

SPARC) and peptidases (i.e. MMP2, cathepsins) suggesting a remodeling of tumor environments in these carcinomas. In the same group the upmodulation of genes specific of NK cells and genes involved in proliferation has been found by using the GSEA software that evaluates microarray data at the level of gene set. Markers emerged from this analysis are under evaluation on tumors section from patients who have received trastuzumab for metastatic disease and categorized as responder or not responder according to the Recist criteria with a medium follow-up of 31 months.

Conclusions: In conclusion, our preliminary results suggest that HER2-amplified breast carcinoma are a heterogeneous group especially concerning extracellular matrix and infiltration composition. Supported by AIRC.

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aCGH analysis of male breast cancers (MBC)

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Background: Male breast cancer (MBC) is a rare disease whose causes are poorly understood. Information on genome alterations by CGH in MBC showed similar pattern of imbalances with female breast cancer (FBC), suggesting a common aetiology. To elucidate the somatic genetic changes of MBC we analysed a series of 30 MBC using array Comparative Genomic Hybridisation (aCGH). aCGH has been successfully used in post-genomic cancer research studies because the screening of gene copy number covers a key role in the understanding of biological pathways involved in the complex tumorigenic process.

Methods: 30 male patients who had received a primary diagnosis of breast cancer and had been analyzed for familial characteristics in Genetic Outpatients Clinics were investigated and compared with aCGH analysis from sporadic and familial FBC.

Genomic DNA was extracted from 20 mg of frozen tumor tissues using the DNeasy tissue kit (Qiagen). The reactions were checked on 0.8% agarose gel and the DNA obtained was quantified by spectrophotometry (Nanodrop, Celbio). aCGH analysis was performed using the Microarray Kit 44B (Agilent). We identified the most significant sequences altered as ones with $p < 0.0001$ and $\log_2(\text{ratio})$ value $+0.5$ and -0.5 for gains and losses, respectively. The data set was analysed with MATLAB software to extrapolate profiles for the 30 MBC in study.

Results: Preliminary results on 20 patients showed the presence of a wide range of chromosome alterations spanning all the genome. The most frequent chromosomes involved in gains were 8q21-24, 17q12, 20q13 in which some interesting genes map, such as PLEC1, a protein involved in cytoskeleton-membrane attachment, DOK5, an adapter protein involved in signal transduction and BCAS1, a candidate oncogene for breast cancers. The most frequent losses were on chromosomes 2p23.2, 19p13, 22q13 and Y, in which the mapped genes were: GSTT1, a Glutathione S-transferase and APOBEC3A, which has a role in growth or cell cycle control. The deletions on Y chromosome encompass TBL1Y gene and PRKY gene, whose function is important in cytotipic differentiation in males.

Conclusions: Our approach allows to identify somatic genetic changes that are specific in MBC. We think it is important to report these alterations because our data could show some possible association functions in tumour development.

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BRCA1, ER-alpha expression and molecular BRCA1 alterations in familial and sporadic breast cancer

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Background: There are major discrepancies concerning the usefulness of various antibodies in detecting BRCA1 protein expression and its subcellular localization. The aim of the present study was to evaluate the performance of immunohistochemical MS110 expression with respect to molecular BRCA1 alterations in a series of familial and sporadic breast cancer patients.

Methods: An immunohistochemical study was performed on TMA samples from 93 sporadic and 94 familial breast cancer patients with (7/94) and without BRCA1 germline mutations. In all 94 patients, BRCA1 alterations gene have been studied by dHPLC and direct sequencing. BRCA1 protein expression level has been evaluated by using the monoclonal MS110 antibody, suitable for immunohistochemical analysis of paraffin-embedded tissue sections.

Results: Immunohistochemistry carried out using the MS110 antibody, showed positive nuclear-staining for BRCA1 protein in 34 (41%) sporadic and 37 familial (44%) breast tumours respectively. All the tumours from patients carrying BRCA1 mutations showed complete loss of both BRCA1 and ER α expression, regardless of the type of mutation ($p = 0.02$). BRCA1 wild type expression resulted similar both in sporadic and in familial tumours, while ER α expression was higher in sporadic tumour patients (62% vs 40% in sporadic and familial cases respectively; $p = 0.012$). Interestingly, the presence of the E1038G polymorphism in BRCA1 exon 11 was significantly associated with protein expression ($p = 0.029$). Furthermore, confirming what found in previous studies, loss or reduction of both BRCA1 and ER-alpha expression in familial patients were correlated both with higher histological grade ($p < 10^{-6}$, $p = 0.004$ respectively) and lower PgR positive rate ($p = 0.001$, $p = 0.022$, respectively). No significant correlation between BRCA1 and ER-alpha expression was found in both familial and sporadic patients.

Conclusions: Lack of MS110-immunostaining is significantly associated with molecular alterations. However, the frequency of MS110 negative cases also detected in BRCA1-wild type tumours, points to the inability of the IHC-BRCA1 expression in discriminating between familial and sporadic breast cancer, further suggesting that ER-alpha more than BRCA1 could be associated with sporadic breast cancer.

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Treatment of locally advanced head-and-neck squamous carcinomas with chemotherapy alternated to radiation and Cetuximab (ALTERCC Phase II study)

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Background: The gold-standard in the treatment of locally advanced squamocellular carcinomas is the combination of cisplatin-based chemotherapy with radiation therapy. To improve the outcome of this approach, the anti-EGF receptor (EGFr) monoclonal antibody C225 (Cetuximab) has been used with radiation alone or chemo-radiotherapy. We have initiated a phase II trial to evaluate the feasibility and activity of a regimen containing Cetuximab and radiation alternated to cisplatin-based chemotherapy (ALTErNating Radiotherapy and Chemotherapy plus Cetuximab, ALTERCC).

Methods: Chemotherapy (Cisplatin 20 mg/mq/day for 5 days plus 5-FU 200 mg/mq/day for 5 days) was given in weeks 1, 4 and 7, while radiation (10 Gy over 5 fractions, 1 fraction per day) in weeks 2, 3, 5, 6, 8, 9. Cetuximab was given at 400 mg/mq loading dose followed by 250 mg/mq weekly, concomitantly with the radiotherapy. Most patients (82%) had a disease stage IV and 92% had nodal involvement. All cases had primary tumours of either the oral cavity, oro-pharynx, hypo-pharynx or larynx, that were immune-positive for EGF receptor when analysed by immune-histochemistry.

Results: To date, we have treated 35 patients. Out of 23 measurable responses, on an intent-to-treat analysis, 15 (65%) were complete and 5 (22%) were partial. Two patients included in the study died, one patient refused treatment. No patient underwent progression during the treatment. Overall, the regimen was tolerable and characterized by the same spectrum of toxicities as in conventional regimens of combined chemo- and radiotherapy. Grade 3-4 neutropenia and mucositis were the most frequent adverse effects and were seen in 13 and 16 out of 24 cases, respectively. Interestingly, two thirds of the patients (18/24 = 75%) developed a benign, humid epidermo-lysis of the neck, which spontaneously regressed after one week of topic nursing procedures. This toxic effect is probably radiation-related and never appeared before an administered cumulative dose of 45 Gy. However, a cutaneous toxic interaction between Cetuximab and fluorouracil is also possible.

Conclusions: All together, our data demonstrate the feasibility and activity of Cetuximab plus radiation alternated to chemotherapy in patients with locally advanced head-and-neck squamocellular carcinomas. Our results also prompt the implementation of phase III studies to compare the ALTERCC protocol with conventional regimens of cisplatin-based chemotherapy combined with radiation.

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Molecular features in locally advanced head-and-neck squamous carcinomas might predict response to treatment with chemotherapy alternated to radiation and Cetuximab

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Background: The anti-EGF receptor (EGFr) monoclonal antibody C225/Cetuximab has shown a promising activity in patients with locally advanced head-and-neck squamocellular carcinoma (HNSCC), when

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 (ALTErning 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.

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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.

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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.

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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

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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