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 reliminary results suggest that HER2-amplified breast carcinoma are a heterogenous group especially concerning extracellular matrix and infiltration composition.
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P29
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 log2(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 APOBECC3A, 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.

P47
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 cattied 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 ERá expression, regardeless of the type of mutation ($p = 0.02$). BRCA1 wild type expression resulted similar both in sporadic and in familial tumours, while ERá 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 unability 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.

P42
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 fluorouracile 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.

P43
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