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## Eradication of chemoresistant ovarian cancer in combination with gene and cytotoxical therapy

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Ovarian cancer is still one of the most fatal cancers among women, because most efforts for improving screening, diagnosis and potential cure have been unsuccessful. The major problem in the failure of cancer treatment consists of multiple drug resistance. Thus, chemoresistant epithelial ovarian cancer cells stage IV obtained from surgicl excision, have been derived utilizing the collagenase method. Immunohistochemical analysis has detected high expression of MDR1 gene of Pgp. Also, sequencing of p53 coding exons 5–8 by PCR, using intron-specific oligonucleotides, has detected a missense mutation in codon 273 (CGT to CAT). Furthermore, immunoblot analysis has detected upregulation of bcl-2 and downregulation of bax. These cells, after incubation with taxol-molecules have not exhibited any apoptotic signs. Liposomes consisting of high Tc phospholipids and equimolar cholesterol have been prepared. Taxol-molecules are entrapped in their lipophilic region, while wild-type p53 cDNA is entrapped in the hydrophilic region of these liposomes. Then we incubate these liposomes with the tumour cells. TEM analysis proves that liposomes adsorb on the cellular membrane for lipid exchange because cancer cells exhibit an enhanced need for cholesterol uptake for membrane synthesis. For the first time ever, we observe an endocytotic mechanism of ovarian tumour cells via caveolae. This way, liposomes are transported into the cytoplasm, avoiding the lysosomes, which constitute another chemoresistant mechanism. Finally, liposomes are fused with the nuclear membrane of the tumour cells, releasing intranuclearly wild-type p53 and the taxol-molecules. After this phenomenon, we observe by immunoblot analysis downregulation of bcl-2 oncogene and upregulation of bax. As a result, we observe apoptotic signs, such as DNA fragmentation, enhanced apoptotic index and phenotypical alterations, such as condensation of chromatin in crescentic caps adjacent to the nuclear membrane, incomplete nuclear membranes and translucent cytoplasmic vacuoles, and other apoptotic vesicles, which are phagocytosed by non-transduced cells causing their cellular death by mediating a bystander effect. This morphological evidence is verified by biochemical assays, where the metabolic activity has been reduced significantly according to MTT analysis, and DNA according to BrdU analysis has been reduced proportionally, compared to the controls consisting of incubating tumour cells with empty liposomes.

**Conclusion:** We have circumvented MDR-1 gene, re-established function of p53, downregulated bcl-2 oncogene and amplified bax, leading to the apoptosis of the ovarian tumour cells. Furthermore, we have protected the therapeutic molecules from biological milieu interactions (i.e. HDL), enhancing their therapeutic index and $t_{1/2}$.

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## Vaccination of colorectal and pancreatic cancer patients with baculovirus-derived extracellular domain of GA733 antigen

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**Purpose:** In patients with gastrointestinal tumors, expression of GA733 antigen up to 90% on primary tumor or metastases has been shown. Since the antigen has been purified and characterized, adjuvant immunization of patients after tumor resection with a vaccine preparation of GA733 antigen is believed to induce tumor-specific immune response.

**Methods:** At the Dpt. of Surgery, University of Ulm, 7 pts. (3 pancreatic cancer, 3 colon cancer, 1 rectal cancer; 3 female, 4 male) after curative resection of the primary tumor were injected intradermally with an ALUM-bound preparation of baculovirus-derived extracellular domain of GA733 antigen provided after FDA-approved purification and safety testing by the *Wistar Institute*. The vaccine was injected at least 4 weeks postoperatively in doses of 50 (2), 200 (3) or 800 $\mu$g (2) once monthly five to seven times. Immune response was monitored for antibody production by MHA and FACS analysis.

Toxicity was noted according to WHO criteria.

**Results:** In MHA, 5/7 pts. (3 pancreas, 2 colon) developed antibodies specifically bound to GA733-positive SW 1116 cells (but not to antigen-negative melanoma WM9 cells) with $\geq$10% cells bound after 3 to 6 vaccinations. Two patients had pre-existing antibodies (2 pancreas). In FACS analysis, 4/7 pts. (3 pancreas, 1 colon) showed GA-733 positive antibodies (>5%). There was no toxicity > WHO2 and no autoimmune reaction (thyroid, kidney). At the injection site, a mild induration was noted.

**Conclusion:** With a baculovirus-derived extracellular domain of GA733 antigen, in patients after curative resection of colorectal or pancreatic cancer, specific antibody production after 3 to 6 vaccinations was induced.

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## Double-blind randomised trial of 105AD7 vaccine

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105AD7 vaccine is a human anti-idiotype which mimics the antigen 791Tgp72. The vaccine was administered to over 150 patients with no associated toxicity. T-cell responses were observed in 80% of the patients in a Phase I trial. The mechanism of action of the vaccine has recently been clarified. The anti-idiotype is rapidly internalized on antigen presenting cells by Fc mediated endocytosis as both Fab and Ab complexed to alum are processed 1,000 fold less efficiently than whole Ab. Sequencing of the Ab have revealed that the CDR-H3 is hypermutated and contains HLA A1, A3 and A24, and HLA-DR1, 3 and 7 MHC binding motifs. All patients showing Tcell responses had the predicted MHC phenotypes.

As patients in the phase I study survived significantly longer than a contemporary group, the vaccine has been evaluated in a double-blind randomised trial. 165 patients with recurrent colorectal cancer were immunised with 10 $\mu$g of 105AD7 (i.d) and 100 $\mu$g of 105AD7 alum (i.m.) or 10 $\mu$g of saline followed by an i.m. injection of alum alone. Patients could receive 3 courses of injections at 6 weekly intervals. 55% of patients completed all three courses, 20% only 2 and 25% received only a single immunisation. There was no Ab related toxicity in any of the patients. The final patient was recruited in October 1996 and 31 patients remain alive. Survival is the primary end-point of this study which will be presented at ECCO.

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## Induction of AG-specific T cells by allogenic mamma carcinoma cells transfected with CD80

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**Introduction/Purpose:** The use of tumor cells genetically modified to express immunostimulatory molecules as vaccine represents a strategy for the induction of anti-tumor immune responses. To circumvent the requirement for generating individual vaccines for each patient, allogeneic cell lines with shared HLA-alles are under investigation. Here we characterized in vitro the potential of a human mamma carcinoma cell line (KS) transfected with the costimulatory molecule CD80 to induce an Ag-specific immune reponse of allogeneic HLA-A2 matched T lymphocytes.

**Methods/Results:** CD80+ KS cells and PBMC, respectively, were pulsed with the antigenic peptide MP57–68 derived from the Influenza Matrix Protein and the generation of MP57–68 specific and allospecific T cells was determined at the single cell level in ELISPOT experiments by IFN-$\gamma$ release. The generation of peptide-specific T cells was not negatively influenced by allo-antigens as determined following T cell stimulation with autologous or allogeneic PBMC. Importantly, allogeneic CD80+ KS cells but not untransfected cells induced a peptide-specific response which could be further augmented by pretreating CD80+ KS cells with IFN-$\gamma$ and TNF-$\alpha$. Moreover, cytokine pretreated CD80+ KS cells can have a similar stimulatory potential as PBMC.

**Conclusion:** This supports the use of immunogenic tumor cell transfectants for the induction of specific immune responses in allogeneic HLA-matched situations. Furthermore, ELISPOT technology may be suitable to monitor the generation of a tumor-specific T cell response following vaccination.