P8

T cell responses in cancer patients after vaccination with a cryptic hTERT peptide (Vx-001)

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Background: The human telomerase reverse transcriptase (hTERT) is highly expressed in most human malignancies and correlates with poor prognosis. A major problem of tumour vaccination is the tolerance to tumour antigens that is directed against their "dominant" (high affinity for HLA) rather than their "cryptic" (low affinity for HLA) peptides. Therefore, "cryptic" peptides can be good candidates for the activation of an anti-tumour immune response. TERT572Y is an HLA-A*0201-restricted cryptic peptide derived from hTERT, where a tyrosine has substituted in P1 in order to increase affinity to HLA. TERT572Y has been shown to induce tumour immunity in HLA-A*0201 transgenic mice. The purpose of this study was to assess the specific-T cell immune response in HLA-A*0201 patients with advanced cancer vaccinated with the TERT572Y peptide.
Methods: 100 patients with various types of cancer were enrolled and were vaccinated subcutaneously twice with the optimized TERT572Y peptide followed by four vaccinations with the native TERT572 peptide at three week intervals. Patients who completed the six-vaccination schedule and maintained disease stability received booster doses with the native peptide every three months. Separated peripheral blood mononuclear cells, from the patients collected after the 2nd and 6th vaccination and before each boost, were screened for reactivity to the TERT572Y and TERT572 peptides using an IFN-gamma ELISpot assay. Also, peptide-specific CD8+ T cells were identified by IFN-gamma staining using flow cytometry. Cytotoxic T lymphocyte activity after peptide-specific stimulations was assessed by analysis of perforin by ELISpot assay.
Results: Seventy-two (72%) of the hundred patients were evaluated for post 2nd vaccination immune response, while thirty-seven (37%) patients were assessed for immune response after the six vaccinations. CD8+ T cell responses were detected in forty-eight patients (67%) out of seventy-two after the 2nd vaccination and thirty-three (89%) out of thirty-seven after the 6th vaccination, as revealed by IFN-gamma ELISpot assay and flow cytometry. Prolonged vaccination maintained the number of peptide-specific CD8+ T cells in the majority of patients. In addition, it was demonstrated that the specific-CD8+ T cells displayed cytotoxic activities following peptide stimulation by producing perforin.
Conclusions: The cryptic hTERT572Y peptide vaccine can induce TERT572-specific CD8+ T cell immune responses in the majority of vaccinated cancer patients.

P45

Identification of proteins associated with cisplatin resistance in ovarian cancer by two proteomic approaches

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Background: Cisplatin is an effective antitumor agent for the treatment of several solid tumors including human ovarian carcinoma. However, the development of cisplatin resistance represents a serious clinical problem; therefore a great deal of investigation has concerned the mechanisms by which tumor cells become resistant to this chemotherapeutic agent. 2008 and C13 cells are human ovarian carcinoma cells sensitive and resistant to cisplatin, respectively. The resistant subline, isolated after prolonged exposure to low drug doses, is approximately 20-fold more resistant to cisplatin.
In the present study we investigate the protein profiles of both cell lines, searching for differentially expressed proteins as possible molecular biomarkers of resistance to the drug.
Methods: We use two proteomic approaches: two-dimensional liquid chromatography (2D LC) and two-dimensional electrophoresis (2D EF). 2D LC is performed on PF-2D platform (Beckman Coulter®) where protein separation takes place on a chromatofocusing column, followed by a reverse phase C18 column.
Absorbance signal, monitoring the second chromatographic step is analyzed to generate the differential protein pattern by a specific software (Delta Vue™). Selected chromatographic peaks (containing proteins over- or under-expressed in cisplatin resistant cells with respect to cisplatin sensitive ones) are further analyzed for protein identification by matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS).
Results: About eighty chromatographic peaks generated from 2D LC show a differential expression consisting in at least 100% variation between the two cell lines, and 85% of peaks were successfully identified by MALDI-MS analysis.
2D EF was applied to compare the results obtained with the former technology. Among differentially expressed proteins we identified: Glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase1, enolase1, HSP27, and ATP-syntase which are under-expressed in C13, cisplatin resistant cells.
Conclusions: Complete differential expression profile could be useful to identify down-/up-regulated pathways in cisplatin resistant cells and, hopefully, some of related proteins could be further investigated as candidates to predict/monitor response to cisplatin treatment in serum and tumor tissue.

P17

EGFR, K-RAS and HER2 mutations as predictive factors for gefitinib sensitivity in non-small cell lung cancer (NSCLC)

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Background: In non-small cell lung cancer (NSCLC), somatic mutations in the EGFR kinase domain correlate with sensitivity to tyrosine kinase inhibitors while KRAS and HER2 mutations are considered to cause resistance to these agents. We sought to examine the correlation between EGFR, KRAS and HER2 mutational status and clinical outcome in NSCLC patients treated with gefitinib.
Methods: Formalin-fixed tissue samples from 60 patients with advanced NSCLC treated with gefitinib were microdissected and DNA was extracted from tumor cells. EGFR, KRAS and HER2 mutation analysis was performed by PCR amplification and sequencing of EGFR exons 18−21, KRAS exon 2 and HER2 exon 20.
Results: Sixty tumor samples were successfully analyzed for EGFR and KRAS and 47 for HER2 mutations. Twenty-four patients (40%) harbored EGFR mutations, 12 (20%) KRAS and 4 (8.3%) HER2. There was a statistically significant association between the presence of EGFR mutations and disease control rate (DCR; partial response [PR] or stable disease [SD]) [DCR 79.2% in mt(+) vs 52.8% in mt(−), $p = 0.038$] and overall survival (OS) [88 wks in mt(+) vs 48 wks in mt(−), $p = 0.049$]. Among the 12 patients with KRAS mutations, 2 developed progressive disease (PD), 9 stable disease and one partial response with gefitinib [response rate 8.3% in mt(+) vs 10.4% in mt(−), $p = 0.83$]. Two patients carried EGFR and KRAS mutations concomitantly. No significant association was found between overall survival and KRAS mutations [OS 88 weeks in mt(+) vs 52 in mt(−), $p = 0.554$]. Among the 4 patients with HER2 mutations, none responded to gefitinib. Three patients with concomitant EGFR and HER2 point mutations had stable disease and 1 patient with an insertion mutation developed progressive disease. The overall survival was 13 weeks in mt(+) vs 64 in mt(−) patients ($p = 0.022$).
Conclusions: Our data support that EGFR mutations confer sensitivity to gefitinib treatment as already reported. KRAS and HER2 mutations per se could not confer resistance to gefitinib. However, given the small number of cases and the retrospective nature of this study, these observations should be interpreted with caution.

P74

Validation of a panel of serological biomarkers of angiogenesis for use in early clinical trials

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Background: Assays that report tumour neo-vasculature are essential for the development of novel drugs that target angiogenesis. Non-invasive imaging modalities are often used in clinical trials to assess vascular permeability and where serial biopsies are available, immuno-histochemical measurements of endothelial cell markers (CD31, CD34, CD105) are performed. Many molecules measured in serum have reported to have potential clinical utility as pharmacodynamic biomarkers of angiogenesis, not least because of the relative ease of collecting bloods both pre- and serially post-therapy. Two classes of molecules have been studied; angiogenic factors and molecules released from neo-vasculature either naturally during of angiogenesis and change as result of therapeutic intervention. The lead candidate biomarkers of angiogenesis are the VEGF family of cytokines but VEGF does not act alone in stimulating