combined to radiation. We have been attempting to find molecular features that might be predictive of the clinical response to Cetuximab, administered with radiation alternated to chemotherapy, in an ongoing phase II trial (ALTErnating Radiotherapy and Chemotherapy plus Cetuximab, ALTERCC) at our Institution.
Methods: The ALTERCC protocol consisted of: Cisplatin 20 mg/mq/day for 5 days plus 5-FU 200 mg/mq/day for 5 days in weeks 1, 4 and 7; radiation (10 Gy over 5 fractions, 1 fraction per day) in weeks 2, 3, 5, 6, 8, 9. Cetuximab 400 mg/mq loading dose followed by 250 mg/mq weekly, concomitantly with the radiotherapy. The status of EGFr in sections of paraffin-embedded primary tumours was evaluated using the EGFr Pharma DX kit (Dako). For mutation screening, the indicated exons of the EGFr, K-Ras and p53 gene were amplified by PCR, the PCR products purified using the QIAquick Gel Extraction Kit (Qiagen) and sent for sequencing to MWG Biotech (Munich, Germany).
Results: EGFr expression was evaluated in 29 cases out of 35 treated so far. Among 15 patients with a complete clinic-pathological response, 13 (87%) had a EGFr score >200 (percentage of positive cells times the intensity of the signal in a scale from 1 to 3). Out of 6 patients with a partial response, only one (17%) had a EGFr score >200, while four (67%) had a score <100. We have also been screening the same tumours for known mutations or polymorphisms in the genes coding for EGFr, K-Ras and p53. In 11 cases examined so far, no mutations were found in exons 18, 19, 20 and 21 of EGFr, as well as in exons 1 and 2 of K-Ras. Also, the status of the 72 polymorphic codon in exon 4 of p53 varied among those cases without any obvious correlation with either the EGFr score or with the response to the therapy. In the same cases, we have also determined the number of polymorphic CA repeats in the first intron of the EGFr gene. This value has been reported to correlate with the extent to which this gene is expressed in tumour samples. However, we did not find any correlation between the total number of CA repeats (in both alleles) and either the EGFr score or the response to the therapy.
Conclusions: Our preliminary results indicate that, among patients with locally advanced HNSCC, those with a high EGFr score (>200) in sections of their primary tumours might be the ones that benefit most from Cetuximab plus radiation alternated to chemotherapy. The other molecular features examined so far do not seem to be predictive of either response or resistance to Cetuximab plus alternated chemo- and radiotherapy, although examination of a larger pool of patients is strictly needed to draw robust, statistical conclusions.

P59

Building a quality assurance (QA) system that is fit for purpose in an academic laboratory

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Background: In Europe, scientists conducting biomarker studies on clinical trials must comply with European Directive (2001/20/EC). These regulations make few specific references to laboratory activities but QA is mandatory so academics must devise QA systems to encompass all aspects of patient sample analysis with little guidance. The 2003 British Association for Research into QA system termed Good Clinical Laboratory Practice (GCLP) covers every facet of trial sample analysis: contractual agreements with study sponsors, staff training, fit for purpose facilities, apparatus qualification, certificated reagents, method validation, study plans, sample tracking, work conduct, data capture/storage, report writing and document archiving. All elements are governed by SOPs and subject to QA audits. Often, in a budget-confined and demanding academic environment, implementing GCLP can only be achieved realistically in incremental steps resulting in an inevitable, intermediate phase where only part of a QA system is in place. Complying with the rigorous dictates of a QA system requires changes to management structures and working practices of academic laboratories. While the academics' tendency is to multi-task, it is preferable that dedicated QA personnel are assigned.
Results: To initiate the process of QA development, we first placed emphasis on patient sample tracking and biomarker method validation, followed by implementation of procedures for data handling, archiving and reporting. Some examples of new developments in our QA system include switching to templates to aid construction of analytical plans and final study report writing and use of pro-forma laboratory notebooks where analysts need only to tick a box or fill out a limited number of fields. The responsibility for the research group to be compliant lies with the project manager, but in our experience dedicated Quality Assurance teams can positively contribute to ease the burden on the project manager. Perhaps the most time consuming process that translational science laboratories undertake is method validation so we are pursuing a "fast-track', yet scientifically rigorous, approach to method validation. We have also placed recent emphasis on developing electronic resources for sample tracking and data analysis.
Conclusions: Our versatile QA system responds to new developments in translational academic research such as high throughput analysis, while always striving towards increased compliance.

P31

Monitoring phenotypical changes of breast cancer cells, sorted in accordance to their CD44 and CD24 marker expression

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Background: Evidence suggests that cancer is initiated and maintained by a subpopulation of cells that only comprise a small proportion of the total tumor cell population named cancer stem cells (CSC's), because they like normal stem cells are able to differentiate and undergo selfrenewal. It has been shown that a low number of breast cancer cells with the phenotype CD44+CD24−/lowLin− were capable of forming tumors in mice, while CD44−CD24+Lin− cells were unable to form tumors. Therefore, the putative breast CSC's can be enriched and distinguished from the bulk of non-tumorigenic breast cancer cells based on cell surface expression. The aim of this study was to examine the phenotype changes over time of cultured breast cancer cells sorted in accordance to CD44 and CD24 expression.
The results were used in planning of our ongoing study, where we try to identify additional markers of breast cancer stem cells (CD44+CD24−/low) by metabolic labeling of the cells followed by FACS sorting, cell content fractionation and mass spectrometry analysis.
Methods: Cells derived from the breast cancer cell line HMT3909 were sorted by FACS and three subpopulations were isolated: CD44+/CD24−/low, CD44−/low/CD24− and CD44−/low/CD24+. The cells were cultured and their respective expression profiles were monitored by FACS. The subpopulations were HE stained and the morphology of the cells examined.
Results: After five passages, the majority of cells obtained from the CD44+/CD24−/low subpopulation, showed declined expression of the CD44 marker and a minor part became CD24+. The two subpopulations containing CD44−/low expressing cells, showed very similar expression profiles after culturing of the cells, despite they had been sorted in a CD24+ and a CD24-/low subpopulation, respectively. Few cells, contained in these two subpopulations, expressed the CD44 marker after five passages. Microscopically, cells from the CD44+/CD24−/low population could be distinguished from other cells by their disconnection to neighbour cells and their oblong morphology.
Conclusions: The CD44/CD24 expression profiles derived from the HMT3909 cell line changed over time to resemble the distribution of HMT3909 cells before sorting. This could be due to an incomplete separation of CSC and more differentiated cancer cells using only the CD44 and CD24 markers. Identification of additional markers may allow a more complete separation of these two cell subsets.

P84

Response monitoring with positron emission tomography (PET) in patients with advanced non-small-cell lung cancer (NSCLC) treated with bevacizumab and erlotinib: a phase II study

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Background: Determining the activity of targeted drugs by RECIST criteria may underestimate their clinical efficiency. The aim of the current study was to evaluate whether early response to treatment with bevacizumab and erlotinib can be detected with 18FDG-PET and H2(15)O-PET in advanced NSCLC patients.
Methods: Patients were enrolled in a multicenter 2-stage phase II study. Primary endpoint was non-progression (NPR) at 6 weeks defined by RECIST criteria. Patients with advanced non-squamous NSCLC who had received no prior chemotherapy were treated with bevacizumab 15 mg/kg q 3 week and erlotinib 150 mg daily. Patients underwent dynamic 18FDG-PET and H2(15)O-PET scanning as well as computed tomography (CT) at baseline and after 3 and 6 weeks of treatment. Standard uptake value (SUV), metabolic rate (MRglu), tumor blood flow (BF) and the volume of distribution (Vd) were defined.
Results: Between 25/1/06 and 30/03/07 47 patients were included. 20 patients underwent both dynamic 18FDG-PET and H2(15)O-PET at baseline, 18 at baseline and after 3 weeks and 15 underwent all scans. Results for these 20 patients: M/F 13/7; median age 60 (range 34−80); stage IIIB/IV 4/16; PS 0/1/2: 7/9/4. Percentage of NPR at 6 weeks was 79%. At the time of analysis 10/20 patients have died and 9/10 remaining patients showed progressive disease. Median time to follow up was 161

days, median time to progression 122 days and median survival 211 days. No significant correlations were found between BF and MRglu per scan as well as between the change in BF and MRglu over time. The residual MRglu after 3 weeks of treatment predicted survival (P = 0.03; 95% CI, 2.18 to 430197). Martingale residual plots showed that the effect of BF is not simply linear. However, the change in BF between baseline and 3 weeks post-treatment showed that a decrease might be indicative of time to progression (P = 0.006; 95% CI, 1.0 to 1.06) and survival (P = 0.076; 95% CI, 1.0 to 1.05).
Conclusions: 18FDG-PET and H2(15)O-PET seem valuable biomarkers in monitoring early response to antiangiogenic treatment in patients with NSCLC. Due to the limited number of patients and the relative short time to follow up, results have to be interpreted with care. Residual MRglu was able to predict survival, whereas tumor blood flow seems to be a promising biomarker for monitoring treatment response.

P16

Biological profiles of two ERBB2-amplified human breast cancer xenografts diversely sensitive to Trastuzumab

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Background: Trastuzumab is a recombinant monoclonal antibody directed against the human growth factor receptor-2(ERBB2/HER2), overexpressed in 25% of breast cancers. However, only 35% of patients with ERBB2-positive cancer respond to trastuzumab and 50% of patients achieving an initial response escape to trastuzumab. Only few ERBB2-breast cancer cell lines are available for preclinical studies. Here, the purpose is to approach the mechanism of trastuzumab resistance using two new models of human breast cancers xenografts (HBCx) with ERBB2 gene amplification, sensitive or resistant to trastuzumab, respectively.
Methods: Tumor samples were directly implanted into nude mice from patients and expanded as xenografts (Marangoni et al, Clin Cancer Res 2007). CGHarray detected ERBB2 amplification and exons of ERBB2 gene were sequenced. Tumor growth and responses to trastuzumab (10 mg/kg ip weekly) were determined. Gene, protein expression and phosphorylation were evaluated by Q-RTPCR and Western Blot, respectively. Coimmunoprecipitation assays were performed for ERBB2/ERBB3 heterodimerisation. ERBB2-positive BT474 cell line was from ATCC.
Results: Both HBCx-13 and HBCx-5 xenografts were canalar infiltrating cancers, HBCx-5 being mucinous. p53 was mutated in HBCx-13 and not in HBCx-5, PTEN expression was higher in HBCx-13 than in HBCx-5. Both displayed an high ERBB2 amplification in CGHarray. No mutation was detected in exons 14, 15, 16, 19 and 20 coding for ERBB2 extracellular domain and tyrosine kinase domain. HBCx-13 was exquisitely sensitive to trastuzumab while HBCx-5 was resistant. AKT and ERK phosphorylation was increased in both HBCx whereas it decreases in BT474 cells after trastuzumab treatment. Trastuzumab induced ERBB2/ ERBB3 dimerisation in both xenografts while it decreased in BT474.
Conclusions: These data indicate that trastuzumab resistance of HBCx-5 is not associated with changes in heterodimerization, ERBB2 mutations in analyzed exons or p53 mutations. The difference observed in PTEN expression could explain the differences in trastuzumab responses. It is showed that these xenografts and BT474 cells have different ways of response to trastuzumab. These new models of ERBB2 amplified breast cancers are the opportunity to explore mechanisms of resistance to Trastuzumab and to test new compounds.

P52

Activated CD8+ T cells radiosensitize EMT-6 mammary carcinoma cells through secretion of interferon-gamma

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Background: Activated CD8+ T cells were shown to be major mediators of anti-tumor immunity, while their effect on radiosensitivity has not yet been explored. The aim of this study was to examine the cytolytic and radiosensitizing activities of CD8+ cells in 1% oxygen, modeling the immunosuppressive and radioprotective microenvironment of solid tumors.
Methods: Splenocytes were isolated from BALB/c mice and activated with immobilized anti-CD3 and soluble anti-CD28. CD8+ T cells were selected by immunomagnetic beads. Syngeneic EMT-6 mammary carcinoma cells were irradiated in 1% oxygen and their radiosensitivity was assessed by colony formation assay. Radiosensitization was determined as a dose enhancement ratio at the level of a surviving fraction of 0.1. To exmanine the cytolitic activity, tumor cell viability was accessed by a 3 h tetrazolium based MTT assay.
Results: Expanded CD8+ T cells secreted high levels of IFN-gamma and increased the radiosensitivity of syngeneic EMT-6 mammary carcinoma cells up to 1.8-fold. This radiosensitization was abrogated by IFN-gamma immunoneutralization and by the metabolic iNOS inhibition in tumor cells. While considerable radiosensitizing effects were observed at a CD8+/EMT-6 cell ratio below 1/1, the cytotoxicity of CD8+ T cells was impaired by hypoxia even at a 10/1 ratio. RT-PCR, FACS and ELISA data in agreement revealed down-regulation of IFN-gamma in hypoxic CD8+ cells. In contrast, hypoxia transcriptionally up-regulated iNOS in EMT-6 tumor cells that were exposed to IFN-gamma+/CD8+ T cells. The latter was essential for preserving the radiosensitizing effects under hypoxic conditions.
Conclusions: Our results for the first time demonstrate the radiosensitizing properties of activated CD8+ cells. This finding warrants further validation of T cell immunity as a prognostic determinant of tumor radioresponse and indicates a rationale for exploring the radiosensitizing potential of immunostimulating strategies.

P65

Radiosensitization by histone deacetylase inhibitors +/− demethylating agents in head and neck cancer cell lines

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Background: Promoter hypermethylation and histone deacetylation are the most important epigenetic changes identified in cancer. Of major interest is the reversibility of these processes that has resulted in the implementation of several new drugs in cancer therapy. The aim of this project was to evaluate the radiosensitizing potential of the demethylating agent decitabine (DAC), either alone or in combination with the histone deacetylase inhibitors (HDAC-I) trichostatin A (TSA) and LBH589 (Novartis) in several head and neck cancer cell lines. Furthermore, a possible relationship between the radiosensitivity and the methylation profile of each individual cell line was investigated.
Methods: For seven head and neck cancer cell lines, hypermethylation of several genes was assessed by conventional methylation-specific PCR (MSP) and by epi-array ("base5-platform", Oncomethylome Science). This included genes involved in response to irradiation (ATM, PARP3, . . .) as well as genes known to be frequently methylated in this cancer type (p16, MGMT, RASSF1A, DAPK, . . .). Sensitivity of all cell lines to radiotherapy +/− optimized doses of DAC +/− TSA or LBH589 was determined by colony assays.
Results: The investigated cell lines show diverse methylation profiles both with conventional and with array-MSP. So far, some cell lines seem to be radiosensitized by the HDAC-I LBH589 and/or TSA. No radiosensitization by DAC has been identified yet.
Conclusions: In this project, the radiosensitizing potential of DAC +/− TSA or LBH589 as well as a link between radiosensitivity and methylation profile of several head and neck cancer cell lines is investigated. The final results of this project will be presented at the time of the congress.

P38

Radioimmunotherapy (RIT) of refractory or relapsed Hodgkin's lymphoma (HL) with ^{90}Yttrium-labelled antiferritin antibody

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Background: The aim of this study was to evaluate the safety and efficacy of radiolabelled DTPA-chelated rabbit polyclonal antiferritin antibody (Ab) in relapsed or refractory HL.
Methods: The protocol included a first intravenous injection of ^{111}Indium-labelled antiferritin Ab followed by immunoscintigraphy at 4, 48, and 72 hours and intravenous injection of ^{90}Yttrium-labelled antiferritin Ab in the case of tumour targeting.
Results: Ten patients were included in the study: median number of chemotherapy regimens: 3; number of autografted pts: 8; number of previously irradiated pts: 9; response to last chemotherapy: 6 PR and 4 progressions. All immunoscintigraphies showed tumour targeting. Nine patients were treated, as the last patient died from progressive HL before therapeutic injection. Median injected activity was 12 MBq/kg (0.32 mCi/kg). Among the ten patients who were included in the study, 1 CR and 6 PR were observed (ORR 70%) with a median duration of response of 8 months (range: 7−12 months). Toxicity was mainly haematological, with grade 1 or 2 neutropenia and anaemia, and grade 2 and 3 thrombocytopenia. The pharmacokinetic study showed that the half-lives of ^{111}Indium and ^{90}Yttrium were almost identical.