of resting energy expenditure (REE), increase of total daily physical activity, decrease of IL-6 and TNF-alpha and improvement of fatigue.

Methods: All patients were given as basic treatment: polyphenols + antioxidant agents alpha lipoic acid, carbocysteine, Vitamins E, A and C, all orally. Patients were then randomised to one of the following 5 arms: (1) Medroxyprogesterone Acetate (MPA)/Megestrole Acetate (MA); (2) Pharmaco-nutritional support containing 2 g EPA; (3) L-carnitine; (4) Thalidomide; (5) MPA/MA + Pharmaco-nutritional support + L-carnitine + Thalidomide. Treatment duration 4 months. The sample size was 475 patients. At May 2007, 160 patients, well balanced for all clinical characteristics, have been included. Body composition has been assessed by dual energy X-ray absorptiometry (DEXA) since January 2007.

Results: No severe side effects were observed. As for efficacy, an interim analysis on 145 patients showed an improvement of at least 1 primary endpoint in arm 3 (significant decrease of REE and fatigue), 4 (significant decrease of IL-6) and 5 (significant decrease of REE and fatigue), whilst arm 2 showed a significant worsening of LBM, REE and MFSI-SF. The t-test for changes demonstrated the worsening of LBM, REE and MFSI-SF in arm 2 versus arms 3, 4 and 5 and therefore it was withdrawn from the study. Arms 1, 3, 4 and 5 showed no statistical significant difference.

Conclusions: The interim results obtained so far seem to suggest that the most effective treatment for CACS/OS should be a combination regimen and L-carnitine alone. The study is still in progress up to completion of final accrual about 400 patients (sample size reduced because of withdrawal of arm 2 from the study).

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P54

Study of ATP7B copper transporter mRNA levels as a prognostic factor in advanced colorectal cancer patients treated with 5-fluorouracil plus oxaliplatin

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Background: It has been demonstrated a correlation between ATP7B protein and/or mRNA levels and response to treatment in ovarian cancer patients treated with cisplatin based chemotherapy. The aim of our work was to determine ATP7B mRNA expression levels in colorectal cancer (CRC) tumor tissue of patients treated with oxaliplatin (OXA) and 5-fluorouracil (5FU) according Spanish TTD group schedule and to correlate with response to treatment.

Methods: mRNA levels were analyzed by using Real Time PCR (RT-QPCR). The housekeeping gene used was â-actin and as a reference sample we used commercial human mRNA from liver. Chi-square and Fisher test were used in order to value differences in response rate to treatment. Time to progression (TTP) was studied by using Kaplan Meyer curves and Log rank test.

Results: Fifty-three advanced CRC patients treated with 5FU plus OXA were analyzed. 62.15% of them were males; primary tumour was localized in colon in a 66.7% of cases. We did not observe any progression to treatment in the group of patients that had ATP7B expression levels under percentile 25 (group 1). In contrast, patients with expression levels upper (group 2) showed 13.2% of progressions. Group 1 patients also had a greater number of complete responses than group 2 (33.3% vs 15.8%). TTP median was 14.66 and 7.18 months for group 1 and 2 patients respectively (Log rank p = 0.035).

Conclusions: According our results, lower expression of ATP7B in tumor tissue of CRC patients treated with 5FU plus OXA correlates with a greater response rate and a better TTP. However, these results should be confirmed in a higher number of patients.

P12

SELDI-TOF MS serum protein profiling predicts poor prognosis renal cancer patients

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Background: Approximately 30% of patients with renal cancer (RCC) present with metastasized disease and only 15−25% of patients respond to anti-tumor treatment. Although treatment outcome is improving due to the development of various targeted therapies, adequate selection of patients for these treatment approaches is important. Profiling of proteins in body fluids to predict patient outcome would be an attractive and

non-invasive approach for utilization in the clinic. Surface-enhanced laser desorption/ionization-time of flight mass spectrometry (SELDI-TOF MS) was used to identify protein signatures in the serum proteome of RCC patients that discriminate between patients with poor or good outcome.

Methods: In this pilot study we analyzed protein profiles in the serum of 57 renal cancer patients (2% stage I, 12% stage II, 7% stage III and 79% stage IV patients according to the American Joint Committee on Cancer) and 59 healthy controls. Denatured serum samples were incubated on CM10 ProteinChip arrays and analyzed using the PBS-IIC ProteinChip Reader. Clinical data was collected and the extended Memorial Sloan-Kettering Prognostic Factors Model for survival was calculated. Ratios discriminating between RCC cases and controls were selected to generate a predictive multi-protein model. Univariate and multivariate Cox Proportional Hazard analyses were performed. Protein masses included in the predictive model were identified.

Results: In RCC serum samples we identified ion masses predictive for patient survival, and built a protein-model consisting of five signature peaks with m/z ratios of 2944, 3331, 6457, 6654, and 9201 Da, that could correctly identify poor prognosis patients with sensitivity and specificity of 80% and 76% for 1-year survival. Cumulative 1-year survival was 93% for low-risk patients, compared to 48% for high-risk patients (P = 0.0001, Log-rank test). Multivariate analysis indicated that our model was an independent predictor of survival when compared to the Memorial Sloan-Kettering Prognostic Factors Model. The tentative protein identities were apolipoprotein C-I (doubly and singly charged, and a singly charged fragment), a haptoglobin-alfa1 fragment and a yet unidentified 2.9 kDa protein fragment.

Conclusions: SELDI-TOF MS can be used to assess the prognosis of RCC patients independent of present prognostic factor models.

P23

Novel computational paradigms in breast cancer familiarity profiling

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Background: Genomic DNA copy number aberrations are frequent in solid tumours although their underlying causes of chromosomal instability in tumours remain obscure. The aim of our work was to individualize a genomic profile characterizing familial breast cancer.

Methods: For this purpose, a series of 124 consecutive breast cancer patients analyzed for aCGH entered the study. The results have been elaborated by an Artificial Immune System paradigm that we hypothesized could be successfully employed in the elucidation of biological dynamics of cancerous processes using a novel fuzzy rule induction system for data mining (IFRAIS) of aCGH data.

The most important characteristic of IFRAIS is that it discovers fuzzy classification rules, naturally comprehensible. It is obvious, of course, that comprehensible knowledge is essential in real-world data mining problems (e.g. in bioinformatics). The accuracy of results are expressed in terms of medians of the extracted values. The selected strategy for training and validation was the KFold cross-validation with K = 5. The global level of accuracy reached by the system nears the 97%; a quite competitive result indeed, even if we consider that algorithms like J48 (C4.5 evolution) is not able to go beyond the 94.34% of accuracy.

Results: We evidenced 3 rules to define familial and 5 rules to define sporadic breast cancer. In particular, rule 1 for familial cases involved genes linked to neurodegenerative pathway and comprised genes involved in important step of cell cycle, including apoptosis and signalling transduction in processes activating NFKB (locus 20p13, locus 11q22.3), or with growth factor activity (locus Xq26.2). Rule 2 of sporadic cases included genes involved in cell metabolism and differentiation (TGIF, CYP2R1, PUM1), while rule 4 involved genes of apoptosis pathway and a gene encoding a protein highly conserved among species, SCOC, probably involved in regulation of important cell cycle processes.

Conclusions: In this work we present the study of a novel rule induction system. We can conclude that novel biologically-inspired data mining techniques seem to be competitive interesting tools in cancer research. However, the full understating of the underlying dynamics in cancer settlement and progression still remains a primary objective.

P82

18F-fluorothymidine(FLT)-PET as a biomarker of the antineoplastic effects of radiation therapy combined to the anti-angiogenic agent Enzastaurin in lung cancer model

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Background: Positron emission tomography (PET) with 15F-fluorodeoxy-glucose is an established tool in diagnosis, staging, and surveillance of cancer. A limitation of this imaging modality, however, is its nonspecificity

between cancer and inflammation. 18F-fluorothymidine (FLT)-PET imaging may be more specific for cancer since it measures cell proliferation. Here, we used FLT-PET to assess the antineoplastic effects of radiation therapy (RT) combined with the antiangiogenic agent Enzastaurin (ENZ).

Methods: Human H460 lung cancer cells were used in an athymic nude mice xenograft model. Treatment groups (each containing 5 mice) consisted of vehicle control (DMSO), 80 mg/kg bid ENZ alone, vehicle+RT and ENZ+RT. DMSO and ENZ alone were administered for 2 days, then either sham or 2 Gy was added prior drug treatment for 5 days. After the 7 days of treatment, fixed tumor sections from each group were analyzed by IHC, using von Willebrand Factor (vWF) or Ki67 staining. Tumor blood vessels were quantified using vWF by randomly selecting 5 separate $400\times$ fields and counting the vessels/field number. Ki67 positive cells number were scored/plotted in similar fashion to vessel quantification. Whole body FLT-PET imaging of hind limb tumors were performed before and after the 7 treatment days

Results: Ki67 proliferation index revealed that ENZ+RT group resulted in 70% (p < 0.001) and 30% (p < 0.001) reduction in proliferating cells relative to control or RT alone groups, respectively. vWF quantification showed that ENZ+RT (3; SD = 1.6) resulted in a 3-fold reduction in average number of vessels/field compared to control (10.2; SD = 2.77; p < 0.007) and 2-fold reduction relative to RT alone (7; SD = 2.1; p < 0.04). Tumor response to therapy was measured in FLT-PET imaging as change in FLT uptake. The ENZ+RT group showed the most significant attenuation of tumor avidity for FLT. When normalized to muscle uptake, only the ENZ+RT group showed a decrease below baseline measurement, which significantly correlated to IHC analysis. Furthermore, the RT+ENZ treatment resulted in a substantially decrease in tumor viability from 97% to 75%, while 90% tumor was viable before treatment

Conclusions: FLT imaging effectively depicted antineoplastic effects of RT combined with ENZ. The measurements of tumor cell proliferation by FLT-PET were significantly correlated to in vivo biomarkers such as Ki67 or vWF. These results demonstrate that FLT-PET may be important in measuring therapeutic response to anti-angiogenic agents and RT in clinical setting.

P40
The Centre of Biological Ressources of Nancy, France: DNA, RNA and proteines quality testing

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Background: Biobanks are now a keystone for the biotechnologies. In the centre for Biological Ressources of Nancy we have collected from 1989, 6,000 samples. They correspond to patients operated upon for lung tumors, mesothelioma and also from healthy controls. All are annoted with the complete clinical follow up and with the results of biological research. The collection is open to the scientific community at large and all samples need therefore to be quality controls certified. These controls are described here.
Methods: RNA was extracted from randomly selected 20 paired samples of lung cancer(tumor and healthy parenchyma) and the mRNA levels for three retinoid receptors were measured with a triplex of taqman probes. DNA was also extracted and the number of copies of the EGF receptor was measured with a duplex of taqman, one being the stable calibrator. Corresponding plasmid calibrators were included. The levels of GAPDH protein was measured with a specific ELISA in the same samples.
Results: GAPDH protein is well conserved in lung tissues but does not reflect the quality of the mRNA. The choice to measure very low expressed genes like retinoid receptors is better than to measure actin mRNA. In the lung, RARalpha is a stable calibrator. The DNAcontrol is a good quality control. Plasmids calibrators calibrate the machines result for harmonization between qPCR machines.
Conclusions: For biobank, quality controls are needed and we tested the validity of such tools. The best quality controls were for RNA and DNA but plasmid reference are also needed to calibrate qPCR machines results since they have different performances while we need controls in kit effective for all machines.

P78
Polymeric immunoglobulin receptor downregulation in lung cancer development

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Background: Epithelial transport of polymeric immunoglobulin A (pIgA) from submucosal tissues to mucosal lumen is mediated by the polymeric immunoglobulin receptor/secretory component (pIgR/SC). Although pIgR/SC is downregulated in lung cancer, its implication in tumorigenesis remains unknown. Our hypothesis is that downregulation of pIgR/SC is an

early event in lung tumor progression and that pIgR/SC contributes to cell proliferation, apoptosis and migration.
Methods: Expression levels of pIgR/SC were evaluated by Western blot (WB) and immunohistochemistry (IHC). WB was performed on 2 normal bronchioepithelial and 8 lung cancer cell lines, and on normal and cancer tissues from 4 different patients. IHC was performed on 11 normal lung tissues, 10 preinvasive lung tissues and 162 lung cancer tissues assembled in tissue microarrays (TMA). Immunostaining was scored by 2 pathologists. The scoring index (0 to 300) was created as product of staining intensity (0: no, 1: low, 2: moderate, 3: strong) and percentage of positive cells (0 to 100%).
Results: WB showed low expression of pIgR/SC in 10/10 lung cell lines and downregulation in 2/2 squamous cell carcinoma (SCC) and in 1/2 adenocarcinoma (ADC) tissues, compared to normal lung tissues from same patients. IHC on normal lung tissues (10/11) revealed a strong staining for pIgR/SC in mucous and serous cells in surface and glandular epithelium (mean score, MS = 250), a weak staining in ciliated cells (MS = 50) and an expected absence of staining in alveolar cells. Preinvasive lung lesions showed no pIgR/SC expression (4 low and 6 high grades). IHC on lung TMA displayed pIgR/SC positive staining only for 10/59 ADC (17%, MS = 48), for 4/11 bronchioloalveolar carcinoma (36%, MS = 61), and for 4/63 SCC (6%, MS = 21). There was no staining for all of the 12 large cell lung cancers, 2 small cell lung cancers, 5 large cell neuroendocrines and 10 carcinoids.
Conclusions: Our results demonstrate downregulation of pIgR/SC expression in a continuum from normal epithelium to invasive lung cancer, in a majority of lung tumors. In addition, absence of pIgR/SC expression in preinvasive lesions suggests that pIgR/SC downregulation is an early event during lung cancer development. Based on these results, we are currently evaluating whether pIgR/SC downregulation represents a marker of tumorigenesis process or could participate in regulation of proliferation, apoptosis and migration in normal, transformed and cancerous airway epithelial cells.

P58
The use of a multi-analyte immunoassay panel (MAIP) to detect potential prognostic biomarkers associated with 2-month progression free survival rate in patients treated with Enzastaurin as 2nd and 3rd line therapy of NSCLC

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Background: Since the development of tumor markers several attempts have been made to discover other plasma or serum based markers to either predict therapy response to anti-tumor treatments or to better anticipate the prognosis of patients with cancer. The advent of flow cytometry has allowed the development of assessing serum or plasma markers using multiplex immunoassay approaches, as applied in a previously presented study.
Methods: Plasma samples were collected from patients participating in a single arm, phase II study of single agent with enzastaurin as a 2nd and 3rd line treatment for NSCLC. Samples collected at baseline, prior to cycle 1, cycle 2, and cycle 3 (1 cycle = 28 days) were banked and stored at −80°C, prior to analysis by standard procedures by Rules Based Medicine (Austin, TX). Univariate mixed effect analyses were performed to determine if there was any evidence of changes in analyte concentrations across cycles or between sub-groups of patients categorized by their progression free survival time – greater than or less than 2 months. For this initial screen for possible markers, it was decided not to adjust results for multiplicity of tests, thus recognizing the potential for false discoveries in the listed results.
Results: 55 patients were enrolled and received 500 mg oral enzastaurin/day. No tumor responses were observed. Thirty-six patients progressed and 19 (35%) patients were free from progression (FFP) at 2 months. 13 analytes were associated with the patients who were FFP ⩾2 months. Majority of the biomarkers, such as IL-6, IL-8, CRP, alpha-1-antitrypsin, complement-3, fibrinogen, growth hormone, PAI-1, TIMP-1 and VEGF, were reduced in patients who were FFP ⩾2 months, while apolipoprotein-A1, IL-7 and TNF-beta increased.
Conclusions: Since this study was not a randomised study and because there was no observed tumor response as defined by RECIST, it was not possible to associate a specific marker with enzastaurin activity. However, the difference in some markers when associated with the 2-mo freedom from progression, suggests that these markers may be prognostic for patients in 2nd/3rd line NSCLC.