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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-