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Novel 2-step desensitization dosing regimen of intravenous PV701, an oncolytic virus, results in improved tolerability: a phase I study of patients with advanced solid tumours

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Background: PV701 is an oncolytic Newcastle disease virus that is active preclinically when given intravenously (IV). The broad spectrum oncolytic activity of PV701 is due to tumour-specific defects in the interferon antiviral response. Prior study [J Clin Oncol 2002; 20: 2251-2266] has shown that IV PV701 can be administered safely to patients (6 doses over a 2 week period q 21 days). Following a 1st dose of 12 billion plaque-forming units (BPFU)/m^2, desensitization to the adverse event profile was observed for the subsequent 5 doses. This allowed the repeat dose to be 10-fold higher than the 1st dose (120 vs. 12 BPU/m^2). Flu-like symptoms were common after the 1st dose, decreasing in severity and frequency with subsequent doses. In an effort to further improve first dose tolerability, the current phase I trial evaluated a 2-dose desensitization methodology. This approach uses intrapatient dose escalations from 1 BPFU/m^2 to 12 BPFU/m^2 to further reduce 1st-dose toxicity and adverse events of subsequent higher doses of up to 120 BPFU/m^2.
Methods: Adults with advanced, incurable solid tumours, good performance status and adequate end-organ function were enrolled. PV701 was given IV over 30 minutes, 6 times in 2 weeks, cycled q 21 days. 4 dose levels were studied. In each cohort, the 1st and 2nd doses were 1 and 12 BPFU/m^2, respectively. Doses 3-6 were constant in each patient (pt), but escalated by cohort: 24, 48, 96, 120 BPFU/m^2. DLT was defined as any drug-related toxicity of grade 3/4 seen during cycle 1. Pts were assessed for response after cycle 2; those benefiting could continue on an extension protocol.
Results: Thirteen pts have been enrolled to date (7 males; median age 58; 5 colorectal; 2 each breast, sarcoma, ovary; 1 each NSCLC, anal). First dose toxicity consisted of flu-like symptoms lasting < 72 hours, all grade 2 or less, and less severe than that seen with the previous phase I study. No DLTs have been observed to date. Median number of cycles delivered = 2 (range <1 - 8). Of 10 pts currently evaluable for response: SD = 5; PD = 5. Analysis of antibody response is ongoing.
Conclusions: Two-step desensitization of IV PV701 is well-tolerated with an improved safety profile compared to the previous phase I trial. Enrollment to dose level 4 (six doses at 1/12/120/120/120/120 billion PFU/m^2 per cycle) continues. This novel intravenous dosing methodology will be utilized in planned phase II studies of PV701.

Drug resistance and modifiers

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Peptide PNC-28 of the p53 binding domain to mdm-2 is a potent inhibitor of growth of carcinoma cells in a novel *in vivo* model of pancreatic carcinoma

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Pancreatic cancer remains one of the most lethal cancers causing death of >30,000 people per year in the US alone with a similar number of new cases diagnosed every year. Other than radical surgery, frequently not a viable option for the patient, there is only little that medical practice can do to prolong significantly a patient's life. Pancreatic cancer is unique in that in >90% of the tumors single point mutations have been identified in the genes of the ras proto-oncogene family as well as mutations in the p53 anti-oncogene in >75% of the tumors. Using conformational analysis on the p53-protein and its interaction with its regulatory protein mdm-2 we have identified peptides from the mdm-2 binding domain of p53 (residues 12-26) that upon introduction into tumor cells cause rapid necrosis in several different tumors including the novel pancreatic cancer cell lines BMRPA1.TUC3 and BMRAP1.NNK. In contrast, the same p53 peptides were not cytotoxic for normal differentiated pancreatic cells, BMRPA1, and did not inhibit the differentiation of human hematopoietic stem cells (PNAS 98:12438-12443, 2001). During synthesis, the peptides were linked to a penetratin leader enabling their direct entry into the cells' cytoplasm. We have now examined the effect of one of the p53-penetratin peptides, PNC-28, in a novel pancreatic carcinoma model established by the xeno-transplantation of BMRPA1.TUC3 cells into Nu/Nu mice. In these mice the tumor tissue displayed a typical desmoplastic reaction, invasive metastatic growth into the surrounding tissue and metastasis to the lungs, liver, pancreas and intestine. Examining the PNC-28 peptide effect on this tumor, we have found that (a) when applied 5ds prior to a subcutaneous or intraperitoneal tumor cell implantation, PNC-28 blocked growth of BMRPA1.TUC3 cells in all but 1 of the 9 mice treated (1/9);(b) PNC-28 arrested tumor growth (0/10) and prevented distant metastases when treatment of the transplanted Nu/Nu mice occurred within 24 h of tumor cell transplantation;(c) PNC-28 exerted its effect irrespective of having been injected either at or distant to the site of tumor implantation. In contrast, simultaneous treatment of tumor-laden Nu/Nu mice with a penetratin-control peptide (PNC-29)neither slowed tumor growth nor delayed metastases. Together with the *in vitro* studies the present results strongly suggest that PNC-28 may provide an effective treatment of pancreatic cancer. Supported by the Lustgarten Foundation.

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Retained sensitivity to brostallicin after loss of DNA mismatch repair

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Defects in DNA mismatch repair (MMR) are associated with predisposition to tumorigenesis and with drug resistance owing to high mutation rates and failure to engage DNA-damage-induced apoptosis. DNA minor groove binders (MGBs) are a class of anticancer agents highly effective in a variety of human cancers. Due to their mode of action, MGBs agents may be targets for MMR induced resistance. This study was aimed at investigating the effect of loss of MMR on the sensitivity to brostallicin (PNU-166196), a novel synthetic alpha-bromoacrylic, second generation DNA MGB, using a proliferation assay (MTT) and the clonogenic assay. We report that MLH1-deficient HCT116 parental colorectal adenocarcinoma cells were 2-fold more sensitive to the antiproliferative effect of brostallicin than the MLH1-proficient HCT116+ch3 counterpart, but that MLH1-deficient cells were as sensitive to the clonogenic survival as the MLH1-proficient cells. Likewise, MSH2-deficient HEC59 parental endometrial adenocarcinoma cells were as sensitive to the clonogenic survival to brostallicin treatment as the MSH2-proficient HEC59+ch2 counterpart. In addition, p53-deficient mouse fibroblasts lacking PMS2 exhibited a 2-fold higher sensitivity to brostallicin than PMS2-proficient cells in the MTT assay, but the clonogenic assay did not reveal any difference in sensitivity to this agent between PMS2-deficient and PMS2-proficient cells. These data demonstrate that, unlike to other MGBs, MMR-deficient cells retain their sensitivity to brostallicin, indicating that brostallicin-induced cytotoxicity does not depend on functional MMR. These findings suggest testing brostallicin in the treatment of MMR-defective tumors.

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Phase I study to determine the safety of MS209 in combination with docetaxel in patients with solid progressive tumor

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MS209 (MS) is an oral dihydroquinoline derivative, which binds directly to P-glycoprotein, and belongs to MDR inhibitors. This new compound is tested in a phase I study in combination with docetaxel (D). D is administered as a 1-hour infusion every 3 weeks, with corticosteroid premedication. At cycle 1, D is administered alone and for further cycles MS is given 30 minutes before D. The dose escalation scheme follows a modified Fibonacci model with 6 steps (D from 60 to 100 mg/m^2 and MS from 300 to 1200 mg/body). Three patients are registered by dose level; the cohort size can be extended up to 6 patients in case of dose-limiting toxicity (DLT). DLT is defined as neutropenia grade (G) 4 for more than 7 days, thrombocytopenia G4, febrile neutropenia and any G3-4 non-hematological toxicity except alopecia, nausea-vomiting - and was evaluated during cycle 2. PK samples are collected during the first 24 hours at cycle 1 and 2. As of May 2002, 26

patients completed treatment with a total of 107 cycles (median: 3.5 cycles, range: 1-9). Four patients are still on treatment. One complete response (breast) and 2 partial responses (larynx and lung) were observed with 7 additional stable diseases. The main toxicity is neutropenia G3-4. The following DLTs were observed: At level 3 (D:80/MS:600): febrile neutropenia G4, infection G3 leading to death, At level 4 (D:80/MS:900): stomatitis G3, dysphagia G3, fatigue G3, At level 5 (D:80/MS:1200): 2 DLT: stomatitis G3/neutropenia G3 and stomatitis G4/fatigue G3 The MTD was reached at level 5. The recommended dose is then level 4 (D:80/MS:900). PK analysis did not demonstrate a strong PK interaction between the two compounds but at the highest dose levels, there is a trend to an increase of docetaxel AUC when this agent is given in combination with MS209. Complete data set and PK analysis will be presented.

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A phase I pharmacokinetic study of ONT-093 in combination with paclitaxel in patients with advanced cancer

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Background: ONT-093 (OC144-093) is a third generation, orally bioavailable inhibitor of P-glycoprotein (P-gp). In pre-clinical studies, inhibition of P-gp and reversal of multidrug resistance (MDR) could be achieved at nM concentrations. ONT-093 does not affect paclitaxel pharmacokinetics (PK) in rodents. Phase I clinical trials of single agent ONT-093 in normal human volunteers showed no dose-limiting toxicities at biologically active serum concentrations in doses ranging from 300 to 500 mg.
Methods: We performed a Phase I pharmacokinetic trial of ONT-093 in doses from 300 to 500 mg administered orally 14 h before, 2 h before and 10 h after intravenous paclitaxel doses of 150 to 225 mg/m^2 repeated every 21 days. All patients received paclitaxel alone for cycle 1 to allow intra-patient comparisons of toxicity and paclitaxel pharmacokinetics.
Results: To date, 18 patients have been enrolled into 4 dose levels and have received doses of ONT-093 up to 500 mg and paclitaxel up to 175 mg/m^2. Toxicities have mainly been attributable to paclitaxel, and included arthralgia, myalgia, neutropenia, and peripheral neuropathy. Toxicities possibly associated with ONT-093 include grade 1-2 headaches and transient grade 1 elevation of liver transaminases in 1 patient. Three patients have had higher-grade neutropenia with cycle 2. One of these patients, who was also heavily pre-treated and had extensive hepatic metastases, had febrile neutropenia on cycle 2, dose level 4 (ONT-093 500 mg and paclitaxel 175 mg/m^2). This cohort is being expanded. Cmax concentrations of ONT-093 given at 500 mg are $> 8\ \mu$M, well above that required in pre-clinical models to inhibit P-gp and completely reverse MDR. Plasma PK of paclitaxel are unchanged between cycle 1 and 2.
Conclusions: Biologically active doses of ONT-093 have been well tolerated in combination with standard doses of paclitaxel. There have been no alterations of paclitaxel PK parameters with the combination at the doses tested. These results support the continued clinical development of ONT-093 as an active, potent, non-toxic inhibitor of P-gp in conjunction with cytotoxic chemotherapy. Patient accrual continues, and final results will be presented.

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Hyperthermia and multidrug resistance: Impact on expression and regulation of MDR genes in human cancer cells

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Hyperthermia is used for the treatment of cancer patients in combination with chemotherapy, cytokines and/or radiotherapy. Since multidrug resistance (MDR)-associated genes are inducible by external stress factors such as heat and drugs used for chemotherapy, we investigated the influence of hyperthermia on expression, regulation and function of the MDR genes MDR1, MRP1, and MVP/LRP in *in vitro* models as well as in clinical specimens. In colon carcinoma cell lines, hyperthermia caused nuclear translocation of the transcription factor YB-1. Nuclear YB-1 interacts with the promoters of the MDR1 and MRP1 genes and is associated with increased MDR1 and MRP1 gene transcription, as well as strong efflux pump activity. However, a combination of hyperthermia and drug treatment effectively reduced cell survival demonstrating that activation of MDR1 and MRP1 gene expression and increased efflux pump activity after hyperthermia did not consequently lead to an increase in a MDR phenotype. The ability of hyperthermia to abrogate drug resistance in the presence of an increase in functional MDR proteins may provide an explanation for the efficacious results seen in the clinic in colon cancer patients treated with hyperthermia and chemotherapy. We investigated MDR1 expression in colon cancer specimens of patients who were treated by radio-chemo-thermo therapy. We find that the levels of MDR1 expression in colon cancer specimens before and 6 weeks after radio-chemo-thermo-therapy were not significantly different in the majority of cases. Since induction of MDR1 gene expression by external stress factors such as heat occur directly after exposure to hyperthermia, we conclude that this increase of MDR1 gene activity has ceased after 6 weeks, the time point of resection when the tumor specimens were analyzed. We then determined the levels of MDR genes sequentially before, during, and after isolated hyperthermic isolated limb perfusion (hILP) with rhTNFa/melphalan in patients with advanced soft tissue sarcoma and locoregional metastatic malignant melanoma. In the majority of patients (> 80%) MVP/LRP expression was induced during hILP, often parallelling the increase in temperature during hILP. This is the first study to investigate expression of MDR genes sequentially during hILP of patients. The result of this study demonstrates that hILP caused selective induction of MVP/LRP expression, whereas MDR1 and MRP1 expressions were rarely affected by the treatment regimen.

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Polymorphisms of MDR1 and MRP2/cMOAT in healthy North Eastern Italian subjects

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MDR1 and MRP2 belong to the ABC transporter genes superfamily encoding for integral membrane glycoproteins that function as ATP-dependent export pumps with substrate specificity. The increased expression of these proteins in cancer cells is associated with the development of cross resistance of tumors to many antiblastic agents. Their activity in normal tissue cells has a protective role towards xenobiotcs and prevents chemotherapy toxicity modulating pharmacokinetics of antineoplastic drugs. At least 15 single nucleotide polymorphisms (SNPs) have been described in a Caucasian population for MDR1, one of them resulted particularly interesting such as C3435T in exon 26, its pharmacological effect has been demonstrated and its frequency is quite variable among different ethnic groups. For MRP2 several mutations have been described in Dubin-Johnson Syndrome patients. Few of them have been detected also in Japanese and Jewish healthy subjects and described as SNPs. Their influence on the protein activity are not yet clarified but they could have a role on MRP2 expression or functionality. No population studies have been reported till now on them. We analysed the frequency of C3435T SNP for MDR1 gene and C24T SNP for MRP2 in 800 healthy blood donors from North East of Italy. Distribution of MDR1 C3435T genotype was as follows: C/C in 191 subjects (23.96%), C/T in 434 subjects (54.26%), and T/T in 175 subjects (21.78%). The allelic frequency was 48.91% for T allele,51.09% for C allele. Distribution of MRP2 C24T genotype was as follows: C/C in 494 subjects (61.78%), C/T in 271 subjects (33.86%), and T/T in 35 patients (4.36%). The allelic frequency was 78.7%1 for C allele, 21.29% for T allele was. In conclusion the population we analysed showed for MDR1 a frequency quite similar to the one described in literature for the Caucasian population. For MRP2 we found out a considerable allelic frequency for C24T in our geographic area and this encourages further investigations to evaluate its impact on pharmacokinetics of drugs excreted by this transporter protein.

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Co-dominance of cisplatin resistance in somatic cell hybrids

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Intrinsic or acquired resistance to cisplatin in cancer cells remains a major obstacle to successful chemotherapy. The relevant genetic and molecular mechanisms of resistance have not yet been identified. We have isolated cisplatin-resistant human KB epidermoid carcinoma cell lines resistant to varying levels of cisplatin after single and multiple selection steps.