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