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Clinical consequences of node negative being positive in breast cancer

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Axillary lymph node metastases of breast cancer of the same side are the most significant prognostic indicator for patients with operable disease, yet the clinical relevance of micrometastases in these lymph nodes is uncertain. Previous studies indicated that axillary lymph node metastases less than 2 mm in diameter are associated with a favorable outcome compared with the node-positive, and with similar outcome compared with node-negative disease. In contrast, some retrospective studies on patients primarily identified as having node-negative disease and subsequently found to have axillary micrometastases, showed an inreased recurrence rate in the latter compared with "true" node-negative status. The prognostic value of micrometastatic disease in sentinel lymph node mapping, of the size of micrometastases (isolated tumor cells or larger) and of the application of highly sensitive immunohistochemical staining, is presently unclear. We retrospectively evaluated adjuvant treatment recommendations on 4195 consecutive patients with first diagnosis of invasive breast cancer (any pT, pN0-N1 and M0), referred to the Division of Medical Oncology after performed surgery at the European Institute of Oncology from April 1997 to September 2002, to evaluate if treatment decision was influenced by the degree of nodal involvement. A total of 3076 patients were classified pN0 (or pNSent neg), 477 had pN1a, and 642 pN1≥bi (AJCC TNM 5th edition). Patients with pN1≥bi disease (compared with patients with micrometastatic and node-negative disease) were prescribed more anthracycline containing chemotherapy (52.6% vs 35.7% vs 7.2% respectively, p<.001) and were less likely to receive endocrine therapy alone (22.4% vs 39.5% vs 55% respectively, p <.001). Axillary lymph node micrometastasis in breast cancer remains a subject for research. Treatment recommendations for patient care should take into consideration estimation of the degree of risk together with assessment of endocrine responsiveness of the tumor and patient's preferences.

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Minimal tumour cell involvement in lymph nodes in GI-and NSCL cancer

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The incidence of local relapse after complete (R 0) resection of solid tumors is largely determined by the skill of the surgeon, whereas distant disease is due to tumor biology. The presence of individual disseminated tumor cells - e.g., in bone marrow and lymph nodes as indicator organs - can be detected by sensitive immunocytochemical and molecular methods and is increasingly considered as clinically relevant and independent prognostic factor. The intention of an adjuvant therapy is the elimination of such occult tumor cells before these cells can establish clinically overt metastases. Therefore an early detection of such micrometastatic cells could identify a group of patients with a high risk of tumor relapse, who might benefit from such therapeutic regimen. Compared to solid metastases, isolated micrometastatic tumor cells are appropriate targets for intravenously applied anti-cancer therapeutics because they are easily accessible for macromolecules and immunologic effector cells. The majority of these tumor cells appear to be nonproliferating, which might explain the extended latency period ("dormancy") between their primary diagnosis and the occurrence of a subsequent metastatic relapse in some tumor entities. Furthermore this "dormancy" might be an explanation for the failure of standard antiproliferative adjuvant chemotherapy. Adjuvant therapeutic strategies aimed at both quiescent and proliferative tumor cells are therefore of increasing interest. In this paper the current state of research in the field of minimal tumor cell dissemination in patients with solid epithelial tumors is discussed.

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Can we trust the sentinel lymph node in melanoma?

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Background: The aim of the study was to determine the reliability of sentinel node biopsy in melanoma.

Methods: Between December 1993 and October 2002, 250 patients with cutaneous melanoma were studied prospectively. Preoperative lymphoscintigraphy was performed after injection of a mean dose of 65.6MBq ^{99m}Tc-nanocolloid (Amersham Cygne, Eindhoven) intradermally around the primary lesion site in a volume of 0.3 ml. The sentinel node was surgically identified with the aid of 1.0ml patent blue dye (Patent Blue, Laboratoire Guerbet, Aulnay-sous-Bois) and a gamma-ray detection probe (Neoprobe 2000, Hamburg).

Results: Lymphoscintigraphic visualization and surgical identification were 100%. In 60 patients (24%), one or more sentinel nodes were tumour-positive. The patients were followed for recurrent disease for a median duration of 72 months (range 13 – 104 months). A total of 105 recurrences were seen in 59 patients (24%). In seven patients, the first recurrence was seen in the lymph node basin from which a tumour-free sentinel node had been removed earlier. The false negative rate was thus 10%. In-transit metastases in sentinel node negative and sentinel node positive patients were seen in thirteen (7%) and fourteen (23%) patients respectively. The five-year overall survival rates for patients with a tumour-negative or tumour-positive sentinel node were 89% and 64% respectively (p<0.001).

Conclusions: The reliability of lymphatic mapping in determining the tumour-status of the basin concerned is limited (sensitivity 90%). The incidence of in transit metastases in sentinel node positive patients is high (23%). These unfavourable factors have to be weighed against the reliable prognostic information and potential survival benefit of the early removal of nodal metastases.

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Micrometastases in lymph nodes in urological tumours

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Using monoclonal antibodies to epithelial cytokeratins (CK) or tumour-associated cell membrane glycoproteins, individual carcinoma cells derived from various types of solid tumours (including urological tumours) can be detected on cytologic preparations at frequencies of 10^{-4} to 10^{-6}. These assays may be used to improve tumour staging with potential consequences for adjuvant therapy. Some clinical studies have suggested that the presence of these immunostained cells in bone marrow and lymph nodes of patients without clinical or histopathological signs of metastases might be prognostically relevant, while other studies failed to do so (for review, see Pantel et al, J. Natl. Cancer Inst. 91: 1113-24, 1999; Mueller & Pantel, Am. J. Cancer 2:77-86, 2003). In addition to immunocytochemistry, new molecular detection methods based on the amplification of a marker mRNA species by the polymerase chain reaction (PCR) technique have been developed. Although this technique is in principle more sensitive than immunocytochemistry, their specificity is lower due the illegitimate low level expression of the marker transcript in the surrounding leukocytes. This can lead to false-positive findings, as shown by the analysis of normal lymphoid and hematopoietic tissue from noncarcinoma control patients. In our hands, only PSA mRNA showed a sufficient specificity as RT-PCR marker (Zippelius et al, J. Clin. Oncol. 15:2701-08, 1997). However, the suitability of PSA as marker is limited by downregulation of PSA expression in prostate tumour cells, which can lead to false-negative findings. Besides tumour biology, variations in the PCR assays may lead to substantial differences in the reported rates of micrometastases in lymph nodes and other organs such as blood or bone marrow (Zippelius et al., Clin. Cancer Res. 6: 2741-50, 2000). This may explain at least in part the discrepant results on the clinical significance of micrometastasis in urological tumours (and other tumour entities as well). The low number of micrometastatic tumor cells hampers approaches to obtain information on their biological properties, which might help to identify new therapeutic targets. The tools established in our laboratory (e.g., micrometastatic cell lines, single cell (RT)PCR, multiple labeling, and FISH) allow to obtain further insights into the phenotype and genotype of micrometastases at the single cell level (e.g., Solagoklu et al., PNAS 99: 2246-51, 2002). In conclusion, there is an urgent need for the development of standardized protocols that can then be used in large scale clinical trials to determine whether nodal micrometastases really matters in urological tumours. Moreover, the identification of the molecular determinants of micrometastasis may help to design new strategies to detect and eliminate minimal residual cancer.

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The importance and validation of molecular imaging

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Molecular imaging has its roots in molecular biology and cell biology as well

as in imaging technology. Molecular-genetic imaging provides visualization in space and time of normal as well as abnormal cellular processes at a molecular or genetic level. Three different noninvasive, in vivo imaging technologies have developed more or less in parallel: 1) magnetic resonance imaging; 2) nuclear imaging (PET and gamma camera); 3) optical imaging of small animals. However, it is the convergence of these disciplines that is at the heart of this success story; it is the wellspring for further advances and has provided the opportunity to address new research questions. Three different imaging strategies are described: 1) "direct molecular imaging", whereby the resultant image of probe localization and magnitude (image intensity) is directly related to its interaction with the target molecule. "Indirect molecular imaging" is a little more complex in that it may involve multiple components. One example of indirect imaging is "reporter imaging", which involves a complimentary "reporter gene" and a "reporter probe". The "reporter gene" product can be an enzyme that converts a "reporter probe" to a metabolite that is selectively trapped within transduced cells. Alternatively, the reporter gene product can be a receptor or transporter that "irreversibly traps" the probe in transduced cells. Indirect reporter imaging paradigms are widely used in molecular imaging and will be discussed in greater detail. "Surrogate" imaging can be used to reflect down-stream effects of one or more endogenous molecular/genetic processes. This latter approach is particularly attractive for potential translation into clinical studies in the near-term, because established radiopharmaceuticals and imaging paradigms that are already in the clinic are used. The development of versatile and sensitive imaging paradigms that do not require tissue samples will be of considerable value and can be used for monitoring molecular-genetic and cellular processes in animal models of human disease, as well as for studies in human subjects in the future. Non-invasive imaging of molecular-genetic and cellular processes will compliment established ex vivo molecular-biological assays that require tissue sampling, and would provide a spatial as well as a temporal dimension to our understanding of various diseases. For a recent review, see: Blasberg RG, Tjuvajev JG. J Clin Invest. 111(11):1620-9, 2003.

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Developments in MRI and MRS for biological/functional imaging

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Magnetic Resonance Imaging (MRI), often combined with i.v. contrast agents, is widely used in anticancer drug trials. Recently, "functional MRI" methods have been developed and this term is now applied to several very different techniques. In oncology, the main functional technique is Dynamic Contrast Enhanced MRI (DCE-MRI), in which blood flow and the permeability of the tumour neovasculature are assessed by monitoring the pharmacokinetics of standard Gd-based contrast agents. To obtain physiologically-meaningful parameters (e.g. Tofts' K_{trans}) one must deconvolve the highly non-linear arterial input function (AIF) from the tumour uptake curve. Measuring the AIF is difficult in the rodent tumour models that are used both for laboratory evaluation of antivascular drugs and for developing methods for monitoring human trials, mainly because of the small blood vessels. We will report a novel method for obtaining an AIF from the rat-tail vessels during the acquisition of a tumour uptake curve, allowing calculation of K_{trans} and other parameters.

Magnetic Resonance Spectroscopy (MRS), uniquely, gives non-invasive measurements of the chemical constituents of body tissues. It has been slow to achieve acceptance in routine medicine, but modern instruments now allow an MR spectrum to be taken in a few minutes during an MRI examination. ^{1}H MRS of the brain is the most extensively developed method, and brain tumours have been found to have reproducibly different spectra that permit both diagnosis and grading. However, few radiologists or oncologists have the skills required to interpret such spectra. The EU **INTERPRET** project (http://carbon.uab.es/INTERPRET/), a four-country collaboration led by Prof Carles Arus, has developed a Decision Support System (DSS) to enable clinicians to diagnose and grade brain tumours. It compares each unknown spectrum with a database of several hundred spectra from tumours and other intracerebral lesions, in each of which the diagnosis has been carefully validated. As well as performing computer-based pattern recognition analysis on an unknown spectrum, the DSS allows the operator to review difficult or ambiguous spectra by studying their associated images and clinical data in comparison with any cases in the database.

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Prioritising PK/PD endpoints for molecular imaging

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Studies of pharmacokinetics (PK, what the body does to the drug) and pharmacodynamics (PD, what the drug does to the body) are an essential component of drug discovery and development. Defining the precise relationship between PK and PD is critical. It is especially important to establish a pharmacological 'audit trail' that links all of the essential parameters of drug action, from the molecular target to the clinical effects. The pharmacological audit trail allows us to answer two critical questions: 1) How much gets there? and 2) What does it do? During drug discovery, it is essential that PK/PD properties are optimised, so that the best compound can be selected for clinical development. Furthermore, as part of contemporary mechanistic, hypothesis-testing clinical trials, the pharmacological PK/PD audit trail facilitates rational decision making. However PK/PD endpoints frequently require invasive sampling of body fluids and tissues. Non-invasive molecular measurements, eg using MRS/MRI or PET, are therefore very attractive. This presentation will highlight the need for PK/PD endpoints in modern drug design and development. The value of PK/PD endpoints will be illustrated, and the importance of non-invasive molecular and functional imaging in drug development will be highlighted. Examples will include the use of PK/PD endpoints in the development of molecular therapeutic drugs such as the Hsp90 inhibitor 17AAG as well as the development of SR4554 as a non-invasive probe for the detection of tumour hypoxia. Because resources are inevitably limiting, PK/PD endpoints must be prioritised. This involves making a choice between PD endpoints that measure whether a specific molecular target (eg a particular kinase) has been inhibited and those that detect whether an entire biochemical pathway (eg the Ras or PI3 kinase pathways) or biological effect (eg apoptosis or angiogenesis) has been modulated. The advantages and disadvantages of each will be described.

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Target identification labelling for molecular imaging

Abstract not received.

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Clinical molecular imaging in oncology drug development and radiotherapy

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In vivo molecular imaging is an emerging discipline that is of importance to the development of anti-cancer therapies. Molecular imaging uses new and emerging quantitative functional imaging technology to look at molecular pathways. Technologies encompassed within molecular imaging include optical, magnetic resonance and nuclear medicine techniques. Positron emission tomography (PET) is the most sensitive and specific technique for imaging molecular pathways in vivo in humans. PET uses positron emitting radionuclides to label molecules, which can then be imaged in vivo. The inherent sentisitivity and specificity of PET is the major strength of this technique. Indeed, PET can image molecular interactions and pathways, providing quantitative kinetic information down to sub-picomolar levels. Molecular imaging can provide pharmacokinetic, pharmacodynamic and mechanistic information. Use of the technique in early clinical trials can: (1) provide information on optimum biological dose and PK/PD relationships; (2) identify tumours containing specific molecular targets; and (3) provide in vivo pharmacodynamic evaluation of compounds. Its use can also be extended to general physiological questions; for example, regarding vascular physiology and in vivo pharmacokinetics. Molecular imaging provides information in vivo in humans: Is the drug hitting the target? Is the target expressed in an accessible way? What are the timing and magnitude of such molecular interactions? Does this molecular interaction have the desired downstream effect? Advantages of knowing this information early in vivo in humans: Speed of drug development. Stopping compounds early if they prove not to have the desired mechanism. In vivo target validation. Identification of new targets. In radiotherapy molecular imaging has potential for quantitating the biological phenotype and thus defining the Biological Target Volume (BTV). There is potential here to define areas for boost treatment and to assess response to therapy. As anti-cancer strategies become more directed towards a defined molecular target, we need information that is relevant to humans about whether the molecular target is expressed, the selectivity and binding of the compound for that target, and the effects of such an interaction.