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Phase I study of HSPPC-96 (Oncophage®) vaccine in patients with completely resected pancreatic adenocarcinoma

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Background: Cancer vaccines based on autologous heat-shock proteins are of particular interest due to their ability to carry a diverse array of peptides representing the individual cancer, against which a subject can be vaccinated.

Materials and Methods: We examined an HSPPC-96 (heat shock protein peptide complex-96, Oncophage® vaccine derived from autologous patient tumor in a phase I study of subjects with resected pancreatic adenocarcinoma. After signing informed consent, patients underwent a complete surgical resection of the primary tumor if possible, if there was no evidence of metastatic disease. If HSPPC-96 could be isolated after resection, patients were vaccinated within 8 weeks of surgery with 5 mcg HSPPC-96 derived from their autologous tumor, every week, for four doses. Subjects were followed during vaccination by ELISpot assay of autologous T cells against antigen presenting cells loaded with autologous HSPPC-96. Patients on this study received neither adjuvant chemotherapy nor radiation. Subjects were followed clinically by CT scan and CA-19-9 every three months for one year for evidence of recurrence, and approximately every six months thereafter.

Results: Only 5 patients of the first 15 had successful generation of HSPPC-96 after surgical resection, owing to proteolysis of the tumor sample in vaccine preparation. A change in the manufacturing process improved yield from patient samples. Thereafter, 5 patients were enrolled and resected and all 5 received vaccine. In total, 10 patients received a full course of vaccinations between 10/97 and 7/01. Subjects had no dose-limiting toxicities. Three patients are alive and without disease at 5.0, 1.7, and 1.6 years. One patient is alive with disease after progressing on the first CT scan following the final vaccination. Median overall survival is 2.0 years, mean 2.0 years. The frequency of autologous anti-HSPPC-96 ELISpots increased substantially in one patient of the first five examined, from < 10 at baseline to ~100 per 10^5 CD8+ cells one week after the final vaccination, and decreased to 20 four weeks after the final vaccination. Two of five patients had increases in ELISpot number of borderline significance.

Conclusion: Despite the possibility of selection bias, these results are promising and support the further evaluation of autologous HSPPC-96 in resected pancreatic cancer.

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Gemcitabine mediates cell death in human pancreatic cancer by recruitment of CD95 and the mitochondrial apoptosis pathway

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Introduction: Pancreatic cancer is a neoplasm whose incidence almost equals its death rate. Due to the inability to detect pancreatic cancer at an early stage, most patients suffer from metastatic disease preventing surgical treatment. Unfortunately, gemcitabine, commonly used for palliative chemotherapy in pancreatic cancer, has little impact on patient survival. It has been revealed that the upregulation of proteins known to inhibit apoptosis might be causal. Therefore, we investigated the impact of the apoptosis inhibitor Bcl-xl and its antagonist Bax in human pancreatic carcinoma cells with respect to gemcitabine-induced apoptosis.

Methods: To determine whether Bcl-xl and Bax expression correlates with the apoptotic index of cells exposed to gemcitabine we carried out Jam-assays and Western-Blot analyses in three established pancreatic cancer cell lines (Colo357, PancTU1, Panc1) expressing different levels of Bcl-xl and Bax. We transduced these genes into Colo357 by retroviral infection to examine gemcitabine-induced apoptosis. As control wild type cells (Colo357wt) or Colo357 expressing the enhanced green fluorescent protein (Colo357EGFP) were employed. Furthermore, we evaluated the differences in tumor growth of either orthotopically inoculated Colo357wt or Colo357 pools expressing Bcl-xl, Bax or EGFP in gemcitabine-treated SCID mice.

Results: There was a strong correlation between Bcl-xl expression and the apoptotic index of cells exposed to gemcitabine. Overexpression of Bcl-xl in Colo357 rescued these cells from gemcitabine-induced apoptosis and prevented mitochondrial permeability transistion and cleavage of death substrate PARP. However, gemcitabine-mediated upregulation of CD95 surface expression, G1-arrest and cleavage of caspase-8 remained unaffected. These data, pointing towards the involvement of death receptors in gemcitabine-induced apoptosis, could be substantiated by transfection of c-Flip. C-Flip rendered Colo357 cells resistant to gemcitabine. Overexpression of Bax failed to increase the susceptibility of Colo357 cells with regard to gemcitabine in vitro. Interestingly, mice bearing Colo357bcl-xl tumors remained unaffected by gemcitabine treatment whereas mice bearing Colo357bax tumors exhibited a 12-fold stronger tumor regression than mice from the Colo357wt and EGFP control group. Here we show for the first time that therapeutic effects of gemcitabine depend on CD95 and the mitochondrial apoptosis pathway in pancreatic cancer.

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Immunotherapeutic application of novel pancreatic cancer-specific tumor marker PaCa-Ag1.

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Cancer of the exocrine pancreas (PaCa) annually claims ~56000 lives in Europe and ~187000 lives worldwide. PaCa has the lowest five-year survival rate of all cancers because of its late diagnosis and its high resistance to chemo- and radiation therapy. Therefore, it is imperative to explore alternative therapies. Previously, we reported the identification of PaCa-Ag1 as an early PaCa marker. PaCa-Ag1, recognized by monoclonal antibody 3C4 (mAb3C4), is a 43kD protein expressed on the surface of rodent and human PaCa cells, and that is absent from rodent and human normal tissues including the pancreas. In addition to its unique specificity several observations make PaCa-Ag1 a potential target for the use of immunotherapy approaches against PaCa: Indirect Immunofluorecence (IIF) and immunoblotting (IB) of a real-time course experiment of treating the rodent PaCa cells, BMRPA.TUC3, with fluorescein-labeled mAb3C4 ($mAb3C4^{FITC}$) showed the endocytic removal from the cell surface of the $mAb3C4^{FITC}$-PaCa-Ag1 complexes within 30min. By 90min, PaCa-Ag1 was undetectable in the cell lysates by both IIF and by IB. Removal of the immune complexes was paralleled by intracellular events seriously affecting cell proliferation. In particular, a rapid reduction by more than 90% of ras-p21 (within 20min) and p-JNK (by 40min), both signal transduction proteins important for inducing cell growth, suggested that binding of mAb3C4 to PaCa-Ag1 may interfere with cell proliferation, and that PaCa-Ag1 may play a role in cell proliferation. In fact, when mAb3C4 was continuously present during culture of human PaCa, i.e. MiaPaCa-2 cells the mAb appeared to interfere with the cells regular growth morphology. In an *in vitro* cytotoxicity assay, mAb3C4 in presence of fresh serum as a source of active complement lysed >70% of Mia PaCa-2 and BMRPA1.TUC3 cells. The *in vivo* cytotoxicity of mAb3C4 was demonstrated in Nu/Nu mice (n=7) growing tumors from xenografted PaCa cells. Within one week of introduction of mAb3C4 into the tumor-bearing animals, induction of tumor necrosis was observed followed by reductions of tumor sizes from ~18mm to less than 4mm (>75%; 18d) while tumors continued to grow in control animals (n=5) ($p<0.001$). Together, the cell surface localization on PaCa cells and the *in vitro* and *in vivo* effects of mAb3C4 on PaCa-Ag1+ cells and not on other cells make PaCa-Ag1 a good candidate to develop a selective immunotherapeutic approach to this devastating disease.