such as proliferation or cell death (apoptosis). IHC is also widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue. Visualising an antibody-antigen interaction can be accomplished in a number of ways. In the most common instance, an antibody is conjugated to an enzyme, such as peroxidase, that can catalyse a colour-producing reaction. Alternatively, the antibody can also be tagged to a fluorophore, (immunofluorescence).

Materials and Methods: In Cameroon, the above techniques are not available. We established a collaboration with a few laboratories in developed countries; mainly in Switzerland and France. Paraffin blocks of diagnosed cancers have been sent abroad since January 2000. Immunohistochemistry has been performed free of charge.

Results: A series of 103 cancer patients was included in this study. There were 40 malignant lymphomas, 20 cases of early stage Kaposi's sarcoma, 20 soft tissue tumours, 15 breast cancers, 5 brain tumours, 3 urethral cancers. There were no HER-2 cases and and no sentinel node biopsies were performed in this series. The delay of sending specimens and receiving results via internet was one month. Sending specimens and receiving results via the Internet was one month. Apart from classifying and clarifying their diagnosis, none of these patients received specifc treatment after their immunohistochemistry result.

Conclusion: Even performed free of charge, immunohistochemistry does not permit specifc treatment for Cameroonian cancer patients because they can't pay for drugs such as monoclonal antibodies. We hope the situation may change in the future.

PP72

Demonstration of dose-dependent target inhibition using a quantitative biomarker assay for SB939, a potent, orally active HDAC inhibitor, in a Phase I clinical study in solid tumors

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Background: SB939 is an orally active, hydroxamic acid based HDAC inhibitor with very favorable pharmaceutical, pharmacokinetic and pharmacodynamic properties that is currently in Phase I clinical trials. A sensitive Western blot assay to quantitate histone H3 acetylation (acH3) on lysine 9 and 14 was developed to measure target efficacy of SB939.

Materials and Methods: The assay was validated in normal or HCT-116 tumor bearing mice treated with 125 mg/kg t.i.w. SB939. Samples were collected on day 1 and day 15 pre-dose, 3 h and 24 h after dosing, corresponding to the time points of sample collection in the Phase I clinical study. To test the linearity of the acH3 signal mice were treated with doses from 25 mg/kg to 200 mg/kg for 3h. Biomarker analysis of Phase I studies were performed on patients PBMCs, isolated with CPT-tubes. Samples were snap-frozen and lysed in the presence of a HAT inhibitor.

Results: The Western blot assay was sensitive enough to detect as little as 22 ng/ml of SB939 in cultured cancer cells, or 44 ng/ml in PBMCs of healthy volunteers. Signals could be detected in liver, spleen, PBMCs as well as tumor tissue sampled from normal or HCT-116 tumor bearing mice treated with 125 mg/kg SB939 orally t.i.w. The highest signals were detected 3 h post-dose, with no background for vehicle treated mice. AcH3 signals were lower on day 15 than on day 1 in all normal tissues, but increased in tumor tissue, where also the highest absolute acH3 levels were detected. The increase in signal was linear in all tissues tested, except in tumor tissue, which showed maximal saturation already at doses of 100 mg/kg. In PBMC samples from SB939-treated patients with advanced solid malignancies, a dose-dependent increase in relative acetylation values was observed: from 0.8 to 1 to 1.5 for patients treated with 20 mg, 40 mg and 60 mg respectively, correlating well with the proportional increase of SB939 in the plasma. Interestingly, patients which stayed on treatment for the longest time without disease progression showed a sustained enhanced signal on day 15.

Conclusion: Using a sensitive and quantitative Western blot assay, we demonstrate that SB939 induces a dose-dependent increase in acH3 levels in normal and tumor tissues in animal models, as well as in PBMCs from patients with solid tumors in a Phase I trial. Furthermore, a prolonged effect on the acH3 signal on d15 could be indicative for response to SB939 treatment and warrants further investigation.

PP32

A unified approach to define incidence of acute kidney injury (AKI) with serum creatinine as biomarker

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Background: Acute kidney injury (AKI) is a life threatening complex disease associated with mortality, morbidity and length of stay in hospital [Gottlieb SS, 2002]. SCr which is currently a main biomarker for AKI, rise normally in 24−72 hours after the inflammation occurred. Constant and slow rate of production of SCr, among cancer inpatients makes it less reliable for AKI classification. This complexity guides our research in the area of classification and modeling to overcome these challenges. We hypothesize that the rate (gradient change) in the SCr as biomarker will better predict the acute renal disease than simple difference

Materials and Methods: To delineate the extent of AKI using unified criteria, we examined the medical records of 5013 patients admitted to MD Anderson Cancer Center for three months. We run random intercept regression model to estimate the AKI for different baseline SCr. We estimated equation for critical SCr values as: $SCr(crit) = SCr(baseline)^{0.95} * \{[3 - \exp(-t/2SCr^{1.2})]/2\}$, where SCr(crit) is critical SCr level (above which AKI is predicted) in that point in time with reference to the baseline SCr at the first observation time t = 0 days. We internally validated the definition and results are in conformity with the AKIN criteria.

Results: Proposed AKI criteria based on gradient change in SCr performs batter in validation and regression analysis. Sensitivity and specificity remained 93% and 83% respectively. We observed minimal false rejection and improved detection of AKI, with even smaller changes in SCr. AKI is highly associated with length of stay (OR: 2.25; 95% CI: 1.85−2.74), ICU admission (OR: 2.7; 95% CI: 2.0−3.7), PACU admission (OR: 5.0; 95% CI: 3.7−7.0), BMT LLM (OR: 2.59; 95% CI: 2.0−2.74), Med Oncology (OR: 2.8;; 95% CI 2.2−3.6), Surgical Oncology (OR: 2.1; 95% CI 1.4−3.2), Pain Symptom (OR: 2.5; 95% CI 1.6−3.8). An odd of having AKI was fivefold increased for the patients who eventually died in hospital with cancer, in contrast to those remained alive after controlling for other covariates. Similarly, cancer patients who were diabetes mellitus (DM) had about 40% increased risk of odds of AKI compared with those without DM with 95% CI being from 15% to 70% with all P-values <0.0001.

Conclusion: We conclude that by estimating the unified equation for AKI based on the gradient change in SCr, it improves the specificity and early prediction of AKI. Even small serum creatinine SCr increase is independently associated with increased risk of mortality [Smith GL, 2003].

PP120

Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis in resected non-small cell lung cancer

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Background: The impact of host immunity on outcome in lung cancer is controversial. We studied the clinical significance of lymphoid infiltration in resected non-small cell lung cancer (NSCLC) specimens.

Materials and Methods: We analysed 196 NSCLC cases for tumour- and stroma-infiltrating CD3+, CD8+ and FOXP3+ cells by immunohistochemistry to assess the relative proportions of total, cytotoxic and regulatory T-lymphocytes (Tregs), respectively. Enumeration of immune subsets was performed using a novel automated image analysis algorithm. To test the influence of lymphocyte distribution pattern on survival, the data were divided into two groups, based on whether the ratio of intratumoral to intrastromal lymphocyte count was greater or less than the median value.

Results: A high CD8+ tumour/stroma infiltration ratio was associated with an increased overall survival (OS) compared to a low tumour/stroma infiltration ratio (P < 0.001). Conversely, there was an inverse association between survival and tumour islet FOXP3+ Treg density (P < 0.001). Multivariate analysis revealed that CD8+ tumour/stroma ratio emerged as an independent predictor of survival (HR 0.38; 95% CI 0.24−0.61, P < 0.001). The combination of a high tumour islet/stroma CD8+ ratio and low tumour islet/stroma FOXP3+ ratio showed the strongest prognostic effect, being associated with a 3 yr OS rate of 91% (HR 1.58; 95% CI 1.25−2.00, P < 0.001).

Conclusion: Microlocalization of infiltrating T-lymphocytes is a powerful predictor of outcome in surgically resected NSCLC. Immune-based

therapies that augment intratumoral effector T lymphocytes recruitment, while inhibiting Treg accumulation, may be worthy of pursuit.

PP138

A whole blood RNA transcript-based model to predict biopsy Gleason score in newly diagnosed prostate cancer patients

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Background: Histologic grading of prostate cancer is a critical determinant of the biology of prostate cancer and is strongly associated with prognosis. Biopsy Gleason scores (GS) are typically assigned by pathologists, but are subject to variable interpretations. In addition, prostate needle biopsies may underestimate the true score in up to 15−20% of patients due to sampling error. Better methods are needed to assess Gleason score and, by extension, aggressiveness of prostate cancer.
Materials and Methods: From August 2006 to October 2008, a prospective cohort of 198 men with newly diagnosed, localized, untreated prostate cancer consented to the collection of whole blood in PAXgene™ Blood RNA tubes for gene expression analysis. 216 inflammation and cancer-related genes (Source MDx Precision Profiles™) were assayed using optimized Q-PCR technology and logistic regression and latent class (LC) methods were used to develop a 5-gene model which distinguished higher Gleason score (4+3 or higher) from lower Gleason score (3+4 or 3+3) cancers.
Results: In evaluating all 1-, 2- and 3-gene models based on 216 target genes, the best 3-gene model distinguishing higher versus lower GS cancers included CD4, TP53, and E2F1. The best 2-gene model which separated Gleason 6 cancers from all 7 or higher cancers included CASP9 and SOCS3. Together, the combined 5-gene model was able to predict GS 4+3 or higher versus 3+4 and 3+3 with sensitivity of 0.75 and specificity of 0.63, and an AUC of the ROC curve of 0.73 ($p = 8.2 \times 10^{-6}$). In an exploratory LC model which assumes that GS is an imperfect reference test for 'aggressiveness', the combined 5-gene model is able to accurately predict 'aggressive' cancers with sensitivity of 0.84 and 'non-aggressive' cancers with specificity of 0.83. ROC curves for the models predicting 'aggressiveness' of cancer demonstrated AUC 0.91 ($p < 8 \times 10^{-6}$).
Conclusion: Models distinguishing between higher and lower GS cancers were developed based on whole blood RNA transcript measurement of inflammation and cancer-related genes. Furthermore, LC models which assume that some prostate biopsies may miss underlying aggressive disease in some patients suggest that molecular tests could improve the diagnostic accuracy of currently available tests. Validation of this and other models predicting GS and 'aggressiveness' is planned.

PP90

Hereditary transmission of polymorphisms in familial breast cancer

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Background: Recently, it has been demonstrate that many SNPs could predispose people to disease. Genetic alterations in BRCA1 and BRCA2 genes lead to a higher predisposition to breast and ovarian cancer and confer a significantly higher risk of endometrial, pancreas, cervix and prostate cancers. Many studies are focalizing the attention about a possible biological interpretation of the unknown and polymorphic variants in BRCA1 and BRCA2 genes to understand if they have a pathogenic role. The aim of our study was to clarify the role of BRCA SNPs as susceptibility markers of risk studying the genealogic transmission of coding and non coding variants of BRCA1 and BRCA2 genes in family's members enrolled by the genetic counselling program.
Materials and Methods: 20 families, in which DNA from at least one first degree relative was available, have been studied for both pathological mutation and polymorphic variants transmission. BRCA1 and BRCA2 variants have been investigated by dHPLC and direct sequencing.
Results: As expected, pathological mutations were mendelian transmitted. BRCA1 5382insC mutation has been individuated in 7 patients but only 4 families showed a mendelian transmission in at least one first-degree relative while BRCA1 R1494M and BRCA2 2150insTA and 6710delACAA mutations have been found to be transmitted in different family members belonging to the same genealogic tree. Interestingly, polymorphic coding and non coding variants were present in relatives of the studied family while transmission for unknown variant was not evidenced. In particular in our series, the BRCA2 Lys3327Stop, an unknown variants, has been individuated only in a female 63 years old with familial breast cancer history but not in any analyzed relatives, while BRCA1 P871L, E1038G and K1183R segregated together and were all transmitted to first and second degree relatives. Patients with these clusterized SNPs seemed to have peculiar pathologic features as higher differentiated tumors (71% was G1−2, $p = 0.05$) and a trend for less probability to present mutation in BRCA1 or BRCA2 (74% of Myriad >10%, $p = 0.06$).
Conclusion: The significant association of some SNPs with tumor aggressiveness or susceptibility risk lead to underline possible polymorphism transmission pathological significance. SNP maps and modality of their transmission could help to identify further susceptibility markers and provide a basis for a better DNA-based cancer classification.

PP43

RCL2 fixation of neurosurgical specimens: well preserved histomorphology and DNA integrity

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Background: Neurosurgical tumour tissue specimens are usually fixed in formalin to allow optimal histopathological tumour typing. However, formalin fixation damages nucleic acids and impairs molecular biomarker research. RCL2 is a commercial alcohol-based fixative that has been described to preserve histomorphology and nucleic acid integrity in non-CNS neoplasms. In this study, we performed comparative evaluation of the effect of formalin- and RCL2-fixation on histomorphology and DNA integrity in neurosurgical specimens.
Materials and Methods: We included neurosurgical specimens of 13 brain tumours (2 diffuse astrocytomas, 1 anaplastic astrocytoma, 1 anaplastic oligoastrocytoma, 5 glioblastomas, 1 pleomorphic xanthoastrocytoma, 1 meningioma, 1 medulloblastoma, 1 metastasis). Of each patient, 1 tumour sample was fixed in standard 4.5% buffered formaldehyde solution (FOR) and 1 tumour sample was fixed in RCL2 solution. Fixation times ranged from 1 to 8 days before paraffin-embedding. Of each tissue block, we performed: (1) hematoxylin and eosin staining and neuropathological evaluation, (2) DNA extraction using the QIAamp DNA Mini Kit, (3) measurement of OD260nm to determine DNA quantity, (4) measurement of OD 260nm and 280nm to determine DNA quality, (5) polymerase chain reaction using primers for DNA fragments of 100, 200, 300, 400 and 600 base pairs (bp) followed by gel electrophoresis to evaluate suitability of the material for PCR amplification.
Results: Histomorphology was comparable between FOR- and RCL2-fixed tissue samples. DNA extraction from RCL2-fixed tissue specimens (DNA-RCL) resulted in significantly higher yield than DNA extraction from FOR-fixed tissue specimens (DNA-FOR) by a median factor of 2 (range 0.33 to 4.44) ($p = 0.006$, paired T-test). OD 260/280 ratio was $\geqslant$1.7 in 13/13 DNA-RCL and 11/13 DNA-FOR samples. DNA was amplifyable up to a length of 600bp in 12/13 DNA-RCL and 9/13 DNA-FOR specimens.
Conclusion: (1) In our hands, the histomorphology of RCL2-fixed neurosurgical specimens has no significant disadvantages compared to FOR-fixed tissue samples and allows tumour typing according to WHO criteria. (2) RCL2-fixation results in higher DNA yield and quality than FOR-fixation. Thus, RCL2-fixation may be of advantage for comprehensive characterization of neurosurgical specimens regarding both histopathology and molecular analyses.

PP98

High expression of hsa-miR-30a-3p, hsa-miR-30c and hsa-miR-182 predict favorable outcome on tamoxifen treatment in patients with recurrent breast cancer

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Background: Altered miRNAs expression levels have been described in breast cancer (BC) and reported to be associated with metastasis, prognosis and treatment response, suggesting that miRNAs play an important role in BC. We have explored the association of selected miRNAs and tamoxifen clinical response.
Materials and Methods: In a series of 246 ER+ recurrent BC patients treated with tamoxifen, five selected miRNAs, hsa-miR-30a-3p, hsa-miR-30c hsa-miR-182, hsa-miR-187 and hsa-miR-422a, were quantified by real time PCR.
Results: Univariate logistic regression analysis, using log-transformed continuous variables, showed that high levels of hsa-miR-30a-3p (odds ratio [OR]: 1.51, 95% confidence interval [95% CI]: 1.16−1.96; $P = 0.002$), hsa-miR-30c (OR: 3.87, 95% CI: 2.16−6.93; $P < 0.001$), and hsa-miR-182 (OR: 1.53, 95% CI: 1.09−2.16; $P = 0.013$), were associated with clinical benefit of tamoxifen therapy. In multivariate analysis, including traditional predictive factors, of the miRNAs tested, only hsa-miR-30c was significantly associated with clinical benefit (OR: 3.14, 95% CI: 1.61−6.12; $P = 0.001$).