of 0.54 ($p < 0.001$) between predicted and measured treatment response, while extracted DCEMRI parameters together with volumes and PSA gave a correlation coefficient of 0.66 ($p < 0.001$). The approach where all parameters (DWMRI, DCEMRI, volumes, PSA) were combined was superior to all other BPNN simulations and successfully predicted ultimate treatment response with a correlation coefficient of 0.85 ($p < 0.001$).

Conclusions: The results indicate that the combination of several functional MRI parameters obtained early in the course of treatment, into an ANN model, may provide additional information about therapy response. If established, this approach may help identifying non-responding patients early during treatment course, allowing these patients to be considered for alternative treatment strategies, and, thus, providing a contribution to the development of individualized cancer therapy.

150 Molecular characterization of apocrine carcinoma of the breast: validation of an apocrine protein signature in a well-defined cohort

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Background: Invasive apocrine carcinomas (IACs), as defined by morphological features, correspond to 0.3−4% of all invasive ductal carcinomas (IDC), and despite the fact that IDC are histologically distinct from other breast lesions there are currently no molecular criteria available for their diagnosis and no unequivocal information as to their prognosis. In an effort to address these concerns we have used proteome technologies and IHC to discover specific biomarkers that could allow the characterization of these lesions as well as to dissect some of the steps in the processes underlying apocrine metaplasia and development of precancerous apocrine lesions.

Material and Methods: A panel of antibodies against components of an apocrine protein signature that includes probes against the apocrine-specific proteins 15-PGDH, ACSM1, in addition to a set of markers that are consistently expressed (AR, CD24) or not expressed (ERa, PgR, Bcl-2, and GATA-3) by apocrine metaplasia in benign lesions and apocrine sweat glands (Celis et al. 2008, MCP, Celis et al. 2007; Mol Oncol) was used to analyze a defined cohort consisting of 14 apocrine ductal carcinoma in situ (ADCIS), and 33 IACs diagnosed at the Cancer Institute Hospital, Tokyo between 1997 and 2001. Samples were originally classified on the basis of cellular morphology with all cases having more than 90% of the tumour cells exhibiting cytological features typical of apocrine cells.

Results: Using the expression of 15-PGDH and/or ACSM1 as the main criterion, but taking into account the expression of other markers, we were able to identify unambiguously 13 out of 14 ADCIS (92.9%) and 20 out of 33 (60.6%) IAC samples, respectively, as being of apocrine origin. Our results demonstrate that IACs correspond to a distinct, even if heterogeneous, molecular subgroup of breast carcinomas that can be readily identified in an unbiased way using a combination of markers that recapitulate the phenotype of apocrine sweat glands (15-PGDH$^+$, ACSM1$^+$, AR$^+$, CD24$^+$, ERa$^-$, PgR$^-$, Bcl-2$^-$, and GATA-3$^-$).

Conclusions: The results pave the way for addressing issues such as prognosis of IACs, patient stratification for targeted therapeutics, as well as research strategies for identifying novel therapeutic targets.

151 EpCAM expression on disseminated tumour cells in cancers of the digestive tract

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Background: Being involved in nuclear signaling and due its association with the WNT-pathway, EpCAM is an established immunotherapeutic target for adjuvant therapies with several therapeutic antibodies available. This surface molecule is also frequently used to identify and to isolate cancer cells with stem cell properties as well as circulating and disseminated tumour cells (DTC).

Material and Methods: To investigate whether EpCAM is a potential molecular target for systemic adjuvant therapies in cancers of the digestive tract, we systematically investigated the prevalence of EpCAM expression directly on the target cell population − the DTC. We established a double-labeling technique to visualize CK18 and EpCAM simultaneously on single DTC. Our double immuno-labeling was applied to over 200 bone marrow aspirates from patients with cancers of the digestive tract (including head & neck, oesophageal, gastric, pancreatic, and colorectal carcinoma.

Results: While CK-positive cells were detected in the expected range of approximately 30% of the patients, EpCAM was infrequently expressed on CK-positive cells and was almost never detected in cells without CK-positivity. Compared to the remaining GIT malignancies investigated, DTC prevalence was significantly higher in colorectal carcinoma.

Conclusions: EpCAM expression is infrequent on CK-positive DTC and significantly lower as anticipated from previously published data on EpCAM expression in primary tumours of the investigated entities. Our unexpected findings should be considered in clinical trials investigating the efficiency of systemic adjuvant therapies directed against EpCAM.

152 MDM2 SNP 309 polymorphism is associated with increased risk of initiation and early age of onset in nasopharyngeal carcinoma development

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Background: Mdm2 is the principal negative regulator of p53 targetting its export from nuclei to be destroyed by the ubiquitin-proteasome pathway. Recent studies refer that a recent polymorphism in the promoter region of *MDM2* (SNP309 T/G) has been associated with higher levels of its protein, thus it favors p53-pathway abolishment, cell cycle escape and development of cancer. We aimed to study the role of MDM2 SNP309 T/G polymorphism in the development of Nasopharyngeal Carcinoma development.

Methods: A cross-sectional case control study was developed with 111 patients with Undifferentiated type (WHO type III) Nasopharyngeal Carcinoma (UNPC) and 509 healthy individuals from the North of Portugal. We determined the genetic distribution of the MDM2 SNP309 polymorphism by PCR-RFLP in DNA extracted from peripheral blood samples. Statistical analysis was performed to calculate the Odds Ratio (OR) and 95% Confidence Interval (95% CI) as a measure of association between the polymorphism and the development of UNPC. The genotype-specific distributions according to age of disease onset were tested by calculating the cumulative hazard function plots computed by the Kaplan–Meier methodology with Log-rank and Breslow test.

Results: This study revealed an increased frequency of MDM2 SNP 309 GG homozygous in patients with the undifferentiated type of nasopharyngeal carcinoma, which revealed increased risk (OR = 2.51; 95% IC 1.45–4.34) particularly in the early clinical stages OR = 3.39; 95% IC 1.83–6.26). Moreover, we found that the median age of onset of UNPC cases in MDMD2 SNP 309 GG homozygous was significantly different from T allele carriers (55.2 years old *vs* 61.9; $p = 0.008$) with more effect in early clinical stages (55.3 *vs* 65.3; $p < 0.001$).

Conclusion: Our study suggests that MDM2 SNP309 can be a surrogate risk marker for the development of NPC mainly in early ages and as a initiation marker for potential cancer development.

153 Urological cancers models derived from patients

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Urologic cancers (kidney, bladder, prostate) represent 20% of all cancers (1 300 000 cases) and 15% of cancer-related deaths (500 000 deaths) per year worlwide, with an incidence increasing steadily up to 10%/year. These cancers remain therapy-resistant despite the development of targeted therapies. The emergence of new therapeutic approaches are thus urgently needed. These cancers show high cytogenetic variabilities and are heterogeneous for a same tumour. Because they do not reflect such variabilities and heterogeneity, current models, i.e cell-derived xenografts in immunodeficient rodents or genetically manipulated mice, are inadequate to developp effective therapies. The tumour-derived xenografts in immunodeficient rodents appear today as the missing link between cell-derived xenografts and clinical trials. Indeed, they reflect this heterogeneity and allow to identify predictive biomarkers.

Until now we obtained from the Urology department of the New Hospital of Strasbourg tumour and normal corresponding tissues from 130, 27 and 14 patients with sporadic renal cell carcinoma (RCC), transitional bladder cancer and prostate cancer, respectively. Informed consent and clinical history is available for all patients. Tumour fragments were xenografted in nude mice sub-cutaneously and orthotopically using an improved method kept secret. Tumours that have grown were then grafted sequentially until the eighth passage in nude mice. Xenografts are pursued at a rythm of 50 (RCC) and 25 (bladder and prostate) per year. Tumours were analyzed at the histopathologic and metabolomic levels. The anti-tumour efficiency of sunitinib (obtained from Pfizer), sorafenib and everolimus was analyzed in 8 patient-derived RCC xenografted models tumours.

The tumour take rate is 13% (RCC) and 22% (bladder). At that time 24 (RCC), 6 (bladder) and 1 (prostate) patients-derived models were established in nude mice. It took 1 to 9 months for tumour to developp at the first graft but only 1 to 2 months for subsequent passages. Histopathological parameters are conserved during passages. Molecular parameters are under investigation. Some models responded to the treatment and some other not depending on the drug used, and according to the patient's treatment (when available), validating our models. Predictive biomarkers are under study.
These models are available to test new or existing therapies in these cancers according to tumour characteristics.

154 C-kit expression as a novel molecular marker to pre-operatively distinguish benign from malignant thyroid lesions

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Background: Thyroid carcinoma represents 90% of all neuroendocrine malignancies. The vast majority of thyroid cancers are papillary thyroid carcinoma (PTC) and their initial presentation is through a thyroid nodule. The best available test in the evaluation of a thyroid nodule is fine needle aspiration which sometimes is not efficient enough to give a specific diagnosis leading to the so called suspicious diagnoses for PTC. Surgery is usually recommended in these cases, but often it is not known what kind of surgery is appropriate: thyroid lobectomy or total thyroidectomy?
It is therefore necessary to develop more accurate early diagnostic assays for the evaluation of thyroid nodules.
Neoplastic processes often result from changes in gene expression patterns. Recent works have documented that *c-kit*, the receptor for stem cell factor, is expressed in a number of nonhematopoietic cell lineages, including normal thyroid epithelium. *Kit* is an important tyrosine kinase receptor in cell differentiation and growth; it functions as an oncogene in many cancers. In our study we found that the transcript level of *c-kit* in PTC tumour cells is extremely low.
Materials and Methods: We analyzed preoperative thyroid FNAs of patients with benign and malignant thyroid lesions selected from archived materials of the Section of Cytopathology, Division of Surgical, Molecular and Ultrastructural Pathology, and performed RNA extraction, cDNA synthesis and finally *c-kit* detection by qualitative and quantitative PCR.
Results: We observed that: while *c-kit* expression was preserved in a large fraction of goiters and benign adenomas (94%), 91% of malignant thyroid tumours had *c-kit* expression ratio value between 0 and 0.5 ($p = 0.0002$).
It has been suggested in the literature that in some cell types *c-kit* expression positively regulates mitogenesis and is selected for in neoplastic transformation, in other tissues the *c-kit* pathway is involved in morphogenesis and differentiation and is, therefore, negatively selected in the course of tumour progression.
Conclusions: In the preoperative settings we could determine a threshold of *c-kit* gene expression levels above or below which it would be possible to give a value indicative of a benign or malignant thyroid lesion. This become more useful in association with other well known molecular markers (BRAF), and also new ones such as LSM7 (a protein involved in pre-mRNA splicing) that we are also investigating in this study.

155 Clinical significance of the expression and amplification of the cortactin gene at 11q13 in head and neck squamous cell carcinomas

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Background: Despite major advancements in cancer diagnosis and treatment, the survival rate for patients with head and neck squamous cell carcinoma (HNSCC) has only marginally improved over the past few decades. It is therefore essential to identify new markers that can distinguish differences in tumour condition and augment the predictive power of the current clinical markers. Amplification of the 11q13 region is a prevalent genetic alteration in head and neck squamous cell carcinoma (HNSCC). We investigated the clinical significance of cortactin (CTTN) and cyclin D1 (CCND1) amplification in HNSCC tumour progression.
Material and Methods: CTTN and CCND1 amplification was analysed by differential and real-time PCR in a prospective series of 202 laryngeal/pharyngeal carcinomas. CTTN mRNA and protein expression were respectively determined by real-time RT-PCR and immunohistochemistry, and correlated with gene status. Molecular alterations were associated with clinicopathological parameters and disease outcome.
Results: CTTN and CCND1 amplifications were respectively found in 75 (37%) and 90 (45%) tumours. Both correlated with advanced disease; however, only CTTN amplification was associated with recurrence and reduced disease-specific survival ($p = 0.0022$). Strikingly, CTTN amplification differentially influenced survival depending on tumour site ($p = 0.0001$ larynx versus $p = 0.68$ pharynx) and was an independent predictor of reduced survival in the larynx ($p = 0.04$). Furthermore, CTTN overexpression correlated significantly with reduced disease-specific survival ($p = 0.018$). *CTTN* gene status strongly correlated with CTTN expression. All tumours harbouring *CTTN* amplification showed elevated mRNA/protein levels; however, CTTN overexpression occurred at a higher frequency (57%, mRNA and protein) indicating that additional mechanisms contribute to the regulation of CTTN expression in HNSCC.
Conclusion: These data indicate that although *CTTN* and *CCND1* amplification may be both biologically relevant features that cooperatively contribute to cancer development and progression, the strong relationship of *CTTN* amplification/overexpression with prognosis and disease outcome reinforces its role as driver of 11q13 amplification in HNSCC. CTTN emerges as a valuable prognostic marker to identify patients with laryngeal tumours at high-risk of recurrence and poor outcome that could benefit from more intensive treatment and follow-up.

156 Profile, target genes and regulation of microRNAs in ovarian carcinoma tumour progression

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Background: MicroRNAs(miRNAs) are small non-coding RNAs, that exert their regulatory effect post-transcriptionally by binding the 3′-UTR oftheir target mRNA and inhibiting gene translation to protein. Depending on whether miRNAs target oncogenes or tumour suppressor genes, they may be referred to as tumour suppressors or oncogenes respectively. Cellular miRNA expression is tightly regulated. One of thepost-transcriptional regulatory mechanisms involves changes in expression of miRNA machinery proteins, i.e., Dicer, and miRISC components such as the Argonaute (Ago) family members. Numerous studies, using different profiling approaches, have unraveled that miRNA expression is deregulated in various human cancers.
Ovarian canceris the leading cause of death from gynecological cancers in western countries. The disease is asymptomatic in the early stages, and is usually diagnosed at anadvance stage. Primary solid tumours, solid metastases, and effusions to theperitoneal and pleural cavities (ascites) characterize the tumour as itprogresses.
The aim of thestudy was to characterize the difference in miRNA expression pattern between primary ovarian solid carcinomas and effusion-derived cells, using freshly frozen samples. We also assessed changes in regulation of miRNA by evaluating theexpression of the machinery proteins at these two sites.
Material and Methods: Using microRNA-array platforms, we identified three sets of miRNAs: one set is highly expressed in both primary solid carcinomas and effusions. The second set is relatively upregulated in effusions, and the third set is relatively downregulated in effusions. The most significant miRNAs were validated byreal-time PCR.
Results: Our results show concordance between the training and the independent test cohortsfor the downregulated miR-145 and miR-214 and for the upregulated let-7f, miR-182, miR-210, miR-200c, miR-222 and miR-23a in effusions. Using in-silicotarget prediction programs we identified potential target genes for the miRNAsof interest listed above, we investigated the changes of those genes in ourcohort. We analyzed the expression levels of Zeb1, a confirmed target ofmiR-200c as well as c-Myc, that was found to be a predicted target of miR-200c. In addition, we analyzed Pak1 and PTEN, both predicted targets of miR-222. We found inverse correlations between the expression levels of the indicated miRNAs and of the predicted target genes.
We further analyzed the miRNA processing machinery genes that regulate miRNA generationand action. We found significantly higher expression of Ago1, Ago2 and Dicer ineffusion-derived cells compared to primary carcinoma tumours. These alterationsin expression levels indicate on difference in miRNA regulation between the two sites.
Conclusions: In summary, miRNAexpression profiles and the changes in miRNA processing machinery genes reveala new level of regulatory elements in ovarian carcinoma tumour progression.