were used for the production of phage displayed-antigen microarray that was applied to survey autoantibody profiles in patients with gastric cancer (n = 176), various gastrointestinal inflammatory diseases (n = 125) and healthy individuals (n = 148). The microarray data were analysed as qualitative – after normalisation the average serum antibody signal intensities in healthy donors (HD) were calculated for each antigen, and a threshold of 5 SD above the average signal intensities in HD was set to define antigens preferentially reacting with patients' sera.

Results: Serum autoantibody profiling of ~1322 element phage-displayed antigen microarray comprising all immunoselected antigens resulted in the identification of a panel of 232 antigens with potential diagnostic significance. The statistical data analysis resulted in the determination of a 60 antigen detector group that was able to discriminate between gastric cancer and healthy individuals with 81% sensitivity and 95% specificity (PPV 95%, NPV 81%), and between gastric cancer and gastrointestinal inflammatory disorders with 77% sensitivity and 90% specificity. Noteworthy, the sensitivity of the detection of stage I and II cancer was 77% and stages III and IV – 90%. Twenty-nine and 33 antigen detector groups were identified that were able to detect diffuse and intestinal type adenocarcinomas with 86% and 81% sensitivity, respectively, reaching 93% specificity in both cases, and the two groups were shown to encompass different sets of antigens.

Conclusions: Results of this study show that the serum autoantibody signatures have a potential to detect the presence of gastric cancer with significantly higher accuracy and earlier than any of the currently known serological markers and are promising candidates for the development of non-invasive serological tests.

147 Detection of circulating tumour cells in gastric cancer patients using telomerase-specific replication-competent adenoviral agent: a prospective feasibility study

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Background: Cancer of the digestive tract causes blood metastasis as well as lymphatic metastasis. Recent advanced techniques make it possible to detect circulating tumour cells (CTCs). CTCs are predicted to be involved in blood metastases. However, the relationships between CTCs and blood metastases are poorly understood.

In this study, we attempted to detect CTCs which may have potential for metastases in gastric cancer patients using telomerase-specific replication-seelctive adenovirus agent.

Material and Methods: Patients with clinical solitary gastric adenocarcinoma, underwent surgery at the Digestive Disease Center, Showa University Northern Yokohama Hospital between September, 2009 and January, 2010 were eligible for study. Patients aged over 81, those who received preoperative treatment, and those with the other organ neoplasm were excluded from this study.

Twenty-two patients (sixteen men and 6 women) fulfilled the inclusion criteria. The study was approved by the Institutional Review Board of the Showa University and each patient gave written, informed consent. The patients ranged in age from 39 to 74 years (average 55.6 years).

Peripheral blood samples (7.5 ml) were obtained from the patients before surgery, and were infected with telomerase-specific replication-competent adenovirus expressing green fluorescent protein (GFP) (OBP-401; Telome Scan) by incubation in the medium for 24 hours. Circulating tumour cells whose fluorescence can be detected were counted under fluorescence microscopy. And, it was confirmed that GFP fluorescence positive cells (GFP positive cells) were cancer cells by Immunohistochemistry staining.

The disease was pathologically staged with TNM staging system. The stage group value comparisons were performed with the Kruskal-Wallis test.

Results: Total 22 samples were examined. All values were presented as median; the value of stage IIIA group was reported as mean.

The pathological stages were IA in 12 patients, IB in 4 patients, IIIA in 2 patients, and IV in 4 patients. We detected GFP positive cells in all 22 samples. The numbers of GFP positive cells in the samples from patients at stage IA, IB, IIIA, and IV were 8.5 (range, 1–518), 3 (range, 1−10), 12.5 (range, 1−24), and 19 (range, 5−42). Although the value tended to increase with stage progression, there was no significant difference (P = 0.37).

The values in 3 patients with pathological multiple lesions at stage IA were 7, 13, and 518. On the other hand, the values in 4 patients with distant metastases were 5, 6, 32, and 42.

Conclusions: The GFP positive cells were detected in all blood samples from 22 gastric cancer patients, independently of cancer stage. There is possibility of early exact diagnosis of gastric cancer from only blood samples. In contrast, the numbers of GFP positive cells did not clearly show cancer stage. Therefore, the next study to investigate changes in numbers of GFP positive cells through a treatment is designed. Furthermore, we will analyze individual CTCs function after GFP positive cell separation.

148 In vivo preclinical evaluation of the topoisomerase I inhibitor camptothecin in human triple negative breast cancer xenografts

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Background: Triple negative breast cancers (TNBC) have a poor outcome and harbor early relapses despite a high chemosensitivity. New treatments are therefore warranted to improve the prognosis of TNBC.

Material and Methods: Using well characterized human primary TNBC xenografts (1), we have investigated the efficacy of the toposisomerase I inhibitor camptothecin (CPT11). CPT11 was intraperitonealy administered at a dosage of 50 mg/kg at day 1 every 3 weeks for 2 to 3 cycles. Tumour volume was measured twice a week and Relative Tumour Volumes (RTVs) from start of treatment were then calculated.

Results: The four human TNBC HBCx-4B, HBCx-11, HBCx-15, and HBCx-12B xenografts have been treated by CPT11, with an optimal tumour growth inhibition (TGI) of 100%, 91%, 100%, and 90%, respectively. In two models, HBCx-4B and HBCx-15, 100% and 87% of complete remission (CR) have been observed. As shown in the Table, CPT11 was as or more efficient than standard chemotherapies [doxorubicin + cyclophosphamide (AC), docetaxel (D), capecitabin (Cap.), or cisplatin (CDDP)], particularly in the HBCx-4B resistant xenograft.

	HBCx-4B		HBCx-11		HBCx-15		HBCx-12B	
	TGI	CR	TGI	CR	TGI	CR	TGI	CR
CPT11	100	100	91	0	100	87	90	0
AC	40	0	75	0	100	100	35	0
D	20	0	40	0	42	0	32	0
Cap.	45	0	57	0	71	0	55	0
CDDP	34	0	/	/	96	93	51	0

Spontaneous lung metastases occurrence is ongoing histopathological assessment in the spontaneously metastatic BC174 xenograft.

Conclusions: Altogether, these results suggest that topoisomerase I inhibitors could be efficiently used in TNBC. Further clinical trials are therefore warranted to confirm in cancer patients the efficacy of these cytotoxic agents.

Reference(s)

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149 Prediction of response to cancer therapy from functional magnetic resonance image parameters – an artificial neural network approach

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Background: In modern cancer medicine, anatomical magnetic resonance imaging (MRI) is routinely used in diagnostics, treatment planning and assessment of therapeutic efficacy. During the past decade, functional imaging techniques like diffusion-weighted (DW) MRI and dynamic contrast-enhanced (DCE) MRI have increasingly been included into imaging protocols, allowing intratumoural information about underlying vascular, molecular and physiological mechanisms, not available in structural images, to be extracted. Separately, pre-treatment and early changes in functional parameters obtained from DWMRI and DCEMRI have shown potential in prediction of ultimate therapy response. We hypothesized that the combined use of several functional parameters may increase the predictive power.

Material and Methods: We challenged this hypothesis by using an artificial neural network (ANN) approach, exploiting nonlinear relationships between individual variables, which is particularly suitable in treatment response prediction involving complex cancer data. A clinical scenario was elicited by human prostate cancer xenografts treated with combinations of androgen-deprivation therapy and radiotherapy. DWMRI and DCEMRI from pre-radiation and on days 1 and 9 following radiation, in addition to tumour volumes and the established biomarker prostate specific antigen (PSA), were used as inputs to a back propagation neural network (BPNN), both separately and combined.

Results: The use of DWMRI parameters together with tumour volumes and PSA as inputs to the BPNN model revealed a correlation coefficient

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.