tumors. Overexpression of members of the Fibroblast growth Factor family (Int2/Fgf3 and Kgf/Fgf7) caused Wnt-pathway like mammary tumors. This result suggests cooperativity between the Wnt and Fgf pathways, which is also supported by virus insertion analyses in these genetically engineered mice. We conclude that genotypes of transgenic mammary tumors are correlated to their histological phenotypes, and that analysis of a tumor's histomorphology can reveal the signaling pathway that induced the tumor. Our observations suggest that the principle of pathway pathology can be applied to tumors of other organs and to the human disease. This work was supported by the DAAD (A.R., individual grant), the State of California, BCRP JB-0014, and RO1CA89140 from NCI.

286

A phase I clinical trial of an oral formulation of the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA)

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SAHA is a potent inhibitor of histone deacetylase activity. A phase I study of SAHA administered by daily intravenous infusion has shown that SAHA can be given at doses that cause an accumulation of acetylated histones in peripheral blood mononuclear (PBM) cells and in tumors, without intolerable adverse effects. A clinical trial of oral SAHA was initiated to define the maximal tolerated dose and the pharmacokinetic profile and oral bioavailability of oral SAHA in patients with refractory solid tumors (group A), lymphomas (group B) and leukemias (group C). All patients were required to have adequate hepatic, renal and hematologic function with the exception of lymphoma and leukemia patients for whom a platelet count >25,000 and a neutrophil count >500 were required. All patients provided informed consent. The dose of oral SAHA was independently escalated in each group of patients with planned doses levels of 200 mg daily, 400 mg daily, 400 mg q12, 800 mg q12 and 1200 mg q12. Pharmacokinetic studies were performed on day 1 (identical dose given intravenously), day 8 (oral dose fasting), day 9 (oral dose non-fasting) and day 29 (oral fasting). Western blot analyses of histones isolated from PBM cells obtained pre- and post-SAHA dosing were performed. Twenty-five patients (A=17, B=6, C= 2) have been entered into 3 dose levels. Myelosuppression and fatigue were dose limiting toxicities at 400 mg q12 for solid tumor and lymphoma patients. Mean SAHA oral bioavailability among patients receiving the 200 mg and 400 mg doses was 56% and 48% respectively. A dose proportional increase in AUC and Cmax was observed when comparing the 200 mg and 400 mg dose levels. A prolonged duration of acetylated histone accumulation was observed following oral SAHA administration compared to the same dose administered intravenously. Reduction in measurable disease has been observed in refractory papillary thyroid cancer (1 patient), squamous cell carcinoma of the larynx (1 patient), renal cell carcinoma (1 patient) and B-cell lymphoma (1 patient). The preliminary results of this Phase I study of oral SAHA demonstrate that this formulation is readily bioavailable and results in prolongation of acetylated histone accumulation in PBM cells relative to an identical dose administered intravenously. This study is currently ongoing with the goal of identifying the optimum dose and schedule for oral SAHA administration. Support: CA 096228-01, CaPCURE, Aton Pharma.

287

The forced reexpression of the keratin 18 gene in human breast cancer cells results in redifferentiation and a dramatic drop in malignancy

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In vitro experiments as well as clinical studies revealed that the expression of keratin 18 (K18) in breast cancer tumors is associated with a favorable prognosis and a less aggressive phenotype of the carcinoma. To prove the principle we transfected the human K18 gene into the aggressive MDA-231 cell line and isolated a permanently overexpressing clone. These cells grow in dense monolayers with epithelial morphology whereas wild type and mock transfected control are of the dedifferentiated, malignant type with cells appearing spindle shaped, motile, and only loosely attached. The K18-transfected clone is characterized by a high expression of the adhesion proteins plakoglobin, desmoplakin, desmoglein, and E-cadherin in contrast to wild type and control which are virtually devoid. In addition, keratin 8 the dimerisation partner of K18 in keratin filament formation is upregulated too. Conversely the mesenchymal filament protein vimentin, forming the intermediate filaments of the cytoskeleton in MDA-231 wild type and control, is completely down regulated in the K18 clone. The high invasiveness of the wild type in the Boyden chamber is dramatically reduced for the K18-clone. In the nude mouse no metastasis could be observed for the K18-cells whereas wt and control metastasized into lung, liver, and bone marrow. In epithelial cells the intermediate filaments of the cytoskeleton are formed by keratins and K18 is a marker of well differentiated mammary lumenal cells. The loss of K18 and its replacement by vimentin is part of a general loss of differentiation along with the malignant transformation. The forced reexpression of K18 in transfected cells obviously induces redifferentiation with a reorganisation of impaired adhesion structures. Moreover, a reorganisation of these structures in adjacent non-transfected wild type cells could be observed after cocultivation for 2 weeks. Taken together, the impressive results of the nude mouse experiments and the bystander effect on non-transfected cells seem to be good prerequisites for a successful gene therapy with a K18 delivery system.

288

Transfection of follicular thyroid cancer cells with thyrotropin receptor cDNA alleviates malignant phenotype *in vitro* and *in vivo*

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Background: Thyroid stimulating hormone (TSH) is commonly seen as a thyroid specific growth factor inducing differentiation and growth of thyroid cells *in vitro*. Loss of TSH receptor expression in thyroid cancer cells is regarded as a sign of de-differentiation and made responsible for a malignant phenotype by escaping the control of differentiating growth factors. Aim: We studied the effect of TSH in the follicular thyroid cancer cell line HTC, a subclone of FTC133 cells, lacking endogenous expression of the TSH receptor (HTC), and HTC cells transfected with TSH receptor cDNA (HTC TSHr +) *in vitro* and *in vivo*.
Methods: By comparative evaluation of naive as well as HTC TSHr + cells, the effect of a functional TSH receptor was determined by its ability to alter proliferation, cell substratum adhesion, migration and invasion *in vitro*, as well as growth of xenotransplanted HTC cells *in vivo* (NCR nude mice, n=9 mice, respectively).
Results: HTC cells transfected with functional TSH receptor cDNA grew faster *in vitro* (doubling time of 1.15 days vs. 1.56 days, $p<0.05$) and TSH caused a dose dependend increase in cell number. After 5 generation times HTCtshr+ cell number had increased between 80 - 150% over HTC cells devoid of the TSH receptor ($p<0.05$). Adhesion to purified proteins of the extracellular matrix as well as migration and invasion through recostituted basement membrane were decreased in HTC TSHr+ cells, but when stimulated by TSH increased to levels comparable to, and with regard to invasion exceeding that of naive HTC cells. *In vivo* tumor latency was 11 days for HTC TSHr- xenografts and 21 days for HTC TSHr + cells. Significantly smaller final tumor volumes were registered for HTC TSHr+ cells (869 ± 427 vs. 250 ± 217 mm3, $p<0.05$).
Conclusion: This is a first demonstration of regained expression of a functional TSH-receptor in a thyroid cancer cell line to cause a decrease of *in vitro* adhesion and invasivenes of tumor cells as well as impaired *in vivo* growth, suggesting a less aggressive phenotype. However, functioning TSH-receptor enables to stimulate growth, adhesion and invasion in thyroid cancer cells *in vitro*, suggesting a key role of the TSH-receptor to affect features of the malignant pheontype *in vitro*.

289

Involvement of glutathione S-transferase pi inhibitor TLK199 in myeloproliferation and myelodifferentiation

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Glutathione S-transferase pi has recently been shown to be a regulator of mitogen activated protein kinases (MAPK) and an inhibitor of c-Jun N-terminal kinase. We observed that a promyelocytic HL60 cell line resistant to TLK199, a peptidomimetic inhibitor specific for GST pi presented among other cellular alterations, a higher p42/44 MAPK activity. This cell line was resistant to PMA-induced cell growth arrest during monocyte/macrophage cytodifferentiation. This phenotype was associated with a transient activation of p42/44 MAPK (as compared to a sustained activation in wild type

cells) and was reversed by tyrosine phosphatase inhibitors orthovanadate and dephostatin. In addition, TLK199 potentiated the granulocytic cytodifferentiation of HL60 cells induced by all trans retinoic acid. KO mice for GSTpi had higher myeloproliferative indices and circulating white blood cells than wild type animals. TLK199 was also able to stimulate myeloproliferation in wild type mice but not in GSTpi KO animals. The mechanism of action of GSTpi and TLK199 in myeloproliferation is still unknown. We have observed that GST* interacts with JAK1 and JAK2, two proteins involved and required for normal hematopoiesis. We are currently studying the effect of cytokines (G-CSF and GM-CSF) and TLK199 on the interaction between JAK proteins and GSTpi and the involvement of this enzyme in cytokine signaling pathways. Taken together, these results show a potential role of GST* expression and modulation in myeloproliferation and myelodifferentiation.

290

Phase I and pharmacokinetic study of LY293111, an orally available small molecule known to be an LTB4 receptor antagonist, 5-lipoxygenase inhibitor and peroxisome proliferator activated receptor-gamma agonist (PPAR gamma)

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Purpose: LY293111 has antineoplastic activity in a variety of preclinical models. LY293111 was demonstrated to have activity consistent with PPARg agonism in the ZDF rat diabetes model (ED50 for glucose reduction = 33 mg/kg, Css = 0.61μM). The EC50 for adipocyte differentiation in 3T3L1 cells was 0.5 μM. These values are consistent with concentrations required to inhibit tumor growth *in vivo*. We studied the tolerability and pharmacokinetics of LY293111 administered continuously, PO, twice daily (BID) for repeat cycles of 21 days.
Patients and Methods: Thirty-three patients with advanced solid tumors have been treated at five dose levels (200 to 800 mg BID) for a total of 89 cycles.
Results: The most common toxicities were diarrhea, abdominal pain and nausea. One patient at 600 mg BID (n=6) and 2 at 800 mg BID (n=9), experienced grade 3 diarrhea. At 600 mg BID, 1 patient experienced grade 1 abdominal pain and 1 patient experienced grade 1 nausea. At 800 mg BID, 1 patient had grade 1/2 abdominal pain and 3 had grade 1/2 nausea. Two patients with progressive chondrosarcoma and melanoma attained stable disease lasting approximately 16 and 8 cycles, respectively. Dose reductions/delays were infrequent. Increases in steady-state Cpeak and area-under-the-curve 0-10 hours were roughly dose-proportional over this 4-fold dose range. The interpatient variability in PK parameters was approximately 60% (CV %). Steady-state plasma levels in patients at 600 mg BID and above exceed levels in:i) rats treated with the ED50 for glucose reduction in the ZDF model and ii) the efficacious dose in the mouse tumor xenograft model.
Conclusions: LY293111 can be safely administered by continuous oral therapy. The toxicities observed to date are mild and manageable. Steady-state concentrations in humans exceed relevant levels observed in preclinical models. LY293111 will be evaluated in future Phase II studies.

291

Phase I and pharmacokinetic trial of ILY293111 in combination with gemcitabine

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LY293111 is an orally available small molecule known to be an LTB4 receptor antagonist, 5-lipoxygenase inhibitor and an agonist of the peroxisome proliferator activated receptor-gamma (PPAR-g). In a single agent dose escalation trial, the maximally tolerated dose (MTD) of LY293111 was determined to be 600 mg BID with a dose limiting toxicity of Gr. 3 diarrhea(1). Combinations of LY293111 and gemcitabine (Gem) have activity in preclinical models of non-small cell lung and pancreatic cancers. We performed a trial of LY293111 in combination with Gem in which LY293111 (100 to 800 mg) was administered continuously, PO, twice daily (BID) in combination with a fixed dose of Gem (1000 mg/m^2 IV, 3 of 4 weeks) for repeat cycles of 28 days. 35 patients with a range of solid tumors were accrued to five dose levels of LY293111. Median age and ECOG performance status were 59 (24-83) and 1, respectively. All but 1 patient had received prior chemotherapy (16 with prior RT as well). The median number of treatment cycles was 2 (range 1 to 12). Toxicity was mild to moderate in general, and included diarrhea, fatigue, myalgias and nausea. One patient treated at 100mg BID experienced Gr. 3 elevation of transaminases: dose-modification of Gem eliminated this problem; and the patient went on to receive 12 cycles of therapy. LY293111-related Gr. 3 toxicity (diarrhea) was observed in one patient at the 800 mg BID dose. Sporadic myelosuppression was unrelated to LY293111 dose. Mild to moderate edema typical of Gem was cumulative. Four patients have had a partial response: 1 with pancreatic cancer previously treated with Gem, 1 with pancreatic cancer previously treated with 5-FU and radiation, 1 with non small cell lung cancer (1 prior regimen), and 1 with anaplastic thyroid carcinoma (2 prior regimens). Preliminary data suggest that Gem does not interact with the pharmacokinetics of LY293111. Analysis of PMN's for induction of genes regulated by PPAR-g is planned. Thus, full dose Gem can be safely combined with the single-agent MTD of LY293111. Phase II studies in combination with Gem will be conducted using a dose of 600 mg/bid. (1)See Abstract entitled -Phase I and Pharmacokinetic Study of LY293111, an orally available small molecule known to be an LTB4 receptor antagonist, 5-lipoxygenase inhibitor and peroxisome proliferator activated receptor-gamma agonist (PPAR g) [Budman et al., 14th EORTC-NCI-AACR submitted abstract].

New drug targets

292

Telomestatin has a mixed type of binding modes with G-quadruplexes: intercalation between G-tetrads and end-stacking in the loop regions of intramolecular G-quadruplexes

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Telomestatin is the first natural product shown to be a telomerase inhibitor by virtue of its ability to interact quite specifically and strongly with the human telomeric intramolecular G-quadruplex (Kim et al., J. Am. Chem. Soc., 2002, 124, 2098). Previously, using a simulated annealing docking approach, we found that the minimized binding energy was found to be lower when two telomestatins were bound per one G-quadruplex than when one telomestatin was bound. Now we provide the experimental evidence for the stoichiometry of the reaction between telomestatin and the human telomeric intramolecular G-quadruplex structure. Further we provide evidence for the mixed binding modes of telomestatin in this complex. A novel modified filtration method was used to determine the stoichiometry of the reaction for telomestatin binding to the G-quadruplex structure which is formed with an oligomer containing four repeats of human telomeric sequence. When telomestatin was titrated against 0.2 mM of the oligomer, 0.4 mM of telomestatin was needed to saturate all the binding sites, while 0.6 mM of telomestatin was needed to saturate the oligomer at 0.3 mM concentration. These results are consistent with two telomestatin molecules binding per one human telomeric intramolecular G-quadruplex and this is in good agreement with the results from the aforementioned modeling study. In order to characterize the binding mode(s) of telomestatin in the G-quadruplex-drug complex, the stoichiometry of this reaction was determined using modified G-quadruplex structures, which have either an increased number of bases in the two loop regions or an increased number of G-tetrads. The stoichiometry of the reaction was unchanged regardless of the modifications made to the number of bases at each of the loop regions. However, more telomestatin molecules were needed to saturate the binding sites of mutated G-quadruplexes that have additional G-tetrads in their structures. The stoichiometry of these reactions between telomestatin and G-quadruplexes was found to be 2:1 (for G4 with 3 G-tetrads), 4:1 (for G4 with 4 G-tetrads), and 6:1 (for G4 with 6 G-tetrads). The results of these studies suggest that telomestatin intercalates between G-tetrads of intramolecular G-quadruplex structures as well as binds in the loop regions via end-stacking mode.