cancer, in 32 sporadic ones before surgery and in 17 healthy donors. For the analysis of SCE, 500 μl of peripheral blood were cultured in 5 ml of Chromosome medium P (Euroclone); after a period of 48 h at 37ºC, 25 μl of a 1 mg/ml 5-bromo-deoxyuridine solution was added to the blood culture. During the incubation, cells were protected from direct light to prevent photolysis of BrdU-containing DNA. Cells were arrested in metaphases by colcemid. After harvesting, they were treated with hypotonic solution at 37ºC and fixed with methanol:acetic acid 3:1. Slides were air dried, placed in 90ºC hot plate for 1 h, stained with Hoechst for 15 min and exposed to UV light for 1 h. Subsequently, the slides were incubated with water at 56ºC for 10 min and stained with Giemsa. For each patient 10−30 metaphases were evaluated. Every metaphase was scored for SCE and individual mean value per cell was calculated. SCE baseline values of familial breast cancer patients were compared to those of sporadic ones and to those of a control group of healthy donors.
Results: Results were expressed as means ± standard deviation (sd). Intergroup comparisons were performed using Kruskall Wallis non parametric test. However, SCE was significantly increased ($P < 0.05$) in familial breast cancer patients (5.3 ±1.2 sd per metaphase) respect to sporadic (3.9 ±1.2 sd) ones and to controls (4 ±1.5 sd). No correlation was found between clinic pathological data and number of SCE.
Conclusion: We suggest that the increased number of SCE in familial patients respect to sporadic ones reflects an intrinsic genomic instability that is an essential process in carcinogenesis.

PP55

A MAGE-A3 specific quantitative RT-PCR assay used for patient accrual in MAGRIT, a Phase III ASCI (Antigen-Specific Cancer Immunotherapeutic) trial in adjuvant NSCLC

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Background: Over the last 20 years, the field of active cancer immunotherapy has been extensively investigated but no products have been registered to date. GSK Biologicals is developing an approach to cancer therapy, Antigen-Specific Cancer Immunotherapy (ASCI) that combines a tumor-specific antigen, delivered as a recombinant protein, with a potent immunological Adjuvant System. The first compound of this new class of anticancer agent contain MAGE-A3 tumor-specific protein that is expressed in a number of cancer types including non-small cell lung cancer (NSCLC). The MAGE-A3 antigen has been produced as a recombinant protein and combined with an immunological Adjuvant to treat NSCLC patients. Since only a fraction of NSCLC tumors (about 35%) express the MAGE-A3 protein, patients' tumor must be screened for MAGE-A3 expression to evaluate patient eligibility for enrolment in clinical trials.
Materials and Methods: A MAGE-A3 specific quantitative RT-PCR assay has been developed to measure mRNA expression of this protein in macro-dissected, formalin-fixed paraffin embedded (FFPE) tumor samples. Since paraffin embedded tissues are the most common source of human tumor samples, a method using this type of material rather than fresh or frozen tissues, has been developed by Roche Molecular Systems. The use of FFPE tumor biopsies reduces the potential for handling challenges related to testing fresh or frozen tissues and enables easy use in the frame of clinical trials. Design, cut-off and performance criteria have been established and verified for the use of this assay in a Phase III trial.
Results: The assay is being applied in MAGRIT, a large randomized, double-blind, placebo controlled Phase III trial evaluating MAGE-A3 ASCI in NSCLC. To date, over 3000 samples have been tested with the assay. The fraction of MAGE-A3-positive patients identified on FFPE material in MAGRIT is comparable to that identified on fresh frozen biopsies in a Phase II trial (Vansteenkiste et al., ASCO 2007). The large number of samples collected in MAGRIT allows a detailed analysis of MAGE-A3 expression in this patient population.
Conclusion: A critical aspect of the development of this immunotherapy approach is the co-development of the ASCI and the dedicated screening assay. This work reports on the feasibility of using quantitative RT-PCR on FFPE material in large Phase III immunotherapy trials.

PP56

Prostate-specific antigen doubling time in metastatic castration-resistant prostate cancer: a clinically useful prognostic factor

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Background: Metastatic castration-resistant prostate cancer (mCRPC) is a heterogenous disease. In the present study PSA-velocity (PSAV) and prostate-specific antigen doubling time (PSA-DT) in mCRPC progressing after first-line chemotherapy was evaluated at time-1 (emergence of castration-resistance), time-2 (before first-line chemotherapy) and time-3 (after further PSA-progression).
Materials and Methods: Data from 25 mCRPC patients treated with chemotherapy between June 2003 and Jun 2009 were retrospectively analyzed. PSAV and PSA-DT were measured by considering at least three determinations of PSA. Survival rates according to PSAV and PSA-DT were reported and the behaviour of PSA-DT in PSA-responders was evaluated.
Results: The majority of patients received a docetaxel-based regimen (20/25 cases). PSA response rate was 31.8% (7/22). Median PSAV is increasing from time-1 (0.07 ng/ml/day) to time-2 (0.48 ng/ml/day) and time-3 (1.29 ng/ml/day), median PSA-DT is stable (71.6 days, 78.3 days and 71.42 days).
Median overall survival (OS) was 18.1 months and median survival from emergence of mCRPC was 28.8 months. Median OS was 28.8 mos in PSA responsive patients vs 19.4 mos in PSA-stable vs 13.6 mos in PSA non-responders. At time-1, median OS was 21.1 mos vs 11.8 mos ($p = 0.08$) in patients with PSA-DT >60 days and <60 days. At time-2, median OS was comparable (19.2 mos vs 18.1 mos) in patients with PSAV <1 ng/mL/day, and PSAV >1 ng/mL/day, respectively. Median OS was 23.4 mos vs 5.6 mos ($p = 0.01$) in patients with PSA-DT >50 days and with PSA-DT <50 days, respectively. At time-3, PSAV was comparable in responders and non responders (1.23 vs 1.29 ng/mL/day). However, PSA-DT was 65 days in responders compared to 88 days in non responders ($p = 0.20$). Median OS was 19.4 mos vs 28.8 mos in patients with PSA-DT >60 days or <60 ($p = 0.14$).
Conclusion: PSAV, despite the progressive increase in this parameter during the course of disease, is not a reliable predictor of individual prognosis at any time. It appears that a significant reduction in PSA-DT prior to first-line chemotherapy (time-2) is the best predictor of OS.

PP25

Correlation between clinical prognostic factors and MR spectroscopy-based biomarkers determined at 3.0 T without endorectal coils for patients with localised prostate cancer

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Background: The value of biomarkers such as Choline, determined with Magnetic Resonance Spectroscopy (MRS) remains questionable. We aimed to investigate if data from MRS could be related to prognostic parameters for patients (pts) with localised prostate cancer (PCa).
Materials and Methods: Seventy-two pts (mean age: 67.8±6.2 years) with biopsy-proven prostate cancer were referred for MRS. Mean PSA value was 12.9 ng/ml (range: 2.8–68 ng/ml). Pts and tumor characteristics were (number of pts between parentheses): TNM: T ⩽ 2a (46), T2b−c (15), T ⩾ 3 (11), Gleason score ⩽6 (50), 7 (20), ⩾8 (2), PSA ⩽10 ng/ml (44), 11–19 ng/ml (15), ⩾20 ng/ml (13).
The following prognostic classes were proposed according to the classification of D'Amico: Low risk (26): T ⩽ 2a, Gleason ⩽6, PSA ⩽10 ng/ml; intermediary risk (27): T2b−c, Gleason 7, PSA 11–19 ng/ml; high risk (19): T ⩾ 3, Gleason ⩾8, PSA ⩾20 ng/ml.
MRS was performed at 3 T using a phased-array coil. The following metabolites ratios were registered: Choline/Citrate (C/C), Choline+Creatine/Citrate (CC/C) and Choline+Creatine+Spermine/C (CCS/C). Mean ratio of the most pathological voxels and number of pathological voxels (i.e. voxels with a C/C ratio >0.5) were also determined. Wilcoxon and Kruskall-Wallis tests were used to compare mean values.
Results: Mean values of C/C, CC/C and CCS/C of the most pathological voxels were significantly higher for tumors ⩾T2b−c vs. ⩽T2a: 7.5 (±13.6) vs. 2.3 (±5.6), $p = 0.018$; 8.9 (±14.5) vs. 2.5 (±5.7), $p = 0.016$ and

10.3 (±15.4) vs. 3.5 (±6.1), p = 0.018, respectively. These mean values were neither correlated with Gleason score, nor PSA, nor risk groups.
Mean number of pathological voxels for patients with a tumor ⩾T2b−c was: 6.31 (±7.43) and 4.09 (±7.64) if ⩽T2a (p = 0.03). The mean number of pathological voxels was significantly correlated with increased PSA: (OR: 1.80 [95% CI 1.016–1.149], p = 0.013). No correlation was found with the Gleason score. Patients with intermediary risk have a significantly higher number of pathological voxels than in the low risk group (OR: 1.075 [95% CI 1.009–1.145], p = 0.025). A similar result was found for high risk pts with respect to the intermediary group.
Conclusion: At 3 T, biomarkers expressing the relative Choline content and number of pathological voxels were powerful parameters for the localisation of PCa and could be helpful for determining the prognosis more accurately.

PP38

Proteomic analysis of plasma from BRCA1/BRCA2 mutation carriers as modifier risk factor of breast cancer

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Background: BRCA1 and BRCA2 mutations were estimated to cause cumulative lifetime risks of breast cancer of 70% by 70 years. However, there is a marked variation of phenotypic expression among BRCA mutation carriers. Identification of markers that can be used to refine estimates of a woman's individual cancer risk appears to be warranted in order to individualizing prevention strategies. The aim of this study was to analyze using proteomics modifications in the plasma protein map from BRCA1/2 mutation carriers with respect to a control group, as well as potential differences between cancer patients (CP) and cancer-free carriers (CFC). Preliminary results were presented at ICACT 2009 (abstract 237).
Materials and Methods: We use two-dimensional gel electrophoresis and mass spectrometry to analyze plasma levels of the following proteins in 40 female BRCA mutation carriers (22 BRCA1 − 10 CP and 12 CFC − and 18 BRCA2 − 12 CP and 6 CFC): fibrinogen (fgg), haptoglobine (hap), serotransferin (ser), convertase (con), alpha1-antitrypsin (AAT), apolipoprotein A-I (APO AI) and A-IV (APO AIV) and C-reactive protein (PCR). The control group included 10 age-matched non-carriers without personal or family history of cancer. We also use the PD-QUEST software to determine the total protein count for each group.
Results: AAT 4 (p = 0.038), 5 (p = 0.023) and 6 (p = 0.037), hap 4 (p = 0.023) and APO AI 2 (p = 0.038) and 5 (p = 0.013) were increased in plasma from BRCA2 mutation carriers with respect to BRCA1 ones. BRCA1 CFC showed increased levels of AAT 6 (p = 0.058) and APO AIV (p = 0.004) when compared to BRCA1 CP. No significant differences were found in the protein map between BRCA2 CP and CFC. BRCA2 CP when compared with BRCA1 showed increased levels of AAT 6 (p = 0.023), APO AI 2 (p = 0.038) and 5 (p = 0.019) and APO AIV (p = 0.010) and decreased levels of con 3 (p = 0.023). Fgg 2 (p = 0.001) and 3 (p = 0.001), ser 4 (p = 0.001) and con 1 (p = 0.041), 2 (p = 0.006), 3 (p = 0.001), 4 (p = 0.001) and 5 (p = 0.001) were found significatively increased in plasma from CFC with respect to the control group. No significant differences were found in the total protein count among groups using PD-QUEST.
Conclusion: BRCA2 mutation carriers showed a favorable plasma protein map when compared with BRCA1 ones, as well as observed in control group with respect to BRCA1/2 carriers. Moreover, BRCA1 CFC had better prognosis proteomic profile than BRCA1 CP. The confirmation of these results in a larger cohort is required before proteomic analysis could be used to guide prevention strategies among BRCA mutation carriers.

PP17

The predictive value of HLA Class I tumor cell expression and tumor infiltration by regulatory T cells for chemotherapy in patients with early breast cancer

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Background: Additional prognostic and predictive factors may direct tailored treatment in early breast cancer and thus decrease morbidity and mortality rates. We hypothesized that T cell immune interactions affect tumor development and therefore clinical outcome. Therefore, we examined the clinical impact of human leukocyte antigen (HLA) class I tumor cell expression and regulatory T cell (Treg) infiltration in breast cancer.
Materials and Methods: Our study population (n = 677) consisted of all early breast cancer patients primarily treated with surgery in our center between 1985 and 1994. Formalin-fixed paraffin-embedded tumor tissue was used for immunohistochemical staining with HCA2 and HC10 (recognizing HLA Class I) and Foxp3 (recognizing Treg) antibodies. HLA class I expression was evaluated by combining results from HCA2 and HC10 antibodies and was classified into three groups: loss, downregulation and expression. Treg infiltration was defined as complete absence or presence of Treg.
Results: Remarkably, only in patients treated with chemotherapy after surgery, both presence of Treg (p = 0.01; Hazard Ratio (HR): 2.04) and expression of HLA class I (p = 0.002; downregulation HR: 2.11; loss HR: 3.34) resulted in less relapses over time, independently of other clinicopathological parameters. Treg and HLA class I were not of influence on outcome in patients who did not receive chemotherapy. An interaction between chemotherapy and HLA class I (p < 0.001) and Treg (p < 0.001) was found in cox regression analysis.
Conclusion: We showed that HLA class I and Treg both affect prognosis, exclusively in chemotherapy-treated patients. In mouse studies it has been shown that chemotherapy can selectively eliminate Treg, therefore possibly enabling Cytotoxic T-lymphocytes to kill tumor cells that have retained HLA class I expression. Therefore, we conclude that HLA class I and Treg could be applied in response prediction to chemotherapy in early breast cancer patients.

PP28

Prediction of response to neo-adjuvant radiotherapy in patients with locally advanced rectal cancer by means of sequential 18F-FDG-PET

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Background: The current approach to curative treatment of locally advanced rectal cancer is comprised of surgery combined with neo-adjuvant (chemo)radiotherapy. Radiosensitivity of rectal cancer however is variable. Morphological imaging techniques are performing poorly in assessing the response because of their inability to differentiate residual neoplastic from scar tissue. The aim of this study was to investigate the potential of sequential 18F-FDG-PET(CT) in assessing the response of rectal cancer to neo-adjuvant radiotherapy (RT), and to determine which parameter(s) can be used as useful metabolic response indice(s).
Materials and Methods: Patients (pts) were treated by Tomotherapy: dose of 46 Gy to the presacral space and integrated boost to 55.2 Gy on the tumor, if circumferential resection margin was <2 mm on magnetic resonance imaging. Pts underwent total mesorectal excision within 6 w after completion of RT, and tumor regression was graded histologically.
18F-FDG-PET or PET/CT scans were acquired prior to and in the 5 w after the end of RT. Tumoral uptake of 18F-FDG was assessed semi-quantitatively using standardized uptake values (SUV). The percentage difference (Δ) between pre- and post-RT scans in SUVmax, SUVmean (average SUV of tumoral pixels with SUV ⩾2.5) metabolic volume (MV, sum of tumor pixels with SUV ⩾2.5) and the total glycolytic volume (tGV, MV × SUVmean) was investigated as possible metabolic response indices.
Results: 45 consecutive pts (34 male and 11 female; age 65.4±12.5) with histologically confirmed rectal adenocarcinoma (cT3/T4) were enrolled.
Following neo-adjuvant RT, of the 45 pts 20 (44.4%) were classified as responders, while the remaining 25 (55.6%) were non-responders. Intense 18F-FDG uptake was seen in all tumors prior to neo-adjuvant RT: average SUVmax 12.9±6.0 (no significant different beween responders and non-responders). When classifying pts according to histology significant differences in average ΔSUVmax (55.8% vs 37.4% decline, p = 0.023) and ΔSUVmean (40.1% vs 21.0%, p = 0.001) between responders and non-responders respectively were observed. For MV and tGV decreases were more prominent in responders (72.9% vs 62.7% and 80.2% vs 68.1%, respectively), but not significantly different from non-responders.
Conclusion: Sequential FDG-PET(CT) is a useful method to evaluate response of rectal cancer to neo-adjuvant RT. ΔSUVmax and ΔSUVmean are parameters that can be used as indices of metabolic response.

PP20

Clinical significance of the epidermal growth factor receptors (EGFR and HER-2) overexpression in esophageal squamous cell cancer

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Background: The present study was aimed to assess EGFR and HER-2 expression in esophageal squamous cell carcinoma (ESCC) and to correlate the results with clinic-pathological findings and prognosis.
Materials and Methods: The immunohistochemical staining of EGFR and HER-2 receptors were retrospectively investigated in biopsy specimens from 31 patients with T (2−3), N-any, and M-0 ESCC. The results were classified according to the Herceptest criteria (Dako): negative (0/1+) and positive (2+/3+).
Results: The EGFR expression was negative in 7/31 (22.6%) and positive in 24/31 (77.4%), of which 12 (38.7%) were 2+ and 12 (38.7%) were 3+.