with a point mutation. Two patients with point mutations eventually experienced progression of their disease. Tumor progression was not observed in the patients with exon 19 deletions. The mean progression free interval was 430 days (CI [294,567]) for the whole series.

***Conclusions*:** The observations add support to the importance of EGFR status as predictors of response to TKIs. Exon 19 deletions seem to predict the best responses to TKIs.

doi:10.1016/j.ejcsup.2006.04.030

S30. ANTI-TUMOR APPLICATIONS OF ACTIVATING TOLL-LIKE RECEPTOR 9 WITH PF-3512676 (FORMERLY CPG 7909)

Arthur M. Krieg. *Coley Pharmaceutical Group.*

Unmethylated CpG dinucleotides are relatively common in viral and bacterial DNA, but are rare in vertebrate DNA. Toll-like receptor 9 (TLR9) detects these "CpG motifs" as a sign of infection, and can be activated for therapeutic purposes by CpG motifs in synthetic oligodeoxynucleotides (CpG ODN), such as PF-3512676 (formerly called CPG 7909, or CpG 2006). PF-3512676 engages TLR9 in B cells and plasmacytoid dendritic cells (pDC), thereby stimulating innate and adaptive immunity, including antigen-specific Th1-like T cell responses. Murine studies showed anti-tumor activity of PF-3512676 as a monotherapy for relatively small tumors. Tumor regression was associated with the induction of a tumor-specific CTL response. PF-3512676 has been administered at various dose levels to more than 1000 humans, and has shown activity as a monotherapy in phase I human clinical trials when administered by intratumoral injection in basal cell carcinoma, by subcutaneous injection in cutaneous T cell lymphoma (CTCL), melanoma, and renal cell carcinoma, and by intravenous injection in non-Hodgkin's lymphoma.

Although the demonstrated activity as a monotherapy provides some proof-of-concept for the use of PF-3512676 in cancer therapy, we considered that combination approaches may provide greater efficacy. We theorized that disruption of the tumor using conventional anti-tumor therapies may reduce the tumors' resistance to immune mediated attack induced through TLR9 activation with PF-3512676. In murine models regression of larger tumors could be induced when PF-3512676 was used in combination with other therapies, including radiotherapy, surgical resection, monoclonal anti-tumor antibodies, and chemotherapy. The combination of PF-3512676 or other CpG ODN with certain chemotherapy regimens, including paclitaxel or gemcitabine, increased the generation of tumor antigen-specific CTL and/or improved tumor regression and survival in metastatic tumor models. These studies also demonstrated the involvement of *T* cells in the synergy between CpG and paclitaxel, consistent with the hypothesis that this combination induces enhanced tumor specific adaptive immune responses. These encouraging results in mouse models have been extended into human therapy in a controlled Phase II trial, where 112 patients with locally advanced or metastatic non-small cell lung cancer were randomized to receive either chemotherapy alone, or in combination with PF-3512676. The combination with chemotherapy provided a statistically significant improvement in objective response rate, and a trend to prolonged survival (1 year survival 33% vs. 50%, $P = 0.08$). The safety and tolerability of these TLR9 agonists has generally been good, with the major adverse events being transient injection site reactions and flu-like symptoms. Phase III trials of PF-3512676 in combination with 2 doublet chemotherapy regimens (paclitaxel plus carboplatin or gemcitabine plus cisplatin) for first-line therapy of locally advanced or metastatic NSCLC in 1600 patients were initiated in late 2005.

doi:10.1016/j.ejcsup.2006.04.031

S31. GENE PROFILING IN MELANOMA – WHAT HAVE WE GAINED?

Keith Hoek. *Department of Dermatology, University Hospital of Zürich, Switzerland.*

In the mid 1990s one of the first targets of DNA microarray analysis was melanoma. For over a decade researchers have made increasing use of this technology in their efforts to understand the molecular biology underlying this disease. Most work has concentrated on class-comparison approaches which assess the transcriptional differences between aggressive and less aggressive variants, or explored the consequences of activating mutations in members of the MAPK pathway. Other studies examined the effects of various in vitro treatments including UV, retinoids, demethylation and hypoxia. The general outcome of these studies have been the generation of ever longer lists of genes nearly all of which are guilty through association. But who are the master criminals in the crowd? Where are the strings which draw these multitudinous factors together and who are the puppetmasters behind them? DNA microarrays may have brought us closer to the facts, but what they all mean is not at all obvious to the majority of researchers. Just where in these details lies the devil of malignancy?

doi:10.1016/j.ejcsup.2006.04.032

S32. TUMOR STROMA-ASSOCIATED ANTIGENS FOR ANTI-CANCER IMMUNOTHERAPY

Jürgen C. Becker, Valeska Hofmeister, David Schrama. *Julius-Maximilians-University of Wuerzburg, Department of Dermatology, Wuerzburg, Germany.*

Immunotherapy has been widely investigated for its potential use in cancer therapy and it becomes more and more apparent that the selection of target antigens is essential for its efficacy. Indeed, limited clinical efficacy is partly due to immune evasion mechanisms of neoplastic cells, e.g. downregulation of expression or presentation of the respective antigens. Consequently, antigens contributing to tumor cell survival seem to be more suitable therapeutic targets. However, even such antigens may be subject to immune evasion due to impaired

processing and cell surface expression. Since development and progression of tumors is not only dependent on cancer cells themselves but also on the active contribution of the stromal cells, e.g. by secreting growth supporting factors, enzymes degrading the extracellular matrix or angiogenic factors, the tumor stroma may also serve as a target for immune intervention. To this end several antigens have been identified which are induced or upregulated on the tumor stroma. Tumor stroma-associated antigens are characterized by an otherwise restricted expression pattern, particularly with respect to differentiated tissues, and they have been successfully targeted by passive and active immunotherapy in preclinical models. Moreover, some of these strategies have already been translated into clinical trials.

The work is supported by the grant KFO 124-1/2 of the Deutsche Forschungsgemeinschaft.

doi:10.1016/j.ejcsup.2006.04.033

S33. MOLECULAR PHENOTYPING OF MINIMAL RESIDUAL DISEASE IN SOLID CANCER – DEFINING THE MARKERS FOR TUMOR PROGRESSION?

Klaus Pantel. *Institut für Tumorbiologie, Universitätsklinikum Hamburg-Eppendorf, Germany.*

Metastasis is the major cause of deaths in patients with solid tumors. Occult micrometastatic spread of tumor cells might be the seed for the occurrence of overt metastases in distant organs within the years following successful surgical resection of the primary tumor. The two major approaches to detect such "minimal residual disease" are immunocytochemical staining and polymerase chain reaction analysis. These assays are sensitive enough to detect a single disseminated tumor cell (DTC) in the background of millions of normal cells [Pantel and Brakenhoff. *Nat Rev Cancer* 2004;**4**:1–9]. For epithelial tumors, cytokeratins have become the best marker for the immunocytochemical detection of DTCs in blood, lymph nodes and bone marrow (BM).

Micrometastatic cells in BM can be easily collected from the iliac crest, and BM seems to be a common homing organ for DTCs derived from various types of malignant epithelial tumors (e.g., breast, lung, prostate or colorectal cancer). DTCs are present in BM samples of 20–40% of patients even in the absence of lymph node metastases (stage N0) or clinical signs of overt distant metastases (stage M0). A pooled analysis on 4703 breast cancer patients [Braun et al., *NEJM* 2005;**353**:793–802] has shown that the detection of DTC in BM is a marker for an increased risk to develop metastatic relapse. The molecular and biochemical characterization of DTCs helps to identify progression markers towards overt metastases and new therapeutic targets for therapies that are specifically directed against minimal residual disease. This information may refine the current DTC detection approaches, which the major goal of the EU-funded consortium "DISMAL" coordinated by K.P.

doi:10.1016/j.ejcsup.2006.04.034

S34. TARGETING Plk1 FOR CANCER THERAPY

Mathias Schmidt, Hans-Peter Hofmann, Karl Sanders, Volker Gekeler, Thomas Beckers. *Altana Pharma AG, Therapeutic Area Oncology, Byk-Gulden Str. 1, D-78467 Konstanz, Germany.*

A stringent control of mitosis is mandatory to warrant the accurate segregation of sister chromatids in dividing cells. Eukaryotic cells have evolved sophisticated mechanisms to monitor faithful progression through each phase of mitosis in order to prevent the occurrence of aneuploid daughter cells. Polo like kinase 1 (Plk1) has been identified to be a key player for G2-M transition and mitotic progression in both, normal and tumor cells. Multiple essential roles have been assigned to Plk1 at the entry into M-phase, mitotic spindle formation, condensation and separation of chromosomes, exit from mitosis by activation of the anaphase-promoting complex and in cytokinesis.

We have employed chimeric antisense oligonucleotides and siRNA to investigate the molecular alterations after targeted interference with Plk1 in a multitude of human cancer cells. Suppression of Plk1 mRNA inevitably resulted in a dramatic increase of the mitotic index followed by the onset of apoptosis. Mitotically arrested cells displayed randomly separated condensed chromosomes and the occurrence of multiple spindle poles with well-formed asters. Induction of apoptosis was strictly dependent on cell cycle progression: Genetically engineered RKO human colon adenocarcinoma cells with inducible expression of the cdk inhibitor p27Kip1 were completely refractory to Plk1 depletion-induced apoptosis when they were arrested in the G1 phase of the cell cycle. Various mitotic markers including MPM-2, cdc25c, cyclinB1, or phospho-histone H3 were investigated to explore the molecular consequences of Plk1 depletion. While most marker proteins only showed alterations typical for mitosis, modifications of cdc25c allowed distinction between mitotic targeting via Plk1 or via other mechanisms, such as microtubule inhibitors. cdc25c was fully phosphorylated solely in paclitaxel-treated cells, while it was only partially phosphorylated in Plk1 depleted cells despite the fact that both treatments caused a profound mitotic arrest. This differential phosphorylation of cdc25c was used to test whether a pharmacological inhibitor of Plk1 kinase activity would exert the same cellular effects as interference with Plk1 on an mRNA level. In deed, pharmacological intervention with Plk1 using a specific Plk1 kinase inhibitor induced exactly the same molecular alterations and displayed the cell cycle-dependent cytotoxicity as Plk1 interference on an mRNA level. Taken together, these data substantiate the attractiveness of Plk1 as candidate for mitotic targeting of cancers.

doi:10.1016/j.ejcsup.2006.04.035

S35. REGULATION OF UCN-01 INDUCED MITOTIC CELL DEATH BY PRO- AND ANTI-APOPTOTIC PATHWAYS

Celia Vogel, Christian Hager, Holger Bastians. *Philipps University Marburg, Institute for Molecular Biology and Tumor Research (IMT), Marburg, Germany.*