scan appears to underestimate the true response rate as indicated by the patients who went on to surgery. The 3 patients who demonstrated complete pathologic responses at surgery, only showed MR (1 patient) or SD (2 patients) on CT scan.

Conclusion: TNFerade + radiation was well tolerated without DLTs or SAEs. The treatment appears to be very active as all patients showed dramatic tumor necrosis. Consequently, TNFerade + radiation could represent a new paradigm in the treatment of soft tissue sarcoma, either as neoadjuvant treatment or for palliation. Final dataset including all patients at all dose levels will be presented at the meeting.

472

Phase I clinical trials with direct intratumoural injection of an adenovirus-nitroreductase (Ad-NTR) vector, CTL102, in liver and prostate tumour patients

S.R. Hill[1], M.J. Horne[1], V. Mautner[2], J.G. Young[3], D.H. Palmer[3], J. Ellis[1], C.J. Wrighton[1], D.J. Kerr[4], N.D. James[3], A. Mountain[1]. [1]*Cobra Therapeutics Ltd, Research & Development, Keele, United Kingdom;* [2]*University of Birmingham, CRC Institute of Cancer Studies, Birmingham, United Kingdom;* [3]*Queen Elizabeth Hospital, Birmingham, United Kingdom;* [4]*University of Oxford, Department of Clinical Pharmacology, Oxford, United Kingdom*

A gene-directed enzyme prodrug therapy approach, using bacterial nitroreductase (NTR) to convert the prodrug CB1954 to a toxic bifunctional alkylating agent, has demonstrated activity in a range of preclinical models. We have constructed an adenovirus coding for NTR (CTL102) and are conducting phase I clinical trials in patients with liver (1° hepatocellular / colorectal 2°) or prostate tumours with a view to defining safe doses which give sufficient NTR expression to provide activation of co-administered CB1954. Escalating CTL102 doses were directly injected into tumours, using ultrasound guidance, with subsequent monitoring for overt toxicity, virus shedding, viral dissemination and immune response. In addition, following tumour resection, NTR expression was assessed by immunohistochemistry. 14 patients have entered the liver trial with a 4-log escalation of CTL102 dose (10e8 - 10e11 particles). Toxicity has been minimal, 1 patient developed transient pyrexia and flu-like symptoms at a low dose. No shedding of intact virus has been detected, although some viral DNA was detected in whole blood up to 24 hours after dosing. All patients showed an increased neutralising anti-adenovirus antibody titre, although there was significant interpatient variation in the isotype and kinetics of these elevated levels. NTR expression was detectable at all dose levels and showed an increasing dose-response relationship. Tumour architecture appeared to influence NTR expression, which was evident in both tumour and non-tumour (stroma, fibroblast and lymphocyte) cells, but not in associated normal liver from the resection margin. The top dose of 10e11 particles produced a level of NTR expression considered adequate to initiate a further arm of the study, co-administering CTL102/CB1954 to patients with inoperable tumours. 3 patients have entered the prostate trial at the initial dose of 10e10 particles. No toxicity was seen and no evidence of virus shedding was detected. Like the liver trial, some viral DNA was detected in whole blood, and also urine, up to 24 hours after dosing. NTR expression was detectable in both tumour and normal epithelial cells of the prostatic ducts in all 3 patients. NTR expression was localised to the peripheral zone, where the majority of prostatic tumours arise, and correlated well to the injection site. Multiple injections may thus be required to maximise the spread of virus throughout the prostate. Dose escalation in this trial continues.

473

Delivery of a c-raf Antisense Oligodeoxynucleotide (LErafAON) by intermittent bolus dosing (Weekly Infusions) in patients with advanced solid tumors: a phase I study

C.M. Rudin[1], J. Marshall[2], C.H. Huang[3], L. Strauss[4], C. Fleming[4], C. Zhang[2], D. Kumar[2], P. Gokhale[2], U. Kasid[2], M.J. Ratain[1]. [1]*University of Chicago, Medical Center, Chicago;* [2]*Georgetown University, Washington, DC, USA;* [3]*Temple University, Philadelphia, PA, USA;* [4]*NeoPharm, Inc., Lake Forest, USA*

Rapid cleavage *in vivo* and inefficient cellular uptake limit the clinical utility of antisense oligonucleotides (AON). The delivery of AON agents has required continuous infusion and large doses. Liposomal encapsulation of an AON to the c-raf proto-oncogene mRNA (LErafAON) using a novel cationic lipid results in prolonged circulation, inhibition of target protein and delayed growth of tumor xenografts after bolus intermittent dosing in pre-clinical studies. Safety and dose-limiting toxicities of LErafAON, administered by weekly 90-180 minute intravenous infusions for 8 weeks, were evaluated in patients with advanced solid tumors. To date, 19 patients have received 139 doses of LErafAON (median 6 doses; range 1-32 doses): 4 at 1 mg/kg/week; 3 at 2 mg/kg/week; 4 at 4 mg/kg/week; and 8 at 6 mg/kg/week. Age range was 29-77 years; M:F ratio was 9:10. Acute infusion-related reactions (IRR, as with other liposomal preparations), including chills, fever, flushing, chest tightness, dyspnea, hypoxemia, back or flank pain, hypertension or hypotension, occurred in 15 patients and required discontinuation in 5. Transient complement activation was observed; IRR were not evidently dose-related. In successive cohorts, increased infusion duration and pre-treatment with corticosteroids, H1- and H2-antagonists reduced the frequency and severity of IRR. Progressive dose-related decline in platelet count, potentially related to c-raf inhibition, was observed. At 4 mg/kg/week, platelet declines of 65% were observed by week 5 with subsequent plateau. Of 6 patients who received at least 3 doses at 6 mg/kg/week, 3 had Grade 2 and 2 had Grade 3 (dose-limiting) thrombocytopenia prior to the next weekly dose; suppression persisted for 2-3 weeks. With pre-treatment, the maximum tolerated dose appears to be 4 mg/kg/week. Plasma levels indicate dose proportionality with end of infusion rafAON levels of 0.3 to 0.9 μg/mL after 1 to 6 mg/kg. RafAON was detectable (sensitivity *10 ng/mL) for up to 24 hours post-infusion. At 4 mg/kg/week, 2 of 4 patients had treatment extended beyond the planned 8 weeks (16 and 32 weeks). Pharmacodynamic studies to assess intracellular c-raf mRNA and Raf-1 protein levels are in progress. Alternative formulation of LErafAON that may reduce IRR is underway. Patient enrollment continues at 4 mg/kg/week.

474

Antisense oligonucleotides targeting ceramide glycosylation overcome multidrug resistance in cancer cells

Y. Liu, J. Yu, A. Bitterman, A. Giuliano, M. Cabot. *John Wayne Cancer Institute, Breast Cancer Research Program, Santa Monica, USA*

Glucosylceramide synthase (GCS) catalyzes ceramide glycosylation, disrupts ceramide-induced apoptosis elicited by chemotherapy, and appears be a major cause of multidrug resistance (MDR) in cancer. Previous studies pinpoint GCS as a therapeutic target for MDR [Liu, Y. Y., Han, T. Y., Giuliano, A. E., and Cabot, M. C. FASEB J. 15, 719-730, (2001)]. In this work, we have synthesized antisense GCS oligodeoxyribonucleotides (asGCS ODNs) to block GCS mRNA transcription, and tested several of the oligos for chemotherapy-enhancing properties in drug resistant cancer cell models. Of the eleven reagents generated, asGCS ODN-7 at low concentrations (EC_{50} 0.3 μM) displayed a dramatic inhibitory influence on cell growth. Antisense GCS ODN-7 suppressed GCS mRNA expression (RT-PCR) by 80%, and GCS protein (Western blot) by 40%. Consistent with down-regulation of GCS and the ceramide mode of anthracycline action, asGCS ODN-7 affected 30- and 10-fold increases in sensitivity to Adriamycin in drug resistant breast cancer MCF-7-AdrR (EC_{50} 0.25 vs. 7.8 μM), and in drug resistant ovarian cancer A2780-AD cells (EC50 0.6 vs. 6.0 mM), respectively. Further, asGCS ODN-7 increased MCF-7-AdrR cell sensitivity to Taxol, Vinblastine, and Actinomycin D by 3-, 9- and 11-fold, respectively. Compared to asGCS ODN-7, the GCS chemical inhibitor, PDMP (D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol), was less efficient and increased Adriamycin sensitivity approximately 4-fold. Subsequent studies revealed that asGCS ODN-7 overcomes drug resistance by enhancing ceramide-induced apoptosis and drug uptake. In conclusion, antisense GCS oligonucleotides effectively depress GCS expression, enhance apoptosis and drug uptake, and increase chemotherapy sensitivity, making them promising agents for cancer therapy.

475

bcl-2 specific siRNA molecules inhibit growth of pancreatic cancer *in vitro* and *in vivo*

M. Ocker[1], A. Geick[2], D. Neureiter[3], P. Hadwiger[2], C. Herold[1], E.G. Hahn[1], H.-P. Vornlocher[2], D. Schuppan[1]. [1]*University Erlangen-Nuernberg, Medicine I, Erlangen, Germany;* [2]*Ribopharma AG, Kulmbach, Germany;* [3]*University Erlangen-Nuernberg, Pathology, Erlangen, Germany*

Aim: Double-stranded oligoribonucleotides (siRNAs) effectively suppress gene expression via the RNA interference (RNAi) mechanism. In cancer cells, a variety of growth promoting and anti-apoptotic genes is overexpressed. Inhibition of bcl-2 expression should shift the bax/bcl-2 ratio towards pro-apoptotic bax and induce apoptosis. Anti-sense approaches have shown that inhibition of bcl-2 induces apoptosis in different tumor cells and enhances sensitivity for chemotherapy.