DNA methylation in the promoter regions of genes is a prominent epigenetic gene silencing mechanism associated with resistance to endocrine therapy in patients with recurrent breast cancer. We have employed a microarray-based technology to investigate the promoter DNA methylation status of 117 candidate genes in tumors of breast cancer patients who received tamoxifen as first-line endocrine treatment for recurrent breast cancer. Of the genes analyzed, phosphoserine aminotransferase (PSA-T1) emerged as the strongest marker to predict progression-free survival. Among the 117 candidate genes, DNA-methylation markers associated with breast cancer patient outcome after adjuvant endocrine therapy were also identified and validated in independent groups of patients. DNA-methylation status of PITX2 showed the strongest correlation with disease recurrence. These results provide validated high-level evidence that DNA-methylation status allows clinically relevant risk assessment in tamoxifen-treated breast cancer, both in the adjuvant and palliative setting.

doi:10.1016/j.ejcsup.2006.04.024

S24. MOLECULAR PATHOGENESIS OF PAPILLOMA VIRUS ASSOCIATED CANCERS

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A number of human cancers are caused by persistent infections with high risk human papilloma viruses (HR-HPV), among them about 100% of cervical cancers, many anogenital cancers, some head and neck cancers, and a subset of skin cancers. HPVs infect basal epithelial cells via microlesions and usually replicate in differentiated superficial epithelial cells. Two HPV oncogenes, E6 and E7, interfere with the hosts cell cycle and apoptosis regulation. Most importantly, E7 disrupts the binding of pRb and E2F and induces continuous cell cycle activation. E6 triggers degradation of p53 and thus abrogates apopotosis. Local immune defense mechanisms lead to spontaneous clearance of HPV infections in the majority of cases. In few infections, however, deregulated expression of E6 and E7 in basal epithelial cells induces major chromosomal instability and can initiate epithelial transformation. Several characteristic changes have been identified in epithelial cells transformed by HPV: Strong p16INK4a expression was found in medium to high grade premalignant lesions as well as in cervical cancer indicating the functional inactivation of pRb. Proliferation associated markers like ki67, telomerase, MCM5 and CDC6 are expressed at various levels in premalignant lesions and invasive cancer. In advanced lesions, high levels of chromosomal imbalances can be observed, a very specific alteration is the gain of 3q. Integration of HPV DNA into the host cell genome specifically indicates advanced lesions with a high probability of progression and is frequently found in cervical cancer. Several biomarkers, especially the detection of HR-HPV DNA and p16INK4a protein, are currently being evaluated in order to improve existing cervical cancer screening procedures.

doi:10.1016/j.ejcsup.2006.04.025

S25. DEVELOPING STRATEGIES FOR TUMOR VACCINATION

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Vaccination against cancer has had a variable history, with claims of success often fading into disappointment. The reasons for this include poor vaccine design, inadequate understanding of the nature of the immune response, and a lack of objective measures to evaluate performance. The characterization of tumor-associated antigens (TAAs) recognized by human T lymphocytes in a MHC-restricted fashion has opened new possibilities for specific vaccine approaches to the treatment of human cancers. Recent findings include vaccine formulation, relevant knowledge concerning mechanisms of induction of effective immunity from preclinical models, and translation into clinical trials. We now have novel vaccine strategies to activate specific attack on tumor cells and we understand more about activation and regulation of immunity against cancer (co-stimulation versus co-inhibition, regulatory T cells). We also have modern assays using surrogate markers (MHC multimer analysis, IFN-γ Elispot assay) to correlate with clinical effects. Although early clinical vaccine trials based on synthetic peptides, proteins, 'naked' DNA, tumor-RNA, dendritic cells, and recombinant vaccinia viruses indicate that vaccines can induce immune responses and tumor regression in some cancer patients, careful study design and use of standardized clinical and immunological criteria are needed. Basic principles of tumor vaccination and clinical trials will be discussed.

doi:10.1016/j.ejcsup.2006.04.026

S26. TARGETING MUC1 WITH LIPOSOMAL BLP25

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MUC1 is a mucin glycoprotein expressed by both normal cells and a wide variety of epithelial carcinomas. Mucins expressed by cancer cells have abnormal glycosylation that results in shorter and simpler carbohydrate chains as well as exposure of normally hidden (cryptic) epitopes on the protein backbone. These changes result in unique antigenicity of cancer cell mucins relative to their normal cell counterparts and make MUC1 an ideal candidate antigen for immunotherapy.

L-BLP25 vaccine is an investigational therapeutic cancer vaccine being studied for the treatment of epithelial carcinomas. L-BLP25 vaccine incorporates a synthetic lipopeptide sequence identical to a portion of the protein backbone of MUC1. The vaccine is a liposomal formulation that consists of the synthetic MUC1 lipopeptide, an immunoadjuvant [monophosphoryl lipid A (MPL)], and three lipids: cholesterol, dimyristoyl phosphatidylglycerol (DMPG) and dipalmitoyl phosphatidylcholine (DPPC). The BLP25 lipopeptide provides the antigenic specificity for a T-cell immune response, while the adjuvant serves to TLR4 to activate APCs. The liposomal delivery system is thought to ensure delivery of peptide antigen and adjuvant to the exact same cell as well as facilitate access to the intracellular antigen presenting machinery of a cell.

So far, 168 patients with non-small cell lung cancer (NSCLC) or prostate cancer have been treated with L-BLP25 vaccine in phase I or II clinical trials. A randomized phase IIb study was performed that compared the safety and efficacy of L-BLP25 in patients with stage IIIB or IV NSCLC against best supportive care (BSC). Data from this study revealed an overall median survival of 17.4 months for patients on the vaccine arm versus 13 months for the patients in the BSC arm. Two-year survival was 43% for the L-BLP25 vaccine arm versus 29% for the BSC arm. The median survival for patients with locoregional Stage IIIB disease (without pleural effusion) was 30.6 months in the L-BLP25 arm and 13.3 months for patients in the control arm. Two-year survival was 60% for the L-BLP25 vaccine arm versus 37% for the BSC arm. Safety data from the phase IIb study showed an incidence of adverse events similar to those previously reported in L-BLP25 clinical trials, i.e. mild to moderate flu-like symptoms and injection site reactions. Although the differences in survival were statistically not significant, these results suggest a survival advantage in locoregional stage IIIB patients treated with L-BLP25 vaccine. A phase III trial in unresectable stage III NSCLC patients will be launched in 2006.

doi:10.1016/j.ejcsup.2006.04.027

S27. SURVIVING – PRECLINICAL RATIONAL AND PRELIMINARY RESULTS

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Prognosis of most disseminated solid tumors remains gloomy as neither chemotherapeutic nor unspecific immune modulatory approaches were able to improve the overall survival of these patients. Hence, specific immunotherapy has received increasing attention. Disappointing clinical results, however, indicate that the choice of suitable antigens is of special importance. To this end, the inhibitor of apoptosis protein survivin, which is over-expressed in several tumours but is largely undetectable in adult tissues, appears to be a promising target for vaccination purposes, since downregulation or loss of expression is associated with impaired tumour progression. Consequently, heavily pretreated patients suffering from advanced, therapy-refractory melanoma, pancreatic, cervical or colorectal cancer were vaccinated with affinity-improved HLA-A1, A2 or B35-restricted survivin-derived peptide epitope together with Monatamide ISA-51 in a compassionate use setting. Preliminary results from an interim analysis of this ongoing clinical trial demonstrated that patients mounted strong survivin specific T cell responses as measured by ELISPOT assay and tetramer-staining. Furthermore, in situ peptide/HLA-A2 multimer staining confirmed that these survivin reactive cells infiltrated both visceral and soft tissue metastases. Most importantly, clinical activity was suggested by both disease stabilisation as well as objective responses.

doi:10.1016/j.ejcsup.2006.04.028

S28. THE ROLE OF EGFR FAMILY IN PRENEOPLASIA AND LUNG CANCER; PERSPECTIVES FOR TARGETED THERAPIES AND SELECTION OF PATIENTS

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The Erb-B family of receptors seems to play an important role in the lung carcinogenesis. Several studies have demonstrated over expression of epidermal growth factor receptor (EGFR) in bronchial dysplasias.[1] We have studied 268 bronchial biopsies from high-risk individuals participating in a high-risk sputum cohort (>30 pack-years of smoking history and COPD defined as FEV1 < 70% of expected), and EGFR protein was strongly expressed in about 60% of patients with normal bronchial histologies, but increased with increased level of dysplasia. Also HER2 was highly expressed in both normal bronchial epithelium as well as in preneoplasias.[2]

The expression of EGFR/HER2 in malignant lung tumors was studied in several studies. Squamous cell carcinomas has most often high expression of EGFR, but less HER2, while adenocarcinomas has more often high expression of HER2 and less EGFR. Of interest is that bronchioloalveolar carcinomas has high expression of both EGFR and HER2, which can contribute to explain why this subtype seems to be more sensitive to EGFR inhibitors than the other non-small cell subtypes.[3]

The *prognostic role* of EGFR and HER2 has been addressed.[4,5] In our study 183 patients with resected NSCLC were studied, and no significant prognostic association was demonstrated in this study. However, other studies have reported that EGFR overexpression is associated with poorer prognosis.[5] In the UCCC study also the EGFR gene copy number was studied by FISH, and a tendency to a shorter survival was seen for increased gene copy number.[4]

The *predictive role* of EGFR and HER2 for sensitivity to EGFR TKIs has been studied at UCCC in two separate cohorts of NSCLC patients. In an Italian cohort of 108 NSCLC patients we found that EGFR protein expression, increased gene copy number detected by FISH and EGFR gene mutations were all associated to treatment outcome after EGFR TKI therapy.[6] Patients with high EGFR gene copy number (high polysomy/amplification) had a high objective response rate (36%), disease control rate (67%) and median survival of 19 months, which was significantly better than the patients with no or low gene gain. The same was found in another study cohort (SWOG 0126) for patients with BAC subtypes.[7] The association between increased EGFR gene copy number and survival has been demonstrated in two randomized placebo controlled studies, one with erlotinib with hazard ratio (HR) = 0.44 in the FISH positive group and in the ISEL study with gefitinib (HR = 0.61), while no differences was seen in the FISH negative groups.[8,9]

In order to identify a panel of markers, which can predict sensitivity to EGFR TKIs we studied the predictive value of *combined markers*. While a combination of positive IHC and positive FISH were associated with very high response rates (41%) and prolonged survival (median 21 months), negative IHC and FISH assessment was associated with no response and very short survival (median 6months) indicating no clinical benefit in this group of patients.[10] We have also performed in vitro NSCLC cell line studies with EGFR TKIs and characterized sensitive and resistant cell lines by Affymetrix gene chips. We have identified several