156

Determination of minimal residual disease in solid tumor patients

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Using monoclonal antibodies to epithelial cytokeratins (CK), individual carcinoma cells can be detected on cytologic preparations at frequencies of 10^{-5} to 10^{-6}. Prospective clinical studies have indicated that the presence of these immunostained cells in bone marrow and lymph nodes of patients without clinical or histopathological signs of metastases is prognostically relevant. In addition to immunocytochemistry, new molecular detection methods based on the amplification of a marker mRNA species by the polymerase chain reaction technique have been developed. The current assays need to be standardized and may then be used to improve tumor staging with potential consequences for adjuvant therapy. Another promising clinical application is monitoring the response of micrometastatic cells to adjuvant therapies which, at present, can only be assessed retrospectively after an extended period of clinical follow-up. The extremely low frequency of bone marrow tumor cells greatly hampers approaches to obtain more specific information on their biological properties. The tools established in the recent years (e.g., micrometastatic cell lines, single cell (RT)PCR, multiple labeling, and FISH) allow to obtain insights into the biology of disseminated tumor cells which may help to design new strategies to detect and eliminate minimal residual cancer. The available data indicate that micrometastatic cells represent a selected population of cancer cells which, however, still express a considerable degree of heterogeneity.

157

Genomic surrogates for cancer drug development

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Surrogate endpoints which determine the molecular efficacy of clinical entity are essential for the early phase of cancer drug development. The ultimate endpoint - survival – is simply too sl prioritise the large number of novel candidate molecules. Surro include the release of tumour DNA fragments into the serum, th quantitation of novel tumour markers and the identification of downstream effects of tumour growth delay such as apoptosis, the interaction with local blood vessels. Genetic indicator syste being developed for direct intra tumoral injection to act as repo constructs for drug activity. Genomics and proteomics offer pr areas in the search for surrogate endpoints.

158

IMMUNOLOGIC ENDPOINTS IN VACCINE DEVELOPMENT.

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We can detect tumor specific immunity after active immunization, yet there are no standard immunologic monitoring methods that will allow comparison of vaccine strategies between labs or even allow accurate assessment of the immunogenicity of a particular vaccine. A major problem now facing tumor immunologists is the standardization and development of reproducible and clinical grade immunologic assays to determine the magnitude of tumor specific immune responses generated in the context of clinical trials of cancer vaccines. New technologies based on the function of antigen specific T cells, the recognition of peptide-MHC complexes, and the interaction of T helper cells with B cell augmentation have allowed the development of highly quantitative methods of T cell and antibody analysis based on antigen specific recognition and function. Clinical development, however, of ELIspot, flow cytometry for intercellular cytokine staining, MHC tetramers, and class and isotype quantitative antibody assays requires different experimental tactics than the development of a laboratory based tool. Accuracy, precision, sensitivity, specificity, and reliability of the technology must be determined. Whereas assay validation is straightforward, in many respects, for serologic studies, validations of T cell based techniques require the expertise of both molecular and cellular immunologists in generating standards for analysis and novel design of validation approaches. Finally, clinical development of techniques and troubleshooting technical issues must take place in well defined antigen systems and principles demonstrated applied to cancer antigen models.

159

CLINICAL RELEVANCE OF CYTOKINE SECRETION IN NON-SMALL CELL LUNG CANCER

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Immunosuppressive molecules are not only produced by immunocompetent cells, but also by a whole variety of tumor cells. Therefore, escape from immune surveillance mediated by tumor-derived immunosuppressive factors may be a very important step in the development and clinical course of malignant disease. Moreover, an impairment of immune defense and a suppression of cytokine secretion capacity in tumor patients may have clinical relevance and influence survival.

Increased production of immunosuppressive IL-10 by non small cell lung cancer (NSCLC) and increased plasma IL-10 concentrations in NSCLC-patients have recently been correlated to reduced survival. We earlier demonstrated suppression of IL-2 secretion also in NSCLC-patients. Analysis of the influence of IL-2 suppression on survival in NSCLC-patients has recently been carried out. In addition, the influence of IL-10 on IL-2 secretion in whole blood cell cultures was investigated.

Concentrations of IL-2 in the supernatant of whole blood cell cultures from 90 NSCLC-patients were measured by ELISA at the time of diagnosis. The correlation of the IL-2-concentration to survival was analysed by using crit-level, the Kaplan-Meier method, the log-rank test and the cox-regression model.

IL-2 secretion capacity at the time of diagnosis significantly influences survival in NSCLC-patients. With a cut-off value for IL-2 of 1100 pg/ml, survival was significantly different between the group with high IL-2 (n=38, 29 failures) and the group with low IL-2 (n=52, 47 failures) with a p-value of 0.014 in the whole patient group. With an observation time from 0.5 to 96.4 months, 5-year-survival was 24.5% and 6.2% in the group with high and low IL-2, respectively. In the subgroup of surgically treated patients (n=33), survival was different with a p-value of 0.011. In 14 patients with surgical resection of the tumor and high IL-2 at diagnosis and 19 patients with surgical resection, but low IL-2 at diagnosis, median survival was 86,2mts and 11.3mts, respectively. Moreover, secretion of IL-2 measured by ELISA was inhibited in a dose dependent manner upon addition of IL-10 in whole blood cell cultures from normal individuals. Thus, suppression of IL-2 secretion in NSCLC-patients may be mediated by tumor-derived IL-10.