Scientific Symposium

Screening and prevention of lung cancer

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New optical methods

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The gradual shift from central to peripheral lung cancers has left a sizeable minority of central, exfoliating lesions, whose cells can be found in sputum. Unfortunately, initial screening studies failed to show a decrease in mortality associated with chest radiographs and/or sputum cytology. Sputum cytology has however improved and additional techniques, including immuno-cytochemistry and computer-based optical imagery have been developed. Preneoplasias and early epithelial cancers are often not discernable during fiberoptic bronchoscopy because, at these early stages, changes of the mucosa are subtle. The performance of flexible bronchoscopes has continuously improved and videobronchoscopy is now widely used. The new videochip bronchoscopes may afford a better image quality allowing a more accurate evaluation of the mucosal surface, with computerized processing. The major recent progress is autofluorescence bronchoscopy (AFB) that was linked to the observation that tumorous tissue could be distinguished from normal by a loss of autofluorescence. For AFB examination, a monochromal light of 442 μm is directed at mucosa, sub-epithelial fluorophores are stimulated to emit light of longer wave lengths and these millions of emitted fluorescence signals are digitalized into a real-time video image of bronchial mucosa, through the AFB systems, allowing distinction between area showing normal and abnormal fluorescence. Biopsies of abnormal areas are performed at the end of the whole procedure. Several studies have reported that addition of AFB to conventional white-light bronchoscopy increases the detection rate of preneoplastic lesions and early cancers. These studies have also pointed out the low positive predictive value of autofluorescence bronchoscopy such that a large number of biopsies in fact show normal histological results and new systems try to solve this problem.
The cost of AFB, its invasiveness and the duration of examination prohibit its use for mass screening purposes. The best field for AFB remains exploration of patients identified on the basis of sputum. AFB technology may also be of clinical value in detecting synchronous occult cancers in patients at very high-risk including lung cancer patients with roentgenographically visible or occult lung cancers prior to their curative treatment or patients with head and neck cancers, as well as workers occupationally exposed to bronchial carcinogens. AFB may also be of use for surveillance.

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Spiral CT screening

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Based on the results of several completed and ongoing lung cancer screening trials in different parts of the world, several new position statements from professional organizations have been released in 2004 and 2005. The recommendations of the Como conference (2003) include an informed-decision making for – more specified now – high risk individuals aged above 45 or 50 years of age who are current or former smokers with at least 20 to 30 pack-years of cumulative exposure and without life-limiting co-morbidities, and not to screen for lung cancer outside clinical trials. The American Cancer Society continues to recommend that CT screening not be performed in asymptomatic at risk persons, but recognizing that many heavy smokers choose on their own to be screened, they recommend informed decision making, and performance of the CT screening test in experienced centers that are linked to multidisciplinary specialty groups for diagnosis and follow-up. In contrast, the US Preventive Services Task Force makes no recommendation for or against the use of CT screening in their latest updated recommendations, and also advice to discuss the pros and cons with the screenee. They also conclude that from the available data we can conclude that spiral CT screening can diagnose lung cancer at a significantly earlier stage than by current clinical practice. Currently, a large number of non-randomised cohort studies on lung cancer spiral CT screening trial have been completed, while several randomised trials are ongoing. In the cohort studies completed 55 to 85 percent of the cancers detected at baseline and 60–100 percent of the cancer detected at annual repeat are stage I tumors. This markedly better than the current state of practice where only 15–20% of all newly diagnosed lung cancer cases are in stage I. Since the introduction of spiral CT screening in the 1990s in Japan, the 5 year survival rate of all stages of lung cancer has improved from 48.8% to 80.4%. Although these data are very promising, they do not prove that lung cancer screening improves lung cancer survival, because it might be possible that by screening people only know their diagnosis earlier without living any longer (lead time bias). Of great interest is, however, the observation by Henschke *et al.* (2005), who found a clear relationship between tumor size and lymph node status in the ELCAP screening cohort. The percentages of cases with no lymph node metastases was 91%, 85%, 63% and 61% for tumor size categories of <15 mm, 16–25 mm, 26–35 mm, and >35 mm, respectively. These percentages are much higher than previously reported in the SEER registry, and confirms the usefulness of asymptomatic lung cancer at small sizes, and supports the hypothesis that the smaller the cancer detected, the better the prognosis is. Invasive procedure for benign lesions have been performed in 4–22% at baseline and 14–55% during incidence screening. Percentages of interval cancers vary between 0 and 33%.

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Molecular markers in lung cancer risk assessment

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A major obstacle in conducting lung cancer prevention studies is the inability to identify truly high-risk cohorts and the inability to predict the course of developing invasive lung cancer. For effective applying lung cancer prevention strategy, we have to select individuals at different risk levels and use different preventive measurements. For example, aggressive preventive strategies, including the use of agents with certain side effects, might be justified if the individuals with very high probability to develop invasive lung cancer within 3 years whereas only well tolerated agents such as natural products should be given to those at low and intermediate risk. Because most individuals at risk of developing lung cancer lack clinical symptoms or identifiable signs, molecular measurements (i.e., biomarkers), which accumulate from the beginning of tumorigenesis and are detectable with current technologies, may prove to be valuable clinical tools in assessing individuals' cancer risk. In applying molecular analysis for predicting lung cancer risk, two major factors should be taken into account, i.e. the level of exposure to tobacco carcinogens and the inherited genetic background. Inherited genetic variables, often in the form of single nucleotide polymorphism (SNP), can be used to determine individual's susceptibility to tobacco carcinogens. Although a number of SNP have been associated with lung cancer risk, the risk predicting value of these SNP are often insufficient for clinical applications. Because these genetic variables could not modulated by preventive agents, they cannot be used to assess treatment efficacy. Therefore, somatic changes contributing to lung tumorigenesis will be better predictors or markers for lung cancer risk assessment as well as intermediate endpoints of lung cancer prevention. Early genetic and epigenetic alterations, such as LOH in regions harboring critical tumor suppressor genes (e.g., 3p14, 9p21, and 17p13) and promoter hypermethylation (p16, DAP-kinase, and FHIT), have been frequently detected in bronchial epithelium of heavy smokers long before lung cancer development. The potential value of these alterations in predicting cancer development is under investigation. Other common abnormalities in tobacco-damaged airways include activation of telomerase, overexpression of tumor antigens, and expression of alternative spliced transcripts. With advances in technologies, we are in the process to develop a risk prediction model based on gene expression profiles obtained at different stages of tumorigenesis. Expression signatures may then allow us to predict lung cancer development and be used to assess efficacy of prevention.

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Early lung cancer detection biomarkers and the molecular profiling of CT screen detected lung nodules

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Decrease in lung cancer mortality will only be possible if reduction of tobacco consumption habits is achieved, and new molecular-targeted therapies and early detection techniques are developed. In my presentation I will review recent progress in the field of biomarkers for early detection of lung cancer and will summarize our work on the molecular profile of small pulmonary nodules resected from lung cancer spiral CT-based screening protocols.
A successful lung cancer screening program will require high levels of sensitivity and specificity. It is also very important to hit upon the subpopulation of individuals with higher risk to develop lung cancer in order to improve detection rates and lower cost/benefit ratios. To achieve these aims, a combination of imaging techniques with well characterized and validated molecular markers may prove very beneficial. Biomarkers may inform about genetic predisposition to lung cancer or suggest the

presence of transformed or preneoplastic cells in the patient's respiratory tract. In recent years some biomarkers have been proposed with potential diagnostic or prognostic value in lung cancer. Unfortunately, most of these biomarkers have not been validated in large populations and still lack an unambiguous demonstration of clinical usefulness. Along the same lines, the identification of biomarkers for the early detection and for premalignant lesions has had a relatively limited success. The study of carcinogenesis models to search for new biomarkers, the development of new highly sensitive molecular techniques, and the availability of large series of patients for which well protocolized and standardized sample collection is organized, may help to solve this urgent need for a robust and validated early detection molecular tool. In clinical settings, the lung cancer early detection biomarkers will be analyzed in non invasive specimens (blood, sputum, exhaled air) with automatized techniques.
Low dose spiral computerized axial tomography (spiral CT) is effective for the detection of small early lung cancers. Although published data seem promising, there has been a significant degree of discussion concerning the potential of overdiagnosis in the context of spiral CT-based screening. We and others have analyzed the phenotypic and genetic alterations in the small pulmonary malignancies resected within research spiral CT screening trials. Our aim was to determine whether their malignant molecular features are similar to those of resected lung tumors diagnosed conventionally. We analyzed 17 biomarkers of lung epithelial malignancy. The molecular alterations and the frequency of phenotypic or genetic aberrations were very similar when screen detected and non-screen detected lung cancers were compared, suggesting that the screen detected tumors are genuine neoplastic growing cells.

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Chemoprevention

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Incidence and mortality associated with lung cancer has not been significantly modified since more than 25 years, despite introduction of new cytotoxic drugs and development of multidisciplinary approaches combining surgery, chemotherapy and radiotherapy. This points out how much chemoprevention approaches are necessary. Complete characterization of molecular determinants of lung carcinogenesis is essential to allow rational and targeted development of chemopreventive agents. Molecularly targeted agents are currently being studied in all treatment settings including that of chemoprevention, which is defined as the use of natural or synthetic agents to interrupt the process of carcinogenesis and to prevent or delay tumour occurrence. Lung cancer chemoprevention trials have being highly disappointing notably when performed in current smokers. Interesting but preliminary results have being obtained in trials focusing at former or never smokers. Progress in chemoprevention is reliant on the collaborative efforts of researchers in basic science and clinical settings who study the biology of lung cancer with the goal of uncovering new mechanisms of carcinogenesis. Small molecules which target specific receptors or mutations such as inhibitors of epidermal growth factor receptor or RAS will have an increasingly significant role as they are associated with more tolerable side-effects and may prove more effective. Development of a risk model with intermediate endpoints is essential for future chemoprevention studies.

Scientific Symposium
Ovarian cancer – an update

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Ovarian cancer diagnostics: update on proteomics

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Proteomics is the study of proteins and protein signaling pathways and how they effect the process, diagnosis, and intervention for science. While many consider cancer a disease of genes, one must remember that it is the protein that is the work horse and the most readily detectable of the subcellular information sources. Key questions are how to harness the information resources of proteomics for optimal use. Multiple approaches of proteomics have evolved for application to ovarian cancer and other cancers. Measurements of proteins and their activated components in tissue and biological materials can provide an insight into the biology and behavior of the cancer. Another application is using proteins and protein patterns for descriptive purposes.
A good screening test is one that has high sensitivity, high specificity, is readily done, and for which there is an intervention that can make a difference. Proteomic pattern development from serum proteins fits the requirement for easy access to source material. Techniques using high throughput mass spectrometry platforms coupled with complex bioinformatics for detection of proteins and/or protein patterns are under development. Results to date show that the process of matrix-associated laser desorption and ionization mass spectrometry can yield datastreams from which protein diagnostics for ovarian and other cancers have been advanced. The general sensitivity and specificity of these experimental screening developments has been good, 85–95%, but inadequate for use as a diagnostic due to the translation to poor positive predictive value, necessitating too many invasive procedures yielding false positive designations. Work is ongoing by our group and others to optimize quality control, robotics, and reproducibility. It is important that any platform(s) selected is valid, reliable, and reproducible across users. Clinical studies are under development to build diagnostic patterns of early disease recurrence and for differentiation of benign disease and unaffected women from those harboring cancer.
A second and advancing approach of proteomics is applied to the classification of protein signals and pathways to assessment of clinical behavior in response to treatment. Many targeted agents are being applied to ovarian cancer other cancers. Their putative mechanisms of action, validated in the laboratory and xenograft or transgenic animal models, needs to be confirmed in patients. One approach to this is through surrogate biomarkers. However, there are very few biomarkers that have been validated as to the demonstration of their accuracy for what happens in the tumor. For example, it is clear that inhibition of epidermal growth factor receptor results in rash, commonly on the face and chest. However, it has not been globally confirmed that inhibition of EGFR in skin resulting in rash is concomitant with inhibition of EGFR in tumor. Hence, there was need to develop technologies that can be applied to small tumor samples, such as needle biopsies, for analysis of biochemical modificaiton within the tumor. Our group has applied laser capture microdissection and reverse phase tissue lysate microarrays and measured changes in biochemical protein profiles before and into therapy with molecular targeted agents. Examples of these will be presented.

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Molecular predictors of ovarian cancer response and progression

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Multiple gene alterations take place during tumor development and progression and could specifically contribute to the treatment response and eventually impact on disease outcome. A large survey of recent published data, integrated with our own data, indicate that loss of TP53 gene function may contribute to a platinum-based chemotherapy resistance and to taxane sensitivity, but in both early and advanced stage patients the impact of TP53 mutation status in overall survival is still debated. However a deeper characterization of mutational spectrum seems to indicate that distinct type of mutations might result in short-term survival benefit. Even in the case of BRCA family genes, the questions of whether epithelial ovarian cancer (EOC) carriers of BRCA mutations have specific prognostic patterns or BRCAness phenotype could account for platinum sensitivity have not yet been solved. Taken together these data suggest that even if these genetic molecular markers cannot be considered the single prognostic driving forces they may influence overall survival in selected populations. Recent technological developments, enabling simultaneous measurement of many parameters ("omic" approaches), hold the promise to more effectively address the multifactorial basis of drug resistance and to help in identifying new molecular predictive markers. The microarray analysis of the transcriptome in advanced stage EOC samples has provided a wealth of data on differential gene expression and has identified sets of dysregulated genes potentially associated to EOC pathogenesis, progression and worse prognosis. Validation of these genes needs to be done at protein level and the importance of their differential expression has to be related to the clinical impact. Tissue microarray (TMA) technology, allowing the simultaneous analysis of hundreds of specimens on a single paraffin block, could greatly improve the reproducibility of immunohistochemistry (IHC). IHC validation of the prognostic significance of two Ig-CAMs, initially identified through the transcriptome analysis, indicated that their differential expression was significantly associated in specific subgroups of patients with poor prognosis. Present data await validation in independent case materials but since now suggest that a deeper understanding of ovarian cancer cell biology may have multiple implications and potentially could allow identification of prognostic markers.