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

J. Cummings, S. St George Smith, T. Ward, M. Ranson, C. Dive.
Paterson Institute, Manchester, United Kingdom

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

N. Dahl, R. Leth-Larsen, H. Ditzel. *Medical Biotechnology Center, Denmark*

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.

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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

A. de Langen[1], O. Hoekstra[1], A. Dingemans[1], H. Groen[2], V. van den Boogaart[3], J. Pruijm[2], P. Kappert[2], E. Smit[1]. *[1]VU University Medical Center, Amsterdam, The Netherlands; [2]University Medical Center Groningen, Groningen, The Netherlands; [3]University Hospital Maastricht, Maastricht, The Netherlands*

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