Methods: Pancreatic carcinoma cell lines YAP C and DAN G as well as human foreskin fibroblasts were transfected with siRNAs against bcl-2 in serum-free media for 4 h using Oligofectamine. The bacterial Neomycin-resistance gene served as control. Final concentrations of siRNAs ranged from 1 to 100 nM. After 24 to 120 h incubation, the number of vital cells was determined by trypan blue exclusion test and apoptosis was quantified by flow cytometry after propidium iodide staining. NMRI-mice transplanted with human pancreatic cancer xenografts were used as an *in vivo* model. Animals received daily intraperitoneal injections of 200 μg/kg siRNAs dissolved in physiologic saline for 28 days. Tumor diameters were determined by daily measurements. After sacrificing the animals, tumor specimens were subjected to conventional histology, immunohistology (bcl-2, Ki-67) and TUNEL staining. Total protein and RNA was extracted for Western and Northern Blot analysis.
Results: siRNA directed against bcl-2 induced apoptosis and reduced the number of viable cells in both tumor cell lines in a time- and dose-dependent manner, while mock-transfected cells or cells receiving control-siRNA remained unaffected. siRNA against bcl-2 delayed growth of pancreatic carcinoma xenografts *in vivo*. Western-Blot analysis revealed a down-regulation of target molecules in responsive cells as well as in tumor specimens.
Conclusions: 1) siRNA against bcl-2 induces apoptosis in human pancreatic cancer cells *in vitro*. 2) Induction of apoptosis is specific, control-siRNA has no effect. 3) siRNAs against bcl-2 is effective *in vivo*. 4) Application of oncogene-specific siRNAs may contribute to therapy of pancreatic carcinoma.

476

Specific Inhibition of bcr-abl Gene Expression by small interfering RNA in bcr-abl+ Cells

M. Scherr[1], K. Battmer[1], T. Winkler[1], O. Heidenreich[2], A. Ganser[1], M. Eder[1]. [1]*Hannover Medical School, Hematology and Oncology, Hannover, Germany;* [2]*University of Tuebingen, Molecular Biology, Tuebingen, Germany*

RNA interference (RNAi) as a protecting mechanism against invasion by foreign genes was first described in Caenorhabditis elegans and has subsequently been demonstrated in diverse organisms such as protozoa, insects, plants, fungi, and mammalian cells. RNAi is the mechanism of sequence-specific, post-transcriptional gene silencing initiated by double-stranded RNAs (dsRNA) homologoues to the gene being suppressed. dsRNAs are cleaved by cellular RNAses such as Dicer, to generate duplexes of 21 nt with 3'-overhangs (short interfering RNA, siRNA) that induce degradation of mRNAs. mRNAs derived as fusion transcripts from chromosomal translocations often found in human leukemias that encode oncogenic proteins are attractive targets for molecular defined therapeutical approaches. As a model system we focused on the bcr-abl mRNA, the fusion transcript arising from the Philadelphia translocation t(9;22)(q34;q11) expressed in about 95% of patients with chronic myelogenous leukemia (CML) and in about 20% of adult patients with acute lymphoblastic leukemia (ALL). In cotransfection assays using a recombinant reporter gene consisting of the bcr-abl-fusion sequence linked to the EGFP-gene (sb3a2-d4EGFP), several siRNAs reduced the number of sb3a2-d4EGFP+ cells by 90% and the fluorescence intensity per cell up to one hundred-fold. Electroporation of K562 cells expressing bcr-abl with anti-laminA/C siRNA inhibited laminA/C protein expression in up to 80% of the cells. Transfection with anti-bcr-abl siRNAs specifically reduced bcr-abl-mRNA expression up to 75% in K562 cells, while c-bcr- and c-abl mRNA expression were unaffected as quantified by real-time RT-PCR. Anti-bcr-abl- siRNA also inhibited proliferation of K562 cells up to 75% after 4 days. To analyze the effects of anti-bcr-abl siRNAs on cell proliferation in more detail we used the murine TonB cell line derived from IL-3 dependent BaF3-cells. In TonB cells that can be induced to express the b3a2-bcr-abl variant under control of a doxycycline-inducible promoter, anti-bcr-abl siRNA reduced bcr-abl mRNA expression by 70% independent of IL-3. Anti bcr-abl siRNA inhibited factor-independent, but not cytokine-dependent proliferation of TonB cells to a similar extent as the tyrosine kinase inhibitor STI571. These data demonstrate that siRNAs can specifically and efficiently interfere with expression of an oncogenic fusion gene and may revert malignant transformation in bcr-abl positive cells.

477

Antitumor activity and I-124 pet imaging based on tumor-localized salmonella

S.A. Soghomonyan[2], M. Doubrovin[2], J. Pike[1], M. Ittensohn[1], J. Runyan[1], Z. Li[1], I. King[1], J. Gelovani Tjuvajev[2], R. Blasberg[2], D. Bermudes[1]. [1]*Vion Pharmaceuticals, Inc., Department of Microbiology;* [2]*Memorial Sloan Kettering Cancer Center, Neurology and Radiology, New York, USA*

Genetically modified Salmonella typhimurium (TAPET-TK) expressing herpes virus thymidine kinase (HSV1-tk) display the ability to replicate in tumors, cause tumor necrosis and to accumulate I-124 labeled 2'-fluoro-1-beta-D-arabino-furanosyl-5-iodo-uracil (FIAU). Therapy with TAPET-TK Salmonella treatment is safe due to genetically stable attenuation of bacterial virulence. The parental strain of bacteria VNP20009 is currently being studied in phase I human clinical trials. Here, we demonstrate selective targeting and replication of TAPET-TK Salmonella in B16-F10 melanoma and its ability to be imaged using positron emission tomography (PET). C57BL/6 mice bearing B16-F10 melanomas were injected i.v. with 10e6 TAPET-TK Salmonella typhimurium or saline (control group). On the fourth day after bacterial injection, the animals received 200 uCi of I-124 labeled FIAU. Twenty four hours after FIAU injection, MicroPet acquisition was obtained. After imaging, tumors and normal tissues were removed and radioactivity and bacterial count assays performed. Immunohistochemistry with anti-Salmonella antibodies and H & E staining were also performed. Mean values of cfu/g for tumors varied from $8.4 \times 10e8 \pm 5.2 \times 10e8$ (sd) to $9.9 \times 10e8 \pm 4.8 \times 10e7$, and were 380, 720, 290 and 690 fold higher than that in muscle, liver, spleen and kidney, respectively. Tumor radioactivity was higher in the treated group than the non-treated control. Tissue radioactivity (% dose/g) correlated with tissue bacterial number in treated animals (cfu./g) and did not vary with tumor size. Tumor mass was less in the treated animals, compared to that in non-treated animals. A linear relationship was found between level of radioactivity and bacterial count in all the tissues assayed. Differences in tumor mass did not appear to alter the bacterial or radioactivity concentration. A difference in tumor mass and level of radioactivity between treated and control groups was observed consistent with treatment response. These results demonstrate that selective targeting of TAPET-TK Salmonella to tumors can be imaged non-invasively with [124I]-FIAU and PET.

478

Antitumor activity and bystander effects of the tumor necrosis factor-related apoptosis-inducing ligand (trail) gene

S. Kagawa[1], T. Fujiwara[1], Y. Tsunemitsu[1], S. Ohtani[1], B. Fang[2], J. Roth[2], N. Tanaka[1]. [1]*Okayama University Graduate School of Medicine and, Department of Surgery, Okayama, Japan;* [2]*The University of Texas, Department of Thoracic and Cardiovascular Surgery, Houston, USA*

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has been reported to specifically kill malignant cells but to be nontoxic to normal cells. To evaluate the antitumor activity and therapeutic value of the TRAIL gene, we constructed adenoviral vectors expressing the human TRAIL gene. The *in vitro* transfer elicited apoptosis, as demonstrated by the quantification of viable or apoptotic cells and by the analysis of activation of pro-caspase-8 and cleavage of poly(ADP-ribose) polymerase. The intratumoral delivery elicited tumor cell apoptosis and suppressed tumor growth. In comparison with Bax gene treatment, which is toxic to normal cells, TRAIL gene treatment caused no detectable toxicity in cultured normal fibroblasts nor in mouse hepatocytes after systemic gene delivery. Furthermore, coculture of cancer cells expressing TRAIL with those expressing green fluorescent protein (GFP) resulted in apoptosis of both cells, whereas coculture of Bax-expressing cells with GFP-expressing cells resulted in the cell death of the Bax-expressing cells only, which suggested that the transfer of the TRAIL gene resulted in bystander effects. Transfection of the GFP-TRAIL gene into cancer cells resulted in the death of GFP-positive cells and their neighbors, whereas GFP-TRAIL genes, transfected into normal human fibroblasts or bronchial epithelial cells, did not kill such cells. Thus, the direct transfer of the TRAIL gene led to selective killing of malignant cells with bystander effect, which suggests that the TRAIL gene could be valuable for treatment for cancers.