gene, designated FCU1, encodes a bifunctional chimeric protein that combines the enzymatic activities of FCY1 and FUR1 and efficiently catalyses the direct conversion of 5-fluorocytosine (5-FC), a non-toxic prodrug, into the cytotoxic metabolites 5-fluorouracil (5-FU) and 5-fluorouridine-5'-monophosphate (5-FUMP). Interestingly, the cytosine deaminase activity is 10-fold higher in the chimeric protein compared to the natural protein.

Results: In this study we demonstrate that a MVA (Modified Vaccinia Virus of Ankara) engineered to express the FCU1 gene significantly enhances the sensitivity of numerous human tumour cells to 5-FC (LD50 5-FC = 1μM in the FCU1 treated cells compared to LD50 5-FC = 10mM in the CDase treated cells; $p<0.01$). Moreover, passive diffusion of the 5-FU ensures an impressive bystander effect with the ability to kill 100% of a in vitro tumour cell population with only 1% FCU1-transduced cells. Intratumoral injections of MVA-FCU1 into human tumour-bearing mice, with concomitant systemic administration of 5-FC, led to a sustained control of tumour growth. The FCU1-induced tumour growth suppression was observed in different human colorectal tumour models whereas 5-FU administered IP at the maximum tolerated dose did not show any anti-tumor effect in the same models. Finally, a 10-fold higher concentration of 5-FU is detected inside the tumour compared to a systemic administration of 5-FU while no detectable 5-FU is found in the circulation, ensuring a higher safety profile with no systemic toxicity.

Conclusions: The FCU1 suicide gene is a unique combination of an innovative approach and a validate and secure chemotherapy that makes it a novel and powerful candidate for treating all 5-FU sensitive tumours.

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Pre-clinical safety and immunogenicity studies of a HER-2 protein vaccine in cynomolgus monkeys

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Background: The HER-2 growth factor receptor has emerged as an attractive target for immunotherapy of breast cancer. Pharmexa A/S has developed a modified recombinant HER-2 protein vaccine (HER-2 AutoVacTM protein) that includes potent helper T cell epitopes to enhance immunogenicity. Pre-clinical safety and immunogenicity studies were performed in cynomolgus monkeys to support clinical trials in patients with HER-2 positive breast cancer.

Methods: A pilot immunogenicity study and a two armed 3-9 month repeated dose toxicity study were performed. In the pilot study, groups of 6 monkeys were injected with 100mg HER-2 protein vaccine formulated in three different adjuvants: two commercial adjuvants (A and B) and a standard aluminum adjuvant The monkeys received either 5 immunizations at 2 week intervals, or 3 immunizations at weeks 0, 2 and 6. In the 3-9 month repeated dose study, groups of 6 monkeys received 20, 100 or 500mg HER-2 protein vaccine formulated in the aluminum adjuvant at weeks 0, 2 and then every 4 weeks for the duration of the trial. A recovery period was included for one group of the 3 month arm of the study. Sera were collected at regular intervals and evaluated for HER-2 specific antibodies by direct ELISA. At the conclusion of treatment, monkeys in the toxicology study were sacrificed and subjected to full necropsy and histological analyses.

Results: Significant HER-2 specific antibody titers were measured in all monkeys in the pilot study. The highest response was seen with adjuvant A after 5 immunizations at 2 week intervals. The responses after 3 immunizations on weeks 0, 2 and 6 were similar between adjuvant A and aluminum. In the 3-9 month study, all animals receiving 500mg HER-2 protein vaccine had significant antibody responses after one immunization, while the 20mg and 100mg dose groups developed titers after 2 immunizations. Titers were maintained by continued boosting and declined rapidly in the recovery group upon cessation of treatment. No vaccine associated toxicity was observed after 3 months treatment (9 month results pending). It was concluded that immunization with the HER-2 AutoVac protein induced significant HER-2 specific antibody titers and was safe in cynomolgus monkeys.

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Increased gap junctional intercellular communication in esophageal cancer augments the bystander effect in HSV-tk/GCV suicide gene therapy

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Background: There is accumulating evidence indicating that gap junction is important in induction of the bystander effect (BE) in a gene therapy through herpes simplex virus thymidine kinase (HSV-tk)/ganciclovir (GCV). We previously reported that substantial BE was observed in esophageal cancer cells with gap junctional intercellular communication (GJIC). Here, we investigated the effect of chemicals, such as all-trans retinoic acid (ATRA), cAMP and glucocorticoid, and transduction of gap junction protein, connexin (Cx), gene on GJIC and BE in esophageal cancer cells.

Materials and Methods: Four esophageal cancer cell lines were selected by the status of Cxs expression and GJIC out of 8 cell lines for subsequent experiments: KE-3, KE-6, KE-8 and KE-10. Expression of Cx26 and Cx43 and GJIC before and after treatment with ATRA, cAMP and glucocorticoid in above cells were examined with Western blot analysis, immunohistochemistry and dye transfer assay. Those chemicals' effect on BE was examined by cell killing assay with GCV treatment by using pretreated mixtures of cells transfected with HSV-tk gene and parental cells at varioius ratios. Finally we transfected cells with Cx32 and HSV-tk gene and then examined the extent of GJIC and BE with the same procudures.

Result: KE series did not express Cx26 mRNA and protein. The localization pattern of Cx43 protein was classified into four types; at cell-cell contact area(KE-8), in the cytoplasm (KE-3), at both cytoplasmic and cell-cell contact area(KE-6), and no expression(KE-10). The excellent GJIC and BE was observed in KE-8, and the moderate in KE-6. Little GJIC and BE was observed in KE-3 and KE- 10. When 10% HSV- tk(+) cells were mixtured, about 50% of cells were killed in KE-8. All three chemicals showed no influence on the Cx expression. Although cAMP and glucocorticoid had no positive effect on GJIC and BE in all cell lines, ATRA enhanced the GJIC in KE-6 by 25%, leading to significant increase of BE by 50%. Mixing experiment with Cx32+tk+ cells and parental cells revealed augmentation of BE, resulting from increase of GJIC; 50% of cell death in the presence of 10% Cx32+tk+ cells.

Conclusion: Our results indicate that the bystander effect in HSV-tk/GCV suicide gene therapy is induced according to the intensity of GJIC in esophageal cancer cells and that enhance of GJIC by ATRA and Cx gene transduction is effective to induce strong bystander effect.

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Additive and synergistic effects on TRAIL/Apo2L induced apoptosis after irradiation and eradication of clonogenic cells in solid tumor cell lines in vitro

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Background: TRAIL (TNF related apoptosis inducing ligand)/Apo2L is a very promising member of the family of death ligands. The ligand preferentially induces apoptotic cell death in a wide range of tumor cells but not in normal cells. TRAIL/Apo2L triggers apoptosis even in cells not undergoing apoptosis in response to radiation, since ionizing radiation induce apoptosis by a different pathway as death ligands although an overlapping set of molecules is involved. Based on the assumption of distinct signalling pathways, combination of TRAIL/APO2L and ionizing radiation seems ideal to increase the therapeutic efficacy. The question in how far the combination of both modalities induces additive or synergistic apoptotic effects and eradication of clonogenic tumor cells is subject of this report.

Material and methods: Tumor cell lines: NCI-H460 (adenocarcinoma of the lung), HCT-15 and Colo-205 (colon carcinoma), MDA-MB-231 (mamma carcinoma), LnCAP (prostate cancer), SCC-4 (squamous cell cancer). Determination of apoptosis was performed 12-48 h after simultaneous application of TRAIL/APO2L and irradiation (2-10 Gy) and after 12h preirradiation of TRAIL/Apo2L treated tumor cells. Rates of apoptosis were determined by flow cytometric evaluation of caspase activation and/or by microscopical observation of morphological changes of Hoechst stained cell nuclei. Clonogenity was determined by colony forming assays.

Results: Both TRAIL/Apo2L and ionizing radiation alone lead to a dose dependent induction of apoptosis in several of the analysed tumor cell lines. Concomitant application of combined treatment leads to an additive effect. Preirradiation (12h prior to TRAIL/Apo2L treatment) leads to supraadditive effects in HCT-15 und Colo-205 tumor cell lines. Clonogenity of the cells was inhibited to a noticeable degree.TRAIL/Apo2L triggers cell death also in cell lines with very low (HCT-15, SCC4, MDA-MB231) rates of irradiation induced apoptosis.

Conclusion: TRAIL/APO2L and irradiation lead to additive and, dependent on the time course of application, partially supraadditive effects after combined treatment. Therefor, combined treatment with TRAIL/Apo2L and irradiation represents a new treatment strategy for tumours based on activation of two distinct cell death mechanisms.