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Srp20, the first transcriptionally up-regulated gene by flavopiridol (flavo), is a pre-mRNA splicing factor that induces apoptosis in human colon cancer cells

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Alternate pre-mRNA splicing contributes significantly to developmental regulation of gene expression and various cellular processes. Changes in pre-mRNA splicing can have profound effects on cellular behavior including sensitivity to chemical stimuli. We identified Srp20 as a gene that is transcriptionally induced during apoptosis by combination treatment of SN-38 (the active metabolite of CPT-11) followed by flavo in human colon cancer Hct116 cells. Srp20 has profound effects on sensitivity to flavo. Inhibition of endogenous Srp20 expression in Hct116 cells by stable expression of an antisense (AS) Srp20 cDNA decreased the sensitivity of cells to flavo as measured by DNA condensation and PARP cleavage. The clonogenic assays showed a 2-fold increase in the IC50 of AS-Srp20 with flavo as compared with vector transfected cells. Transient expression of Srp20 induces apoptosis with significant PARP cleavage and Caspase-8 activation after 72 h in Hct116 cells. Additionally, when xenografted in mice, AS-Srp20-expressing Hct116 cells were significantly more resistant to CPT-11 followed by flavo as compared with vector transfected Hct116 cells. Flavo induces Srp20 mRNA in a time dependent manner. To investigate the regulation of Srp20 gene, we cloned 2.7 kb fragment of human genomic DNA immediately 5′ of the Srp20 coding region. This 5′-flanking region contain a TATA box and consensus binding sequences for CREB and E2F-1 transcription factors. To identify the DNA elements in the Srp20 promoter region responsible for transcriptional up-regulation by flavo, we fused the 2.7 kb and serially truncated human Srp20 5′-flanking region to a luciferase reporter gene. The transient transfection of Srp20 promoter vectors indicated that the −137 bp Srp20 promoter (Srp137) was the minimal region required to show enhancement in activity by flavo treatment in Hct116 cells. This region of promoter contains a consensus sequence for binding site for transcription factor CREB. Site directed mutagenesis of CREB binding sequences in the Srp20 promoter completely abrogated the induction of its activity by flavopridol. Furthermore, chromatin immunoprecipitation analysis indicates higher binding of CREB protein to Srp20 promoter following flavo treatment. Our studies suggest that transcriptional up-regulation of pre-m-RNA splicing factor, Srp20, may be a mechanism by which flavo induces apoptosis or augments the CPT-11/SN-38 effect. The increased Srp20 expression may result in altered splicing of genes that mediate the process of apoptosis. Flavo is a drug that suppresses transcription of numerous genes. Srp20 represents the first gene to be transcriptionally induced by flavopridol and induce apoptosis. Plans are underway in both the laboratory and the clinic to investigate the importance of this gene relative to response to flavo based therapies.

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A phase I and pharmacokinetic trial ARQ 501, an Activated Checkpoint Therapy (TM) agent, in patients with advanced solid tumors

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ARQ 501 is an investigational anticancer agent that works through the novel process of activation of cellular checkpoints, termed Activated Checkpoint Therapy (ACT). ARQ 501 selectively induces sustained elevation of E2F-1 followed by activation of the S phase checkpoint and apoptosis in cancer cells. Potent and highly selective anticancer activity has been demonstrated in a broad range of tumor models.

To test the anticancer activity of ARQ 501 in human disease, a phase I dose escalation study was commenced in September 2003 with the aim of defining the Maximum Tolerated Dose (MTD) and to characterize the pharmacokinetic parameters of a weekly one hour infusion of ARQ 501 given for a minimum of 4 weeks (one cycle). Single subject cohorts were evaluated between 10 and 140 mg/m^2; 3 subject cohorts have been enrolled at doses beginning at 200 mg/m^2. To date, 11 pts have been enrolled and have received a total of 25 cycles of treatment between the dose range of 10 to 280 mg/m^2. Provisional data on these patients is reported here. The patient characteristics are as follows: 5M/6F; median age: 57 years (range 38–73). Tumor types are pancreatic (1), breast (1), NSCLC (1), sarcoma (5), adenocarcinoma of unknown primary (2) and papillary thyroid (1). All patients had received prior chemotherapy.

Pharmacokinetic data showed a dose proportional increase in c_{max} and AUC for ARQ 501, with no evidence of drug accumulation. No objective responses have been observed to date, although stable disease greater than 6 months has been observed in a sarcoma patient treated at a dose of 20 mg/m^2 (dose was escalated from 10 mg/m^2 after 8 weeks). Six patients were taken off study for disease progression after 5 to 16 weeks of therapy. Only one serious adverse event has been reported, a disease related pulmonary embolism in a pancreatic carcinoma patient. Adverse events have been minimal and have included pruritis, rash, injection site reaction, anaemia, myalgia, fatigue, sweating and loss of appetite.

In conclusion, rapid dose escalation of ARQ 501 has been possible with minimal toxicity. Dose escalation of ARQ 501 continues, with a 1.4-fold increment for each dose level. Additional pharmacokinetic and safety data will be presented.

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FasL, but not TRAIL, induces apoptosis in human hepatocytes in chimeric mice

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Introduction: While tumor necrosis factor (TNF) family ligands TNFα and FasL can kill solid tumors, their clinical usage has been limited by their hepatotoxicity. TNF-related apoptosis-inducing ligand (TRAIL), a new TNF member, is currently in development as a potential antitumor agent because it kills tumor cells but spares normal cells in cultures and animals. However, a polyhistidine-tagged human TRAIL was reported to kill isolated human hepatocytes. In this study, we examined a recombinant non-tagged native sequence of human TRAIL for its toxicity and antitumor effects in the chimeric mice with human hepatocytes.

Methods: The chimeric mice were generated through a crossing homozygous Alb-*uPA*transgenic mouse with homozygous SCID/*bg* mouse. The litters with Alb-*uPA* homozygosity were injected with freshly isolated human hepatocytes.

Results: To test the TRAIL toxicity, two-month-old chimeric mice were injected intravenously either with 500 μg of the TRAIL or 30 μg of the antibody cross-linked Flag-FasL (30 μg of Flag-FasL mixed with 12 mg antibody). The chimeric mice that received the FasL injection succumbed within 90 minutes whereas the chimeric mice injected with 500 μg TRAIL remained healthy. α 1-antitrypsin (hAAT) concentrations before and after TRAIL injection in the mice. Histologic examination revealed extensive necrosis, severe edema, hemorrhage, and caspase-3 cleavage in the livers of the chimeric mice treated with FasL, but not TRAIL. Western blots detected caspase-8, -3 and DFF45 cleavage products in the liver tissues from the mice injected with FasL, but not the TRAIL. To show TRAIL selective antitumor activity, we injected two-month-old chimeric mice with 8×10^6 tumor cells either intraperitoneally or subcutaneously and then treated the mice with intraperitoneal injections of 100 μg TRAIL or 100 μl normal saline, twice per day for consecutive 10 days. Analysis of the peritoneal and subcutaneous tumor sizes indicated TRAIL treatment either eliminated or inhibited the tumor growth. Serum tests showed no difference in human α1-antitrypsin concentrations between the TRAIL treated and untreated mice. Histologically, the human hepatocytes appeared to be normal. Biochemically, caspase-8, -3 and DFF45 cleavage was not detected in the liver tissues from the TRAIL treated or untreated mice.

Conclusion: The evidence presented here demonstrates that the recombinant non-tagged soluble human TRAIL (amino acids 114–281) has a profound apoptotic effect on tumors but is non-toxic to human hepatocytes *in vivo*. This form of TRAIL may prove to be a safe and effective biological agent for cancer therapy in future human clinical trials.

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Identification of the molecular target for MX2167, a novel anticancer agent

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We have previously reported that derivatives of gambogic acid (GA) demonstrate good pharmacokinetic properties and anti-tumor efficacy in several rodent tumor models with tumor growth inhibition ranging from 60 to 90% using various dosing schedules. MX2167 is our lead drug candidate derived from GA and is a novel inducer of apoptosis with demonstrated activity in different cancer cell lines including breast, prostate