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Role of soluble HER2 extracellular domain in HER2-mediated cell growth

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The HER receptor family contains naturally occurring isoforms consisting of various portions of the extracellular domain (ECD). These isoforms can self-dimerize/codimerize with full-length receptors, potentially mediating therapeutic effects against human cancers driven by HER receptor overexpression. A 110-kDa HER2/ECD isoform shed by proteolytic cleavage is detectable in cell culture medium of HER2-overexpressing cells and in serum of HER2-positive metastatic breast cancer patients (MBC). The potential clinical relevance of the circulating HER2/ECD fragment in disease progression or treatment response has been widely reported. Indeed, high serum levels of HER2/ECD correlate with poor prognosis, increased metastasis and decreased responsiveness to conventional therapies in MBC patients. However, contrary to the expected inhibition of trastuzumab binding to tumor cells through formation of immune complexes between the drug and serum HER2/ECD, data indicate that elevated serum ECD levels (>15 ng/ml) before treatment initiation are a predictor of a good response to trastuzumab. Toward the goal of improving the clinical management of HER2-positive patients and of clarifying the predictive value of HER2/ECD, we initiated pre-clinical studies to define the role of the shed ECD in HER2-mediated cell growth. We produced and purified on a trastuzumab affinity column a recombinant human soluble HER2/ECD (rECD) as a surrogate of human native shed HER-2/ECD (nECD) by transfection of pcDNA3.1HygroHER2/ECD plasmid vector in HER2-overexpressing SKOV3 carcinoma cells. The cell clones grew more slowly than control cells both in vitro and in vivo. Parental SKOV3 cells co-cultured in the presence of 3 different cell clones releasing different amounts of rECD and different concentrations of soluble purified rECD were significantly growth-inhibited as compared to the same cells co-cultured with mock-transfected cells and RPMI with 10% FCS. Moreover, proliferation of parental cells significantly decreased as a function of increasing levels of rECD in the incubation medium. Immune-depletion of nECD from SKOV3 culture supernatant significantly increased tumor cell proliferation compared to the same cells cultured in the presence of SKOV3-conditioned medium containing high levels of nECD. (Partially supported by AIRC).

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SPC2996 - a potent, specific, long-lived and safe inhibitor of Bcl-2 in cultured cells and experimental animals shows efficacy in patients with chronic lymphocytic leukaemia

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Cell survival by abolishing programmed cell death in cancer cells has been closely linked to high Bcl-2 expression[1,2]. The therapeutic potential of reducing Bcl-2 in cancer cells has been documented and resistance to existing cancer therapies has been linked to Bcl-2.

We have generated a Bcl-2 inhibitor - SPC2996, a 16-mer oligonucleotide incorporating Locked Nucleic Acid (LNA), which has unique high-affinity binding to Bcl-2 mRNA and enhanced resistance to nuclease digestion. SPC2996 is currently evaluated for clinical safety and initial efficacy in a phase I/II study in patients with advanced Chronic Lymphocytic Leukaemia (CLL).

The in vitro effects of SPC2996 were analyzed in human cancer cell lines by Western Blotting, quantitative polymerase chain reaction (qPCR) and biochemical assays. Low nanomolar concentrations of SPC2996 substantially reduced intracellular levels of Bcl-2 mRNA and protein and induced caspase 3/7 activation and apoptosis in a dose-dependant manner.

In vivo, the bio-distribution of SPC2996 was assessed with tritium-labelled compound given intravenously in mice. Anti-tumour efficacy was assessed using human prostate (PC3) and melanoma (518A2) tumour cell line xenografts in immune deficient mice.

Systemically administered SPC2996 accumulated in liver, kidney, ovaries, bone marrow, lymph nodes and skin. After administration of SPC2996 at doses ranging between 5-10 mg/kg/day, significant tumour growth reduction was exhibited in the xenograft tumour models.

In a dose escalation study, 35 CLL patients were treated with SPC2996. In patients treated with 6 x 4.0 mg/kg infusions, regression analyses of Bcl-2 mRNA levels in whole blood extracts showed a significant trend towards downregulation over the period of treatment. In addition, a decrease in lymphocyte count was observed in all patients in this treatment group (6 patients). Five out of six patients in the group showed a maximal reduction in lymphocyte count ≥ 50%, indicating a clinically beneficial response.

SPC2996 is the first member of a new generation of specific RNA antagonising oligonucleotides with enhanced properties derived from LNA, being evaluated and showing efficacy in a phase I/II study.

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Magnetic resonance imaging study of carmustin and sorafenib antitumor efficacy evaluation in orthotopic human glioblastoma models xenografted in nude rats

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Glioblastoma is the most aggressive subtype of brain tumors. Monitoring changes in glioma microvasculature should help to evaluate the efficacy of new antitumor therapy. The aim of this study was to assess the sensitivity of magnetic resonance imaging (MRI) biomarkers to the antitumor activity of Carmustin and Sorafenib in human glioblastoma model.

Nude rats were orthotopically injected at D0 with U87-MG glioma cells. Rats were randomized at D14 to receive either one injection of 10 mg/kg Carmustin (BCNU) i.v. or 14 daily administrations of 100 mg/kg Sorafenib (SORA) p.o. or no treatment (CTL). Rat survival was monitored daily. Blood volume (BV), vessel size index (VSI), apparent diffusion coefficient (ADC) and blood brain barrier permeability to a contrast agent (BBB perm.) were mapped, in tumor, at 2.35T one day before treatment and 1, 4 and 14 days after treatment onset (respectively D13, D15, D18 and D28). Tumor volumes were measured on T2-weighted images. VSI/BV and BBB perm. parameters were computed from T2, T2* and T1-weighted images using an intravascular contrast agent (ferumoxtran-10) and P846, a Gd-based contrast agent, both provided by Dr P. Robert, (Guerbet/AMAG Pharmaceuticals). In each group, the same four rats were imaged at each time point. Four additional rats were also imaged per time point and euthanized at the end of the imaging session for Collagen IV immunohistochemistry studies.

SORA and BCNU treatments strongly inhibited the tumor growth of both models (T/C=25% and 6% at D28 respectively). At D28, ADC in SORA and BCNU groups were 21 and 23% higher than in the CTL group, respectively. At any time, VSI did not differ between BCNU ant CTL groups. VSI in SORA group was significantly increased by 24 to 42% when compared to CTL group at D15 and D28, respectively. BV was not modified by BCNU treatment but was strongly decreased by SORA treatment (4.6±0.5 at D13 to 1.86±0.2% at D28). While BBB remained permeable in BCNU and CTL groups, SORA-treated tumor became impermeable to P846 as early as 4 days after treatment onset. Despite tumor growth inhibition and vasculature modification, BCNU and SORA displayed a moderate increase of U87-MG tumor-bearing rats survival (ILS=18% and 23%, respectively). Collagen IV staining demonstrate a strong decrease of vessel number in the SORA-treated tumors.

MRI demonstrated a tumor growth inhibition induced by SORA and BCNU treatments despite the poor effect of these 2 treatments on the survival of U87-MG-bearing rats. ADC appeared sensitive to both treatment but VSI and BV were sensitive to the effect of SORA treatment only. These results are consistent with the anti-angiogenic activity of SORA (confirmed by Collagen IV staining). Together, these results indicate that VSI, BV and ADC markers measured by MRI would be of value to combine anti-angiogenic with cytotoxic therapies in glioblastomas.

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Molecular changes in histopatologically normal prostate tissue adjacent to cancer

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Cancer begins with multiple genetic alterations that sequentially transform a cell, or a group of cells in a particular organ. As a result of this transfor-

mation, according to field cancerization concept, genetically altered but histologically normal appearing cells predate the development of neoplasia or coexist with malignant cells. Prostate cancer is often multifocal, and it is likely that multiple tumors arise from an organ which has been earlier genetically altered by a particular carcinogen. Aim of our study was to identify molecular signature of genetically changed but histologically normal prostate cells.

In this study we performed a comprehensive gene expression analysis on 36 human prostate biopsy samples including prostate cancer tissue, prostate tissue adjacent to tumor and benign prostatic hyperplasia, using U133 Plus 2.0 Affymetrix arrays.

In the first step of analysis genetic profiles of prostate cancer samples and benign prostatic hyperplasia samples were compared. We have found 279 genes which differentiate the groups, among them were genes found in other studies as changed in prostate cancer: AMACR, hepsin, EZH2, which demonstrates that microarray analysis of biopsy specimens gives similar results to the studies performed using prostatectomy specimens. In the next step we compared the genetic profiles of benign prostatic hyperplasia and normal-appearing prostate tissue adjacent to cancer. We obtained 98 probesets differentiating those two groups, and this difference was significant (p=0.054) according to the global test of difference. We also compared gene expression values of genes belonging to molecular pathways described in Biocarta database. This analysis revealed that pathway: “Chromatin Remodeling by hSWI/SNF ATP-dependent Complexes” seemed to be particularly down-regulated in prostate tissue adjacent to cancer (p<0.0001), with seven genes showing expression decrease (p<0.05). Genes identified by us has yet to be validated by RT-PCR and immunohistochemical analysis.

Molecular changes in prostate tissue adjacent to cancer found in our study appear to have potential utility as early diagnostic markers.

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Clinical and biological significance of CDK4 amplification in well-differentiated and dedifferentiated liposarcomas

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BACKGROUND: MDM2 (12q15), HMGA2 (12q14.3) and CDK4 (12q14.1) are the main target genes of the 12q14-15 amplicon in well-differentiated and dedifferentiated liposarcomas (WDLPS/DDLPS). While MDM2 and HMGA2 are consistently amplified, CDK4 is not amplified in approximately 10% of WDLPS/DDLPS. Our aim was to determine whether the absence of CDK4 amplification was –i) associated with specific clinico-pathological features –ii) compensated by another genomic event involved in the p16-CDK4/cyclinD1-pRb pathway. MATERIAL AND METHODS: We compared the clinical characteristics of a series of 44 WDLPS/DDLPS with amplification of both MDM2 and CDK4 (MDM2+/CDK4+) to a series of 38 WDLPS/DDLPS with amplification of MDM2 but no CDK4 amplification (MDM2+/CDK4-). We have used fluorescence in situ hybridization (FISH) and real-time quantitative RT-PCR analysis to determine the status of the CDKN2A (9p21.3), RB1 (13q14.2) and CCND1 (CYCLIND1, 11q13.3) genes. RESULTS: A higher proportion of MDM2+/CDK4- WDLPS/DDLPS were low-grade lesions belonging to the lipoma-like subtype of WDLPS (58% versus 32%, p= 0.03). Moreover, MDM2+/CDK4- WDLPS/DDLPS were smaller in size than MDM2+/CDK4+ WDLPS/DDLPS (proportion of tumors ≥ 20 cm: 21% versus 45.5%, p=0.03) and occured almost exclusively in the deep soft tissues of the extremities. They were very rarely located in the retroperitoneum (10.5% versus 52%, p=0,0002). In order to determine whether CDKN2A or RB1 deletions or CCND1 amplification were alternative mechanisms for CDK4 amplification, we have analyzed by FISH the status of these 3 genes in 18 cases. We have detected neither CDKN2A and RB1 deletion nor CCND1 amplification. We have found a strong overexpression of CDKN2A in all the 8 cases analyzed by quantitative RT-PCR whereas the expression of RB1 was not significantly altered.. CONCLUSIONS: Although deletions of the CDKN2A locus is among the most frequent sites of genetic loss in human cancer, our results show that this aberration is not involved in the pathogenesis of WDLPS/DDLPS even in those lacking CDK4 amplification. A high level of CDKN2A mRNA has already been reported in several other tumor types and represents a well-known response of cells to oncogenic alterations such as impairment of the P53 pathway resulting from the amplification of MDM2. Altogether, our findings suggest that the absence of CDK4 amplification might not be counterbalanced by a genomic alteration of the p16-CDK4/cyclinD1-pRb pathway. CDK4 amplification could not be as indispensable as the amplification of MDM2 and HMGA2 in WDLPS/DDLPS and may only represent a secondary genomic aberration occurring more frequently in retroperitoneal lesions which have a prolonged evolution before clinical diagnosis.

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Temozolomide and radiotherapy antitumor efficacy evaluation with magnetic resonance imaging and proton magnetic resonance spectroscopy in human glioma models in nude rats

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Malignant glioblastoma remain uniformly fatal despite aggressive therapeutic protocols. Validation of more predictive biomarkers of treatment efficacy in experimental human glioblastoma models would greatly benefit from the establishment of additional quantitative endpoints. The aim of this study was to validate proton Magnetic Resonance Spectroscopy (1H-MRS) and Diffusion-weighted MR Imaging (DwMRI) to evaluate the anti-tumor activity of Temozolomide (TMZ) and radiotherapy (RT) in 2 human glioblastoma models.

CGL9 and U87-MG glioma cells were inoculated at D0 by stereotactic injection in the right caudate nucleus of 2 groups of 22 nude rats. Tumor-bearing rats were ranked according to body weight and randomized at D12 (U87-MG) or at D19 (CGL9) to receive either 5 administrations of 16.5 mg/kg TMZ per os daily or 5 tumor-localized irradiations of 2Gy daily (D12-D16 and D19-D23 for U87-MG and CGL9 respectively), or no treatment (CTL). Imaging was performed on a Bruker Pharmascan 4.7 T at D12, D13, D16, D19, D23 (U87-MG) and D19, D20, D23, D26, D33 (CGL9). Tumor volume was measured using T2-weighted images (U87-MG) or T1-weighted, contrast-enhanced images (CGL9). DwMRI and 1H-MRS were performed at the same timepoints.

Apparent Diffusion Coefficient (ADC) maps were computed from DwMRI volumes, and distributions of ADC analyzed within regions of interest within the tumor and the contralateral lesion-free tissue. Spectroscopic data were acquired using a SVS PRESS sequence, with voxel sizes adapted to the dimensions of the glioma in order to avoid partial volume effects with normal cerebral tissue. A spectrum was also acquired on the contralateral tissue. Spectral data were analyzed using LC-Model.

TMZ increased the life span of both U87-MG and CGL9 tumor-bearing rats (ILS = 126% and >200% for U87-MG and CGL9 respectively). The TMZ-treated to CTL tumor volume ratios (T/C%) were 8 and 2% for U87-MG and CGL9 at the last imaging timepoint, respectively. Radiotherapy (RT) increased the life span of 27% of U87-MG and 10% of CGL-9 tumor-bearing rats

ADC was increased by 34% in TMZ compared to CTL group for U87-MG tumors, whereas ADC was not modified by TMZ in CGL9 tumors.

In the U87-MG CTL group, a progressive reduction in NAA and creatine was observed during the study period. The ratio of total choline and total creatine increased from 0.5±0.2 to 2.5±0.3 in the CTL group, while it decreased from 0.8±0.3 to 0.3±0.2 in the TMZ group. Analysis of MRS data on the CGL9 model and on RT group is pending.

Using MRI, we observed a strong inhibition of tumor growth by TMZ treatment on both models, together with increased survival. ADC is a sensitive parameter to the effect of TMZ on U87-MG, but not on CGL9 tumors. Monitoring tumor metabolism using 1H-MRS is well suited to follow the growth of U87-MG tumors and allows quantification of the antitumor effect of TMZ with choline being the most obvious candidate as a pertinent biomarker.

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Identification of the best molecular markers for early detection of melanoma metastases

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Melanoma, the deadliest of skin cancers, is typified by its high propensity to metastasize and its refractoriness to treatments thereafter. Metastasis occurs mostly through the lymphatic system, and the extent of lymph node metastasis involvement is considered as the best prognostic indicator. Unfortunately, the lymphatic metastatic process is still poorly understood, and the present immunohistological analyses underestimate the number of