Symposia

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Molecular pathology of breast carcinomas

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The biologic and pathologic features of breast carcinomas have beer extensively studied with the goal of identifying markers that might accurately predict the clinical outcome, and or the response to anti cancer treatment of breast cancer patients. Unfortunately, the usefulness of biologic information in tumor management remains highly controversial. Indeed, the Clinical Practice Guidelines for the use of tumor markers in breast carcinoma, proposed in late 1997 by the American Society of Clinical Oncology, conclude that data are still insufficient to recommend the use of biologic markers for prognosis. When considered singly, parameters such as c-erbB-2 overexpression, p53 alteration, and hormone receptor expression appear to yield relevant information about disease progression and/or response to therapy but not enough reliable for the clinical use. For their use in association, however difficulties in interpretation arise whenever parameters of good and poor prognosis are found in the same tumor. Furthermore, many prognostic variables identified in univariate analyses as relevant for breast carcinoma lose their prognostic significance upon multivariate analyses, indicating their association to other factors with a higher prognostic power. To investigate the association between variables, we studied a large retrospective series of breast carcinoma patients using the multiple correspondence analysis, which enables graphical examination of associations among categories. By this analysis, two distinct subsets of breast carcinomas were identified. These two subsets display different clinical outcomes and different responses to chemotherapy.

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Progress in the molecular diagnostics of lymphoma

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Molecular diagnostics find two main applications in the management of lymphoma: as adjuncts to morphology and immunohistology in diagnosis, and as means to detect low level residual disease despite clinical remission.

In diagnosis immunoglobulin and T-cell receptor gene rearrangements have long been used to determine B- or T-cell lineage. With the REAL classification it has become easier to associate molecular findings with specific illnesses. CD30+ anaplastic large cell lymphoma associated with the t(2;5) carries a favourable prognosis, whilst rearrangements involving c-Myc at 8q24 suggest a more aggressive tumour type, frequently Burkitt's lymphoma. Subdivision of diffuse large cell lymphomas according to the presence of Bcl-6 rearrangements distinguishes those with more favourable prognosis. To date few studies have demonstrated prognostic significance from molecular findings alone. In most diseases it is the patterns of protein expression rather than genetic alteration which provide the best prognostic information.

Extensive studies of consistent chromosomal rearrangements have yielded a variety of targets for detection of small numbers of lymphoma cells at the molecular level, mainly using the PCR. The rearranged immunoglobulin or T-cell receptor genes provide widely applicable targets, whilst specific rearrangements such as those involving Bcl-1, Bcl-2, Bcl-6, Bcl-10, c-Myc or ALK/NPM are more sensitive but constrained by the illness concerned.

The t(14;18) translocation of follicular lymphoma has been most studied but even here the association between the detection of Bcl-2/IgH and the presence of active lymphoma is not straightforward. Patients with prolonged remissions may still have the translocation detectable without developing recurrent disease, the PCR gives positive results in a small proportion of healthy donors and PCR studies of the blood become negative in some patients in whom only a partial clinical response is achieved. There is nonetheless good evidence to suggest that therapy which makes the translocation undetectable is better than therapy which does not.

Newer methodologies hold considerable promise for molecular diagnostics: dense array hybridisation will permit the analysis of complex patterns of gene expression as an aid to diagnosis. Real-time quantitative PCR will allow better use to be made of information about minimal residual disease. Comparative Genomic Hybridisation will allow the identification of new targets for these studies. An important next step along the way to using these techniques for guiding therapy will be the development of standard methodologies to allow the results of different centres to be compared.

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New prognostic and predictive factors in colorectal cancer

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The improved pre-, per-, and postoperative imaging techniques presently used in colorectal cancer have as yet not made tumour staging good enough for safe selection of patients to additional therapy and appropriate surveillance regimes. During the latest two decades much effort has been spent on developing methods to identify minimal residual disease (MRD). Predominantly, tumour cells in the bone marrow have been in focus as the major indicator organ, but also tumour cells at other sites of much interest, that is the macroscopically negative regional lymph nodes of the resected specimen, the peritoneal cavity, and the peripheral blood, have been investigated. Only a few out of several studies using immunohistochemistry with antibodies mainly against cytokeratins or CEA in single or a few sections from dissected regional lymph nodes have been able to show any prognostic impact of detected single or small clusters of tumour cells. Tumour cell enrichment by gradient centrifugation followed by positive or negative cell separation using magnetic beads targeted with antibodies against tumour-associated antigens present on the cell surface, in the cytoplasm, or in the nucleus, has improved the possibility to detect few turnout cells among a huge number of benign cells in the bone marrow, blood, or peritoneal cavity. Immunocytochemistry on cytospins using double-staining techniques or multiparameter flow-cytometry has made it possible to further characterize the fenotype. There are studies reporting on the prognostic significance of tumour cells predominantly in the bone marrow. Common antibodies, for example the 17–1A, anti-cytokeratins, and anti-CEA antibodies, are only tumour-associated and not tumour-specific, leading to the occurrence of some false positives in benign control samples, why results must be interpreted with caution. More lately, the use of molecular-based techniques, such as FISH and RT-PCR, have been developed for true diagnosis of MRD. Besides using primers for CEA and cytokeratins, the RT-PCR technique has shown most promising results when using primers for Ki-ras and p53.

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New prognostic and predictive factors in ovarian cancer

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In ovarian cancer, only a few features such as residual tumor, stage, histologic type and grade, performance status and age have gained general acceptance as factors of independent prognostic value on the basis of multivariate analyses with some of these factors, the discriminating power of which is restricted to an already partly determined patient situation such as histologic grade in early stage and residual tumor in advanced stage disease. The only clinically established factor predictive for response to first line therapy is the early fall in CA-125. Beyond patient- and tumor-related factors, the quality of treatment defined as center factor or the degree of specialization of the surgeon can be of additional prognostic relevance. No established factor exists so far to select primary standard chemotherapy. Chemosensitivity testing based choice of primary therapy has yet to demonstrate its superiority over standard drug choice. Response to subsequent chemotherapy in platinum pretreated patients is significantly dependent on tumor burden and histology only, but not on time from last treatment. The

next "generation" of prognostic factors has been characterized by better measurability and reproducibility and therefore a higher degree of objectivity as e.g. morphometric criteria such as mean nuclear area (MNA), volume percentage of epithelium (VPE) or proliferation criteria such as mitotic index, DNA-index or S-phase fraction. Representants of the latter have proven to be of independent prognostic value when simultaneously tested together with new molecular targets. No definitive positioning and sometimes even contradictory data are available yet regarding the new prognostic markers: anemia, thrombocytosis, alpha-catenin, collagen IV, VEGF, tumor vascularity, IL-12, IL-6, CSF-1, PgR, CASA, sialyl-TN, p53, p21, CD 44v6, Bcl-2, c-erb B-2, p 27 KIP1, HSP 27, K-ras mutants, MCP-1, PDGF-alpha, uPA, uPAR, PAI-1, PAI-2 or clonogenic growth. Factors predictive for therapy have not been elaborated more conclusively as yet and comprise the following: chemosensitivity in vitro, p53, Bcl-2, c-erb B-2, INT-2, P-gp, MRP, LRP, excision repair, laminin expression, GSTpi, BAX and nm23. A critical appraisal with regard to the actual status of the single factors and their putative interrelationship discriminating prognosis and prediction will be presented.

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Prognostic implications of tumor cell infiltration of the hematopoietic system in pediatric and adult tumor patients

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In order to create a more individualized therapy, worldwide efforts exist to better define biological risk groups. Circulating tumor cells in the hematopoietic system could serve as important prognostic markers, however, the data available so far are controversial. This fact can be explained by biological differences between the individual tumor entities but could also be based on methodical problems. In carcinoma patients, the presence of tumor cells in the bone marrow (BM) and peripheral blood (PB) at diagnosis is believed to reflect a worse prognosis in patients with "localized" disease as compared to patients without BM involvement. Moreover, the prognostic effect of the dynamics of BM or PB clearing during the course of the disease was demonstrated for neuroblastoma and ALL patients by different reports. In order to circumvent diagnostic errors or problems caused by fluctuations of the mRNA or protein expression of the tumor cells, a method combining the detection of tumor-specific immunological and DNA aberrations was developed allowing the visualization of tumor cells (e.g. neuroblastomas, Ewing tumors and breast carcinomas) in a hematopoietic surrounding. In addition, this system allows for the first time to exactly quantify the number of infiltrating tumor cells. The sensitivity of this method is set by the cells available for analysis, thus allowing the unambiguous identification of extremely low tumor cell infiltrates (e.g. 1 tumor cell in 107 MNCs). In stage 4 NB patients with genetically aggressive tumors, we observed that the delayed tumor cell clearance of the bone marrow indicates a more serious development of the disease. Rapid bone marrow clearance seems to be associated with a decreased risk of death (RR = 0.09; 95% CI: 0.008–1.068). Thus, this new method allows both, an accurate diagnosis, quantification and functional characterization of low tumor cell infiltrates in the hematopoietic system besides offering an ideal way to monitor the response of the tumor cells to cytotoxic treatment.

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New prognostic factors in childhood cancers

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Cytogenetic and molecular analysis of childhood cancers has lead to the identification of a number of genetic markers which are becoming instrumental for the management of patients. In Ewing tumour (ET), the presence of specific fusion genes between EWS and various members of the Ets family constitutes a tumour specific marker which can be used for the diagnosis of ET. Depending on the type of fusion transcript which is observed in the tumours, authors have suggested that this marker could also have a prognostic significance, the more frequent EWS-FLI-1 type 1 fusion being of better prognosis than other fusion types. The extreme sensitivity and specificity of the detection of this gene fusion has enabled to develop assays for a better evaluation of minimal metastatic disease in ET. Indeed, preliminary results indicate that micrometastatic patients with otherwise localized tumours share the same unfavourable outcome than patients with clinically detectable metastasis. In alveolar rhabmyosarcoma, a specific fusion between the PAX3 or 7 gene with FKHR is observed and similar assays for diagnosis and detection of micrometastasis are currently being developed. In neuroblastoma, the N-myc amplification, the loss of 1p chromosome fragment and the over-representation of 17q, constitute three genetic alteration which are frequently associated, and which have been shown to distinguish a particularly aggressive group of neuroblastomas. Finally, the clinical significance of mutations of the hSNF5/INI1 gene, a gene recently shown to be the target of loss-of-function mutations in malignant rhadboid tumours, among paediatric cancer will be discussed.

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High dose chemotherapy with hematopoietic stem cell support (HDCT) in germ cell cancer (GCC)

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Background: Even after the introduction of dose-intensified and drug-alternating cisplatin based conventional chemotherapy (CT), the 5 year survival rates for patients (pts) with intermediate and poor prognosis GCC remain 70% and 45%, respectively. In pts failing initial cisplatin based CT, conventional salvage treatment leads to a 30% long-term survival rate. Further dose intensification of the CT by HDCT has been introduced as a new treatment option for GC. In most schedules HDCT is applied by 1, 2 or 3 high dose cycles after 1 or 2 conventional courses of CT.

Results: In GCC HDCT has been evaluated as part of the primary treatment in pts with poor prognosis (risk assessment based on initial disease manifestations or on insufficient early decline in tumor markers) and in pts failing primary CT. Based on phase II studies long-term survival rates are 70–80% in pts with poor prognosis who receive HDCT as part of their primary treatment. In pts with relapsing/progressive disease long-term survival rates after HDCT are 0–50%, dependent on prognostic factors (degree of cisplatin-responsiveness, metastatic burden). In experienced institutions the overall toxic death rate is <5%. Currently ongoing phase III studies aim to confirm the above promising phase II results of HDCT (EORTC 30974, US Intergroup study, European study IT94).

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High-dose chemotherapy for solid tumors – The Spanish experience

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The SOLTI Group (Spanish Cooperative Group for the Study of Intensive Chemotherapy Treatment in Solid Tumors) was created in 1993. Originally comprising four centers, today the Group has eleven participating hospitals. At the time of its inception, the Group initiated five confirmatory studies in high-risk breast cancer patients using conventional high-dose chemotherapy regimen with peripheral blood progenitor cell (PBPC) support. Three of this studies have now been concluded and the remaining are due to the end of this year. The Group has already embarked on other new studies and other are being proposed for breast cancer and other solid tumors. At the end of 1998, 8 studies are ongoing and a total of 666 patients were enrolled.

Ongoing Studies: Patients with more than four positive and less than ten axillary nodes were included since april 1997 in a randomized *study 9606* designed to evaluate the use of adjuvant conventional accelerated doses of chemotherapy with or without high-dose as consolidation. Patients were allocated to receive four two-weekly cycles of epirubicin 120 mg/m^2 and cyclophosphamide 1 g/m^2 supported by G-CSF, followed or not by STAMP-V. Patients with 10 or more axillary lymph-nodes positives were included in *protocol 9701* replacing the original 9301 closed on April 1998. On this protocol patients receive two courses of doxorubicin, 80 mg/m^2 followed by two courses of paclitaxel 200 mg/m^2 every two weeks supported with GCSF, followed by one course of cyclophosphamide, 3 g/m^2 and paclitaxel 200 mg/m^2 with GCSF for stem-cell mobilization. After recovery patients receive STAMP-V consolidation.

The *Study 9608* investigate the use of paclitaxel given in weekly schedule at 80 mg/m^2 in patients relapsing after high-dose adjuvant treatment Forty patients were included and in a preliminary evaluation clone on the first 28 measurable patients there were 8 CR and 11 PR (68%) with an median of time to progression of 10 months

Studies 9301 and 9302: Preliminary data from this two studies were presented at the ASCO meeting this year as a poster in one joint analysis from 235 patients included between 1994 and 1996 (2); 168 breast cancer patients with 10 or more affected axillary lymph nodes after surgery (study 9301:group 1) and 37 locally advanced tumors with 4 or more N+ (group 2) or inflammatory breast cancer (30 patients) with chemo-sensitive disease