative index (PI) of 80% or greater identified a group of patients (18%) with a poor outcome. Survival in these patients with a high Ki-67 PI was reduced considerably when compared with those with a low PI. One-year survival estimates were reported as 82% (low PI) versus 18% (high PI). Szczuraszek *et al.*, in a separate study evaluated the prognostic and predictive value of Ki-67 expression in NHL (13). They utilized an immunohistochemical approach to determine Ki-67 expression in archival paraffin-embedded sections sampled from 56 previously untreated patients with diagnosed primary NHL. In a similar manner Ki-67 expression was noted in 75% of the tumor cases and shorter survival times were observed in the group manifesting higher Ki-67 indices. Interestingly no significant correlation was detected between Ki-67 expression and the clinical or pathological parameters of the patients. In a recent study Broyde *et al.*, evaluated the role and prognostic significance of the Ki-67 PI in NHL (14). They monitored the Ki-67 PI in 319 patients with newly-diagnosed non-Hodgkin's lymphoma. In this population, 268 patients were observed with a Ki-67 PI that was correlated with clinical course and outcome. The mean Ki-67 PI ranged from 26.6% in indolent lymphomas to 97.6% in very aggressive lymphomas. Their findings suggested that the mean Ki-67 PI differed by lymphoma type. A cut-off value of 45% was recommended to help differentiate indolent from aggressive disease. In diffuse large B-cell lymphoma, a cut-off value of 70% can distinguish patients with a good and bad prognosis when combined with other prognostic factors.

This novel cKi-67 assay described by Bruey and colleagues is highly reproducible with a low coefficient of variation (CV<10%) and is sufficiently sensitive to detect the low levels of cKi-67 present in healthy individuals. In their study Bruey *et al.*, demonstrated that Ki-67 protein is present in the plasma of both normal control subjects and patients with ALL. This data correlated with an earlier report on ALL functional markers where cellular Ki-67 was elevated. Circulating Ki-67 is additionally a promising pharmacodynamics marker for evaluating the efficacy of chemotherapy therapy regimes. Analysis of tissue sections have revealed that sulindac sulfide inhibits the proliferation of colon cancer cells by reducing the expression of Ki-67 and another proliferation marker PCNA [15]. A quantitative cKi-67 assay would circumvent biopsy procedures and permit direct assessment of drug efficacy in clinical trials. Finally Ki-67 is also a potential drug target for pharmaceutical oncology programs. Zheng *et al.*, have shown recently that renal cancer cell growth can be inhibited both *in vitro* and *in vivo* with oncolytic adenovirus expressing a short hairpin RNA targeting Ki-67 mRNA [16]. The cKi-67 assay developed by Bruey and colleagues may have long term utility in monitoring the response to this therapeutic intervention.

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