

Session 6: Cancer III

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[11.00–11.30]

Monoclonal antibodies that mimic the T cell receptor and human disease

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Abstract: Antibodies endowed with binding specificity for peptide-major histocompatibility complexes (MHC) have broad application. We have designated monoclonal antibodies with T cell receptor- (TCR) like binding specificity as TCRmimics. The MHC system binds peptides and presents them on the cell surface for T cell surveillance. Since peptides presented by MHC complexes reflect cellular status the MHC class I system is considered nature's proteomic scanning chip. We have developed technology to create TCRm antibodies that recognize unique peptide-MHC class I complexes and have applied these novel reagents to investigate protein processing and peptide presentation by tumor cells. In addition, we are developing TCRm for diagnostic and therapeutic applications and have shown recently the elimination of tumor cells through targeting peptide-MHC targets.

[11.30–12.00]

The human antibody SAM-6 defines a new tumour-specific variant of the heat shock protein GRP78

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Heat-shock proteins (HSPs) are critical components of a cell's defense mechanism against injury associated with adverse stresses. Although HSPs are very benefi-

cial to the normal cell, cancer cells often over-express HSPs on the membrane and use them in response to stresses associated with various therapies (hyperthermia, chemotherapy, radiation), diminishing the treatment effects. The fully human monoclonal antibody SAM-6 is a germ-line coded IgM, isolated from a gastric cancer patient by TRIOMA technology. SAM-6 induces an excess of intracellular lipids by overfeeding malignant cells with oxidized LDL. The treated cells over-accumulate depots of cholesterol-esters and triglycerides and undergo apoptosis. Here we show that the SAM-6 antibody binds to a tumor-specific O-linked carbohydrate moiety expressed on a membrane-bound variant of GRP78, which is a member of the HSP70 family. These data show that cancer-specific modifications of cell surface protection molecules are ideal targets for immuno-therapeutical approaches.

[12.00–12.20]

In vitro and in vivo antitumor activities of anti HERV-K antibody against breast cancer

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The expression of human endogenous retrovirus-K (HERV-K) surface envelope (SU) env proteins in several breast cancer (Clinical Can Res. 2001 and Oncogene, 2003) and ovarian cancer (Int. J. Cancer, 2006) cell lines and tissues has been reported by us. In this study, we have produced monoclonal antibodies (mAbs) against HERV-K SU protein and have characterized the antitumor effects of the antibodies against breast cancer *in vitro* and *in vivo*. Anti-HERV-K mAb 6H5 bound most breast cancers cells and tissues, but not normal or benign breast cells and tissues. More than 85% of breast cancer biopsies, but no benign or normal breast biopsies were positive for HERV-K expres-

sion. The immunohistochemical staining score was increased in the order benign, ductal carcinoma *in situ*, infiltrating ductal carcinoma, and metastatic ductal carcinoma. The expression score of HERV-K env proteins was correlated with patient age, stage, nuclear grade, ER, PR and Her/2 status, and lymph node involvement. The mAbs inhibited growth of several breast cancer cell lines *in vitro* and *in vivo*. The mechanism of inhibition by anti-HERV-K mAbs includes inhibition of cancer cell proliferation and induction of cancer cells to undergo apoptosis. The cytotoxicity of mAb 6H5 toward cancer cells, but not normal or benign breast cells, was determined by MTT assay, cytotoxicity assay, flow cytometry, and apoptosis assay. The 6H5 mAb was able to protect mice bearing MCF-7 breast cancer cells, including MCF-7 and MDA-MB-231 cells. Tumor sizes were significantly reduced in mice treated with 6H5 mAb, in comparison to mice treated with saline. Thus, HERV-K env protein is a unique tumor target, and anti-HERV-K mAbs could be of therapeutic value for human cancers.

[12.20–12.40]

The 14F7, Monoclonal antibody that mediated non-apoptotic cell death

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Gangliosides are prominent members of glycosphingolipids membrane constituents that are distinguished by the presence of one or more sialic acid residues. Gangliosides have been involved in multiple processes such as growth, differentiation, adhesion and more recently as regulators of cell death pathways. Some of these molecules can be considered as tumour-associated antigens, leading to the use of anti-gangliosides mAbs in the diagnosis and therapy of cancer. In this study we analyze the induction of cell death by an anti-NeuGc-GM3 monoclonal antibody that we call 14F7, and elucidate for the first time the essential role of this NeuGc-GM3 ganglioside in the initiation of cell death signaling. Interestingly, this complement-

independent mechanism does not resemble apoptosis, since no DNA fragmentation, Fas mediation or caspase substrate activation was observed. However NeuGc-GM3 ganglioside mediated cell death is accompanied by cell aggregation, membrane damage and caspase 9-activation and also pore formation in the membrane. This induction of cell death leads us to support further therapeutic application of anti-NeuGc-GM3 monoclonal antibodies in cancer treatment.

[12.40–13.00]

Anti-ErbB2 human immunoagents

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We have engineered a series of human immunoagents, directed to one of the most convenient tumor-associated antigens, the RTK ErbB2 (Her2/neu). Overexpression of ErbB2 in carcinomas, frequent in mammary carcinoma, marks an adverse prognosis. We produced and characterized: Erbicin, a single-chain human anti-ErbB2 immunoagent; Erb-hRNase, a human immunoRNase made up of Erbicin fused to a human RNase; Erb-hcAb, a human, reduced size ("compact") antibody, in which two Erbicin molecules are fused to the CH2 and CH3 regions of a human IgG1.

All these immunoagents selectively bind ErbB2 with 1-4 nM affinity, and exert a strong, selective cytotoxic action towards ErbB2-positive carcinoma cells. Both, Erb-hRNase and Erb-hcAb are effective also *in vivo* on tumor xenografts implanted in laboratory animals. Preliminary studies suggest that they bind an epitope different from that targeted by other anti-ErbB2 mAbs; are not immunogenic, and do not display cardiotoxic effects *in vitro*. More interestingly, they appear to be active also on Herceptin-resistant tumors.

Recent investigations of the mechanism of antitumor action of these anti-ErbB2 immunoagents have indicated that: (i) Erb-hcAb induces the homodimerization of ErbB2, which leads to its down regulation and lysosomal degradation; (ii) the antitumor action of Erb-hRNase depends on its ribonuclease activity, which is exerted in the cytosol, where the immunoagent builds up to such levels as to neutralize the endogenous cytosolic RNase inhibitor.

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