Adjuvant Pancreatic Cancer Treatment Strategies

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The following table categorizes suggested adjuvant measures for pancreatic cancer control with respect to their likely utility as retardants of cancer growth and spread, and as adjuvants to chemotherapy. Suggested dose schedules are provided for many of these agents; these are provisional and may change in light of future research. Metformin and berberine have very similar activities (activation of the enzyme AMPK), so one or the other can be used; metformin is a prescription drug, whereas berberine is a nutraceutical. I.v. ascorbate can be used alone or as an adjuvant to chemotherapy. Note that many of these agents are prescription drugs, and hence require the active cooperation and approval of your doctor. Doctor approval of low-dose aspirin is also wise, and use of spirulina at the same time as chemotherapy or i.v. ascorbate is not recommended. GcMAF (macrophage activating factor) must be administered by subcutaneous injection; although it does not have drug approval, it can be obtained by mail-order from Europe or Japan. Importantly, these measures should be considered as adjuvants to, *not substitutes for*, recommended surgery, gemcitabine chemotherapy, or other therapies employed by your doctor. Abstracts of pertinent research are appended below. This list does not pretend to be exhaustive.

	Growth Control	Chemo Adjuvant	Prescription Drug	Suggested Dose
Metformin or	X	X	X	500 mg 3 times daily
Berberine	X	X		500 mg 2-3 times daily
Fish Omega-3	X	X		2-5 g daily
Green Tea Catechins	X	X		250 mg twice daily
Melatonin	X	X		10-20 mg at bedtime
Low-Dose Aspirin	X			81 mg daily
Vitamin D	X			5,000-10,000 IU daily
Spirulina	X	--	\	15-30 g daily
Salsalate	X	X	X	1500 mg 2-3 times daily
DIM (BioResponse)	X	X		300 mg twice daily
Tocotrienols	X	X		125 mg twice daily
Low-Dose Naltrexone	X		X	3-5 mg before bedtime
GcMAF	X			100 ng i.m.once weekly

I.V. Ascorbate	X	X	X
Celecoxib	X	X	X
Zileuton		X	X
Nelfinavir		X	X
Hydroxychloroquine	X	X	X
Itraconazole	X	X	X
Met Cyclophophamide	X		X

Metformin or Berberine

Sadeghi N, Abbruzzese JL, Yeung SC, Hassan M, Li D. Metformin use is associated with better survival of diabetic patients with pancreatic cancer. *Clin Cancer Res* 2012 May 15;18(10):2905-12.
Abstract: PURPOSE: Accumulating evidence suggests that metformin has antitumor activity. The aim of this study was to determine whether metformin use has a survival benefit in patients with pancreatic cancer. EXPERIMENTAL DESIGN: We conducted a retrospective study of patients with diabetes and pancreatic cancer treated at The University of Texas MD Anderson Cancer Center (Houston, TX). Information on diabetes history, including treatment modalities and clinical outcome of pancreatic cancer, was collected using personal interviews and medical record review. Survival analysis was carried out using a Kaplan-Meier plot, log-rank test, and Cox proportional hazards regression models. RESULTS: Among the 302 patients identified, there were no significant differences in demographic or major clinical characteristics between the patients who had received metformin (n = 117) and those who had not (n = 185). The 2-year survival rate was 30.1% for the metformin group and 15.4% for the non-metformin group (P = 0.004; chi(2) test). The median overall survival time was 15.2 months for the metformin group, and 11.1 months for the non-metformin group (P = 0.004, log-rank test). Metformin users had a 32% lower risk of death; the HR (95% confidence interval) was 0.68 (0.52-0.89) in a univariate model (P = 0.004), 0.64 (0.48-0.86) after adjusting for other clinical predictors (P = 0.003), and 0.62 (0.44-0.87) after excluding insulin users (P = 0.006). Metformin use was significantly associated with longer survival in patients with nonmetastatic disease only. CONCLUSIONS: Our finding that metformin use was associated with improved outcome of patients with diabetes and pancreatic cancer should be confirmed in independent studies. Future research should prospectively evaluate metformin as a supplemental therapy in this population

Pollak M. Metformin and pancreatic cancer: a clue requiring investigation. *Clin Cancer Res* 2012 May 15;18(10):2723-5.
Abstract: Laboratory models show antineoplastic activity of metformin under certain conditions, and pharmacoepidemiologic studies have reported reduced cancer burden among diabetics taking metformin. Therefore, the hypothesis that metformin has antineoplastic activity is receiving increasing attention. However, gaps in knowledge must be addressed before metformin can be "repurposed" for oncologic indications

Li W, Yuan Y, Huang L, Qiao M, Zhang Y. Metformin alters the expression profiles of microRNAs in human pancreatic cancer cells. *Diabetes Res Clin Pract* 2012 May;96(2):187-95.

Abstract: AIMS: To investigate the effect of metformin on the expression profiles of microRNAs in human pancreatic cancer cells. METHODS: MicroRNAs real-time PCR Array was applied to investigate differentially expressed miRNAs in Sw1990 cells treated with or without metformin. Stem-loop real time RT-PCR was used to confirm the results of the array assay in Sw1990 and Panc-1 cells. The effects of miR-26a on cell growth, apoptosis, invasion and migration abilities were respectively examined by CCK8 assay, Apoptosis assay, Matrigel invasion and migration assay. HMGA1 was proved to be a target of miR-26a by Luciferase reporter assay, Real-time PCR and Western-blotting. RESULTS: Nine miRNAs were significantly up-regulated in metformin treated cells. Metformin up-regulated the expression of miR-26a, miR-192 and let-7c in a dose-dependent manner. Forced expression of miR-26a significantly inhibited cell proliferation, invasion, migration and increased cell apoptosis, whereas knockdown of miR-26a obtained the opposite effect. Furthermore, we demonstrated that HMGA1, an oncogene, is a direct target of miR-26a. Nude mice xenograft models confirmed that metformin up-regulated the level of miR-26a and surpressed the expression of HMGA1 in vivo. CONCLUSION: These observations suggested that modulation of miRNA expression may be an important mechanism underlying the biological effects of metformin

Bao B, Wang Z, Ali S et al. Metformin inhibits cell proliferation, migration and invasion by attenuating CSC function mediated by deregulating miRNAs in pancreatic cancer cells. *Cancer Prev Res (Phila)* 2012 March;5(3):355-64.
Abstract: Pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States, which is, in part, due to intrinsic (de novo) and extrinsic (acquired) resistance to conventional therapeutics, suggesting that innovative treatment strategies are required for overcoming therapeutic resistance to improve overall survival of patients. Oral administration of metformin in patients with diabetes mellitus has been reported to be associated with reduced risk of pancreatic cancer and that metformin has been reported to kill cancer stem cells (CSC); however, the exact molecular mechanism(s) has not been fully elucidated. In the current study, we examined the effect of metformin on cell proliferation, cell migration and invasion, and self-renewal capacity of CSCs and further assessed the expression of CSC marker genes and microRNAs (miRNA) in human pancreatic cancer cells. We found that metformin significantly decreased cell survival, clonogenicity, wound-healing capacity, sphere-forming capacity (pancreatospheres), and increased disintegration of pancreatospheres in both gemcitabine-sensitive and gemcitabine-resistant pancreatic cancer cells. Metformin also decreased the expression of CSC markers,CD44, EpCAM,EZH2, Notch-1, Nanog and Oct4, and caused reexpression of miRNAs (let-7a,let-7b, miR-26a, miR-101, miR-200b, and miR-200c) that are typically lost in pancreatic cancer and especially in pancreatospheres. We also found that reexpression of miR-26a by transfection led to decreased expression of EZH2 and EpCAM in pancreatic cancer cells. These results clearly suggest that the biologic effects of metformin are mediated through reexpression of miRNAs and decreased expression of CSC-specific genes, suggesting that metformin could be useful for overcoming therapeutic resistance of pancreatic cancer cells

Kisfalvi K, Eibl G, Sinnett-Smith J, Rozengurt E. Metformin disrupts crosstalk between G protein-coupled receptor and insulin receptor signaling systems and inhibits pancreatic cancer growth. *Cancer Res* 2009 August 15;69(16):6539-45.
Abstract: Recently, we identified a novel crosstalk between insulin and G protein-coupled receptor (GPCR) signaling pathways in human pancreatic cancer cells. Insulin enhanced GPCR signaling through a rapamycin-sensitive mTOR-dependent pathway. Metformin, the most widely used drug in the treatment of type 2 diabetes, activates AMP kinase (AMPK), which negatively regulates mTOR. Here, we determined whether metformin disrupts the crosstalk between insulin receptor and GPCR signaling in pancreatic cancer cells. Treatment of human pancreatic cancer cells (PANC-1, MIAPaCa-2, and BxPC-3) with insulin (10 ng/mL) for 5 minutes markedly enhanced the increase in intracellular [Ca(2+)] induced by GPCR agonists (e.g., neurotensin, bradykinin, and angiotensin II). Metformin pretreatment completely abrogated insulin-induced potentiation of Ca(2+) signaling but did not

interfere with the effect of GPCR agonists alone. Insulin also enhanced GPCR agonist-induced growth, measured by DNA synthesis, and the number of cells cultured in adherent or nonadherent conditions. Low doses of metformin (0.1-0.5 mmol/L) blocked the stimulation of DNA synthesis, and the anchorage-dependent and anchorage-independent growth induced by insulin and GPCR agonists. Treatment with metformin induced striking and sustained increase in the phosphorylation of AMPK at Thr(172) and a selective AMPK inhibitor (compound C, at 5 micromol/L) reversed the effects of metformin on [Ca(2+)](i) and DNA synthesis, indicating that metformin acts through AMPK activation. In view of these results, we tested whether metformin inhibits pancreatic cancer growth. Administration of metformin significantly decreased the growth of MIAPaCa-2 and PANC-1 cells xenografted on the flank of nude mice. These results raise the possibility that metformin could be a potential candidate in novel treatment strategies for human pancreatic cancer

Wang LW, Li ZS, Zou DW, Jin ZD, Gao J, Xu GM. Metformin induces apoptosis of pancreatic cancer cells. *World J Gastroenterol* 2008 December 21;14(47):7192-8.
Abstract: AIM: To assess the role and mechanism of metformin in inducing apoptosis of pancreatic cancer cells. METHODS: The human pancreatic cancer cell lines ASPC-1, BxPc-3, PANC-1 and SW1990 were exposed to metformin. The inhibition of cell proliferation and colony formation via apoptosis induction and S phase arrest in pancreatic cancer cell lines of metformin was tested. RESULTS: In each pancreatic cancer cell line tested, metformin inhibited cell proliferation in a dose dependent manner in MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tet razolium assays). Flow cytometric analysis showed that metformin reduced the number of cells in G1 and increased the percentage of cells in S phase as well as the apoptotic fraction. Enzymelinked immunosorbent assay (ELISA) showed that metformin induced apoptosis in all pancreatic cancer cell lines. In Western blot studies, metformin induced poly-ADP-ribose polymerase (PARP) cleavage (an indicator of caspase activation) in all pancreatic cancer cell lines. The general caspase inhibitor (VAD-fmk) completely abolished metformin-induced PARP cleavage and apoptosis in ASPC-1 BxPc-3 and PANC-1, the caspase-8 specific inhibitor (IETD-fmk) and the caspase-9 specific inhibitor (LEHD-fmk) only partially abrogated metformin-induced apoptosis and PARP cleavage in BxPc-3 and PANC-1 cells. We also observed that metformin treatment dramatically reduced epidermal growth factor receptor (EGFR) and phosphorylated mitogen activated protein kinase (P-MAPK) in both a time- and dose-dependent manner in all cell lines tested. CONCLUSION: Metformin significantly inhibits cell proliferation and apoptosis in all pancreatic cell lines. And the metformin-induced apoptosis is associated with PARP cleavage, activation of caspase-3, -8, and -9 in a time- and dose-dependent manner. Hence, both caspase-8 and -9-initiated apoptotic signaling pathways contribute to metformin-induced apoptosis in pancreatic cell lines

Pinto-Garcia L, Efferth T, Torres A, Hoheisel JD, Youns M. Berberine inhibits cell growth and mediates caspase-independent cell death in human pancreatic cancer cells. *Planta Med* 2010 August;76(11):1155-61.
Abstract: Pancreatic cancer is one of the most aggressive human malignancies with an increasing incidence worldwide. In addition to the poor survival rates, combinations using gemcitabine as a backbone have failed to show any benefit beyond monotherapy. These facts underscore an urgent need for novel therapeutic options and motivated us to study the effect of berberine on pancreatic cancer cells. Here, we undertook an mRNA-based gene expression profiling study in order to get deeper insight into the molecular targets mediating the growth inhibitory effects of berberine on pancreatic cancer cells compared to normal ones. Twenty-four hours after treatment, berberine showed preferential selectivity toward pancreatic cancer cells compared to normal ones. Moreover, expression profiling and Ingenuity pathway analysis results showed that the cytotoxicity of berberine was accompanied with an activation of BRCA1-mediated DNA damage response, G1/S and G2/M cell cycle checkpoint regulation, and P53 signalling pathways. The activation of these signalling pathways might be

explained by the fact that berberine intercalates DNA and induces DNA strand break through inhibition of topoisomerases and induction of DNA lesions

Fish Omega-3s

Song KS, Jing K, Kim JS et al. Omega-3-polyunsaturated fatty acids suppress pancreatic cancer cell growth in vitro and in vivo via downregulation of Wnt/Beta-catenin signaling. *Pancreatology* 2011;11(6):574-84.
Abstract: BACKGROUND/AIMS: omega3-polyunsaturated fatty acids (omega3- PUFAs) are known to possess anticancer properties. However, the relationship between omega3-PUFAs and beta-catenin, one of the key components of the Wnt signaling pathway, in human pancreatic cancer remains poorly characterized. METHODS: Human pancreatic cancer cells (SW1990 and PANC-1) were exposed to two omega3-PUFAs, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), to investigate the relationship between omega3-PUFAs and the Wnt/beta-catenin signaling pathway in vitro. Mouse pancreatic cancer (PANC02) cells were implanted into fat-1 transgenic mice, which express omega3 desaturases and result in elevated levels of omega3-PUFAs endogenously. The tumor size, levels of Wnt/beta-catenin signaling molecules and apoptosis levels were analyzed to examine the influence of omega3-PUFAs in vivo. RESULTS: DHA and EPA significantly inhibited cell growth and increased cell death in pancreatic cancer cells. DHA also reduced beta-catenin expression, T cell factor/lymphoid-enhancing factor reporter activity and induced beta-catenin/Axin/GSK-3beta complex formation, a known precursor to beta-catenin degradation. Furthermore, Wnt3a, a natural canonical Wnt pathway ligand, reversed DHA-induced growth inhibition in PANC-1 cells. Immunohistochemical analysis showed aberrant upregulation and increased nuclear staining of beta-catenin in tumor tissues from pancreatic cancer patients. However, beta-catenin levels in tumor tissues from fat-1 transgenic mice were reduced with a significant increase in apoptosis compared with those from control mice. CONCLUSION: omega3-PUFAs may be an effective therapy for the chemoprevention and treatment of human pancreatic cancer.

Arshad A, Al-Leswas D, Stephenson J, Metcalfe M, Dennison A. Potential applications of fish oils rich in n-3 fatty acids in the palliative treatment of advanced pancreatic cancer. *Br J Nutr* 2011 September;106(6):795-800.
Abstract: The palliative treatment of patients with advanced pancreatic cancer (APC) has undergone little advancement in the last 15 years. Novel therapies that have been investigated to extend survival have shown little benefit over existing chemotherapy regimens. Patients with APC often experience significant weight loss, which is one of the primary factors involved in declining quality of life. Recently, the ability of n-3 fatty acid rich oral preparations to attenuate or reverse tumour-related weight loss has been investigated in this patient group with encouraging results. Laboratory investigation has also yielded promising results suggesting a potential direct tumouricidal effect of n-3 fatty acids as well as the putative potentiation of existing chemotherapy regimes. The present review aims to examine the potential applications of fish oils rich in n-3 fatty acids in patients with APC, present a selection of the studies carried out to date and outline avenues of possible further clinical investigation

Boutros C, Somasundar P, Razzak A, Helton S, Espat NJ. Omega-3 fatty acids: investigations from cytokine regulation to pancreatic cancer gene suppression. *Arch Surg* 2010 June;145(6):515-20.
Abstract: Omega-3 (omega-3) fatty acids have been clinically and experimentally associated with the amelioration of chronic and acute inflammation; however, the mechanisms for these observations have not been well defined. During the past decade, laboratories of nutrition and inflammation have demonstrated that the anti-inflammatory activities of omega-3 fatty acids occur at least in part

through the inhibition of macrophage-elaborated tumor necrosis factor production and through inactivation of the nuclear factor-kappaB signaling pathway subsequently altering proinflammatory cytokine transcription. These observations led to further experiments that support a role for omega-3 fatty acids in the restoration of apoptosis in various chemoresistant tumor models through a similar inactivation of the nuclear factor-kappaB signaling pathway. The potential for nutritional modulation of host inflammation has been an ongoing and expanding area of investigation. An increased emphasis has been placed on the potential for diet and dietary supplements to serve as modulators of host response to disease, injury, and infection

Spencer L, Mann C, Metcalfe M et al. The effect of omega-3 FAs on tumour angiogenesis and their therapeutic potential. *Eur J Cancer* 2009 August;45(12):2077-86.
Abstract: Omega-3 fatty acid (omega-3 FA) consumption has long been associated with a lower incidence of colon, breast and prostate cancers in many human populations. Human trials have demonstrated omega-3 FA to have profound anti-inflammatory effects in those with cancer. In vitro and small animal studies have yielded a strong body of evidence establishing omega-3 FA as having anti-inflammatory, anti-apoptotic, anti-proliferative and anti-angiogenic effects. This review explores the evidence and the mechanisms by which omega-3 FA may act as angiogenesis inhibitors and identifies opportunities for original research trialling omega-3 FAs as anti-cancer agents in humans. The conclusions drawn from this review suggest that omega-3 FAs in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found principally in oily fish have potent anti-angiogenic effects inhibiting production of many important angiogenic mediators namely; Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF), Platelet-Derived Endothelial Cell Growth Factor (PDECGF), cyclo-oxygenase 2 (COX-2), prostaglandin-E2 (PGE2), nitric oxide, Nuclear Factor Kappa Beta (NFKB), matrix metalloproteinases and beta-catenin

Hering J, Garrean S, Dekoj TR et al. Inhibition of proliferation by omega-3 fatty acids in chemoresistant pancreatic cancer cells. *Ann Surg Oncol* 2007 December;14(12):3620-8.
Abstract: BACKGROUND: Pancreatic cancer-gemcitabine (GEM) chemoresistance has been demonstrated to be associated with enhanced NF-kB activation and antiapoptotic protein synthesis. The well-known capacity of omega-3 fatty acids (n-3 FAs) to inhibit NF-kB activation and promote cellular apoptosis has the potential to restore or facilitate gemcitabine chemosensitivity. METHODS: Four pancreatic cancer cell lines (MIA PaCa-2, BxPC-3, PANC-1, and L3.6), each with distinct basal NF-kB and differing GEM sensitivity profiles, were administered: 100 uM of (1) n-3FA, (2) n-6FA, (3) GEM, (4) n-3FA + GEM, or (5) n-6FA + GEM for 24 and 48 hours. Proliferation was assessed using the WST-1 assay. To define the mechanism(s) of altered proliferation, electron mobility shift assay for NF-kB activity, western blots of phoshoStat3, phosphoIkappaB, and poly(ADP-ribose) polymerase (PARP) cleavage were performed in the MIA PaCa-2 cell line. RESULTS: All cell lines demonstrated a time/dose-dependent inhibition of proliferation in response to n-3FA. For MIA PaCa-2 cells, n-3FA and n-3FA + GEM treatment resulted in reduction of I-kB phosphorylation and NF-kB activation when compared with n-6FA control. n-3FA and combination treatment also significantly decreased Stat3 phosphorylation, whereas GEM alone had no effect. n-3FAs and n-3FA + GEM groups demonstrated increased PARP cleavage, mirroring NF-kB activity and Stat3 phosphorylation. CONCLUSIONS: n-3 FA treatment is specifically associated with inhibition of proliferation in these four pancreatic cell lines irrespective of varied gemcitabine resistance. An experimental paradigm to screen for potential contributory mechanism(s) in altered pancreatic cancer cellular proliferation was defined, and using this approach the co-administration of n-3 FA with GEM inhibited GEM-induced NF-kB activation and restored apoptosis in the MIA PaCa-2 cell-line

Funahashi H, Satake M, Hasan S et al. Opposing effects of n-6 and n-3 polyunsaturated fatty acids on pancreatic cancer growth. *Pancreas* 2008 May;36(4):353-62.
Abstract: OBJECTIVES: Epidemiologic studies suggest that fish oil, rich in n-3 polyunsaturated fatty

acids (PUFA), possesses antitumor activity, whereas n-6 PUFAs may stimulate the development of cancers. The aim of this study was to evaluate the effects of n-6 and n-3 PUFAs on the growth of pancreatic cancer. METHODS: The n-6 PUFA arachidonic acid (AA) stimulated the growth of cyclooxygenase (COX) 2 positive human pancreatic cancer (PaCa) cells, which was mediated by COX-2 generated prostaglandin E2 (PGE2) binding to EP2 and EP4 receptors. In contrast, the n-3 PUFA eicosapentaenoic acid decreased the growth of COX-2-positive and COX-2-negative PaCa cells. The COX-2-dependent mechanism of eicosapentaenoic acid was mediated by binding of PGE3 to EP2 and EP4 receptors. Dietary intake of n-3 PUFAs decreased the growth of pancreatic cancers in a xenograft model, which was accompanied by a decrease of PGE2 and an increase of PGE3 in the tumors. CONCLUSIONS: Our studies provide evidence that n-3 PUFAs possess antitumor activities, whereas n-6 PUFAs stimulate pancreatic tumor growth. The opposite effects of n-3 and n-6 PUFAs are mediated by the formation of different prostaglandin species. n-3 PUFAs may prove beneficial as monotherapy or combination therapy with standard chemotherapeutic agents in pancreatic cancer patients

Brown TT, Zelnik DL, Dobs AS. Fish oil supplementation in the treatment of cachexia in pancreatic cancer patients. *Int J Gastrointest Cancer* 2003;34(2-3):143-50.
Abstract: Patients with pancreatic cancer often experience a loss of weight and appetite, known as the anorexia-cachexia syndrome, which is associated with decreased quality of life and reduced survival. Research into the biological mechanisms of cachexia has demonstrated that an array of inflammatory mediators and tumor-derived factors cause appetite suppression, skeletal muscle proteolysis, and lipolysis,producing an overall hypercatabolic state that contributes to loss of fat and lean body mass. Omega-3 polyunsaturated fatty acids (n-3 PUFAs) have been shown to modulate levels of proinflammatory cytokines, hepatic acute phase proteins, eicosanoids, and tumor-derived factors in animal models of cancer and may reverse some aspects of the process of cachexia. Results of clinical trials of n-3 PUFAs in the form of fish oils have been mixed, but should encourage further investigation into dietary fish oil supplementation, including the most effective route of administration and the proper dosage to promote optimal weight maintenance and to limit side effects. Concerns about standardization and quality control should also be considered. With the current available evidence, a recommendation for the use of omega 3 polyunsaturated fatty acids in pancreatic cancer cachexia is premature

Barber MD, Fearon KC, Tisdale MJ, McMillan DC, Ross JA. Effect of a fish oil-enriched nutritional supplement on metabolic mediators in patients with pancreatic cancer cachexia. *Nutr Cancer* 2001;40(2):118-24.
Abstract: Weight loss in advanced cancer patients is refractory to conventional nutritional support. This may be due to metabolic changes mediated by proinflammatory cytokines, hormones, and tumor-derived products. We previously showed that a nutritional supplement enriched with fish oil will reverse weight loss in patients with pancreatic cancer cachexia. The present study examines the effect of this supplement on a number of mediators thought to play a role in cancer cachexia. Twenty weight-losing patients with pancreatic cancer were asked to consume a nutritional supplement providing 600 kcal and 2 g of eicosapentaenoic acid per day. At baseline and after 3 wk, patients were weighed and samples were collected to measure serum concentrations of interleukin (IL)-6 and its soluble receptor tumor necrosis factor receptors I and II, cortisol, insulin, and leptin, peripheral blood mononuclear cell production of IL-1 beta, IL-6, and tumor necrosis factor, and urinary excretion of proteolysis inducing factor. After 3 wk of consumption of the fish oil-enriched nutritional supplement, there was a significant fall in production of IL-6 (from median 16.5 to 13.7 ng/ml, P = 0.015), a rise in serum insulin concentration (from 3.3 to 5.0 mU/l, P = 0.0064), a fall in the cortisol-to-insulin ratio (P = 0.0084), and a fall in the proportion of patients excreting proteolysis inducing factor (from 88% to 40%, P = 0.008). These changes occurred in association with weight gain (median 1 kg, P = 0.024). Various mediators of catabolism in cachexia are modulated by

administration of a fish oil-enriched nutritional supplement in pancreatic cancer patients. This may account for the reversal of weight loss in patients consuming this supplement

Wigmore SJ, Barber MD, Ross JA, Tisdale MJ, Fearon KC. Effect of oral eicosapentaenoic acid on weight loss in patients with pancreatic cancer. *Nutr Cancer* 2000;36(2):177-84.
Abstract: Eicosapentaenoic acid (EPA) has been shown to modulate aspects of the inflammatory response that may contribute to weight loss in cancer. This study aimed to evaluate the acceptability and effects of oral supplementation with high-purity EPA in weight-losing patients with advanced pancreatic cancer. Twenty-six patients were entered into the study. EPA (95% pure) was administered as free acid starting at 1 g/day; the dose was increased to 6 g/day over four weeks, and then a maintenance dose of 6 g/day was administered. Patients were assessed before EPA and at 4, 8, and 12 weeks while receiving EPA, for weight, body composition, hematologic and clinical chemistry variables, acute-phase protein response, and performance status. Overall survival was noted. Supplementation was well tolerated, with only five patients experiencing side effects possibly attributable to the EPA. Before starting EPA, all patients had been losing weight at a median rate of 2 kg/mo. In general, after EPA supplementation, weight was stable. After four weeks of EPA supplementation, patients had a median weight gain of 0.5 kg ($p = 0.0009$ vs. rate of weight loss at baseline), and this stabilization of weight persisted over the 12-week study period. Total body water as a percentage of body weight remained stable, as did the proportion of patients with an acute-phase protein response, patients' nutritional intake, and performance status. Overall median survival from diagnosis in this study was 203 days. This study suggests that EPA is well tolerated, may stabilize weight in cachectic pancreatic cancer patients, and should be tested as an anticachectic agent in controlled trials

Barber MD, Ross JA, Voss AC, Tisdale MJ, Fearon KC. The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer. *Br J Cancer* 1999 September;81(1):80-6.
Abstract: Previous studies have suggested that administration of oral eicosapentaenoic acid (EPA) will stabilize weight in patients with advanced pancreatic cancer. The aim of the present study was to determine if a combination of EPA with a conventional oral nutritional supplement could produce weight gain in these patients. Twenty patients with unresectable pancreatic adenocarcinoma were asked to consume two cans of a fish oil-enriched nutritional supplement per day in addition to their normal food intake. Each can contained 310 kcal, 16.1 g protein and 1.09 g EPA. Patients were assessed for weight, body composition, dietary intake, resting energy expenditure (REE) and performance status. Patients consumed a median of 1.9 cans day(-1). All patients were losing weight at baseline at a median rate of 2.9 kg month(-1). After administration of the fish oil-enriched supplement, patients had significant weight-gain at both 3 (median 1 kg, $P= 0.024$) and 7 weeks (median 2 kg, $P = 0.033$). Dietary intake increased significantly by almost 400 kcal day(-1) ($P = 0.002$). REE per kg body weight and per kg lean body mass fell significantly. Performance status and appetite were significantly improved at 3 weeks. In contrast to previous studies of oral conventional nutritional supplements in weight-losing cancer patients, this study suggests that an EPA-enriched supplement may reverse cachexia in advanced pancreatic cancer

Green Tea Polyphenols

Shankar S, Marsh L, Srivastava RK. EGCG inhibits growth of human pancreatic tumors orthotopically implanted in Balb C nude mice through modulation of FKHRL1/FOXO3a and neuropilin. *Mol Cell Biochem* 2012 September 13.
Abstract: Human pancreatic cancer is currently one of the fourth leading causes of cancer-related

mortality with a 5-year survival rate of less than 5 %. Since pancreatic carcinoma is largely refractory to conventional therapies, there is a strong medical need for the development of novel and innovative cancer preventive strategies. The forkhead transcription factors of the O class (FOXO) play a major role in cell proliferation, angiogenesis, metastasis, and tumorigenesis. The objectives of this study were to examine whether FKHRL1/FOXO3a modulates antitumor activity of (-)-epigallocatechin-3-gallate (EGCG), an active ingredient in green tea, in pancreatic cancer model in vivo. PANC-1 cells were orthotopically implanted into Balb c nude mice and gavaged with EGCG after tumor formation. Cell proliferation and apoptosis were measured by Ki67 and TUNEL staining, respectively. The expression of PI3K, AKT, ERK, and FOXO3a/FKHRL1 and its target genes were measured by the western blot analysis and/or q-RT-PCR. FOXO-DNA binding was measured by gel shift assay. EGCG-treated mice showed significant inhibition in tumor growth which was associated with reduced phosphorylation of ERK, PI3K, AKT, and FKHRL1/FOXO3a, and modulation of FOXO target genes. EGCG induced apoptosis by upregulating Bim and activating caspase-3. EGCG modulated markers of cell cycle (p27/KIP1), angiogenesis (CD31, VEGF, IL-6, IL-8, SEMA3F, and HIF1alpha), and metastasis (MMP2 and MMP7). The inhibition of VEGF by EGCG was associated with suppression of neuropilin. EGCG inhibited epithelial-mesenchymal transition by upregulating the expression of E-cadherin and inhibiting the expression of N-cadherin and Zeb1. These data suggest that EGCG inhibits pancreatic cancer orthotopic tumor growth, angiogenesis, and metastasis which are associated with inhibition of PI3K/AKT and ERK pathways and activation of FKHRL1/FOXO3a. As a conclusion, EGCG can be used for the prevention and/or treatment of pancreatic cancer

Kostin SF, McDonald DE, McFadden DW. Inhibitory effects of (-)-epigallocatechin-3-gallate and pterostilbene on pancreatic cancer growth in vitro. *J Surg Res* 2012 October;177(2):255-62.
Abstract: BACKGROUND: It has been previously shown that the naturally occurring antioxidant (-)-epigallocatechin-3-gallate (EGCG), found in green tea, and pterostilbene, a stilbenoid derived from blueberries, inhibit pancreatic cancer in vitro when used individually. We hypothesized that the combination of EGCG and pterostilbene would reveal additive effects in vitro. METHODS: Using the pancreatic cancer cell lines MIA PaCa-2 and PANC-1, efficacy and synergism were evaluated for cell proliferation and viability (3-(4,5-dimethyltiazol-2-y1)-2,5-diphenltetrazolium bromide assays, cell cycle analysis) and mitochondrial apoptosis (mitochondrial depolarization, cytochrome C release, caspase-3/7 activity, cell death detection using enzyme-linked immunosorbent assay). RESULTS: Cell proliferation assays revealed significant additive antiproliferative effects with pterostilbene and EGCG in both cell lines at the later, 72-h, point ($P < 0.05$). MIA underwent S-phase arrest with the combination (10-12% increase); however, cell cycle arrest was not observed in PANC. The combination induced mitochondrial depolarization and upregulated cytochrome C ($P < 0.05$) in MIA, but these effects were not observed in PANC. EGCG increased caspase-3/7 in MIA; however, the combination did not significantly increase the activity in either cell line ($P < 0.05$). Apoptosis was only observed in PANC ($P < 0.05$). The reduction in proliferation in MIA in the 3-(4,5-dimethyltiazol-2-y1)-2,5-diphenltetrazolium bromide assays with the combination indicated that cell death occurs, possibly through another mechanism. CONCLUSIONS: Our results are encouraging regarding the future use of EGCG and pterostilbene to improve traditional pancreatic cancer therapies. In conclusion, EGCG and pterostilbene have additive, antiproliferative effects in vitro and alter the apoptotic mechanisms in both cell lines by modulation at different points in the mechanism

Vu HA, Beppu Y, Chi HT et al. Green tea epigallocatechin gallate exhibits anticancer effect in human pancreatic carcinoma cells via the inhibition of both focal adhesion kinase and insulin-like growth factor-I receptor. *J Biomed Biotechnol* 2010;2010:290516.
Abstract: The exact molecular mechanism by which epigallocatechin gallate (EGCG) suppresses human pancreatic cancer cell proliferation is unclear. We show here that EGCG-treated pancreatic cancer cells AsPC-1 and BxPC-3 decrease cell adhesion ability on micro-pattern dots, accompanied by dephosphorylations of both focal adhesion kinase (FAK) and insulin-like growth factor-1 receptor

(IGF-1R) whereas retained the activations of mitogen-activated protein kinase and mammalian target of rapamycin. The growth of AsPC-1 and BxPC-3 cells can be significantly suppressed by EGCG treatment alone in a dose-dependent manner. At a dose of 100 muM which completely abolishes activations of FAK and IGF-1R, EGCG suppresses more than 50% of cell proliferation without evidence of apoptosis analyzed by PARP cleavage. Finally, the MEK1/2 inhibitor U0126 enhances growth-suppressive effect of EGCG. Our data suggests that blocking FAK and IGF-1R by EGCG could prove valuable for targeted therapy, which can be used in combination with other therapies, for pancreatic cancer

Shankar S, Ganapathy S, Hingorani SR, Srivastava RK. EGCG inhibits growth, invasion, angiogenesis and metastasis of pancreatic cancer. *Front Biosci* 2008;13:440-52.
Abstract: We have shown that epigallocatechin-3-gallate (EGCG), a polyphenolic compound from green tea, inhibits growth and induces apoptosis in human pancreatic cancer cells. However, the preclinical potential of EGCG in a suitable mouse model has not been examined. In this study, we examined the molecular mechanisms by which EGCG inhibited growth, invasion, metastasis and angiogenesis of human pancreatic cancer cells in a xenograft model system. EGCG inhibited viability, capillary tube formation and migration of HUVEC, and these effects were further enhanced in the presence of an ERK inhibitor. In vivo, AsPC-1 xenografted tumors treated with EGCG showed significant reduction in volume, proliferation (Ki-67 and PCNA staining), angiogenesis (vWF, VEGF and CD31) and metastasis (MMP-2, MMP-7, MMP-9 and MMP-12) and induction in apoptosis (TUNEL), caspase-3 activity and growth arrest (p21/WAF1). EGCG also inhibited circulating endothelial growth factor receptor 2 (VEGF-R2) positive endothelial cells derived from xenografted mice. Tumor samples from EGCG treated mice showed significantly reduced ERK activity, and enhanced p38 and JNK activities. Overall, our data suggest that EGCG inhibits pancreatic cancer growth, invasion, metastasis and angiogenesis, and thus could be used for the management of pancreatic cancer prevention and treatment

Takada M, Nakamura Y, Koizumi T et al. Suppression of human pancreatic carcinoma cell growth and invasion by epigallocatechin-3-gallate. *Pancreas* 2002 July;25(1):45-8.
Abstract: INTRODUCTION: The consumption of green tea is associated with a lower risk of several types of human carcinomas. A number of studies have focused on the possible mechanisms of cancer prevention by tea extracts, especially polyphenols such as epigallocatechin-3-gallate (EGCG). AIMS AND METHODOLOGY: Green tea-derived EGCG was tested in human pancreatic carcinoma cells. The cells (PANC-1, MIA PaCa-2, and BxPC-3) were treated with different doses of EGCG (0, 25, 50, 100, and 200 micromol/L) for 48 hours in culture medium. Proliferation of pancreatic carcinoma cells was measured by means of the WST-1 colorimetric assay. For the study of cell invasion, the cells were incubated with 100 micromol/L EGCG for 2 hours. Then, the cells were added into the cell insert, coated with Matrigel basement membrane matrix. After incubation at 37 degrees C for 24 hours, the cells that had invaded through the Matrigel were counted visually under the microscope. RESULTS: The growth of all three pancreatic carcinoma cells was significantly suppressed by EGCG treatment in a dose-dependent manner. EGCG treatment caused significant suppression of the invasive ability of pancreatic carcinoma cells PANC-1, MIA PaCa-2, and BxPC-3 but did not affect the cell cycle protein cyclin D1. CONCLUSION: EGCG may be a potent biologic inhibitor of human pancreatic carcinomas, reducing their proliferative and invasive activities

Melatonin

Lissoni P, Brivio F, Fumagalli L et al. Neuroimmunomodulation in medical oncology: application of psychoneuroimmunology with subcutaneous low-dose IL-2 and the pineal hormone melatonin in

patients with untreatable metastatic solid tumors. *Anticancer Res* 2008 March;28(2B):1377-81.

Abstract: BACKGROUND: Anticancer immunity is under psychoneuroendocrine regulation, mainly via the pineal gland and brain opioid system, which may stimulate and inhibit antitumor immunity respectively. Cancer-related immuno-suppression does not depend only on functional damage of immune cells, but also on alterations of systems responsible for the neuroimmunomodulation, the most frequent of wich is a decline in blood levels of the pineal hormone melatonin (MLT). PATIENTS AND METHODS: A study was performed to evaluate the influence of an exogenous administration of MLT alone or MLT plus subcutaneous (SC) low-dose interleukin-2 on tumor progression and survival time in patients with untreatable metastatic solid tumors. The study included 846 patients with metastatic solid tumor (non-small cell lung cancer or gastrointestinal tract tumors) randomized to receive the best supportive care only, supportive care plus MLT (20 mg/day, orally in the evening), or MLT plus SC low-dose IL-2 (3 MIU/day for 5 days/week, for 4 consecutive weeks). RESULTS: The MLT alone was able to induce a significant increase of disease stabilization and survival time with respect to supportive care alone. The association of lL-2 with MLT provided a further improvement in the percentage of tumor regressions and of 3-year survival with respect to MLT alone. CONCLUSION: The administration of IL-2 and the pineal hormone MLT may induce control of neolplastic growth and a prolonged survival time in patients with metastatic solid tumors, for whom no other conventional anticancer therapy is available

Lissoni P, Tancini G, Barni S et al. Treatment of cancer chemotherapy-induced toxicity with the pineal hormone melatonin. *Support Care Cancer* 1997 March;5(2):126-9.

Abstract: Experimental data have suggested that the pineal hormone melatonin (MLT) may counteract chemotherapy-induced myelosuppression and immunosuppression. In addition, MLT has been shown to inhibit the production of free radicals, which play a part in mediating the toxicity of chemotherapy. A study was therefore performed in an attempt to evaluate the influence of MLT on chemotherapy toxicity. The study involved 80 patients with metastatic solid tumors who were in poor clinical condition (lung cancer: 35; breast cancer: 31; gastrointestinal tract tumors: 14). Lung cancer patients were treated with cisplatin and etoposide, breast cancer patients with mitoxantrone, and gastrointestinal tract tumor patients with 5-fluorouracil plus folates. Patients were randomised to receive chemotherapy alone or chemotherapy plus MLT (20 mg/day p.o. in the evening). Thrombocytopenia was significantly less frequent in patients concomitantly treated with MLT. Malaise and asthenia were also significantly less frequent in patients receiving MLT. Finally, stomatitis and neuropathy were less frequent in the MLT group, albeit without statistically significant differences. Alopecia and vomiting were not influenced by MLT. This pilot study seems to suggest that the concomitant administration of the pineal hormone MLT during chemotherapy may prevent some chemotherapy-induced side-effects, particularly myelosuppression and neuropathy. Evaluation of the impact of MLT on chemotherapy efficacy will be the aim of future clinical investigations

Lissoni P. Is there a role for melatonin in supportive care? *Support Care Cancer* 2002 March;10(2):110-6.
Abstract: Melatonin (MLT) is the main hormone released from the pineal gland and has proved to have physiological antitumor activity. MLT has been shown to exert anticancer activity through several biological mechanisms: antiproliferative action, stimulation of anticancer immunity, modulation of oncogene expression, and anti-inflammatory, anti-oxidant and anti-angiogenic effects. Several experimental studies have shown that MLT may inhibit cancer cell growth, and preliminary clinical studies seem to confirm its anticancer property in humans. In addition, MLT may have other biological effects, which could be useful in the palliative therapy of cancer, namely anticachectic, anti-asthenic and thrombopoietic activities. On this basis, the present clinical investigation was performed in an attempt at better definition of the therapeutic properties of MLT in human neoplasms. In a first clinical study, we evaluated the effects of MLT in a group of 1,440 patients with untreatable advanced solid

tumors, who received supportive care alone or supportive care plus MLT. In a second study, we evaluated the influence of MLT on the efficacy and toxicity of chemotherapy in a group of 200 metastatic patients with chemotherapy-resistant tumor histotype, who were randomized to receive chemotherapy alone or chemotherapy plus MLT. In both studies, MLT was given orally at 20 mg/day during the dark period of the day. The frequency of cachexia, asthenia, thrombocytopenia and lymphocytopenia was significantly lower in patients treated with MLT than in those who received supportive care alone. Moreover, the percentage of patients with disease stabilization and the percentage 1-year survival were both significantly higher in patients concomitantly treated with MLT than in those treated with supportive care alone. The objective tumor response rate was significantly higher in patients treated with chemotherapy plus MLT than in those treated with chemotherapy alone. Moreover, MLT induced a significant decline in the frequency of chemotherapy-induced asthenia, thrombocytopenia, stomatitis, cardiotoxicity and neurotoxicity. These clinical results demonstrate that the pineal hormone MLT may be successfully administered in medical oncology in the supportive care of untreatable advanced cancer patients and for the prevention of chemotherapy-induced toxicity

Lissoni P, Paolorossi F, Tancini G et al. Is there a role for melatonin in the treatment of neoplastic cachexia? *Eur J Cancer* 1996 July;32A(8):1340-3.
Abstract: It is known that neoplastic cachexia shows metabolic characteristics different from other common causes of malnutrition, and that it is mainly due to an abnormal secretion of TNF, whose levels are often high in patients with advanced neoplasia. Previous clinical studies have suggested that the pineal hormone melatonin (MLT), which plays an essential role in the neuroendocrine regulation of biological systems, may improve the clinical status of advanced cancer patients and inhibit TNF secretion. To investigate the relationship between MLT, TNF and cancer-related weight loss, 100 untreatable metastatic solid tumour patients entered this study to receive either supportive care alone, or supportive care plus MLT (20 mg/day orally in the evening). Patients were observed for 3 months, and were considered evaluable when they were observed for at least 2 months. There were 86 evaluable patients, the other 14 patients having died from rapid progression of disease. The per cent of weight loss greater than 10% was significantly higher in patients treated by supportive care alone than in those concomitantly treated by MLT, with no difference in food intake ($P < 0.01$). Mean serum levels of TNF progressively increased in the supportive care group, but to levels that were not significantly different from pretreatment values. In contrast, TNF mean concentrations significantly decreased ($P < 0.05$) in patients concomitantly treated by MLT. These results suggest that the pineal hormone MLT may be effective in the treatment of the neoplastic cachexia by decreasing TNF blood concentrations

Ruiz-Rabelo J, Vazquez R, Arjona A et al. Improvement of capecitabine antitumoral activity by melatonin in pancreatic cancer. *Pancreas* 2011 April;40(3):410-4.
Abstract: OBJECTIVE: The purpose of our study was to evaluate the effects of the addition of melatonin and capecitabine on experimental pancreatic cancer. METHODS: Fifty Syrian hamsters were randomized in 5 groups: group 1: no tumor induction (control group); group 2: tumor induction with BOP [N-nitrosobis(2-oxopropyl) amine]; group 3: tumor induction with BOP and melatonin administration; group 4: tumor induction with BOP and capecitabine administration; and group 5: tumor induction with BOP and administration of combined capecitabine and melatonin therapy. The evaluation of pathological tumor evolution and oxidative stress markers in pancreatic tissue was carried out. RESULTS: All animals under BOP exposure presented poorly or moderately differentiated pancreatic adenocarcinoma associated with increased lipoperoxide levels and decreased antioxidant activity in pancreatic tissue. Pancreatic cancer was shown in only 66% of the capecitabine-treated group and 33% of melatonin-treated group ($P < 0.05$), most of them moderately differentiated adenocarcinoma. When capecitabine and melatonin were combined, a well-differentiated pancreatic adenocarcinoma was observed in 10% of animals. The beneficial effect was associated with a decrease in lipoperoxide levels and increased antioxidant activity in pancreatic tissue. CONCLUSIONS: The

combined administration of capecitabine and melatonin provided an improvement in antioxidant status as well as a synergistic antitumoral effect in experimental pancreatic cancer

Cui P, Yu M, Peng X, Dong L, Yang Z. Melatonin prevents human pancreatic carcinoma cell PANC-1-induced human umbilical vein endothelial cell proliferation and migration by inhibiting vascular endothelial growth factor expression. *J Pineal Res* 2012 March;52(2):236-43.
Abstract: Melatonin is an important natural oncostatic agent, and our previous studies have found its inhibitory action on tumor angiogenesis, but the mechanism remains unclear. It is well known that vascular endothelial growth factor (VEGF) plays key roles in tumor angiogenesis and has become an important target for antitumor therapy. Pancreatic cancer is a representative of the most highly vascularized and angiogenic solid tumors, which responds poorly to chemotherapy and radiation. Thus, seeking new treatment strategies targeting which have anti-angiogenic capability is urgent in clinical practice. In this study, a co-culture system between human umbilical vein endothelial cells (HUVECs) and pancreatic carcinoma cells (PANC-1) was used to investigate the direct effect of melatonin on the tumor angiogenesis and its possible action on VEGF expression. We found HUVECs exhibited an increased cell proliferation and cell migration when co-cultured with PANC-1 cells, but the process was prevented when melatonin added to the incubation medium. Melatonin at concentrations of 1 mum and 1 mm inhibited the cell proliferation and migration of HUVECs and also decreased both the VEGF protein secreted to the cultured medium and the protein produced by the PANC-1 cells. In addition, the VEGF mRNA expression was also down-regulated by melatonin. Taken together, our present study shows that melatonin at pharmacological concentrations inhibited the elevated cell proliferation and cell migration of HUVECs stimulated by co-culturing them with PANC-1 cells; this was associated with a suppression of VEGF expression in PANC-1 cells

Leja-Szpak A, Jaworek J, Pierzchalski P, Reiter RJ. Melatonin induces pro-apoptotic signaling pathway in human pancreatic carcinoma cells (PANC-1). *J Pineal Res* 2010 October;49(3):248-55.
Abstract: Pancreatic cancer is a highly lethal disease with a poor prognosis for long-term survival rate at all stages of invasiveness. It responds poorly to radio- and chemotherapy because the tumor cells are resistant to apoptosis. Melatonin has been reported to inhibit pancreatic cancer growth in experimental studies in animals but the effect of melatonin on cultured human pancreatic carcinoma cells has not been tested. Moreover, we have recently shown that melatonin stimulates production of two major anti-apoptotic heat shock proteins, HSP27 and HSP 90, in pancreatic carcinoma cells. This study investigated the changes in intrinsic pathway of apoptosis at the mitochondrial level and cascade of caspases in human pancreatic carcinoma cells (PANC-1) cells subjected to melatonin and/or luzindole. Melatonin (10(-)(8) -10(-)(1)(2) m), the nonselective melatonin receptor antagonist, luzindole (10(-)(8) -10(-)(1)(2) m) or a combination of both agents were added to PANC-1 cell cultures. Cells were harvested, and the cytoplasmic proteins were isolated after 24 and 48 hr of incubation and analyzed employing co-immunoprecipitation and western blot. Administration of melatonin to the PANC-1 cells resulted in the stimulation of Bcl-2/Bax and caspase-9 proteins levels. The strongest signal of these pro-apoptotic factors was observed at the low concentration (10(-)(1)(2) m) of melatonin. Pretreatment with luzindole alone and prior to the addition of melatonin reversed the stimulatory effect of this indoloamine on Bcl-2/Bax and caspase-9 proteins expression in PANC-1 cells. This is the first study to demonstrate a pro-apoptotic effect of low (physiological) concentration of melatonin on the pancreatic carcinoma cells. In conclusion, melatonin induced pro-apoptotic pathways in human pancreatic carcinoma, probably by interaction with the Mel-1 A/B receptors

Padillo FJ, Ruiz-Rabelo JF, Cruz A et al. Melatonin and celecoxib improve the outcomes in hamsters with experimental pancreatic cancer. *J Pineal Res* 2010 October;49(3):264-70.
Abstract: Pancreatic cancer is a major health problem because of the aggressiveness of the disease and the lack of effective systemic therapies. Melatonin (MEL) has antioxidant activity and prevents experimental genotoxicity. The specific inhibitor of cyclooxygenase-2 (COX-2), celecoxib (CEL),

increases the efficacy of chemoradiotherapy in advanced pancreatic cancer. The objective of the study was the comparison and synergic effect of MEL and CEL during either the induction or progression phases of the tumor process, measuring parameters of oxidative stress, number of tumor nodules and survival of animals with pancreatic cancer. Pancreatic cancer was induced by N-nitrosobis (2-oxopropyl)amine) (BOP) in Syrian hamsters. Melatonin and/or CEL were administered during the induction, postinduction as well as during both phases. The presence of tumor nodules were observed macroscopically in pancreatic and splenic areas, and the levels of lipoperoxides (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in pancreatic tissue were measured. The increases in tumor nodules and LPO as well as the reductions in GSH and enzymatic antioxidants in the pancreas induced by BOP were related to a lower survival rate of animals. The administration of MEL exerted a more potent beneficial effect than CEL treatment on the reduction in tumor nodules, oxidative stress and death of experimental BOP-treated animals. The combined treatment only exerted a synergistic beneficial effect when administered during the induction phase. Melatonin by itself had significant beneficial actions in improving the survival of hamsters

Ruiz-Rabelo JF, Vazquez R, Perea MD et al. Beneficial properties of melatonin in an experimental model of pancreatic cancer. *J Pineal Res* 2007 October;43(3):270-5.
Abstract: Pancreatic cancer is a major health problem because of the aggressiveness of the disease and the lack of effective systemic therapies. Melatonin has antioxidant activity and prevents experimental genotoxicity. However, the effect of melatonin in pancreatic cancer has not been tested. Pancreatic carcinogenesis was induced by N-nitrosobis (2-oxopropyl)amine (BOP) in Syrian hamsters. Melatonin was administered during the BOP-induction phase (12 wk) and/or following the postinduction phase (12 wk). Different parameters of oxidative stress including lipid peroxides (LPO) and antioxidants (superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase) were determined in pancreatic tissue. Also, the presence of atypical hyperplasia (AH), well and moderately differentiated adenomacarcinoma (ADC-WD and ADC-MD, respectively) were studied. The administration of BOP induced an intense oxidative stress and ADC induction in the pancreas. The administration of melatonin during the induction or postinduction phase reduced LPO and improved the antioxidant status, as well as drastically reducing the presence of ADC but some AH remained. In conclusion, treatment with melatonin reduced oxidative damage and cancer nodules induced by BOP in the pancreas

Lissoni P, Ardizzoia A, Barni S et al. Efficacy and tolerability of cancer neuroimmunotherapy with subcutaneous low-dose interleukin-2 and the pineal hormone melatonin - a progress report of 200 patients with advanced solid neoplasms. *Oncol Rep* 1995 November;2(6):1063-8.
Abstract: The recent advances in psychoneuroimmunology have demonstrated the existence of a psychoneuroendocrine control of the antitumor immunity. Our previous preliminary studies indicated the possibility of amplifying the biological and therapeutic efficacy of IL-2 cancer immunotherapy by immunomodulating neurohormones, mainly the pineal indole melatonin (MLT), in most advanced solid tumors, including those which generally do not respond to IL-2 alone. This study reports on the results obtained by low-dose IL-2 plus MLT in 200 patients with advanced solid neoplasms, for whom no other effective standard therapy was available. Non-small cell lung cancer, pancreatic adenocarcinoma, hepatocarcinoma, colon cancer and gastric cancer were the neoplasms most frequently detected in our patients. In addition, all patients had a life expectancy less than 6 months. IL-2 was given subcutaneously at 3 million IU/day for 6 days/week for 4 weeks; MLT was given orally at 40 mg/day. In non-progressing patients, a second cycle was given after a 21-day rest period; then, patients underwent a maintenance period consisting of one week of therapy every month until progression. A complete response (CR) was achieved in 4 patients (hepatocarcinoma 2; pancreas 1; gastric cancer 1), a partial reasponse (PR) was achieved in 36 patients (lung 12; liver 6; stomach 4; pancreas 3; colon 3; breast 2; miscellaneous 6). Tumor response rate (CR+PR) was 40/200 (20%) patients. Longer than one year survival was achieved in 79 (39%) patients. Toxicity was mild in all patients, and therapy was administered as a home therapy. The present study confirms in a great number of patients the possibility

to induce objective tumor regressions in most advanced solid tumor histotypes by low-dose IL-2 plus MLT. Thus, immunotherapy with IL-2 and MLT may be considered as a new well tolerated and effective therapy of almost all advanced solid tumors, including those which do not respond to IL-2 alone or to chemotherapy

Low-Dose Aspirin

Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet* 2012 April 28;379(9826):1591-601.

Abstract: BACKGROUND: Daily aspirin reduces the long-term incidence of some adenocarcinomas, but effects on mortality due to some cancers appear after only a few years, suggesting that it might also reduce growth or metastasis. We established the frequency of distant metastasis in patients who developed cancer during trials of daily aspirin versus control. METHODS: Our analysis included all five large randomised trials of daily aspirin (>/=75 mg daily) versus control for the prevention of vascular events in the UK. Electronic and paper records were reviewed for all patients with incident cancer. The effect of aspirin on risk of metastases at presentation or on subsequent follow-up (including post-trial follow-up of in-trial cancers) was stratified by tumour histology (adenocarcinoma vs other) and clinical characteristics. FINDINGS: Of 17,285 trial participants, 987 had a new solid cancer diagnosed during mean in-trial follow-up of 6.5 years (SD 2.0). Allocation to aspirin reduced risk of cancer with distant metastasis (all cancers, hazard ratio [HR] 0.64, 95% CI 0.48-0.84, p=0.001; adenocarcinoma, HR 0.54, 95% CI 0.38-0.77, p=0.0007; other solid cancers, HR 0.82, 95% CI 0.53-1.28, p=0.39), due mainly to a reduction in proportion of adenocarcinomas that had metastatic versus local disease (odds ratio 0.52, 95% CI 0.35-0.75, p=0.0006). Aspirin reduced risk of adenocarcinoma with metastasis at initial diagnosis (HR 0.69, 95% CI 0.50-0.95, p=0.02) and risk of metastasis on subsequent follow-up in patients without metastasis initially (HR 0.45, 95% CI 0.28-0.72, p=0.0009), particularly in patients with colorectal cancer (HR 0.26, 95% CI 0.11-0.57, p=0.0008) and in patients who remained on trial treatment up to or after diagnosis (HR 0.31, 95% CI 0.15-0.62, p=0.0009). Allocation to aspirin reduced death due to cancer in patients who developed adenocarcinoma, particularly in those without metastasis at diagnosis (HR 0.50, 95% CI 0.34-0.74, p=0.0006). Consequently, aspirin reduced the overall risk of fatal adenocarcinoma in the trial populations (HR 0.65, 95% CI 0.53-0.82, p=0.0002), but not the risk of other fatal cancers (HR 1.06, 95% CI 0.84-1.32, p=0.64; difference, p=0.003). Effects were independent of age and sex, but absolute benefit was greatest in smokers. A low-dose, slow-release formulation of aspirin designed to inhibit platelets but to have little systemic bioavailability was as effective as higher doses. INTERPRETATION: That aspirin prevents distant metastasis could account for the early reduction in cancer deaths in trials of daily aspirin versus control. This finding suggests that aspirin might help in treatment of some cancers and provides proof of principle for pharmacological intervention specifically to prevent distant metastasis. FUNDING: None

Vitamin D

Persons KS, Eddy VJ, Chadid S, Deoliveira R, Saha AK, Ray R. Anti-growth effect of 1,25-dihydroxyvitamin D3-3-bromoacetate alone or in combination with 5-amino-imidazole-4-carboxamide-

1-beta-4-ribofuranoside in pancreatic cancer cells. *Anticancer Res* 2010 June;30(6):1875-80.
Abstract: 1,25-Dihydroxyvitamin D(3)-3-bromoacetate (1,25(OH)(2)D(3)-3-BE) is a vitamin D receptor-alkylating derivative of 1,25(OH)(2)D(3). The strong dose-dependent antiproliferative and apoptotic effects of this compound in androgen-sensitive and androgen-insensitive prostate cancer cells have been reported. In this communication, it is reported that 1,25(OH)(2)D(3)-3-BE strongly inhibits the growth of several pancreatic cancer cell lines. This effect is further accentuated by combination with 5-amino-imidazole-4-carboxamide-1-beta-4-ribofuranoside (AICAR), an activator of AMP-activated protein kinase (AMPK)/acetyl-Co-enzyme A carboxylase (ACC) phosphorylation pathways and an inhibitor of Akt phosphorylation. It was observed that the anti-growth property of 1,25(OH)(2)D(3)-3-BE, either alone or in combination with AICAR resulted in the inhibition of Akt phosphorylation in BxPC-3 cells. In conclusion, 1,25(OH)(2)D(3)-3-BE displays a strong therapeutic potential, alone and in combination with AICAR, in pancreatic cancer

Bruggemann LW, Queiroz KC, Zamani K, van SA, Spek CA, Bijlsma MF. Assessing the efficacy of the hedgehog pathway inhibitor vitamin D3 in a murine xenograft model for pancreatic cancer. *Cancer Biol Ther* 2010 July;10(1):79-88.
Abstract: The developmental Hedgehog (Hh) pathway has been shown to cause malignancies in the adult organism, specifically in the proximal gastrointestinal tract. Previous studies have used the Hh-inhibitory alkaloid cyclopamine to treat Hh-dependent tumor growth. The present study aimed to determine the efficacy and specificity of the recently discovered endogenous inhibitor of the Hh pathway, vitamin D3, on inhibition of pancreatic adenocarcinoma cell growth in vitro and in vivo. Vitamin D3 was found to inhibit cell growth specifically through inactivation of Smo and the downstream Hh pathway, rather than activation of the vitamin D3 receptor. However, in in vivo models vitamin D3 was not found to be effective against tumor cell growth

Schwartz GG, Eads D, Naczki C, Northrup S, Chen T, Koumenis C. 19-nor-1 alpha,25-dihydroxyvitamin D2 (paricalcitol) inhibits the proliferation of human pancreatic cancer cells in vitro and in vivo. *Cancer Biol Ther* 2008 March;7(3):430-6.
Abstract: 1,25-dihydroxyvitamin D(3), (1,25(OH)(2)D(3); calcitriol), the hormonal form of vitamin D, exerts growth-inhibitory, pro-apoptotic and anti-metastatic effects on tumor cells in vitro and in vivo but its clinical use is limited by its calcemic effects. Previous studies have shown that the antiproliferative effects of the less calcemic calcitriol analog 19-nor-1,25-(OH)(2)D(2) (paricalcitol) on prostate tumor cell lines are indistinguishable from those of 1,25(OH)(2)D(3). We therefore investigated the anti-proliferative effects of paricalcitol on the growth of pancreatic tumor cell lines in vitro and in vivo. Both 1,25(OH)(2)D(3) and paricalcitol inhibited the growth of BxPC-3, Hs700T and AsPC-1 lines in a dose-dependent manner. This antiproliferative activity correlated with upregulation of the cell cycle inhibitors p21 (Waf1/CIP1) and p27(Kip1). A fourth pancreatic cell line, Hs766T was unresponsive to both paricalcitol and calcitriol. Hs766T cells also failed to upregulate p21/Waf-1/Cip1 or p27/KiP in response to treatments with these agents. Paricalcitol, given three times per week inhibited the growth of AsPC-1 pancreatic tumor cell xenografts in nude mice at a dose that did not cause hypercalcaemia. Tumor inhibition was accompanied by in vivo upregulation of p21 and p27 expression. Given the few therapeutic options for patients with pancreatic cancer, further exploration of paricalcitol, an FDA-approved medication, is warranted

Kawa S, Yoshizawa K, Nikaido T, Kiyosawa K. Inhibitory effect of 22-oxa-1,25-dihydroxyvitamin D3, maxacalcitol, on the proliferation of pancreatic cancer cell lines. *J Steroid Biochem Mol Biol* 2005 October;97(1-2):173-7.
Abstract: Effective chemotherapy for pancreatic cancer is urgently needed. The aim of this study was to compare the anti-proliferative activity on pancreatic cancer cell lines of the vitamin D(3) analog, 22-oxa-1,25-dihydroxyvitamin D(3), maxacalcitol, with that of 1,25-dihydroxyvitamin D(3), calcitriol, with analysis of vitamin D receptor status and the G(1)-phase cell cycle-regulating factors.

Antiproliferative effects of both agents were compared using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method and by measuring the tumor size of xenografts inoculated into athymic mice. Scatchard analysis of vitamin D receptor contents, and mutational analysis of receptor complementary DNA were performed. Levels of expression of cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors, p21 and p27, were analysed by western blotting. In vitro, maxacalcitol and calcitriol markedly inhibited the proliferation and caused a G(1) phase cell cycle arrest with the appearance of numerous domes. In vivo, maxacalcitol inhibited the growth of BxPC-3 xenografts more significantly than calcitriol, without inducing hypercalcemia. Responsive cells had abundant functional vitamin D receptors. However, Hs 766T, showing no response to either agent, had the second highest receptor contents with no abnormalities in its primary structure deduced by receptor complementary DNA. In the responsive cells, p21 and p27 were markedly up-regulated after 24h of treatment with both agents. In non-responsive cells, no such changes were observed. In conclusion, maxacalcitol and calcitriol up-regulate p21 and p27 as an early event, which in turn could block the G(1)/S transition and induce growth inhibition in responsive cells, and maxacalcitol may provide a more useful tool for the chemotherapy of pancreatic cancer than calcitriol because of its low toxicity

Schwartz GG, Eads D, Rao A et al. Pancreatic cancer cells express 25-hydroxyvitamin D-1 alpha-hydroxylase and their proliferation is inhibited by the prohormone 25-hydroxyvitamin D3. *Carcinogenesis* 2004 June;25(6):1015-26.
Abstract: The steroid hormone 1,25-dihydroxyvitamin D(3), [1,25(OH)(2)D(3), calcitriol], the active metabolite of vitamin D, exerts pleiotropic antitumor effects against several malignancies. However, the clinical use of this hormone is limited by hypercalcemia. 25-Hydroxyvitamin D(3), the prohormone of 1,25(OH)(2)D(3), is hydroxylated to the active hormone by the enzyme 25-hydroxyvitamin-1-alpha-hydroxylase [1 alpha(OH)ase]. 1 alpha(OH)ase is found primarily in the kidney, but also is expressed in the prostate, colon and other tissues. Using immunohistochemistry, we report that 1 alpha(OH)ase is highly expressed in both normal and malignant pancreatic tissue. Expression of this enzyme and enzymatic activity was also detected in four pancreatic tumor cell lines. 25(OH)D(3) inhibited the growth of three of four pancreatic cell lines in a manner that correlated with the level of induction of the cyclin-dependent kinase inhibitors p21 and p27 and with the induction of cell cycle arrest at the G(1)/S checkpoint. The growth of a cell line stably transfected with a mutant Ki-ras allele and of a second cell line with an endogenous Ki-ras activating mutation was also inhibited by 25(OH)D(3), indicating that activating Ki-Ras mutations, which occur in almost 90% of pancreatic adenocarcinomas, do not interfere with the growth-inhibitory effects of 25(OH)D(3). The expression of 1 alpha(OH)ase in normal and malignant pancreatic tissue and the antiproliferative effects of the prohormone in these cells, suggest that 25(OH)D(3) may offer possible therapeutic and chemopreventive options for pancreatic cancer

Albrechtsson E, Jonsson T, Moller S, Hoglund M, Ohlsson B, Axelson J. Vitamin D receptor is expressed in pancreatic cancer cells and a vitamin D3 analogue decreases cell number. *Pancreatology* 2003;3(1):41-6.
Abstract: BACKGROUND AND AIM: The vitamin D-receptor (VDR) has been detected in both normal and malignant cells of different tissues. Treatment with vitamin D(3) has been suggested as a possible therapy in malignant diseases such as pancreatic cancer. Synthetic analogues of vitamin D(3) have a less hypercalcemic effect than native vitamin D(3). The aim was to study the expression of the VDR in human pancreatic cancers and to study the in vitro effect of an analogue to vitamin D(3) on cell lines established from these cancers. METHODS: The pancreatic cancer cell lines were established from primary cultures with only cancer cells. A probe specific for the human VDR was used. After reverse-transcriptase PCR and Northern blotting, the expression of the VDR in normal pancreas and in pancreatic cancers was compared. The cell lines were incubated with EB 1089, a synthetic analogue vitamin of D(3), in dose-response studies. The cell number was measured by the XTT colorimetric method. RESULTS: The VDR was expressed in all cancers and in six of the cell lines the expression

was increased more than 3-fold compared to normal pancreas. All cell lines developed from human pancreatic cancers responded with a decreased cell number to the vitamin D(3) analogue at concentrations of 10(-5) M or higher. CONCLUSION: The VDR was expressed in all pancreatic cancers studied. Cell lines derived from these cancers responded with a decrease in cell number to high concentrations of a vitamin D(3) analogue. These results, and the doses to use, have to be confirmed with in vivo studies

Evans TR, Colston KW, Lofts FJ et al. A phase II trial of the vitamin D analogue Seocalcitol (EB1089) in patients with inoperable pancreatic cancer. *Br J Cancer* 2002 March 4;86(5):680-5.
Abstract: Inoperable cancer of the exocrine pancreas responds poorly to most conventional anti-cancer agents, and new agents are required to palliate this disease. Seocalcitol (EB1089), a vitamin D analogue, can inhibit growth, induce differentiation and induce apoptosis of cancer cell lines in vitro and can also inhibit growth of pancreatic cancer xenografts in vivo. Thirty-six patients with advanced pancreatic cancer received once daily oral treatment with seocalcitol with dose escalation every 2 weeks until hypercalcaemia occurred, following which patients continued with maintenance therapy. The most frequent toxicity was the anticipated dose-dependent hypercalcaemia, with most patients tolerating a dose of 10-15 microg per day in chronic administration. Fourteen patients completed at least 8 weeks of treatment and were evaluable for efficacy, whereas 22 patients were withdrawn prior to completing 8 weeks' treatment and in 20 of these patients withdrawal was due to clinical deterioration as a result of disease progression. No objective responses were observed, with five of 14 patients having stable disease in whom the duration of stable disease was 82-532 days (median=168 days). The time to treatment failure (n=36) ranged from 22 to 847 days, and with a median survival of approximately 100 days. Seocalcitol is well tolerated in pancreatic cancer but has no objective anti-tumour activity in advanced disease. Further studies are necessary to determine if this agent has any cytostatic activity in this malignancy in minimal disease states

Colston KW, James SY, Ofori-Kuragu EA, Binderup L, Grant AG. Vitamin D receptors and anti-proliferative effects of vitamin D derivatives in human pancreatic carcinoma cells in vivo and in vitro. *Br J Cancer* 1997;76(8):1017-20.
Abstract: The GER human pancreatic carcinoma cell line possesses receptors for 1,25-dihydroxyvitamin D3. We report that the vitamin D analogue EB 1089 inhibits the growth of these cells in vitro and when grown as tumour xenografts in immunodeficient mice. Tumour-bearing mice were given EB 1089 at a dose of 5 microg kg(-1) body weight i.p. thrice weekly for 4-6 weeks. Tumour growth was significantly inhibited in treated animals compared with controls in the absence of hypercalcaemia. These findings may have therapeutic implications in pancreatic cancer

Spirulina (inhibitor of NADPH oxidase)

Lu W, Hu Y, Chen G et al. Novel role of NOX in supporting aerobic glycolysis in cancer cells with mitochondrial dysfunction and as a potential target for cancer therapy. *PLoS Biol* 2012;10(5):e1001326.
Abstract: Elevated aerobic glycolysis in cancer cells (the Warburg effect) may be attributed to respiration injury or mitochondrial dysfunction, but the underlying mechanisms and therapeutic significance remain elusive. Here we report that induction of mitochondrial respiratory defect by tetracycline-controlled expression of a dominant negative form of DNA polymerase gamma causes a metabolic shift from oxidative phosphorylation to glycolysis and increases ROS generation. We show that upregulation of NOX is critical to support the elevated glycolysis by providing additional NAD+. The upregulation of NOX is also consistently observed in cancer cells with compromised mitochondria due to the activation of oncogenic Ras or loss of p53, and in primary pancreatic cancer tissues. Suppression of NOX by chemical inhibition or genetic knockdown of gene expression selectively

impacts cancer cells with mitochondrial dysfunction, leading to a decrease in cellular glycolysis, a loss of cell viability, and inhibition of cancer growth in vivo. Our study reveals a previously unrecognized function of NOX in cancer metabolism and suggests that NOX is a potential novel target for cancer treatment

Du J, Nelson ES, Simons AL et al. Regulation of pancreatic cancer growth by superoxide. *Mol Carcinog* 2012 March 5.
Abstract: K-ras mutations have been identified in up to 95% of pancreatic cancers, implying their critical role in the molecular pathogenesis. Expression of K-ras oncogene in an immortalized human pancreatic ductal epithelial cell line, originally derived from normal pancreas (H6c7), induced the formation of carcinoma in mice. We hypothesized that K-ras oncogene correlates with increased non-mitochondrial-generated superoxide (O 2.-), which could be involved in regulating cell growth contributing to tumor progression. In the H6c7 cell line and its derivatives, H6c7er-Kras+ (H6c7 cells expressing K-ras oncogene), and H6c7eR-KrasT (tumorigenic H6c7 cells expressing K-ras oncogene), there was an increase in hydroethidine fluorescence in cell lines that express K-ras. Western blots and activity assays for the antioxidant enzymes that detoxify O 2.- were similar in these cell lines suggesting that the increase in hydroethidine fluorescence was not due to decreased antioxidant capacity. To determine a possible non-mitochondrial source of the increased levels of O 2.-, Western analysis demonstrated the absence of NADPH oxidase-2 (NOX2) in H6c7 cells but present in the H6c7 cell lines expressing K-ras and other pancreatic cancer cell lines. Inhibition of NOX2 decreased hydroethidine fluorescence and clonogenic survival. Furthermore, in the cell lines with the K-ras oncogene, overexpression of superoxide dismutases that detoxify non-mitochondrial sources of O 2.-, and treatment with the small molecule O 2.- scavenger Tempol, also decreased hydroethidine fluorescence, inhibited clonogenic survival and inhibited growth of tumor xenografts. Thus, O 2.- produced by NOX2 in pancreatic cancer cells with K-ras, may regulate pancreatic cancer cell growth. (c) 2012 Wiley Periodicals, Inc

Edderkaoui M, Nitsche C, Zheng L, Pandol SJ, Gukovsky I, Gukovskaya AS. NADPH oxidase activation in pancreatic cancer cells is mediated through Akt-dependent up-regulation of p22phox. *J Biol Chem* 2011 March 11;286(10):7779-87.
Abstract: We recently showed that Nox4 NADPH oxidase is highly expressed in pancreatic ductal adenocarcinoma and that it is activated by growth factors and plays a pro-survival, anti-apoptotic role. Here we investigate the mechanisms through which insulin-like growth factor I and serum (FBS) activate NADPH oxidase in pancreatic cancer (PaCa) cells. We show that in PaCa cells, NADPH oxidase is composed of Nox4 and p22(phox) catalytic subunits, which are both required for NADPH oxidase activity. Insulin-like growth factor I and FBS activate NADPH oxidase through transcriptional up-regulation of p22(phox). This involves activation of the transcription factor NF-kappaB mediated by Akt kinase. Up-regulation of p22(phox) by the growth factors results in increased Nox4-p22(phox) complex formation and activation of NADPH oxidase. This mechanism is different from that for receptor-induced activation of phagocytic NADPH oxidase, which is mediated by phosphorylation of its regulatory subunits. Up-regulation of p22(phox) represents a novel pro-survival mechanism through which growth factors and Akt inhibit apoptosis in PaCa cells

Du J, Liu J, Smith BJ, Tsao MS, Cullen JJ. Role of Rac1-dependent NADPH oxidase in the growth of pancreatic cancer. *Cancer Gene Ther* 2011 February;18(2):135-43.
Abstract: K-ras mutations occur in as high as 95% of patients with pancreatic cancer. K-ras activates Rac1-dependent NADPH oxidase, a key source of superoxide. Superoxide has an important function in pancreatic cancer cell proliferation, and scavenging or decreasing the levels of superoxide inhibits pancreatic cancer cell growth both in vitro and in vivo. DNA microarray analysis and RT-PCR has demonstrated that Rac1 is also upregulated in pancreatic cancer. The aim of this study was to determine whether inhibiting Rac1 would alter pancreatic tumor cell behavior. Human pancreatic cancer cells with

mutant K-ras (MIA PaCa-2), wild-type K-ras (BxPC-3) and the immortal H6c7 cell line (pancreatic ductal epithelium) expressing K-ras oncogene (H6c7eR-KrasT) that is tumorigenic, were infected with a dominant/negative Rac1 construct (AdN17Rac1). In cells with mutant K-ras, AdN17Rac1 decreased rac activity, decreased superoxide levels and inhibited in vitro growth. However, in the BxPC-3 cell line, AdN17Rac1 did not change rac activity, superoxide levels or in vitro cell growth. Additionally, AdN17Rac1 decreased superoxide levels and inhibited in vitro growth in the KrasT tumorigenic cell line, but had no effect in the immortalized H6c7 cell line. In human pancreatic tumor xenografts, intratumoral injections of AdN17Rac1 inhibited tumor growth. These results suggest that activation of Rac1-dependent superoxide generation leads to pancreatic cancer cell proliferation. In pancreatic cancer, inhibition of Rac1 may be a potential therapeutic target

Lee JK, Edderkaoui M, Truong P et al. NADPH oxidase promotes pancreatic cancer cell survival via inhibiting JAK2 dephosphorylation by tyrosine phosphatases. *Gastroenterology* 2007 November;133(5):1637-48.
Abstract: BACKGROUND & AIMS: Growth factors, such as insulin-like growth factor-1 (IGF-I), protect pancreatic cancer (PaCa) cells from death. We recently showed that reactive oxygen species (ROS) produced by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase Nox4 mediate the antiapoptotic effect of growth factors. Here, we examine the mechanisms of the antiapoptotic role of NADPH oxidase. We hypothesized that ROSs produced by NADPH oxidase inhibit key protein tyrosine phosphatases (PTPs) and thus sustain the activation of kinases mediating antiapoptotic pathways in PaCa cells. METHODS: Transfections and pharmacologic inhibition were used to assess the effects of NADPH oxidase on Janus kinase 2 (JAK2) kinase, the low molecular weight-protein tyrosine phosphatase (LMW-PTP), and apoptosis. RESULTS: We found that 1 target of ROSs is JAK2, an important antiapoptotic kinase in PaCa cells. Both serum-induced and IGF-I biphasic JAK2 phosphorylation, with a rapid (minutes) and transient first phase, and a slow and sustained (24-72 hours) second phase. Nox4 mediated the sustained phase of JAK2 phosphorylation, which was required for the antiapoptotic effects of IGF-I and serum. Transfection experiments identified the LMW-PTP as a negative regulator of sustained JAK2 phosphorylation. Growth factors inhibited LMW-PTP through its oxidation by NADPH oxidase. LMW-PTP colocalizes with Nox4 both in PaCa cells and in human pancreatic adenocarcinoma. CONCLUSIONS: The results suggest a novel signaling pathway, in which NADPH oxidase activation results in inhibition of PTPs, such as LMW-PTP, leading, in turn, to enhanced and sustained phosphorylation of kinases, such as JAK2, and suppression of apoptosis. This pathway mediates the prosurvival effect of ROSs and suggests new targets for pancreatic cancer treatment

Mochizuki T, Furuta S, Mitsushita J et al. Inhibition of NADPH oxidase 4 activates apoptosis via the AKT/apoptosis signal-regulating kinase 1 pathway in pancreatic cancer PANC-1 cells. *Oncogene* 2006 June 22;25(26):3699-707.
Abstract: Pancreatic adenocarcinoma is an aggressive human malignancy and is characterized by resistance to apoptosis. Recently, NADPH oxidase (Nox) 4-mediated generation of intracellular reactive oxygen species (ROS) was proposed to confer antiapoptotic activity and thus a growth advantage to pancreatic cancer cells. The signaling mechanism by which Nox4 transmits cell survival signals remains unclear. Here, we show that both a flavoprotein inhibitor, diphenylene iodonium (DPI), and small interfering RNAs designed to target Nox4 mRNA (siNox4RNAs) inhibited superoxide production in PANC-1 pancreatic cancer cells, and depletion of ROS by DPI or siNox4RNAs induced apoptosis. Parallely, DPI treatment and siNox4RNA transfection blocked activation of the cell survival kinase AKT by attenuating phosphorylation of AKT. Furthermore, AKT phosphorylation of apoptosis signal-regulating kinase 1 (ASK1) on Ser-83 was reduced by DPI and siNox4RNAs. When ASK1Ser83Ala (an AKT phosphorylation-defective ASK1 mutant) was introduced into PANC-1 cells, this mutant alone induced apoptosis. But, addition of DPI or co-transfection of siNox4RNA had no additive effect, indicating that the mutant can substitute for these reagents in apoptosis induction. Taken

together, these findings suggest that ROS generated by Nox4, at least in part, transmit cell survival signals through the AKT-ASK1 pathway in pancreatic cancer cells and their depletion leads to apoptosis

Vaquero EC, Edderkaoui M, Pandol SJ, Gukovsky I, Gukovskaya AS. Reactive oxygen species produced by NAD(P)H oxidase inhibit apoptosis in pancreatic cancer cells. *J Biol Chem* 2004 August 13;279(33):34643-54.
Abstract: One reason why pancreatic cancer is so aggressive and unresponsive to treatments is its resistance to apoptosis. We report here that reactive oxygen species (ROS) are a prosurvival, antiapoptotic factor in pancreatic cancer cells. Human pancreatic adenocarcinoma MIA PaCa-2 and PANC-1 cells generated ROS, which was stimulated by growth factors (serum, insulin-like growth factor I, or fibroblast growth factor-2). Growth factors also stimulated membrane NAD(P)H oxidase activity in these cells. Both intracellular ROS and NAD(P)H oxidase activity were inhibited by antioxidants tiron and N-acetylcysteine and the inhibitor of flavoprotein-dependent oxidases, diphenylene iodonium, but not by inhibitors of various other ROS-generating enzymes. Using Rho(0) cells deficient in mitochondrial DNA, we showed that a nonmitochondrial NAD(P)H oxidase is a major source of growth factor-induced ROS in pancreatic cancer cells. Among proteins that have been implicated in NAD(P)H oxidase activity, MIA PaCa-2 and PANC-1 cells do not express the phagocytic gp91(phox) subunit but express several nonphagocytic oxidase (NOX) isoforms. Transfection with Nox4 antisense oligonucleotide inhibited NAD(P)H oxidase activity and ROS production in MIA PaCa-2 and PANC-1 cells. Inhibiting ROS with the antioxidants, Nox4 antisense, or MnSOD overexpression all stimulated apoptosis in pancreatic cancer cells as measured by internucleosomal DNA fragmentation, phosphatidylserine externalization, cytochrome c release, and effector caspase activation. The results show that growth factor-induced ROS produced by NAD(P)H oxidase (probably Nox4) protect pancreatic cancer cells from apoptosis. This mechanism may play an important role in pancreatic cancer resistance to treatment and thus represent a novel therapeutic target

McCarty MF. Clinical potential of Spirulina as a source of phycocyanobilin. *J Med Food* 2007 December;10(4):566-70.
Abstract: Recent research reveals that free bilirubin functions physiologically as a potent inhibitor of NADPH oxidase activity. The chromophore phycocyanobilin (PCB), found in blue-green algae and cyanobacteria such as Spirulina, also has been found to be a potent inhibitor of this enzyme complex, likely because in mammalian cells it is rapidly reduced to phycocyanorubin, a close homolog of bilirubin. In light of the protean roles of NADPH oxidase activation in pathology, it thus appears likely that PCB supplementation may have versatile potential in prevention and therapy -- particularly in light of rodent studies demonstrating that orally administered Spirulina or phycocyanin (the Spirulina holoprotein that contains PCB) can exert a wide range of anti-inflammatory effects. Until PCB-enriched Spirulina extracts or synthetically produced PCB are commercially available, the most feasible and least expensive way to administer PCB is by ingestion of whole Spirulina. A heaping tablespoon (about 15 g) of Spirulina can be expected to provide about 100 mg of PCB. By extrapolating from rodent studies, it can be concluded that an intake of 2 heaping tablespoons daily would be likely to have important antioxidant activity in humans -- assuming that humans and rodents digest and absorb Spirulina-bound PCB in a comparable manner. An intake of this magnitude can be clinically feasible if Spirulina is incorporated into "smoothies" featuring such ingredients as soy milk, fruit juices, and whole fruits. Such a regimen should be evaluated in clinical syndromes characterized and in part mediated by NADPH oxidase overactivity in affected tissues

McCarty MF, Barroso-Aranda J, Contreras F. A two-phase strategy for treatment of oxidant-dependent cancers. *Med Hypotheses* 2007;69(3):489-96.
Abstract: In many cancers, a chronic increase in oxidant stress - associated with elevated levels of hydrogen peroxide - contributes to the increased proliferative rate, diminished apoptosis, increased

angiogenic and metastatic capacity, and chemoresistance that often characterize advanced malignancies. This oxidant stress often reflects up-regulation of expression and activity of NADPH oxidase, and/or decreased activity of catalase, which functions as suppressor gene in oxidant-dependent cancers. These characteristics of oxidant-dependent cancers suggest a dual strategy for treatment of these cancers. Since ascorbate can react spontaneously with molecular oxygen to generate hydrogen peroxide, high-dose intravenous ascorbate should be selectively toxic to tumors that are low in catalase activity - as suggested by numerous cell culture studies. Measures which concurrently improve the oxygenation of hypoxic tumor regions would be expected to boost the efficacy of such therapy; calcitriol and high-dose selenium might also be useful in this regard. Secondly, during the intervals between sessions of ascorbate therapy, administration of agents which can safely inhibit NADPH oxidase would be expected to slow the proliferation and spread of surviving tumor cells - while providing selection pressure for a further decline in catalase activity. In effect, cancers treated in this way would be whipsawed between lethally excessive and inadequately low oxidant stress. An additional possibility is that ascorbate-induced oxidant stress in tumors might potentiate the cell kill achieved with concurrently administered cytotoxic drugs, inasmuch as oxidant mechanisms appear to play a mediating role in the apoptosis induced by many such drugs, largely via activation of c-Jun NH(2)-terminal kinase; cell culture studies would be useful for evaluating this possibility

NF-kappaB Inhibitors - Salsalate and Anti-inflammatory Phytochemicals

Perugini RA, McDade TP, Vittimberga FJ, Jr., Duffy AJ, Callery MP. Sodium salicylate inhibits proliferation and induces G1 cell cycle arrest in human pancreatic cancer cell lines. *J Gastrointest Surg* 2000 January;4(1):24-32, discussion.
Abstract: The mutations most common in pancreatic cancer decrease the ability to control G1 to S cell cycle progression and cellular proliferation. In colorectal cancer cells, nonsteroidal anti-inflammatory drugs inhibit proliferation and induce cell cycle arrest. We examined whether sodium salicylate, an aspirin metabolite, could inhibit proliferation in human pancreatic cancer cell lines (BxPC3 and Panc-1). Quiescent cells were treated with medium containing 10% fetal calf serum, with or without salicylate. Cellular proliferation was measured by MTT assay and bromodeoxyuridine incorporation. The fractions of cells in G0/G1, S, and G2/M phases of the cell cycle were quantitated by fluorescence-activated cell sorting. Results were compared between groups by two-tailed t test. Cyclin D1 expression was determined by Western blot analysis and prostaglandin E2 expression by enzyme-linked immunosorbent assay. Serum-starved cells failed to proliferate, with most arrested in the G1 phase. Salicylate significantly inhibited serum-induced progression from G1 to S phase, cellular proliferation, and the expression of cyclin D1. The concentrations at which 50% of serum-induced proliferation was inhibited were 1.2 mmol/L (Panc-1) and 1.7 mmol/L (BxPC3). The antiproliferative effect of sodium salicylate was not explained by inhibition of prostaglandin E2 production. This study provides further evidence in a noncolorectal cancer model for the antineoplastic effects of nonsteroidal anti-inflammatory drugs

McDade TP, Perugini RA, Vittimberga FJ, Jr., Carrigan RC, Callery MP. Salicylates inhibit NF-kappaB activation and enhance TNF-alpha-induced apoptosis in human pancreatic cancer cells. *J Surg Res* 1999 May 1;83(1):56-61.
Abstract: INTRODUCTION: Tumor necrosis factor (TNF-alpha)-induced apoptosis is limited by its coactivation of nuclear factor kappa B (NF-kappaB)-dependent anti-apoptotic genes. Sodium salicylate (NaSal) inhibits NF-kappaB activation by limiting phosphorylation and degradation of its bound inhibitor protein, IkappaB-alpha. We examined whether NaSal enhances TNF-alpha-induced apoptosis in cultured human pancreatic cancer cell lines. METHODS: Two cultured human pancreatic

cancer cell lines were studied. PANC-1 and BxPC-3 cells were serum-starved for 12 h, pretreated or not for 1 h with NaSal (5-20 mM), and then stimulated with recombinant human TNF-alpha (400 units/ml). Western blots of cytoplasmic lysates were performed to demonstrate IkappaB-alpha phosphorylation and degradation. Western blots of nuclear extracts were performed to assess nuclear translocation of NF-kappaB. In separate cultures, apoptosis was measured 4.5 h after TNF-alpha stimulation by both ELISA detection of interhistone DNA fragments and flow cytometry with propidium iodide staining. RESULTS: TNF-alpha induced IkappaB-alpha phosphorylation and degradation, which was inhibited by NaSal in both cell lines. TNF-alpha-induced apoptosis (DNA fragmentation) increased significantly when BxPC-3 cells were pretreated with NaSal. Flow cytometry confirmed this, demonstrating increases in apoptotic cell fractions: 8.5% (untreated), 9.3% (TNF-alpha alone), 14.9% (15 mM NaSal), and 22.9% (NaSal and TNF-alpha). In contrast, no increases in apoptosis were measured in the PANC-1 cell line among the various treatment groups. CONCLUSIONS: NaSal enhances TNF-alpha-induced apoptosis while inhibiting IkappaB-alpha phosphorylation and degradation in BxPC-3 human pancreatic cancer cells

Carbone C, Melisi D. NF-kappaB as a target for pancreatic cancer therapy. *Expert Opin Ther Targets* 2012 April;16 Suppl 2:S1-10.
Abstract: INTRODUCTION: Pancreatic cancer is the fourth leading cause of adult cancer mortality in the USA. It represents one of the greatest challenges in cancer treatment. The NF-kappaB transcriptional factors are constitutively activated in the majority of pancreatic cancers and are involved in the regulation of numerous aspects of tumor development and progression. NF-kappaB and the signaling cascades that regulate its activity have thus become attractive targets for novel therapeutic approaches for pancreatic cancer. AREAS COVERED: This review describes and discusses the most important advances in the comprehension of the complex molecular biology of NF-kappaB, as well as the development of novel NF-kappaB-targeting strategies for the treatment of pancreatic cancer. EXPERT OPINION: Although the inhibition of NF-kappaB, especially when combined with more classic chemotherapeutic drugs, could be a promising therapeutic strategy, direct targeting NF-kappaB still faces important challenges. In the future, targeting nonredundant cytosolic mediators of the activation of NF-kappaB - such as TNF receptor associated factor family member-associated NF-kappaB activator -binding kinase 1 (TBK1) and TGF-beta activated kinase 1 (TAK1) - could represent a better approach to inhibit key processes in pancreatic tumor cells and make a difference for this devastating disease

Bin HB, Jamal MS, Fischer JW, Mustafa A, Verma AK. Plumbagin, a plant derived natural agent inhibits the growth of pancreatic cancer cells in in vitro and in vivo via targeting EGFR, Stat3 and NF-kappaB signaling pathways. *Int J Cancer* 2012 November 1;131(9):2175-86.
Abstract: Pancreatic cancer (PC) is the most aggressive malignant disease, ranks as the fourth most leading cause of cancer-related death among men and women in the United States. We present here that plumbagin (PL), a quinoid constituent isolated from the roots of the medicinal plant Plumbago zeylanica L, inhibits the growth of PC cells both in vitro and in vivo model systems. PL treatment induces apoptosis and inhibits cell viability of PC cells (PANC1, BxPC3 and ASPC1). In addition, i.p. administration of PL (2 mg/kg body weight, 5 days a week) in severe combined immunodeficiency (SCID) mice beginning 3 days after ectopic implantation of PANC1 cells resulted in a significant ($P < 0.01$) inhibition of both tumor weight and volume. PL treatment inhibited (1) constitutive expression of epidermal growth factor receptor (EGFR), pStat3Tyr705 and pStat3Ser727, (2) DNA binding of Stat3 and (3) physical interaction of EGFR with Stat3, in both cultured PANC1 cells and their xenograft tumors. PL treatment also inhibited phosphorylation and DNA-binding activity of NF-kappaB in both cultured PC cells (PANC1 and ASPC1) and in PANC1 cells xenograft tumors. Downstream target genes (cyclin D1, MMP9 and Survivin) of Stat3 and NF-kappaB were similarly inhibited. These results suggest that PL may be used as a novel therapeutic agent against

human PC. Published 2012 Wiley-Liss, Inc. This article is a US Government work, and, as such, is in the public domain in the United States of America

Husain K, Francois RA, Yamauchi T, Perez M, Sebti SM, Malafa MP. Vitamin E delta-tocotrienol augments the antitumor activity of gemcitabine and suppresses constitutive NF-kappaB activation in pancreatic cancer. *Mol Cancer Ther* 2011 December;10(12):2363-72.
Abstract: The NF-kappaB transcription factor functions as a crucial regulator of cell survival and chemoresistance in pancreatic cancer. Recent studies suggest that tocotrienols, which are the unsaturated forms of vitamin E, are a promising class of anticancer compounds that inhibit the growth and survival of many cancer cells, including pancreatic cancer. Here, we show that tocotrienols inhibited NF-kappaB activity and the survival of human pancreatic cancer cells in vitro and in vivo. Importantly, we found the bioactivity of the four natural tocotrienol compounds (alpha-, beta-, delta-, and gamma-tocotrienol) to be directly related to their ability to suppress NF-kappaB activity in vitro and in vivo. The most bioactive tocotrienol for pancreatic cancer, delta-tocotrienol, significantly enhanced the efficacy of gemcitabine to inhibit pancreatic cancer growth and survival in vitro and in vivo. Moreover, we found that delta-tocotrienol augmentation of gemcitabine activity in pancreatic cancer cells and tumors is associated with significant suppression of NF-kappaB activity and the expression of NF-kappaB transcriptional targets (Bcl-X(L), X-linked inhibitor of apoptosis, and survivin). Our study represents the first comprehensive preclinical evaluation of the activity of natural vitamin E compounds in pancreatic cancer. Given these results, we are conducting a phase I trial of delta-tocotrienol in patients with pancreatic cancer using pancreatic tumor cell survival and NF-kappaB signaling components as intermediate biomarkers. Our data also support future clinical investigation of delta-tocotrienol to augment gemcitabine activity in pancreatic cancer

Cheng ZX, Sun B, Wang SJ et al. Nuclear factor-kappaB-dependent epithelial to mesenchymal transition induced by HIF-1alpha activation in pancreatic cancer cells under hypoxic conditions. *PLoS ONE* 2011;6(8):e23752.
Abstract: BACKGROUND: Epithelial to mesenchymal transition (EMT) induced by hypoxia is one of the critical causes of treatment failure in different types of human cancers. NF-kappaB is closely involved in the progression of EMT. Compared with HIF-1alpha, the correlation between NF-kappaB and EMT during hypoxia has been less studied, and although the phenomenon was observed in the past, the molecular mechanisms involved remained unclear. METHODOLOGY/PRINCIPAL FINDINGS: Here, we report that hypoxia or overexpression of hypoxia-inducible factor-1alpha (HIF-1alpha) promotes EMT in pancreatic cancer cells. On molecular or pharmacologic inhibition of NF-kappaB, hypoxic cells regained expression of E-cadherin, lost expression of N-cadherin, and attenuated their highly invasive and drug-resistant phenotype. Introducing a pcDNA3.0/HIF-1alpha into pancreatic cancer cells under normoxic conditions heightened NF-kappaB activity, phenocopying EMT effects produced by hypoxia. Conversely, inhibiting the heightened NF-kappaB activity in this setting attenuated the EMT phenotype. CONCLUSIONS/SIGNIFICANCE: These results suggest that hypoxia or overexpression of HIF-1alpha induces the EMT that is largely dependent on NF-kappaB in pancreatic cancer cells

Veeraraghavan J, Natarajan M, Lagisetty P, Awasthi V, Herman TS, Aravindan N. Impact of curcumin, raspberry extract, and neem leaf extract on rel protein-regulated cell death/radiosensitization in pancreatic cancer cells. *Pancreas* 2011 October;40(7):1107-19.
Abstract: OBJECTIVES: Nuclear factor kappaB (NF-kappaB) plays an intrinsic role in promoting growth, angiogenesis, and metastasis in pancreatic cancer (PC) and serves as a mechanism underlying therapeutic resistance. Accordingly, we investigated the efficacy of bioactive phytochemicals in inhibiting radiotherapy (RT)-induced NF-kappaB activity, signaling, and NF-kappaB-dependent regulation of cell death. METHODS: Panc-1, BxPC-3, and MIA PaCa-2 cells exposed to 10 Gy (single high dose [SDR]) or 2 Gy/d for 5 days (fractionated radiation [FIR]) with or without curcumin

(CUR), neem leaf extract (NLE), or black raspberry extract (RSE) were analyzed. RESULTS: Radiotherapy profoundly induced NF-kappaB-DNA-binding activity with relatively robust activation after FIR. Curcumin, NLE, and RSE significantly inhibited both constitutive and RT-induced NF-kappaB. Furthermore, quantitative polymerase chain reaction profiling of 88 NF-kappaB pathway molecules demonstrated that CUR, NLE, and RSE comprehensively, yet differentially inhibited FIR/SDR-induced genes. Functionally, CUR, NLE, and RSE markedly conferred RT-inhibited cell viability/survival, robustly activated caspase-3/7 activity, and subsequent cell death. More importantly, NF-kappaB overexpression and silencing studies demonstrate that these compounds potentiate RT-induced cell death by targeting RT-induced NF-kappaB. CONCLUSIONS: These data strongly imply that CUR, NLE, and RSE may serve as effective "deliverables" to potentiate RT in PC cure and further throw light that these phytochemicals-induced cell killing may involve selective regulation of RT-induced NF-kappaB

Ahmad A, Wang Z, Wojewoda C et al. Garcinol-induced apoptosis in prostate and pancreatic cancer cells is mediated by NF- kappaB signaling. *Front Biosci (Elite Ed)* 2011;3:1483-92.
Abstract: Garcinol, obtained from Garcinia indica, is a potent antioxidant. Its anticancer activity has been investigated; however, there is no published report on its action against prostate and pancreatic cancer cells. We have earlier reported its activity against breast cancer cells, and here we tested our hypothesis that garcinol could inhibit cell proliferation and induce apoptosis in prostate as well as pancreatic cancer cells. Using multiple techniques such as MTT, Histone-DNA ELISA, activated caspase assays, clonogenic assays and EMSA, we investigated the mechanism of apoptosis-inducing effect of garcinol in prostate (LNCaP, C4-2B and PC3) and pancreatic (BxPC-3) cancer cells. We found that garcinol inhibited cell growth of all the cell lines tested with a concomitant induction of apoptosis in a dose-dependent manner. Down-regulation of NF-kappaB signaling pathway appears to be the mechanism of apoptosis-induction because garcinol inhibited constitutive levels of NF-betaB activity, which was consistent with down-regulation of NF-betaB-regulated genes. A significant decrease in the colony forming ability of all the cell lines was also observed, suggesting the possible application of this compound against metastatic disease. In summary, our results provide pre-clinical evidence to support the use of garcinol against human prostate and pancreatic cancer, thus meriting its further investigation as a potential chemo-preventive and/or therapeutic agent

Wang SJ, Sun B, Cheng ZX et al. Dihydroartemisinin inhibits angiogenesis in pancreatic cancer by targeting the NF-kappaB pathway. *Cancer Chemother Pharmacol* 2011 December;68(6):1421-30.
Abstract: PURPOSE: Dihydroartemisinin (DHA) has recently shown antitumor activity in human pancreatic cancer cells. However, its effect on antiangiogenic activity in pancreatic cancer is unknown, and the mechanism is unclear. This study was aimed to investigate whether DHA would inhibit angiogenesis in human pancreatic cancer. METHODS: Cell viability and proliferation, tube formation of human umbilical vein endothelial cells (HUVECs), nuclear factor (NF)-kappaB DNA-binding activity, expressions of vascular endothelial growth factor (VEGF), interleukin (IL)-8, cyclooxygenase (COX)-2, and matrix metalloproteinase (MMP)-9 were examined in vitro. The effect of DHA on antiangiogenic activity in pancreatic cancer was also assessed using BxPC-3 xenografts subcutaneously established in BALB/c nude mice. RESULTS: DHA inhibited cell proliferation and tube formation of HUVECs in a time- and dose-dependent manner and also reduced cell viability in pancreatic cancer cells. DHA significantly inhibited NF-kappaB DNA-binding activity, so as to tremendously decrease the expression of NF-kappaB-targeted proangiogenic gene products: VEGF, IL-8, COX-2, and MMP-9 in vitro. In vivo studies, DHA remarkably reduced tumor volume, decreased microvessel density, and down-regulated the expression of NF-kappaB-related proangiogenic gene products. CONCLUSIONS: Inhibition of NF-kappaB activation is one of the mechanisms that DHA inhibits angiogenesis in human pancreatic cancer. We also suggest that DHA could be developed as a novel agent against pancreatic cancer

Lu Z, Li Y, Takwi A et al. miR-301a as an NF-kappaB activator in pancreatic cancer cells. *EMBO J* 2011 January 5;30(1):57-67.
Abstract: NF-kappaB is constitutively activated in most human pancreatic adenocarcinoma, which is a deadly malignancy with a 5-year survival rate of about 5%. In this work, we investigate whether microRNAs (miRNAs) contribute to NF-kappaB activation in pancreatic cancer. We demonstrate that miR-301a down-regulates NF-kappaB-repressing factor (Nkrf) and elevates NF-kappaB activation. As NF-kappaB promotes the transcription of miR-301a, our results support a positive feedback loop as a mechanism for persistent NF-kappaB activation, in which miR-301a represses Nkrf to elevate NF-kappaB activity, which in turn promotes miR-301a transcription. Nkrf was found down-regulated and miR-301a up-regulated in human pancreatic adenocarcinoma tissues. Moreover, miR-301a inhibition or Nkrf up-regulation in pancreatic cancer cells led to reduced NF-kappaB target gene expression and attenuated xenograft tumour growth, indicating that miR-301a overexpression contributes to NF-kappaB activation. Revealing this novel mechanism of NF-kappaB activation by an miRNA offers new avenues for therapeutic interventions against pancreatic cancer

Furukawa K, Iida T, Shiba H et al. Anti-tumor effect by inhibition of NF-kappaB activation using nafamostat mesilate for pancreatic cancer in a mouse model. *Oncol Rep* 2010 October;24(4):843-50.
Abstract: Constitutive NF-kappaB activation plays a key role in the aggressive behavior of pancreatic cancer. We have reported that nafamostat mesilate, a serine-protease inhibitor, inhibited NF-kappaB activation and induced apoptosis in human pancreatic cancer cells. The aim of this study is to evaluate the therapeutic efficacy of nafamostat mesilate against pancreatic cancer. In vitro, nafamostat mesilate inhibited NF-kappaB activation of human pancreatic cancer cell line (Panc-1) by suppressing IkappaBalpha phosphorylation and induced caspase-8 mediated apoptosis. In vivo, Panc-1 was implanted into the back of nude mice. Five weeks after implantation, nafamostat mesilate was injected intraperitoneally as the treatment group (n=11) three times a week for six weeks, while the control group (n=13) received vehicle only. At the end of six-week treatment, the tumors grew up to 12.89+/-4.27 mm (mean +/- SD) in the treatment group, and 17.93+/-4.45 mm in the control group, respectively. The tumor volume and weight of the treatment group were reduced by 43 and 61% as compared with the control group. The tumor growth was significantly inhibited in the treatment group ($p<0.0001$). Assays of primary tumors also indicated that nafamostat mesilate inhibited NF-kappaB activation by suppressing IkappaBalpha phosphorylation, resulting in caspase-8 mediated apoptosis. These results suggested that nafamostat mesilate has anti-neoplastic property against experimental pancreatic cancer

Jutooru I, Chadalapaka G, Lei P, Safe S. Inhibition of NFkappaB and pancreatic cancer cell and tumor growth by curcumin is dependent on specificity protein down-regulation. *J Biol Chem* 2010 August 13;285(33):25332-44.
Abstract: Curcumin activates diverse anticancer activities that lead to inhibition of cancer cell and tumor growth, induction of apoptosis, and antiangiogenic responses. In this study, we observed that curcumin inhibits Panc28 and L3.6pL pancreatic cancer cell and tumor growth in nude mice bearing L3.6pL cells as xenografts. In addition, curcumin decreased expression of p50 and p65 proteins and NFkappaB-dependent transactivation and also decreased Sp1, Sp3, and Sp4 transcription factors that are overexpressed in pancreatic cancer cells. Because both Sp transcription factors and NFkappaB regulate several common genes such as cyclin D1, survivin, and vascular endothelial growth factor that contribute to the cancer phenotype, we also investigated interactions between Sp and NFkappaB transcription factors. Results of Sp1, Sp3, and Sp4 knockdown by RNA interference demonstrate that both p50 and p65 are Sp-regulated genes and that inhibition of constitutive or tumor necrosis factor-induced NFkappaB by curcumin is dependent on down-regulation of Sp1, Sp3, and Sp4 proteins by this compound. Curcumin also decreased mitochondrial membrane potential and induced reactive oxygen species in pancreatic cancer cells, and this pathway is required for down-regulation of Sp

proteins in these cells, demonstrating that the mitochondriotoxic effects of curcumin are important for its anticancer activities

Maier HJ, Schmidt-Strassburger U, Huber MA, Wiedemann EM, Beug H, Wirth T. NF-kappaB promotes epithelial-mesenchymal transition, migration and invasion of pancreatic carcinoma cells. *Cancer Lett* 2010 September 28;295(2):214-28.
Abstract: The transcription factor NF-kappaB is constitutively active in pancreatic adenocarcinoma. Here we explore the contribution of NF-kappaB to the malignant phenotype of pancreatic cancer cells in addition to its anti-apoptotic role. Block of NF-kappaB signalling by non-destructible IkappaBalpha rendered cells resistant to TGF-beta-induced epithelial-mesenchymal transition (EMT). In contrast, NF-kappaB activation by TNF-alpha or expression of constitutively active IKK2 induced an EMT-phenotype with up-regulation of vimentin and ZEB1, and down-regulation of E-cadherin. EMT could also be induced in cells with defective TGF-beta signalling. Functional assays demonstrated reduced or strongly enhanced migration and invasion upon NF-kappaB inhibition or activation, respectively

Ali S, Banerjee S, Schaffert JM, El-Rayes BF, Philip PA, Sarkar FH. Concurrent inhibition of NF-kappaB, cyclooxygenase-2, and epidermal growth factor receptor leads to greater anti-tumor activity in pancreatic cancer. *J Cell Biochem* 2010 May;110(1):171-81.
Abstract: Inactivation of survival pathways such as NF-kappaB, cyclooxygenase (COX-2), or epidermal growth factor receptor (EGFR) signaling individually may not be sufficient for the treatment of advanced pancreatic cancer (PC) as suggested by recent clinical trials. 3,3'-Diindolylmethane (B-DIM) is an inhibitor of NF-kappaB and COX-2 and is a well-known chemopreventive agent. We hypothesized that the inhibition of NF-kappaB and COX-2 by B-DIM concurrently with the inhibition of EGFR by erlotinib will potentiate the anti-tumor effects of cytotoxic drug gemcitabine, which has been tested both in vitro and in vivo. Inhibition of viable cells in seven PC cell lines treated with B-DIM, erlotinib, or gemcitabine alone or their combinations was evaluated using 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Significant inhibition in cell viability was observed in PC cells expressing high levels of COX-2, EGFR, and NF-kappaB proteins. The observed inhibition was associated with an increase in apoptosis as assessed by ELISA. A significant down-regulation in the expression of COX-2, NF-kappaB, and EGFR in BxPC-3, COLO-357, and HPAC cells was observed, suggesting that simultaneous targeting of EGFR, NF-kappaB, and COX-2 is more effective than targeting either signaling pathway separately. Our in vitro results were further supported by in vivo studies showing that B-DIM in combination with erlotinib and gemcitabine was significantly more effective than individual agents. Based on our preclinical in vitro and in vivo results, we conclude that this multi-targeted combination could be developed for the treatment of PC patients whose tumors express high levels of COX-2, EGFR, and NF-kappaB

Kong R, Sun B, Jiang H et al. Downregulation of nuclear factor-kappaB p65 subunit by small interfering RNA synergizes with gemcitabine to inhibit the growth of pancreatic cancer. *Cancer Lett* 2010 May 1;291(1):90-8.
Abstract: The clinical benefit of gemcitabine for pancreatic cancer is low due to chemoresistance. Nuclear factor (NF)-kappaB, constitutively activated in pancreatic cancer, is a therapeutic target as it upregulates expression of genes controlling proliferation, apoptosis and angiogenesis. This study aimed to investigate whether downregulation of the p65 subunit of NF-kappaB by siRNA could enhance the efficacy of gemcitabine to treat pancreatic cancer. p65 siRNA synergized with gemcitabine to inhibit the proliferation and induce the apoptosis of pancreatic cancer cells in vitro and in vivo, and suppress the growth and angiogenesis of pancreatic tumors in nude mice. The mechanisms involved inhibition of NF-kappaB activity and consequent inhibition of Bcl-2, cyclin D1

and VEGF, and activation of caspase-3. The results suggest that downregulation of NF-kappaB p65 potentiates the efficacy of gemcitabine in combating pancreatic cancer

Murtaza I, Adhami VM, Hafeez BB, Saleem M, Mukhtar H. Fisetin, a natural flavonoid, targets chemoresistant human pancreatic cancer AsPC-1 cells through DR3-mediated inhibition of NF-kappaB. *Int J Cancer* 2009 November 15;125(10):2465-73.
Abstract: Death receptors of the tumor necrosis factor (TNF) receptor super family have been implicated in constitutive activation of nuclear factor-kappa B (NF-kappaB) in pancreatic cancer (PaC) cells. In this study, we demonstrate that fisetin, a natural flavonoid, induces apoptosis and inhibits invasion of chemoresistant PaC AsPC-1 cells through suppression of DR3-mediated NF-kappaB activation. Fisetin treatment resulted in dose-dependent inhibition of PaC cell growth and cell proliferation with concomitant induction of apoptosis. A cDNA array analysis revealed that fisetin modulates expression of more than 20 genes at transcription level with maximum decrease observed in DR3 expression and a parallel increase observed in the expression levels of IkappaBalpha, an NF-kappaB inhibitor. Down-regulation of DR3 in PaC cells was found to down regulate activated pNF-kappaB/p65, pIkBalpha/beta kinases (pIKK's), MMP9 and XIAP that mostly impart chemoresistance in PaC. Immunoblotting and EMSA analysis showed a marked decrease in pNF-kappaB and NF-kappaB DNA binding activity, respectively, with modest decrease in NF-kappaB promoter activity and significant decrease in MMP9 promoter activity with fisetin treatment. Importantly, consistent with these findings, we further found that transient down-regulation of DR3 by RNA interference significantly augmented fisetin induced changes in cell proliferation, cell invasion and apoptosis paralleled with decrease in pNF-kappaB, pIKKalpha/beta, MMP9, XIAP and NF-kappaB DNA binding activity. Blocking of DR3 receptor with an extra cellular domain blocking antibody demonstrated similar effects. These data provide evidence that fisetin could provide a biological rationale for treatment of pancreatic cancer or as an adjuvant with conventional therapeutic regimens

Pan X, Arumugam T, Yamamoto T et al. Nuclear factor-kappaB p65/relA silencing induces apoptosis and increases gemcitabine effectiveness in a subset of pancreatic cancer cells. *Clin Cancer Res* 2008 December 15;14(24):8143-51.
Abstract: PURPOSE: Nuclear factor kappaB (NFkappaB) activity may increase survival and protect cancer cells from chemotherapy. Therefore, NFkappaB activity may be prognostic, and inhibition of NFkappaB may be useful for pancreatic cancer therapy. To test these hypotheses, we examined NFkappaB activity and the effects of inhibiting NFkappaB in several pancreatic cancer cell lines with differing sensitivities to gemcitabine. EXPERIMENTAL DESIGN: The gemcitabine sensitivity of pancreatic cancer cell lines BxPC-3, L3.6pl, CFPAC-1, MPanc-96, PANC-1, and MIA PaCa-2 were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and fluorescence-activated cell sorting assays. NFkappaB levels were determined by electrophoretic mobility shift assay and reporter assays. The effects of gemcitabine on NFkappaB activity were determined in vitro and in vivo. NFkappaB was inhibited by silencing of the p65/relA subunit using small interfering RNA in vitro and by neutral liposomal delivery of small interfering RNA in vivo, and the effects were evaluated on gemcitabine sensitivity. RESULTS: The cell lines L3.6pl, BxPC-3, and CFPAC-1 were sensitive, whereas MPanc-96, PANC-1, and MIA PaCa-2 were resistant to gemcitabine. No significant correlation was observed between basal NFkappaB activity and gemcitabine sensitivity. Gemcitabine treatment did not activate NFkappaB either in vitro or in vivo. Silencing of p65/relA induced apoptosis and increased gemcitabine killing of all gemcitabine-sensitive pancreatic cancer cells. No significant effects, however, were observed on gemcitabine-resistant pancreatic cancer cell lines either in vitro or in vivo. CONCLUSIONS: NFkappaB activity did not correlate with sensitivity to gemcitabine. Silencing of p65/relA was effective alone and in combination with gemcitabine in gemcitabine-sensitive but not gemcitabine-resistant pancreatic cancer cells. Thus, NFkappaB may be a useful therapeutic target for a subset of pancreatic cancers

Dhillon N, Aggarwal BB, Newman RA et al. Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clin Cancer Res* 2008 July 15;14(14):4491-9.
Abstract: PURPOSE: Pancreatic cancer is almost always lethal, and the only U.S. Food and Drug Administration-approved therapies for it, gemcitabine and erlotinib, produce objective responses in <10% of patients. We evaluated the clinical biological effects of curcumin (diferuloylmethane), a plant-derived dietary ingredient with potent nuclear factor-kappaB (NF-kappaB) and tumor inhibitory properties, against advanced pancreatic cancer. EXPERIMENTAL DESIGN: Patients received 8 g curcumin by mouth daily until disease progression, with restaging every 2 months. Serum cytokine levels for interleukin (IL)-6, IL-8, IL-10, and IL-1 receptor antagonists and peripheral blood mononuclear cell expression of NF-kappaB and cyclooxygenase-2 were monitored. RESULTS: Twenty-five patients were enrolled, with 21 evaluable for response. Circulating curcumin was detectable as drug in glucuronide and sulfate conjugate forms, albeit at low steady-state levels, suggesting poor oral bioavailability. Two patients showed clinical biological activity. One had ongoing stable disease for >18 months; interestingly, one additional patient had a brief, but marked, tumor regression (73%) accompanied by significant increases (4- to 35-fold) in serum cytokine levels (IL-6, IL-8, IL-10, and IL-1 receptor antagonists). No toxicities were observed. Curcumin down-regulated expression of NF-kappaB, cyclooxygenase-2, and phosphorylated signal transducer and activator of transcription 3 in peripheral blood mononuclear cells from patients (most of whom had baseline levels considerably higher than those found in healthy volunteers). Whereas there was considerable interpatient variation in plasma curcumin levels, drug levels peaked at 22 to 41 ng/mL and remained relatively constant over the first 4 weeks. CONCLUSIONS: Oral curcumin is well tolerated and, despite its limited absorption, has biological activity in some patients with pancreatic cancer

Holcomb B, Yip-Schneider M, Schmidt CM. The role of nuclear factor kappaB in pancreatic cancer and the clinical applications of targeted therapy. *Pancreas* 2008 April;36(3):225-35.
Abstract: Pancreatic cancer is one of the leading causes of cancer mortality in the United States. Current therapy for pancreatic cancer involves surgery, chemotherapy, and radiation therapy; however, the 5-year survival rate remains less than 5%. New strategies for treating pancreatic cancer include targeting intracellular signaling that provides survival advantages to cancer cells. One of these targets is the transcription factor nuclear factor (NF) kappaB, which is activated by a variety of mechanisms. Data demonstrate that increased NF-kappaB activity can promote growth and tumorigenesis, inhibit apoptosis, promote angiogenesis, promote invasion and metastasis, and promote chemoresistance in pancreatic cancer. This review explores the roles of NF-JB in these processes and examines the evidence that different NF-kappaB-inhibiting drugs can improve the treatment of pancreatic cancer

Sebens S, Arlt A, Schafer H. NF-kappaB as a molecular target in the therapy of pancreatic carcinoma. *Recent Results Cancer Res* 2008;177:151-64.
Abstract: The constitutive activation of the transcription factor nuclear-factor kappa B (NF-kappaB) is a hallmark of many highly malignant tumours such as the pancreatic ductal adenocarcinoma and accounts for profound chemoresistance. Inhibition of NF-kappaB activation has been shown to be a useful strategy for increasing the sensitivity towards cytostatic drug treatment in vitro and in vivo. Moreover, various pharmacological substances (e.g. thalidomide, bortezomib, sulphasalazine) have already entered clinical studies partially showing promising results for certain types of cancer. Further studies will be needed, in particular for pancreatic ductal adenocarcinoma, to evaluate the therapeutic efficacy of appropriate combinations of a NF-kappaB inhibitor and cytostatic drugs

Li L, Aggarwal BB, Shishodia S, Abbruzzese J, Kurzrock R. Nuclear factor-kappaB and IkappaB kinase are constitutively active in human pancreatic cells, and their down-regulation by curcumin (diferuloylmethane) is associated with the suppression of proliferation and the induction of apoptosis.

Cancer 2004 November 15;101(10):2351-62.
Abstract: BACKGROUND: Pancreatic carcinoma is a lethal malignancy, with the best available therapeutic option-gemcitabine-yielding response rates of < 10%. Because nuclear factor-kappaB (NF-kappaB) has been determined to play a role in cell survival/proliferation in human pancreatic carcinoma, this transcription factor is a potential therapeutic target. METHODS: The authors investigated the ability of curcumin (diferuloylmethane), an agent that is pharmacologically safe in humans, to modulate NF-kappaB activity. RESULTS: NF-kappaB and IkappaB kinase (IKK) were constitutively active in all human pancreatic carcinoma cell lines examined, and curcumin consistently suppressed NF-kappaB binding (as assessed using an electrophoretic mobility gel-shift assay) and IKK activity. Curcumin decreased the expression of NF-kappaB-regulated gene products, including cyclooxygenase-2 (as assessed using immunoblot analysis), prostaglandin E2, and interleukin-8 (as assessed using an enzyme-linked immunoassay), all of which have been implicated in the growth and invasiveness of pancreatic carcinoma. These changes were associated with concentration- and time-dependent antiproliferative activity (as assessed using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide [MTT] assay) and proapoptotic effects (as assessed via annexin V/propidium iodide staining [fluorescence-activated cell sorting, as well as with the induction of polyadenosine-5'-diphosphate-ribose polymerase cleavage). CONCLUSIONS: Curcumin down-regulated NF-kappaB and growth control molecules induced by NF-kappaB in human pancreatic cells. These effects were accompanied by marked growth inhibition and apoptosis. Through these findings, the authors provided a biologic rationale for the treatment of patients with pancreatic carcinoma using this nontoxic phytochemical

Xiong HQ, Abbruzzese JL, Lin E, Wang L, Zheng L, Xie K. NF-kappaB activity blockade impairs the angiogenic potential of human pancreatic cancer cells. *Int J Cancer* 2004 January 10;108(2):181-8.
Abstract: The effect of blockade of NF-kappaB activity on human pancreatic cancer angiogenesis was determined in an orthotopic xenograft model. Highly metastatic L3.3 human pancreatic cancer cells, which expressed an elevated level of constitutive NF-kappaB activity, were transfected with a mutated IkappaBalpha (IkappaBalphaM). After implantation in the pancreas of nude mice, parental (L3.3) and control vector-transfected (L3.3-Neo) cells produced rapidly growing tumors and liver metastases, whereas IkappaBalphaM-transfected (L3.3-IkappaBalphaM) cells had decreased tumorigenicity and metastatic potential. NF-kappaB signaling blockade significantly inhibited the in vitro and in vivo expression of the major proangiogenic molecules vascular endothelial growth factor and interleukin-8 and decreased tumor vascular formation. These events were correlated with retarded tumor growth and suppression of metastasis. Collectively, these data suggest that suppression of tumorigenicity and metastasis by NF-kappaB blockade is due to impaired angiogenic potential of tumor cells

Sclabas GM, Fujioka S, Schmidt C, Evans DB, Chiao PJ. NF-kappaB in pancreatic cancer. *Int J Gastrointest Cancer* 2003;33(1):15-26.
Abstract: Although the genetic profile of pancreatic cancer is emerging as a result of much research, the role of specific genetic alterations that initiate tumorigenesis and produce its cardinal clinical features of locally aggressive growth, metastasis, and chemotherapy resistance remains unresolved. Recently, a number of studies have shown that the inhibition of constitutive NF-kappaB activation, one of the frequent molecular alterations in pancreatic cancer, inhibits tumorigenesis and metastasis. It also sensitizes pancreatic cancer cell lines to anticancer agent-induced apoptosis. Therefore because of the crucial role of NF-kappaB in pancreatic cancer, it is a potential target for developing novel therapeutic strategies for the disease. In vivo and in vitro models that mimic the tumorigenic phenotypes in the appropriate histological and molecular concert would be very useful for confirming the suspected role of the pancreatic cancer signature genetic lesions and better understanding the molecular basis of this disease

Lloyd FP, Jr., Slivova V, Valachovicova T, Sliva D. Aspirin inhibits highly invasive prostate cancer cells. *Int J Oncol* 2003 November;23(5):1277-83.
Abstract: Cell adhesion, proteolytic degradation and cell migration are interrelated processes responsible for the invasion and metastasis of cancer. One of the crucial molecules involved in cancer metastasis is urokinase-type plasminogen activator (uPA). An elevated concentration of uPA is a strong indicator of poor prognosis. In addition to the proteolytic activity of uPA, which degrades the extracellular matrix, uPA also binds to its receptor (uPAR) and controls cell adhesion and migration through the reorganization of actin cytoskeleton. We have recently demonstrated that constitutively active nuclear factor-kappa B (NF-kappaB) is responsible for the increased secretion of uPA and that inhibition of NF-kappaB suppresses secretion of uPA and cell migration of highly invasive cancer cells. Aspirin and other nonsteroidal anti-inflammatory drugs have been recently shown to have a chemopreventive effect in colon and pancreatic cancers. Here we show that aspirin inhibits NF-kappaB, resulting in the suppression of uPA secretion from the highly invasive human prostate cancer cells PC-3. Furthermore, aspirin inhibited migration of PC-3 cells, suggesting an effect on the uPA-uPAR signaling complex. Finally, aspirin suppressed adhesion of PC-3 cells to fibronectin (FN), which binds to an alpha3beta1 integrin receptor, and to vitronectin (VN), which binds to alphavbeta3 integrin receptor. Altogether, our data suggests that aspirin inhibits the formation of uPA-uPAR-FN-alpha3beta1 and uPA-uPAR-VN-alphavbeta3 complexes, resulting in the suppression of cell adhesion and cell motility of the highly invasive prostate cancer cells PC-3. These results indicate that aspirin may contribute directly to reducing invasion and metastasis of prostate cancers by inhibiting cell migration and invasion

Arlt A, Gehrz A, Muerkoster S et al. Role of NF-kappaB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death. *Oncogene* 2003 May 22;22(21):3243-51.
Abstract: Pancreatic cancer is resistant to almost all cytotoxic drugs. Currently, gemcitabine appears to be the only clinically active drug but, because of pre-existing or acquired chemoresistance of most of the tumor cells, it failed to significantly improve the outcome of pancreatic carcinoma patients. The current study examined the relevance of nuclear factor kappaB (NF-kappaB) and PI3K/Akt in the resistance of five pancreatic carcinoma cell lines towards gemcitabine. Treatment for 24 h with gemcitabine (0.04-20 micro M) led to a strong induction of apoptosis in PT45-P1 and T3M4 cells but not in BxPc-3, Capan-1 and PancTu-1 cells. These resistant cell lines exhibited a high basal NF-kappaB activity in contrast to the sensitive cell lines. Furthermore, gemcitabine showed a dose-dependent induction of NF-kappaB. At a dose of 0.04 micro M, gemcitabine still induced apoptosis in the sensitive cell lines, but did not induce NF-kappaB. In addition, NF-kappaB inhibition by MG132, sulfasalazine or the IkappaBalpha super-repressor strongly diminished the resistance against gemcitabine (0.04-20 micro M). In contrast to this obvious correlation between basal NF-kappaB activity and gemcitabine resistance, PI3K/Akt seems not to be involved in gemcitabine resistance of these cell lines. Neither did the basal Akt activity correlate with the sensitivity towards gemcitabine treatment, nor did the inhibition of PI3K/Akt by LY294002 alter gemcitabine-induced apoptosis. These results indicate that constitutive NF-kappaB activity confers resistance against gemcitabine and that modulation of this activity by pharmacological or genetic approaches may have therapeutical potential when combined with gemcitabine in the treatment of pancreatic carcinoma

Muerkoster S, Arlt A, Witt M et al. Usage of the NF-kappaB inhibitor sulfasalazine as sensitizing agent in combined chemotherapy of pancreatic cancer. *Int J Cancer* 2003 April 20;104(4):469-76.
Abstract: Sulfasalazine is commonly used as an anti inflammatory agent and is known as a potent inhibitor of NF-kappaB. Some pancreatic carcinomas are characterized by a constitutively elevated NF-kappaB activity accounting for chemoresistance. To elucidate whether blockade of NF-kappaB activity with sulfasalazine is suitable for overcoming this chemoresistance in vivo, we employed a mouse model with subcutaneously xenotransplanted human Capan-1 pancreatic carcinoma cells. Fourteen days upon tumor inoculation, animals were randomized in 6 groups, receiving no treatment,

treatment with gemcitabine or with etoposide, either alone or in combination with sulfasalazine, or with sulfasalazine alone. Two therapy regimens were given with a 7-day interval in between. Upon treatment with etoposide or gemcitabine alone, tumor sizes were moderately reduced to 65-68% and 50-65%, respectively, as compared to untreated tumors. Sulfasalazine alone only decreased temporarily the tumor sizes. Sulfasalazine in combination with gemcitabine showed only partially higher reduction in tumor sizes than gemcitabine alone, whereas the combination with etoposide reduced significantly the tumor sizes in all experiments (down to 20%). TUNEL-staining showed higher numbers of apoptotic cells in tumors from the combination groups, in particular with etoposide, and proliferation as indicated by Ki67 staining was strongly reduced. Furthermore, combined treatment of sulfasalazine with the cytostatic drugs led to a decreased blood vessel density. Immunohistochemical staining of the activated p65 subunit showed that sulfasalazine treatment abolished the basal NF-kappaB activity in tumor xenografts. These data imply that the well established anti-inflammatory drug sulfasalazine sensitizes pancreatic carcinoma cells to anti cancer drugs, in particular to etoposide in vivo by inhibition of NF-kappaB. This combined chemotherapy offers great potential for improved anti-tumor responses in pancreatic carcinomas

Fujioka S, Sclabas GM, Schmidt C et al. Function of nuclear factor kappaB in pancreatic cancer metastasis. *Clin Cancer Res* 2003 January;9(1):346-54.
Abstract: PURPOSE: We seek to elucidate the role of constitutive nuclear factor kappaB (NFkappaB) activity in human pancreatic cancer cells. We have demonstrated that the transcription factor NFkappaB is activated constitutively in human pancreatic adenocarcinoma and human pancreatic cancer cell lines but not in normal pancreatic tissues or in immortalized/nontumorigenic pancreatic epithelial cells, suggesting that NFkappaB plays a critical role in development of pancreatic adenocarcinoma. EXPERIMENTAL DESIGN: By pooling all of the puromycin resistant clones after inhibitor of nuclear factor-kappaB phosphorylation mutant (IkappaBalphaM) retroviral infection, we generated pancreatic tumor cell lines that express a IkappaBalphaM (S32, 36A) that blocks NFkappaB activity. Inhibition of metastatic phenotype was assayed in an orthotopic nude mouse model. NFkappaB activity was determined by electrophoretic mobility shift assay, and the expression of NFkappaB downstream target genes was analyzed by Northern, Western, and immunohistochemical analyses. RESULTS: We showed that inhibiting constitutive NFkappaB activity by expressing IkappaBalphaM suppresses liver metastasis, but not tumorigenesis, from the metastatic human pancreatic tumor cell line AsPc-1 in an orthotopic nude mouse model. Furthermore, inhibiting NFkappaB activation by expressing IkappaBalphaM significantly reduced in vivo expression of a major proangiogenic molecule, vascular endothelial growth factor, and, hence, decreased neoplastic angiogenesis. Inhibiting NFkappaB activation by expressing IkappaBalphaM and using pharmacologic NFkappaB inhibitor PS-341 also significantly reduced cytokine-induced vascular endothelial growth factor and interleukin-8 expression in AsPc-1 pancreatic cancer cells. CONCLUSION: These results demonstrated that the inhibition of NFkappaB signaling can suppress the angiogenic potential and metastasis of pancreatic cancer, and suggest that the NFkappaB signaling pathway is a potential target for anticancer agents

McCarty MF, Block KI. Preadministration of high-dose salicylates, suppressors of NF-kappaB activation, may increase the chemosensitivity of many cancers: an example of proapoptotic signal modulation therapy. *Integr Cancer Ther* 2006 September;5(3):252-68.
Abstract: NF-kappaB activity is elevated in a high proportion of cancers, particularly advanced cancers that have been treated previously. Cytotoxic treatment selects for such up-regulation inasmuch as NF-kappaB promotes transcription of a large number of proteins that inhibit both the intrinsic and extrinsic pathways of apoptosis; NF-kappaB also boosts expression of mdr1, which expels many drugs from cells. Indeed, high NF-kappaB activity appears to be largely responsible for the chemo- and radioresistance of many cancers. Thus, agents that suppress NF-kappaB activity should be useful as adjuvants to cytotoxic cancer therapy. Of the compounds that are known to be

NF-kappaB antagonists, the most practical for current use may be the nonsteroidal anti-inflammatory drugs aspirin, salicylic acid, and sulindac, each of which binds to and inhibits Ikappa kinase- beta, a central mediator of NF-kappa activation; the low millimolar plasma concentrations of salicylate required for effective inhibition of this kinase in vivo can be achieved with high-dose regimens traditionally used to manage rheumatic disorders. The gastrointestinal toxicity of such regimens could be minimized by using salsalate or enteric-coated sodium salicy-late or by administering misoprostol in conjunction with aspirin therapy. Presumably, best results would be seen if these agents were administered for several days prior to a course of chemo- or radiotherapy, continuing throughout the course. This concept should first be tested in nude mice bearing xenografts of chemoresistant human tumors known to have elevated NF-kappa activity. Ultimately, more complex adjuvant regimens can be envisioned in which salicylates are used in conjunction with other NF-kappa antagonists and/or agents that target other mediators of down-regulated apoptosis in cancer, such as Stat3; coadministration of salicylate and organic selenium may have intriguing potential in this regard. These strategies may also have potential as adjuvants to metronomic chemotherapy, which seeks to suppress angio-genesis by targeting cycling endothelial cells in tumors

Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature* 1998 November 5;396(6706):77-80.
Abstract: NF-kappaB comprises a family of cellular transcription factors that are involved in the inducible expression of a variety of cellular genes that regulate the inflammatory response. NF-kappaB is sequestered in the cytoplasm by inhibitory proteins, I(kappa)B, which are phosphorylated by a cellular kinase complex known as IKK. IKK is made up of two kinases, IKK-alpha and IKK-beta, which phosphorylate I(kappa)B, leading to its degradation and translocation of NF-kappaB to the nucleus. IKK kinase activity is stimulated when cells are exposed to the cytokine TNF-alpha or by overexpression of the cellular kinases MEKK1 and NIK. Here we demonstrate that the anti-inflammatory agents aspirin and sodium salicylate specifically inhibit IKK-beta activity in vitro and in vivo. The mechanism of aspirin and sodium salicylate inhibition is due to binding of these agents to IKK-beta to reduce ATP binding. Our results indicate that the anti-inflammatory properties of aspirin and salicylate are mediated in part by their specific inhibition of IKK-beta, thereby preventing activation by NF-kappaB of genes involved in the pathogenesis of the inflammatory response

Diindolylmethane

Yoon K, Lee SO, Cho SD, Kim K, Khan S, Safe S. Activation of nuclear TR3 (NR4A1) by a diindolylmethane analog induces apoptosis and proapoptotic genes in pancreatic cancer cells and tumors. *Carcinogenesis* 2011 June;32(6):836-42.
Abstract: NR4A1 (Nur77, TR3) is overexpressed in pancreatic tumors and activation of TR3 by 1,1-bis(3'-indolyl)-1-(p-methoxyphenyl)methane (DIM-C-pPhOCH(3)) inhibits cell and tumor growth and induces apoptosis. Microarray analysis demonstrates that in L3.6pL pancreatic cancer cells DIM-C-pPhOCH(3) induces genes associated with metabolism, homeostasis, signal transduction, transcription, stress, transport, immune responses, growth inhibition and apoptosis. Among the most highly induced growth inhibitory and proapoptotic genes including activating transcription factor 3 (ATF3), p21, cystathionase, dual specificity phosphatase 1 and growth differentiation factor 15, RNA interference studies demonstrated that induction of all but the later gene by DIM-C-pPhOCH(3) were TR3-dependent. We also observed that DIM-C-pPhOCH(3) induced Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and induction of TRAIL was ATF3 dependent. Results of this and previous studies demonstrate that TR3 is unique among nuclear receptors since nuclear TR3 is activated or deactivated by diindolylmethane derivatives to induce different apoptotic and growth inhibitory pathways that inhibit pancreatic cancer cell and tumor growth

Ali S, Banerjee S, Schaffert JM, El-Rayes BF, Philip PA, Sarkar FH. Concurrent inhibition of NF-kappaB, cyclooxygenase-2, and epidermal growth factor receptor leads to greater anti-tumor activity in pancreatic cancer. *J Cell Biochem* 2010 May;110(1):171-81.
Abstract: Inactivation of survival pathways such as NF-kappaB, cyclooxygenase (COX-2), or epidermal growth factor receptor (EGFR) signaling individually may not be sufficient for the treatment of advanced pancreatic cancer (PC) as suggested by recent clinical trials. 3,3'-Diindolylmethane (B-DIM) is an inhibitor of NF-kappaB and COX-2 and is a well-known chemopreventive agent. We hypothesized that the inhibition of NF-kappaB and COX-2 by B-DIM concurrently with the inhibition of EGFR by erlotinib will potentiate the anti-tumor effects of cytotoxic drug gemcitabine, which has been tested both in vitro and in vivo. Inhibition of viable cells in seven PC cell lines treated with B-DIM, erlotinib, or gemcitabine alone or their combinations was evaluated using 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Significant inhibition in cell viability was observed in PC cells expressing high levels of COX-2, EGFR, and NF-kappaB proteins. The observed inhibition was associated with an increase in apoptosis as assessed by ELISA. A significant down-regulation in the expression of COX-2, NF-kappaB, and EGFR in BxPC-3, COLO-357, and HPAC cells was observed, suggesting that simultaneous targeting of EGFR, NF-kappaB, and COX-2 is more effective than targeting either signaling pathway separately. Our in vitro results were further supported by in vivo studies showing that B-DIM in combination with erlotinib and gemcitabine was significantly more effective than individual agents. Based on our preclinical in vitro and in vivo results, we conclude that this multi-targeted combination could be developed for the treatment of PC patients whose tumors express high levels of COX-2, EGFR, and NF-kappaB

Banerjee S, Wang Z, Kong D, Sarkar FH. 3,3'-Diindolylmethane enhances chemosensitivity of multiple chemotherapeutic agents in pancreatic cancer. *Cancer Res* 2009 July 1;69(13):5592-600.
Abstract: Clinical management of pancreatic cancer is a major problem, which is in part due to both de novo and acquired resistance to conventional therapeutics. Here, we present in vitro and in vivo preclinical evidence in support of chemosensitization of pancreatic cancer cells by 3,3-diindolylmethane (DIM), a natural compound that can be easily obtained by consuming cruciferous vegetables. DIM pretreatment of pancreatic cancer cells led to a significantly increased apoptosis ($P < 0.01$) with suboptimal concentrations of chemotherapeutic agents (cisplatin, gemcitabine, and oxaliplatin) compared with monotherapy. It is known that resistance to chemotherapy in pancreatic cancer is associated with constitutively activated nuclear factor-kappaB (NF-kappaB), which becomes further activated by chemotherapeutic drugs. Our data provide mechanistic evidence for the first time showing that DIM potentiates the killing of pancreatic cancer cells by down-regulation of constitutive as well as drug-induced activation of NF-kappaB and its downstream genes (Bcl-xL, XIAP, cIAP, and survivin). Most importantly, using an orthotopic animal model, we found reduction in tumor size ($P < 0.001$) when DIM was given in combination with oxaliplatin compared with monotherapy. This was accompanied by loss of phospho-p65 and down-regulation of NF-kappaB activity and its downstream genes (Bcl-xL, survivin, and XIAP), which correlated with reduced cell proliferation (as assessed by Ki-67 immunostaining of tumor specimens) and evidence of apoptosis [as assessed by poly(ADP-ribose) polymerase cleavage and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining]. These results provide strong in vivo evidence in support of our hypothesis that DIM could abrogate chemotherapeutic drug (cisplatin, gemcitabine, and/or oxaliplatin)-induced activation of NF-kappaB, resulting in the chemosensitization of pancreatic tumors to conventional therapeutics

Li Y, VandenBoom TG, Kong D et al. Up-regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. *Cancer Res* 2009 August 15;69(16):6704-12.
Abstract: Pancreatic cancer is the fourth most common cause of cancer death in the United States, and the aggressiveness of pancreatic cancer is in part due to its intrinsic and extrinsic drug resistance characteristics, which are also associated with the acquisition of epithelial-to-mesenchymal transition

(EMT). Emerging evidence also suggests that the processes of EMT are regulated by the expression status of many microRNAs (miRNA), which are believed to function as key regulators of various biological and pathologic processes during tumor development and progression. In the present study, we compared the expression of miRNAs between gemcitabine-sensitive and gemcitabine-resistant pancreatic cancer cells and investigated whether the treatment of cells with "natural agents" [3,3'-diindolylmethane (DIM) or isoflavone] could affect the expression of miRNAs. We found that the expression of miR-200b, miR-200c, let-7b, let-7c, let-7d, and let-7e was significantly down-regulated in gemcitabine-resistant cells, which showed EMT characteristics such as elongated fibroblastoid morphology, lower expression of epithelial marker E-cadherin, and higher expression of mesenchymal markers such as vimentin and ZEB1. Moreover, we found that reexpression of miR-200 by transfection studies or treatment of gemcitabine-resistant cells with either DIM or isoflavone resulted in the down-regulation of ZEB1, slug, and vimentin, which was consistent with morphologic reversal of EMT phenotype leading to epithelial morphology. These results provide experimental evidence, for the first time, that DIM and isoflavone could function as miRNA regulators leading to the reversal of EMT phenotype, which is likely to be important for designing novel therapies for pancreatic cancer

Abdelrahim M, Newman K, Vanderlaag K, Samudio I, Safe S. 3,3'-diindolylmethane (DIM) and its derivatives induce apoptosis in pancreatic cancer cells through endoplasmic reticulum stress-dependent upregulation of DR5. *Carcinogenesis* 2006 April;27(4):717-28.
Abstract: 3,3'-Diindolylmethane (DIM), ring-substituted DIMs and 1,1-bis(3'-indolyl)-1-(p-substitutedphenyl)methanes (C-DIMs) inhibit growth of Panc-1 and Panc-28 pancreatic cancer cells. Although DIMs (diarylmethanes) and selected C-DIMs (triarylmethanes), such as the p-t-butyl derivative (DIM-C-pPhtBu), activate the aryl hydrocarbon receptor and peroxisome proliferator-activated receptor gamma, respectively, this study shows that both DIM and DIM-C-pPhtBu induce common receptor-independent pathways. Both DIM and DIM-C-pPhtBu increased endoplasmic reticulum (ER) staining and ER calcium release in Panc-1 cells, and this was accompanied by increased expression of glucose related protein 78 and C/EBP homologous transcription factor (CHOP/GADD153) proteins. Similar results were observed after treatment with thapsigargin (Tg), a prototypical inducer of ER stress. The subsequent downstream effects of DIM/DIM-C-pPhtBu- and Tg-induced ER stress included CHOP-dependent induction of death receptor DR5 and subsequent cleavage of caspase 8, caspase 3, Bid and PARP. Activation of both receptor-dependent and receptor-independent (ER stress) pathways by DIM and DIM-C-pPhtBu in pancreatic cancer cells enhances the efficacy and potential clinical importance of these compounds for cancer chemotherapeutic applications

Tocotrienols

Husain K, Francois RA, Yamauchi T, Perez M, Sebti SM, Malafa MP. Vitamin E delta-tocotrienol augments the antitumor activity of gemcitabine and suppresses constitutive NF-kappaB activation in pancreatic cancer. *Mol Cancer Ther* 2011 December;10(12):2363-72.
Abstract: The NF-kappaB transcription factor functions as a crucial regulator of cell survival and chemoresistance in pancreatic cancer. Recent studies suggest that tocotrienols, which are the unsaturated forms of vitamin E, are a promising class of anticancer compounds that inhibit the growth and survival of many cancer cells, including pancreatic cancer. Here, we show that tocotrienols inhibited NF-kappaB activity and the survival of human pancreatic cancer cells in vitro and in vivo. Importantly, we found the bioactivity of the four natural tocotrienol compounds (alpha-, beta-, delta-, and gamma-tocotrienol) to be directly related to their ability to suppress NF-kappaB activity in vitro and in vivo. The most bioactive tocotrienol for pancreatic cancer, delta-tocotrienol, significantly enhanced the efficacy of gemcitabine to inhibit pancreatic cancer growth and survival in vitro and in vivo. Moreover, we found that delta-tocotrienol augmentation of gemcitabine activity in pancreatic

cancer cells and tumors is associated with significant suppression of NF-kappaB activity and the expression of NF-kappaB transcriptional targets (Bcl-X(L), X-linked inhibitor of apoptosis, and survivin). Our study represents the first comprehensive preclinical evaluation of the activity of natural vitamin E compounds in pancreatic cancer. Given these results, we are conducting a phase I trial of delta-tocotrienol in patients with pancreatic cancer using pancreatic tumor cell survival and NF-kappaB signaling components as intermediate biomarkers. Our data also support future clinical investigation of delta-tocotrienol to augment gemcitabine activity in pancreatic cancer

Shin-Kang S, Ramsauer VP, Lightner J et al. Tocotrienols inhibit AKT and ERK activation and suppress pancreatic cancer cell proliferation by suppressing the ErbB2 pathway. *Free Radic Biol Med* 2011 September 15;51(6):1164-74.
Abstract: Tocotrienols are members of the vitamin E family but, unlike tocopherols, possess an unsaturated isoprenoid side chain that confers superior anti-cancer properties. The ability of tocotrienols to selectively inhibit the HMG-CoA reductase pathway through posttranslational degradation and to suppress the activity of transcription factor NF-kappaB could be the basis for some of these properties. Our studies indicate that gamma- and delta-tocotrienols have potent antiproliferative activity in pancreatic cancer cells (Panc-28, MIA PaCa-2, Panc-1, and BxPC-3). Indeed both tocotrienols induced cell death (>50%) by the MTT cell viability assay in all four pancreatic cancer cell lines. We also examined the effects of the tocotrienols on the AKT and the Ras/Raf/MEK/ERK signaling pathways by Western blotting analysis. gamma- and delta-tocotrienol treatment of cells reduced the activation of ERK MAP kinase and that of its downstream mediator RSK (ribosomal protein S6 kinase) in addition to suppressing the activation of protein kinase AKT. Suppression of activation of AKT by gamma-tocotrienol led to downregulation of p-GSK-3beta and upregulation accompanied by nuclear translocation of Foxo3. These effects were mediated by the downregulation of Her2/ErbB2 at the messenger level. Tocotrienols but not tocopherols were able to induce the observed effects. Our results suggest that the tocotrienol isoforms of vitamin E can induce apoptosis in pancreatic cancer cells through the suppression of vital cell survival and proliferative signaling pathways such as those mediated by the PI3-kinase/AKT and ERK/MAP kinases via downregulation of Her2/ErbB2 expression. The molecular components for this mechanism are not completely elucidated and need further investigation

Kunnumakkara AB, Sung B, Ravindran J et al. {Gamma}-tocotrienol inhibits pancreatic tumors and sensitizes them to gemcitabine treatment by modulating the inflammatory microenvironment. *Cancer Res* 2010 November 1;70(21):8695-705.
Abstract: Pancreatic cancers generally respond poorly to chemotherapy, prompting a need to identify agents that could sensitize tumors to treatment. In this study, we investigated the response of human pancreatic cells to gamma-tocotrienol (gamma-T3), a novel, unsaturated form of vitamin E found in palm oil and rice bran oil, to determine whether it could potentiate the effects of gemcitabine, a standard of care in clinical treatment of pancreatic cancer. gamma-T3 inhibited the in vitro proliferation of pancreatic cancer cell lines with variable p53 status and potentiated gemcitabine-induced apoptosis. These effects correlated with an inhibition of NF-kappaB activation by gamma-T3 and a suppression of key cellular regulators including cyclin D1, c-Myc, cyclooxygenase-2 (COX-2), Bcl-2, cellular inhibitor of apoptosis protein, survivin, vascular endothelial growth factor (VEGF), ICAM-1, and CXCR4. In an orthotopic nude mouse model of human pancreatic cancer, p.o. administration of gamma-T3 inhibited tumor growth and enhanced the antitumor properties of gemcitabine. Immunohistochemical analysis indicated a correlation between tumor growth inhibition and reduced expression of Ki-67, COX-2, matrix metalloproteinase-9 (MMP-9), NF-kappaB p65, and VEGF in the tissue. Combination treatment also downregulated NF-kappaB activity along with the NF-kappaB-regulated gene products, such as cyclin D1, c-Myc, VEGF, MMP-9, and CXCR4. Consistent with an enhancement of tumor apoptosis, caspase activation was observed in tumor tissues. Overall, our

findings suggest that gamma-T3 can inhibit the growth of human pancreatic tumors and sensitize them to gemcitabine by suppressing NF-kappaB-mediated inflammatory pathways linked to tumorigenesis

Hussein D, Mo H. d-delta-Tocotrienol-mediated suppression of the proliferation of human PANC-1, MIA PaCa-2, and BxPC-3 pancreatic carcinoma cells. *Pancreas* 2009 May;38(4):e124-e136.
Abstract: OBJECTIVE: The rate-limiting activity of the mevalonate pathway, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, provides intermediates essential for growth. Competitive inhibitors of HMG CoA reductase, such as the statins, and down-regulators of reductase, such as the tocotrienols, suppress tumor growth. We evaluated the impact of d-delta-tocotrienol, the most potent vitamin E isomer, on human MIA PaCa-2 and PANC-1 pancreatic carcinoma cells and BxPC-3 pancreatic ductal adenocarcinoma cells. METHODS: Cell proliferation was measured by using CellTiter 96 Aqueous One Solution (Promega, Madison, Wis). Cell cycle distribution was determined by flow cytometry. Apoptosis was evaluated by Annexin V staining and fluorescence microscopy after dual staining with acridine orange and ethidium bromide. RESULTS: d-delta-Tocotrienol induced concentration-dependent suppression of cell proliferation with 50% inhibitory concentrations of 28 (6) micromol/L (MIA PaCa-2), 35 (7) micromol/L (PANC-1), and 35 (8) microL (BxPC-3), respectively. These effects are attributable to cell cycle arrest at the G1 phase and apoptosis. Mevalonate attenuated d-delta-tocotrienol-mediated growth inhibition. A physiologically attainable blend of d-delta-tocotrienol and lovastatin synergistically suppressed the proliferation of MIA PaCa-2 cells. CONCLUSIONS: Suppression of mevalonate pathway activities, be it by modulators of HMG CoA reductase (statins, tocotrienols, and farnesol), farnesyl transferase (farnesyl transferase inhibitors), and/or mevalonate pyrophosphate decarboxylase (phenylacetate) activity, may have a potential in pancreatic cancer chemotherapy

Low-Dose Naltrexone

Donahue RN, McLaughlin PJ, Zagon IS. Low-dose naltrexone targets the opioid growth factor-opioid growth factor receptor pathway to inhibit cell proliferation: mechanistic evidence from a tissue culture model. *Exp Biol Med (Maywood)* 2011 September 1;236(9):1036-50.
Abstract: Naltrexone (NTX) is an opioid antagonist that inhibits or accelerates cell proliferation in vivo when utilized in a low (LDN) or high (HDN) dose, respectively. The mechanism of opioid antagonist action on growth is not well understood. We established a tissue culture model of LDN and HDN using short-term and continuous opioid receptor blockade, respectively, in human ovarian cancer cells, and found that the duration of opioid receptor blockade determines cell proliferative response. The alteration of growth by NTX also was detected in cells representative of pancreatic, colorectal and squamous cell carcinomas. The opioid growth factor (OGF; [Met(5)]-enkephalin) and its receptor (OGFr) were responsible for mediating the action of NTX on cell proliferation. NTX upregulated OGF and OGFr at the translational but not at the transcriptional level. The mechanism of inhibition by short-term NTX required p16 and/or p21 cyclin-dependent inhibitory kinases, but was not dependent on cell survival (necrosis, apoptosis). Sequential administration of short-term NTX and OGF had a greater inhibitory effect on cell proliferation than either agent alone. Given the parallels between short-term NTX in vitro and LDN in vivo, we now demonstrate at the molecular level that the OGF-OGFr axis is a common pathway that is essential for the regulation of cell proliferation by NTX

Berkson BM, Rubin DM, Berkson AJ. Revisiting the ALA/N (alpha-lipoic acid/low-dose naltrexone) protocol for people with metastatic and nonmetastatic pancreatic cancer: a report of 3 new cases. *Integr Cancer Ther* 2009 December;8(4):416-22.
Abstract: The authors, in a previous article, described the long-term survival of a man with pancreatic cancer and metastases to the liver, treated with intravenous alpha-lipoic acid and oral low-dose

naltrexone (ALA/N) without any adverse effects. He is alive and well 78 months after initial presentation. Three additional pancreatic cancer case studies are presented in this article. At the time of this writing, the first patient, GB, is alive and well 39 months after presenting with adenocarcinoma of the pancreas with metastases to the liver. The second patient, JK, who presented to the clinic with the same diagnosis was treated with the ALA/N protocol and after 5 months of therapy, PET scan demonstrated no evidence of disease. The third patient, RC, in addition to his pancreatic cancer with liver and retroperitoneal metastases, has a history of B-cell lymphoma and prostate adenocarcinoma. After 4 months of the ALA/N protocol his PET scan demonstrated no signs of cancer. In this article, the authors discuss the poly activity of ALA: as an agent that reduces oxidative stress, its ability to stabilize NF(k)B, its ability to stimulate pro-oxidant apoptosic activity, and its discriminative ability to discourage the proliferation of malignant cells. In addition, the ability of lowdose naltrexone to modulate an endogenous immune response is discussed. This is the second article published on the ALA/N protocol and the authors believe the protocol warrants clinical trial

Berkson BM, Rubin DM, Berkson AJ. The long-term survival of a patient with pancreatic cancer with metastases to the liver after treatment with the intravenous alpha-lipoic acid/low-dose naltrexone protocol. *Integr Cancer Ther* 2006 March;5(1):83-9.
Abstract: The authors describe the long-term survival of a patient with pancreatic cancer without any toxic adverse effects. The treatment regimen includes the intravenous alpha-lipoic acid and low-dose naltrexone (ALA-N) protocol and a healthy lifestyle program. The patient was told by a reputable university oncology center in October 2002 that there was little hope for his survival. Today, January 2006, however, he is back at work, free from symptoms, and without appreciable progression of his malignancy. The integrative protocol described in this article may have the possibility of extending the life of a patient who would be customarily considered to be terminal. The authors believe that life scientists will one day develop a cure for metastatic pancreatic cancer, perhaps via gene therapy or another biological platform. But until such protocols come to market, the ALA-N protocol should be studied and considered, given its lack of toxicity at levels reported. Several other patients are on this treatment protocol and appear to be doing well at this time

Zagon IS, McLaughlin PJ. Targeting opioidergic pathways as a novel biological treatment for advanced pancreatic cancer. *Expert Rev Gastroenterol Hepatol* 2012 April;6(2):133-5.

Smith JP, Bingaman SI, Mauger DT, Harvey HH, Demers LM, Zagon IS. Opioid growth factor improves clinical benefit and survival in patients with advanced pancreatic cancer. *Open Access J Clin Trials* 2010 March 1;2010(2):37-48.
Abstract: BACKGROUND: Advanced pancreatic cancer carries the poorest prognosis of all gastrointestinal malignancies. Once the tumor has spread beyond the margins of the pancreas, chemotherapy is the major treatment modality offered to patients; however, chemotherapy does not significantly improve survival. OBJECTIVE: Opioid growth factor (OGF; [Met(5)]-enkephalin) is a natural peptide that has been shown to inhibit growth of pancreatic cancer in cell culture and in nude mice. The purpose of this study was to evaluate the effects of OGF biotherapy on subjects with advanced pancreatic cancer who failed chemotherapy. METHODS: In a prospective phase II open-labeled clinical trial, 24 subjects who failed standard chemotherapy for advanced pancreatic cancer were treated weekly with OGF 250 microg/kg intravenously. Outcomes measured included clinical benefit, tumor response by radiographic imaging, quality of life, and survival. RESULTS: Clinical benefit response was experienced by 53% of OGF-treated patients compared to historical controls of 23.8% and 4.8% for gemcitabine and 5-fluorouracil (5-FU), respectively. Of the subjects surviving more than eight weeks, 62% showed either a decrease or stabilization in tumor size by computed tomography. The median survival time for OGF-treated patients was three times that of untreated patients (65.5 versus 21 days, $p < 0.001$). No adverse effects on hematologic or chemistry parameters were noted, and quality of life surveys suggested improvement with OGF. LIMITATIONS:

Measurements other than survival were not allowed in control patients, and clinical benefit comparisons were made to historical controls. CONCLUSION: OGF biotherapy improves the clinical benefit and prolongs survival in patients with pancreatic cancer by stabilizing disease or slowing progression. The effects of OGF did not adversely alter patient quality of life. The use of OGF biotherapy at earlier stages of disease or in combination with other chemotherapeutic agents may further improve the outcome of this malignancy

Cheng F, McLaughlin PJ, Verderame MF, Zagon IS. The OGF-OGFr axis utilizes the p21 pathway to restrict progression of human pancreatic cancer. *Mol Cancer* 2008;7:5.
Abstract: BACKGROUND: Pancreatic cancer is the 4th leading cause of death from cancer in the U.S. The opioid growth factor (OGF; [Met5]-enkephalin) and the OGF receptor form an inhibitory growth regulatory system involved in the pathogenesis and treatment of pancreatic cancer. The OGF-OGFr axis influences the G0/G1 phase of the cell cycle. In this investigation, we elucidate the pathway of OGF in the cell cycle. RESULTS: Using BxPC-3 cells, OGF decreased phosphorylation of retinoblastoma (Rb) protein without changing total Rb. This change was correlated with reduced cyclin-dependent kinase protein (Cdk) 2 kinase activity, but not total Cdk2. OGF treatment increased cyclin-dependent kinase inhibitor (CKI) p21 protein expression in comparison to controls, as well levels of p21 complexed with Cdk2. Naloxone abolished the increased expression of p21 protein by OGF, suggesting a receptor-mediated activity. p21 specific siRNAs blocked OGF's repressive action on proliferation in BxPC-3, PANC-1, and Capan-2 cells; cells transfected with negative control siRNA had no alteration in p21 expression, and therefore were inhibited by OGF. CONCLUSION: These data are the first to reveal that the target of cell proliferative inhibitory action of OGF in human pancreatic cancer is a p21 CKI pathway, expanding strategies for diagnosis and treatment of these neoplasias

Zagon IS, Jaglowski JR, Verderame MF, Smith JP, Leure-Dupree AE, McLaughlin PJ. Combination chemotherapy with gemcitabine and biotherapy with opioid growth factor (OGF) enhances the growth inhibition of pancreatic adenocarcinoma. *Cancer Chemother Pharmacol* 2005 November;56(5):510-20.
Abstract: Gemcitabine is the standard of care for advanced pancreatic neoplasia, and exerts its effect through inhibition of DNA synthesis. However, gemcitabine has limited survival benefits. Opioid growth factor (OGF) is an autocrine-produced peptide that interacts with the nuclear receptor, OGFr, to inhibit cell proliferation but is not cytotoxic or apoptotic. The present study was designed to examine whether a combination of chemotherapy with gemcitabine and biotherapy with OGF is more effective than either agent alone in inhibiting pancreatic cancer growth in vitro and in vivo. The combination of OGF (10(-6) M) and gemcitabine (10(-8) M) reduced MIA PaCa-2 cell number from control levels by 46% within 48 h, and resulted in a growth inhibition greater than that of the individual compounds. OGF in combination with 5-fluorouracil also depressed cell growth more than either agent alone. The action of OGF, but not gemcitabine, was mediated by a naloxone-sensitive receptor, and was completely reversible. OGF, but no other endogenous or exogenous opioids, altered pancreatic cancer growth in tissue culture. The combination of OGF and gemcitabine also repressed the growth of another pancreatic cancer cell line, PANC-1. MIA PaCa-2 cells transplanted into athymic mice received 10 mg/kg OGF daily, 120 mg/kg gemcitabine every 3 days; 10 mg/kg OGF daily and 120 mg/kg gemcitabine every 3rd day, or 0.1 ml of sterile saline daily. Tumor incidence, and latency times to tumor appearance, of mice receiving combined therapy with OGF and gemcitabine, were significantly decreased from those of the control, OGF, and gemcitabine groups. Tumor volumes in the OGF, gemcitabine, and OGF/gemcitabine groups were markedly decreased from controls beginning on days 14, 12, and 8, respectively, after tumor cell inoculation. Tumor weight and tumor volume were reduced from control levels by 36-85% in the OGF and/or gemcitabine groups on day 45 (date of termination), and the group of mice exposed to a combination of OGF and gemcitabine had decreases in tumor size of 70% and 63% from the OGF or the gemcitabine alone groups, respectively. This preclinical evidence shows that combined chemotherapy (e.g. gemcitabine) and biotherapy (OGF) provides an enhanced therapeutic benefit for pancreatic cancer

Macrophage Activating Factor - GcMAF

Kisker O, Onizuka S, Becker CM et al. Vitamin D binding protein-macrophage activating factor (DBP-maf) inhibits angiogenesis and tumor growth in mice. *Neoplasia* 2003 January;5(1):32-40.
Abstract: We have isolated a selectively deglycosylated form of vitamin D binding protein (DBP-maf) generated from systemically available DBP by a human pancreatic cancer cell line. DBP-maf is antiproliferative for endothelial cells and antiangiogenic in the chorioallantoic membrane assay. DBP-maf administered daily was able to potently inhibit the growth of human pancreatic cancer in immune compromised mice (T/C=0.09). At higher doses, DBP-maf caused tumor regression. Histological examination revealed that treated tumors had a higher number of infiltrating macrophages as well as reduced microvessel density, and increased levels of apoptosis relative to untreated tumors. Taken together, these data suggest that DBP-maf is an antiangiogenic molecule that can act directly on endothelium as well as stimulate macrophages to attack both the endothelial and tumor cell compartment of a growing malignancy

Intravenous Ascorbate Therapy

Espey MG, Chen P, Chalmers B et al. Pharmacologic ascorbate synergizes with gemcitabine in preclinical models of pancreatic cancer. *Free Radic Biol Med* 2011 June 1;50(11):1610-9.
Abstract: Conventional treatment approaches have had little impact on the course of pancreatic cancer, which has the highest fatality rate among cancers. Gemcitabine, the primary therapeutic agent for pancreatic carcinoma, produces minimal survival benefit as a single agent. Therefore, numerous efforts have focused on gemcitabine combination treatments. Using a ratio design, this study established that combining pharmacologically achievable concentrations of ascorbate with gemcitabine resulted in a synergistic cytotoxic response in eight pancreatic tumor cell lines. Sensitization was evident regardless of inherent gemcitabine resistance and epithelial-mesenchymal phenotype. Our analysis suggested that the promiscuous oxidative actions of H(2)O(2) derived from pharmacologic ascorbate can culminate in synergism independent of the cancer cell's underlying phenotype and resistance to gemcitabine monotherapy. Gemcitabine-ascorbate combinations administered to mice bearing pancreatic tumor xenografts consistently enhanced inhibition of growth compared to gemcitabine alone, produced 50% growth inhibition in a tumor type not responsive to gemcitabine, and demonstrated a gemcitabine dose-sparing effect. These data support the testing of pharmacologic ascorbate in adjunctive treatments for cancers prone to high failure rates with conventional therapeutic regimens, such as pancreatic cancer

Chen Q, Espey MG, Sun AY et al. Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice. *Proc Natl Acad Sci U S A* 2008 August 12;105(32):11105-9.
Abstract: Ascorbic acid is an essential nutrient commonly regarded as an antioxidant. In this study, we showed that ascorbate at pharmacologic concentrations was a prooxidant, generating hydrogen-peroxide-dependent cytotoxicity toward a variety of cancer cells in vitro without adversely affecting normal cells. To test this action in vivo, normal oral tight control was bypassed by parenteral ascorbate administration. Real-time microdialysis sampling in mice bearing glioblastoma xenografts showed that a single pharmacologic dose of ascorbate produced sustained ascorbate radical and hydrogen peroxide formation selectively within interstitial fluids of tumors but not in blood. Moreover, a regimen of daily pharmacologic ascorbate treatment significantly decreased growth rates of ovarian ($P < 0.005$),

pancreatic ($P < 0.05$), and glioblastoma ($P < 0.001$) tumors established in mice. Similar pharmacologic concentrations were readily achieved in humans given ascorbate intravenously. These data suggest that ascorbate as a prodrug may have benefits in cancers with poor prognosis and limited therapeutic options

Cullen JJ, Spitz DR, Buettner GR. Comment on "Pharmacologic ascorbate synergizes with gemcitabine in preclinical models of pancreatic cancer," i.e., all we are saying is, give C a chance. *Free Radic Biol Med* 2011 June 15;50(12):1726-7.

Chen Q, Espey MG, Sun AY et al. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo. *Proc Natl Acad Sci U S A* 2007 May 22;104(21):8749-54.
Abstract: Ascorbate (ascorbic acid, vitamin C), in pharmacologic concentrations easily achieved in humans by i.v. administration, selectively kills some cancer cells but not normal cells. We proposed that pharmacologic ascorbate is a prodrug for preferential steady-state formation of ascorbate radical (Asc(*-)) and H(2)O(2) in the extracellular space compared with blood. Here we test this hypothesis in vivo. Rats were administered parenteral (i.v. or i.p.) or oral ascorbate in typical human pharmacologic doses (approximately 0.25-0.5 mg per gram of body weight). After i.v. injection, ascorbate baseline concentrations of 50-100 microM in blood and extracellular fluid increased to peaks of >8 mM. After i.p. injection, peaks approached 3 mM in both fluids. By gavage, the same doses produced ascorbate concentrations of <150 microM in both fluids. In blood, Asc(*-) concentrations measured by EPR were undetectable with oral administration and always <50 nM with parenteral administration, even when corresponding ascorbate concentrations were >8 mM. After parenteral dosing, Asc(*-) concentrations in extracellular fluid were 4- to 12-fold higher than those in blood, were as high as 250 nM, and were a function of ascorbate concentrations. By using the synthesized probe peroxyxanthone, H(2)O(2) in extracellular fluid was detected only after parenteral administration of ascorbate and when Asc(*-) concentrations in extracellular fluid exceeded 100 nM. The data show that pharmacologic ascorbate is a prodrug for preferential steady-state formation of Asc(*-) and H(2)O(2) in the extracellular space but not blood. These data provide a foundation for pursuing pharmacologic ascorbate as a prooxidant therapeutic agent in cancer and infections

Chen Q, Espey MG, Krishna MC et al. Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. *Proc Natl Acad Sci U S A* 2005 September 20;102(38):13604-9.
Abstract: Human pharmacokinetics data indicate that i.v. ascorbic acid (ascorbate) in pharmacologic concentrations could have an unanticipated role in cancer treatment. Our goals here were to test whether ascorbate killed cancer cells selectively, and if so, to determine mechanisms, using clinically relevant conditions. Cell death in 10 cancer and 4 normal cell types was measured by using 1-h exposures. Normal cells were unaffected by 20 mM ascorbate, whereas 5 cancer lines had EC(50) values of <4 mM, a concentration easily achievable i.v. Human lymphoma cells were studied in detail because of their sensitivity to ascorbate (EC(50) of 0.5 mM) and suitability for addressing mechanisms. Extracellular but not intracellular ascorbate mediated cell death, which occurred by apoptosis and pyknosis/necrosis. Cell death was independent of metal chelators and absolutely dependent on H(2)O(2) formation. Cell death from H(2)O(2) added to cells was identical to that found when H(2)O(2) was generated by ascorbate treatment. H(2)O(2) generation was dependent on ascorbate concentration, incubation time, and the presence of 0.5-10% serum, and displayed a linear relationship with ascorbate radical formation. Although ascorbate addition to medium generated H(2)O(2), ascorbate addition to blood generated no detectable H(2)O(2) and only trace detectable ascorbate radical. Taken together, these data indicate that ascorbate at concentrations achieved only by i.v. administration may be a pro-drug for formation of H(2)O(2), and that blood can be a delivery system of the pro-drug to tissues. These findings give plausibility to i.v. ascorbic acid in cancer treatment, and have unexpected implications for treatment of infections where H(2)O(2) may be beneficial

Celecoxib

Hill R, Li Y, Tran LM et al. Cell Intrinsic Role of COX-2 in Pancreatic Cancer Development. *Mol Cancer Ther* 2012 October;11(10):2127-37.
Abstract: COX-2 is upregulated in pancreatic ductal adenocarcinomas (PDAC). However, how COX-2 promotes PDAC development is unclear. While previous studies have evaluated the efficacy of COX-2 inhibition via the use of nonsteroidal anti-inflammatory drugs (NSAID) or the COX-2 inhibitor celecoxib in PDAC models, none have addressed the cell intrinsic versus microenvironment roles of COX-2 in modulating PDAC initiation and progression. We tested the cell intrinsic role of COX-2 in PDAC progression using both loss-of-function and gain-of-function approaches. Cox-2 deletion in Pdx1+ pancreatic progenitor cells significantly delays the development of PDAC in mice with K-ras activation and Pten haploinsufficiency. Conversely, COX-2 overexpression promotes early onset and progression of PDAC in the K-ras mouse model. Loss of PTEN function is a critical factor in determining lethal PDAC onset and overall survival. Mechanistically, COX-2 overexpression increases p-AKT levels in the precursor lesions of Pdx1(+); K-ras(G12D)(/+); Pten(lox)(/+) mice in the absence of Pten LOH. In contrast, Cox-2 deletion in the same setting diminishes p-AKT levels and delays cancer progression. These data suggest an important cell intrinsic role for COX-2 in tumor initiation and progression through activation of the PI3K/AKT pathway. PDAC that is independent of intrinsic COX-2 expression eventually develops with decreased FKBP5 and increased GRP78 expression, two alternate pathways leading to AKT activation. Together, these results support a cell intrinsic role for COX-2 in PDAC development and suggest that while anti-COX-2 therapy may delay the development and progression of PDAC, mechanisms known to increase chemoresistance through AKT activation must also be overcome. Mol Cancer Ther; 11(10); 2127-37. (c)2012 AACR

Ding X, Zhu C, Qiang H, Zhou X, Zhou G. Enhancing antitumor effects in pancreatic cancer cells by combined use of COX-2 and 5-LOX inhibitors. *Biomed Pharmacother* 2011 October;65(7):486-90.
Abstract: Cyclooxygenase (COX)-2 and lipoxygenase (LOX)-5 are involved in carcinogenesis of pancreatic cancer. COX-2 inhibitor celecoxib displays inhibitory effects in pancreatic cancer cell growth. Recently, it has been reported that COX-2 inhibitor may not be able to suppress pancreatic tumor growth in vivo and its application is further limited by untoward side effects. The present study provides evidence that combined use of celecoxib and 5-LOX inhibitor MK886 markedly suppresses pancreatic tumor cell growth in vitro. Compared to the single inhibitor treatment, dual treatment with celecoxib and MK886 exerted additive antitumor effects in pancreatic tumor cells. We found that MK886 reversed celecoxib-induced increases in 5-LOX gene expression and Erk1/2 activation in pancreatic tumor cells. Moreover, Dual treatment of pancreatic tumor cells with celecoxib and MK886 inhibited the levels of LBT4 receptor BLT1 and vascular endothelial growth factor. Our results imply that combined use of celecoxib and MK886 might be an effective way to treat clinical patients with pancreatic cancer

Arjona-Sanchez A, Ruiz-Rabelo J, Perea MD et al. Effects of capecitabine and celecoxib in experimental pancreatic cancer. *Pancreatology* 2010;10(5):641-7.
Abstract: INTRODUCTION: Pancreatic cancer is a major health problem because of its aggressiveness and the lack of effective systemic therapies. The aim of the study was the identification of beneficial properties of combined celecoxib and capecitabine treatment during an experimental pancreatic cancer model. METHODS: N-nitrosobis (2-oxopropyl)amine (BOP) was used as a tumoral agent for 12 weeks. Celecoxib and capecitabine were administered either as monotherapy or combined 12 weeks after cancer induction for a period of 24 weeks. The presence of well-developed or moderate adenocarcinoma was evaluated in the pancreas. Several markers of stress, such as lipoperoxides, reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GHS-Px) were determined. RESULTS: BOP induced the presence of pancreatic tumors associated with a rise in lipoperoxides and the reduction of the antioxidant status

in the pancreas. The administration of celecoxib and capecitabine reduced the number of animals with tumors (33 and 66%, respectively). This antitumoral effect was associated with a recovery of GSH, SOD and CAT activity in the pancreas of BOP-treated animals. The combined treatment exerted a synergic antitumoral effect and reduced pancreatic oxidative stress. CONCLUSION: The combined administration of celecoxib and capecitabine exerted a synergistic antitumoral effect and increased the antioxidant status restoration in pancreatic cancer

Padillo FJ, Ruiz-Rabelo JF, Cruz A et al. Melatonin and celecoxib improve the outcomes in hamsters with experimental pancreatic cancer. *J Pineal Res* 2010 October;49(3):264-70.
Abstract: Pancreatic cancer is a major health problem because of the aggressiveness of the disease and the lack of effective systemic therapies. Melatonin (MEL) has antioxidant activity and prevents experimental genotoxicity. The specific inhibitor of cyclooxygenase-2 (COX-2), celecoxib (CEL), increases the efficacy of chemoradiotherapy in advanced pancreatic cancer. The objective of the study was the comparison and synergic effect of MEL and CEL during either the induction or progression phases of the tumor process, measuring parameters of oxidative stress, number of tumor nodules and survival of animals with pancreatic cancer. Pancreatic cancer was induced by N-nitrosobis (2-oxopropyl)amine) (BOP) in Syrian hamsters. Melatonin and/or CEL were administered during the induction, postinduction as well as during both phases. The presence of tumor nodules were observed macroscopically in pancreatic and splenic areas, and the levels of lipoperoxides (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in pancreatic tissue were measured. The increases in tumor nodules and LPO as well as the reductions in GSH and enzymatic antioxidants in the pancreas induced by BOP were related to a lower survival rate of animals. The administration of MEL exerted a more potent beneficial effect than CEL treatment on the reduction in tumor nodules, oxidative stress and death of experimental BOP-treated animals. The combined treatment only exerted a synergistic beneficial effect when administered during the induction phase. Melatonin by itself had significant beneficial actions in improving the survival of hamsters

Lipton A, Campbell-Baird C, Witters L, Harvey H, Ali S. Phase II trial of gemcitabine, irinotecan, and celecoxib in patients with advanced pancreatic cancer. *J Clin Gastroenterol* 2010 April;44(4):286-8.
Abstract: GOALS AND BACKGROUND: Cyclooxygenase-2 (COX-2) has been shown to be expressed in a variety of tumors including pancreatic cancer. The combination of gemcitabine and irinotecan is active in pancreatic cancer. The purpose of this study is to determine the toxicity and response rate to the addition of the selective oral COX-2 inhibitor, celecoxib, to gemcitabine and irinotecan in patients with inoperable pancreatic cancer. STUDY: Twenty-one patients with previously untreated inoperable pancreatic cancer were entered on this trial. Seven patients had localized disease, 8 had metastatic disease, and 6 patients were inevaluable. RESULTS: Twenty percent of the patients had a partial response and 80% of the patients had a stable response with a median response rate of 9 months. The median overall survival was 18 months with 80% of the patients achieving 1-year survival and 20% achieving 2-year survival. Using the FACT-PA scale to measure the quality of life (QOL), 13 of the 15 patients reported an improvement in their QOL and 2 patients reported no change. The median CA19-9 levels for the 13 patients with measurable CA19-9 values, decreased by 71% by cycle 2. Adverse events were acceptable and included neutropenia, thrombocytopenia, nausea, fatigue, and anemia. CONCLUSIONS: The combination of gemcitabine, irinotecan, and celecoxib is an active therapy for inoperable pancreatic cancer. A marked reduction in CA19-9 is observed in all evaluable patients by cycle 2. Toxicity is tolerable and a majority of patients reported a decrease in pain and a significant improvement in their QOL

Pino MS, Milella M, Gelibter A et al. Capecitabine and celecoxib as second-line treatment of advanced pancreatic and biliary tract cancers. *Oncology* 2009;76(4):254-61.

Abstract: OBJECTIVE: An increasing number of patients with advanced pancreatic or biliary tract cancer who progress after a gemcitabine-containing regimen are candidates for further chemotherapy. We therefore evaluated a fully oral regimen of capecitabine and celecoxib (CapCel) as second-line treatment in these patients. METHODS: Thirty-five patients with documented progressive disease after first-line treatment were enrolled. Capecitabine was administered at a dose of 1,000 mg/m(2) b.i.d. for 2 consecutive weeks followed by 1 week of rest; celecoxib was given continuously at 200 mg b.i.d. Progression-free survival at 3 months was the primary study endpoint. RESULTS: The CapCel combination was associated with an overall response rate of 9% and median survival duration of 19 weeks. Sixty percent of patients were free from progression 3 months after the start of treatment. Multivariate analysis identified a positive clinical benefit response and a decline in CA 19.9 serum levels >25% compared with baseline levels as independent predictors of prolonged survival. The treatment protocol was well tolerated with negligible hematological toxicity. The most common grade 3 non-hematological toxicities were hypertransaminasemia, diarrhea and asthenia. CONCLUSIONS: The CapCel combination is a safe treatment option with moderate activity in patients with pancreatic/biliary tract cancer after failure of a previous gemcitabine-containing regimen

Mukherjee P, Basu GD, Tinder TL et al. Progression of pancreatic adenocarcinoma is significantly impeded with a combination of vaccine and COX-2 inhibition. *J Immunol* 2009 January 1;182(1):216-24.
Abstract: With a 5-year survival rate of <5%, pancreatic cancer is one of the most rapidly fatal malignancies. Current protocols for the treatment of pancreas cancer are not as effective as we desire. In this study, we show that a novel Mucin-1 (MUC1)-based vaccine in combination with a cyclooxygenase-2 inhibitor (celecoxib), and low-dose chemotherapy (gemcitabine) was effective in preventing the progression of preneoplastic intraepithelial lesions to invasive pancreatic ductal adenocarcinomas. The study was conducted in an appropriate triple transgenic model of spontaneous pancreatic cancer induced by the KRAS(G12D) mutation and that expresses human MUC1 as a self molecule. The combination treatment elicited robust antitumor cellular and humoral immune responses and was associated with increased apoptosis in the tumor. The mechanism for the increased immune response was attributed to the down-regulation of circulating prostaglandin E(2) and indoleamine 2, 3,-dioxygenase enzymatic activity, as well as decreased levels of T regulatory and myeloid suppressor cells within the tumor microenvironment. The preclinical data provide the rationale to design clinical trials with a combination of MUC1-based vaccine, celecoxib, and gemcitabine for the treatment of pancreatic cancer

Xu XF, Xie CG, Wang XP et al. Selective inhibition of cyclooxygenase-2 suppresses the growth of pancreatic cancer cells in vitro and in vivo. *Tohoku J Exp Med* 2008 June;215(2):149-57.
Abstract: Cyclooxygenase-2 (COX-2), a prostaglandin synthetase, is involved in development of certain tumors. We therefore analyzed COX-2 expression in pancreatic cancer tissues (53 samples) and Panc-1 human pancreatic cancer cells by immunohistochemistry, RT-PCR and western-blotting analyses. Also, immunohistochemistry of proliferating cell nuclear antigen (PCNA) was performed. We found expression of COX-2 was dramatically upregulated in 36 of 53 cases (67.9%) and the expression of COX-2 was associated with the diameter (> 3 cm) of the tumors ($p < 0.05$), but not with the age, gender, tumor location, differentiation, lymph-node metastases and TNM stage. The positivity rate of PCNA expression in the pancreatic cancer cells of the COX-2 positive group (32.88 +/- 13.26%) was significantly higher than that in the COX-2 negative group (24.56 +/- 11.51%) ($p < 0.05$). Then we investigated the effect of selective inhibitors of COX-2 (NS398 and celecoxib) on proliferation of Panc-1 cells by 3-(4,5 dimethyl-2-thiazolyl)-2.5-diphenyl-2H-tetrazolium bromide (MTT) assay. Either NS398 or celecoxib suppressed proliferation of Panc-1 cells dose-dependently in vitro. Furthermore, Panc-1 cells were implanted into nude mice, and celecoxib was administrated orally with feed. The volume of the tumor xenografted into nude mice was decreased by 51.6% in the celecoxib group ($p < 0.01$). In conclusion, the increased expression of COX-2 may be responsible for

rapid proliferation of pancreatic cancer, and specific inhibition of COX-2 suppresses proliferation of Panc-1 cells in vitro and in nude mice. The selective inhibitor of COX-2 may be an effectual agent for pancreatic cancer chemoprevention

Dragovich T, Burris H, III, Loehrer P et al. Gemcitabine plus celecoxib in patients with advanced or metastatic pancreatic adenocarcinoma: results of a phase II trial. *Am J Clin Oncol* 2008 April;31(2):157-62.
Abstract: OBJECTIVES: Cycloxygenase-2 (COX-2) is overexpressed in pancreatic tumors where it may be involved in inflammation, carcinogenesis, and the regulation of neoangiogenesis. The purpose of this trial was to evaluate the combination of intravenous gemcitabine with selective COX-2 inhibitor, celecoxib for effect on survival, disease progression, and tolerability in patients with advanced pancreatic cancer. In addition, limited pharmacokinetic and pharmacodynamic analyses were preformed. MATERIALS AND METHODS: Eligible patients included those with locally advanced or metastatic pancreatic cancer with no prior chemotherapy and ECOG performance status 0-2. The treatment consisted of intravenous gemcitabine 1000 mg/m weekly x 7 weeks and concurrent daily oral celecoxib 400 mg orally twice a day. Daily oral low-dose aspirin 81 mg was administered throughout the study as a precaution for increased risk of thrombotic events. Those with stable or responsive disease were continued on intravenous gemcitabine 1000 mg/m weekly x 3 weeks and concurrent oral celecoxib. RESULTS: Twenty five patients have been enrolled at 3 centers. Five patients had locally advanced cancer; 20 had metastatic disease. The most common grade 3/4 hematological toxicities were neutropenia (32%) and anemia (20%). Four patients (17%) had partial response and 7 (35%) demonstrated stable disease. The estimated 12-month survival rate was 15%, which did not reach the predetermined efficacy end point. There was a trend suggestive of correlation between a decrease in serum vascular endothelial growth factor and patient survival. CONCLUSION: The addition of celecoxib to gemcitabine therapy did not demonstrate significant improvement in measured clinical outcomes, in patients with advanced pancreatic cancer. Higher doses of celecoxib may be needed to observe significant antitumor activity

Ferrari V, Valcamonico F, Amoroso V et al. Gemcitabine plus celecoxib (GECO) in advanced pancreatic cancer: a phase II trial. *Cancer Chemother Pharmacol* 2006 January;57(2):185-90.
Abstract: INTRODUCTION: Single agent gemcitabine (GEM) is the standard treatment of pancreatic adenocarcinoma. Celecoxib is a selective cyclooxygenase-2 (COX-2) inhibitor. Recent studies in human pancreatic tumor cell lines suggest an involvement of COX-2 in tumor-dependent angiogenesis and provide the rational for inhibition of the COX pathway as an effective therapeutic approach. The aim of this study is to evaluate the toxicity and activity of gemcitabine plus celecoxib. PATIENTS AND METHODS: Forty-two consecutive patients with histologically or cytologically confirmed pancreatic adenocarcinoma entered the trial. Twenty-six patients (pts) were metastatic, 16 pts had locally advanced disease. The schedule consisted of GEM 1,000 mg/m2 (as a 30 min iv infusion) on days 1, 8 every 3 weeks and celecoxib 400 mg bid. RESULTS: Four pts (9%) achieved a partial response and 26 (62%) had stable disease, gaining a total disease control in 30 pts (71% [95% CI, 58-84%]). Overall clinical benefit response was experienced by 23 pts (54.7% [95%CI, 38.6-70.1%]). Neither grade 4 neutropenia nor grade 3-4 thrombocytopenia was observed. Grade 3 neutropenia was detected in 19% of pts. Grade 3 non-hematological toxicity was as follows: hepatic toxicity 7%, nausea 2.3%. Three pts (7%) and 5 pts (12%) had respectively a minimum creatinine increase and edema. Median survival was 9.1 months (95% CI, 7.5-10.6 months). CONCLUSION: GEM in combination with celecoxib showed low toxicity, good clinical benefit rate and good disease control. Further clinical investigation is warranted

El-Rayes BF, Zalupski MM, Shields AF et al. A phase II study of celecoxib, gemcitabine, and cisplatin in advanced pancreatic cancer. *Invest New Drugs* 2005 December;23(6):583-90.
Abstract: BACKGROUND: Pancreatic cancer is amongst the most chemoresistant malignancies.

Expression of the cyclooxygenase-2 (COX-2) enzyme plays a major role in tumor progression and resistance to therapy. A Phase II study was undertaken to determine the effect of gemcitabine by fixed-dose rate infusion (FDR), cisplatin and the COX-2 inhibitor, celecoxib, on the 6-month survival rate in patients with metastatic pancreatic cancer. METHODS: The eligibility criteria included a pathologically or cytologically confirmed diagnosis of adenocarcinoma of the pancreas. No prior gemcitabine therapy was allowed. Patients received a combination of gemcitabine 1,000 mg/m(2) over 100 minutes, cisplatin 35 mg/m(2) I.V. on days 1 and 8, and celecoxib continuously at a daily dose of 800 mg. Cycles were repeated every 21 days. RESULTS: Twenty-two patients with metastatic pancreas cancer were enrolled (median age, 59.5 years; M:F, 13:9). The median number of cycles was 2 per patient. The median survival time was 5.8 months (90% CI, 3.6-7.6 months). The probability of survival at 6 months was 46% (90% CI, 27-62%). The major toxicity was neutropenia with grade 3 or 4 toxicities seen in 65% of patients. CONCLUSIONS: The addition of celecoxib to gemcitabine (by FDR) and cisplatin did not appear to increase activity of the chemotherapy doublet in patients with advanced pancreatic cancer. Celecoxib alone may not be sufficient to sensitize pancreatic cancer to the effects of conventional cytotoxic therapy

Wu G, Yi J, Di F, Zou S, Li X. Celecoxib inhibits proliferation and induces apoptosis via cyclooxygenase-2 pathway in human pancreatic carcinoma cells. *J Huazhong Univ Sci Technolog Med Sci* 2005;25(1):42-4.
Abstract: In order to evaluate the effects and mechanisms of celecoxib in inhibiting proliferation and inducing apoptosis on human pancreatic carcinoma cells, the anti-proliferative effect was measured by using methabenzthiazuron (MTT) assay. Cell cycle and apoptosis were analyzed by using flow cytometry (FCM), and the PGE2 levels in the supernatant of cultured pancreatic carcinoma cells were quantitated by enzyme-linked immunoabsordent assay (ELISA). Our results showed that celecoxib suppressed the production of PGE2 and inhibited the growth of JF-305 cells, and the anti-proliferative effect of celecoxib could be abolished by addition of PGE2. FCM revealed that celecoxib could inhibit proliferation and induce apoptosis by Gl- S cell cycle arrest. It was concluded that cyclooxygenase-2 specific inhibitor celecoxib could inhibit proliferation and induced apoptosis of human pancreatic carcinoma cells via suppression of PGE2 production in vitro

Lipton A, Harvey H, Witters L, Kerr S, Legore K, Campbell C. Gemcitabine/Irinotecan/celecoxib in pancreatic cancer. *Oncology (Williston Park)* 2004 December;18(14 Suppl 14):43-5.
Abstract: Unresectable pancreatic cancer has few therapeutic options and a dismal prognosis. Cyclooxygenase-2 (COX-2) expression is increased at the RNA and protein levels in most human pancreatic cancers. The purpose of this trial was to determine whether the addition of a COX-2 inhibitor to chemotherapy was beneficial. To date, 11 patients with inoperable pancreatic cancer have been treated with the combination of gemcitabine (Gemzar), irinotecan (Camptosar), and celecoxib (Celebrex) at 400 mg orally twice daily. Encouraging pain relief, improvement in performance status, and decreases in CA 19-9 and carcinoembryonic antigen levels have been observed

Zileuton (5-Lipoxygenase Inhibitor)

Ding X, Zhou X, Zhang H, Qing J, Qiang H, Zhou G. Triptolide augments the effects of 5-lipoxygenase RNA interference in suppressing pancreatic tumor growth in a xenograft mouse model. *Cancer Chemother Pharmacol* 2012 January;69(1):253-61.
Abstract: PURPOSE: Pancreatic cancer has one of the highest fatality rates of all cancers, and new strategies or reagents to tackle this disease are needed. Triptolide (TL) is able to potently inhibit the growth of pancreatic tumor cells in vitro. On the other hand, blockage of 5-LOX pathway might be useful for treatment of pancreatic cancer. In the current study, we tested the effects of 5-LOX RNA interference and TL individually or in combination in suppressing human pancreatic tumor growth in

xenograft mouse model. METHODS: 5-LOX short hairpin RNA (shRNA) vectors were developed and screened out for their efficacy in human pancreatic cancer cell line SW1990 in vitro. Their antitumor effects were also evaluated by measuring cell proliferation and apoptosis. An effective 5-LOX shRNA was given alone or in combination with TL to treat pancreatic tumor xenograft. Expression levels of 5-LOX and VEGF were measured with Western blotting and immunohistology. RESULTS: Knocking down 5-LOX gene suppressed cancer cell growth in vitro and intra-tumoral delivering of 5-LOX shRNA inhibited growth of transplanted tumor in vivo. TL treatment induced tumor suppression and greatly enhanced antitumor effects of 5-LOX shRNA in the mouse model. 5-LOX RNA interference or TL treatment suppresses VEGF expression in tumor tissue, and combined treatment further reduces its expression. CONCLUSIONS: Both treatments exerted antitumor effects in vivo, and combined use of the two approaches produced more powerful antitumor effects. Synergistic effects of combined treatment in VEGF expression may contribute to the mechanisms of the strong antitumor effects

Tong WG, Ding XZ, Talamonti MS, Bell RH, Adrian TE. LTB4 stimulates growth of human pancreatic cancer cells via MAPK and PI-3 kinase pathways. *Biochem Biophys Res Commun* 2005 September 30;335(3):949-56.
Abstract: We have previously shown the importance of LTB4 in human pancreatic cancer. LTB4 receptor antagonists block growth and induce apoptosis in pancreatic cancer cells both in vitro and in vivo. Therefore, we investigated the effect of LTB4 on proliferation of human pancreatic cancer cells and the mechanisms involved. LTB4 stimulated DNA synthesis and proliferation of both PANC-1 and AsPC-1 human pancreatic cancer cells, as measured by thymidine incorporation and cell number. LTB4 stimulated rapid and transient activation of MEK and ERK1/2 kinases. The MEK inhibitors, PD98059 and U0126, blocked LTB4-stimulated ERK1/2 activation and cell proliferation. LTB4 also stimulated phosphorylation of p38 MAPK; however, the p38 MAPK inhibitor, SB203580, failed to block LTB4-stimulated growth. The activity of JNK/SAPK was not affected by LTB4 treatment. Phosphorylation of Akt was also induced by LTB4 and this effect was blocked by the PI-3 kinase inhibitor wortmannin, which also partially blocked LTB4-stimulated cell proliferation. In conclusion, LTB4 stimulates proliferation of human pancreatic cancer cells through MEK/ERK and PI-3 kinase/Akt pathways, while p38 MPAK and JNK/SAPK are not involved

Hennig R, Grippo P, Ding XZ et al. 5-Lipoxygenase, a marker for early pancreatic intraepithelial neoplastic lesions. *Cancer Res* 2005 July 15;65(14):6011-6.
Abstract: Pancreatic cancer has an abysmal prognosis because of late diagnosis. Therefore, it is important to identify risk factors if we are to be able to prevent and detect this cancer in an early, noninvasive stage. Pancreatic intraepithelial neoplasias (PanIN) are the precursor lesions which could be an ideal target for chemoprevention. This study shows up-regulation of 5-lipoxygenase (5-LOX) in all grades of human PanINs and early lesions of pancreatic cancer in two different animal models (EL-Kras mice and N-nitrosobis(2-oxopropyl)amine-treated hamsters) by immunohistochemistry. The results were consistent in all tissues examined, including seven chronic pancreatitis patients, four pancreatic cancer patients, one multiorgan donor, nine EL-Kras mice, and three N-nitrosobis(2-oxopropyl)amine-treated hamsters, all with PanINs. Overexpression of 5-LOX in NIH3T3 cells resulted in greater sensitivity of these cells to the growth inhibitory effects of the 5-LOX inhibitor Rev5901. These findings provide evidence that 5-LOX plays a key role in the development of pancreatic cancer. Furthermore, the lipoxygenase pathway may be a target for the prevention of this devastating disease

Kennedy TJ, Chan CY, Ding XZ, Adrian TE. Lipoxygenase inhibitors for the treatment of pancreatic cancer. *Expert Rev Anticancer Ther* 2003 August;3(4):525-36.
Abstract: Pancreatic cancer has a dismal prognosis with no effective medical therapy. Therefore, there is a need to search for novel targets for cancer prevention and treatment. The lipoxygenases

oxygenate arachidonic acid and other 20-carbon fatty acids and their downstream metabolites have been found to mediate several aspects of pancreatic cancer development and growth. Therapeutic agents have been developed against various targets in the lipoxygenase pathways. Many of these were first developed for their anti-inflammatory properties and were subsequently found to have anticancer effects. Such agents include lipoxygenase and 5-lipoxygenase-activating protein inhibitors, leukotriene receptor antagonists and natural products with inhibitory effects on these pathways. Dual lipoxygenase and cyclooxygenase inhibition represents an exciting area of research and drug development

Wenger FA, Kilian M, Bisevac M et al. Effects of Celebrex and Zyflo on liver metastasis and lipidperoxidation in pancreatic cancer in Syrian hamsters. *Clin Exp Metastasis* 2002;19(8):681-7.
Abstract: Selective inhibition of eicosanoid synthesis is thought to have effects on carcinogenesis in lung and colon cancer. However, it is still unknown whether pancreatic cancer might also be influenced. Therefore we evaluated the impact of selective cyclooxygenase-2 inhibitor Celebrex and selective 5-lipoxygenase inhibitor Zyflo on liver metastasis in a solid model of pancreatic adenocarcinoma in Syrian hamster. In week 33, the animals were sacrificed and incidence of pancreatic carcinomas and number and size of liver metastases were determined. Activities of antioxidative enzymes (GSHPX/SOD) and concentrations of products of lipidperoxidation were measured in liver metastases and non-metastatic hepatic tissue. The incidence (54.5 vs. 100%), number (3.17 +/- 0.98 vs. 6.75 +/- 0.71) and size (2.67 +/- 1.97 vs. 11.75 +/- 1.98 mm2) of liver metastases were decreased by combined therapy of Zyflo and Celebrex ($P < 0.05$). Furthermore, activities of GSHPX ([73.77 +/- 5.67]*10(5) vs. [15.49 +/- 4.02]*10(5) U/mg prot.; $P < 0.05$) and SOD (474.92 +/- 108.8 vs. 127.89 +/- 38.75 U/mg prot.; $P < 0.05$) were increased, while lipidperoxidation (0.31 +/- 0.08 nmol/mg prot. vs. 1.54 +/- 0.55 nmol/mg prot.; $P < 0.05$) was decreased by combination therapy, in non-metastatic hepatic tissue. Moreover, combined therapy increased lipidperoxidation in liver metastases (0.47 +/- 0.09 vs. 1.95 +/- 0.12 nmol/mg prot.; $P < 0.05$). Thus, a combination of Celebrex and Zyflo might be a new concept to decrease tumour growth in liver metastases in advanced pancreatic cancer

Tong WG, Ding XZ, Witt RC, Adrian TE. Lipoxygenase inhibitors attenuate growth of human pancreatic cancer xenografts and induce apoptosis through the mitochondrial pathway. *Mol Cancer Ther* 2002 September;1(11):929-35.
Abstract: Several studies have suggested that high dietary fat intake, particularly essential fatty acids, is associated with pancreatic cancer development and growth. Our previous studies have demonstrated that blockade of either the 5-lipoxygenase (LOX) or 12-LOX pathway of arachidonic acid metabolism inhibited pancreatic cancer cell proliferation and induced apoptosis. This study investigated the underlying mechanisms for LOX inhibitor-induced apoptosis and the potential of LOX inhibitors as antipancreatic cancer agents using the athymic mice xenograft model. Apoptosis of pancreatic cancer cells induced by LOX inhibitors (including the nonselective LOX inhibitor nordihydroguaiaretic acid, the 5-LOX inhibitor Rev-5901, and the 12-LOX inhibitor baicalein) was confirmed by growth inhibition, annexin V binding, and terminal deoxynucleotidyl transferase-mediated nick end labeling assay in MiaPaCa-2 and AsPC-1 human pancreatic cancer cells. Expression of the antiapoptotic proteins Bcl-2 and Mcl-1 was significantly decreased after LOX inhibitor treatment while that of the proapoptotic protein bax was increased. LOX inhibitors also markedly induced the release of cytochrome c from mitochondria into the cytosol. Caspase-9, caspase-7, and caspase-3 but not caspase-8 were activated after treatment, concomitant with cleavage of the capase-3 substrate poly(ADP-ribose) polymerase. In vivo studies in the athymic mice xenograft model also confirmed the growth inhibitory effect and induction of apoptosis by these LOX inhibitors in pancreatic cancer. In conclusion, LOX inhibitors block pancreatic cancer cell proliferation and induce apoptosis through the mitochondrial pathway both in vivo and in vitro. LOX inhibitors are likely to be valuable for the treatment of human pancreatic cancer

Hennig R, Ding XZ, Tong WG et al. 5-Lipoxygenase and leukotriene B(4) receptor are expressed in human pancreatic cancers but not in pancreatic ducts in normal tissue. *Am J Pathol* 2002 August;161(2):421-8.
Abstract: The 5-lipoxygenase (5-LOX) pathway is critical for pancreatic cancer cell growth and escape from apoptosis. Inhibition of 5-LOX blocks proliferation and induces apoptosis in human pancreatic cancer cells. However, the expression of 5-LOX and its downstream signaling pathway have not been investigated in human pancreatic adenocarcinoma. Reverse transcriptase-polymerase chain reaction revealed expression of 5-LOX mRNA in all pancreatic cancer cell lines tested including, PANC-1, AsPC-1, and MiaPaCa2 cells, but not in normal pancreatic ductal cells. The expression of 5-LOX protein in pancreatic cancer cell lines was demonstrated by Western blotting. Finally, 5-LOX up-regulation in human pancreatic cancer tissues was verified by intense positive staining in cancer cells by immunohistochemistry. Staining for the 5-LOX protein was particularly evident in the ductal components of the more differentiated tumors but not in ductal cells in normal pancreatic tissues from cadaver donors. Immunohistochemistry also revealed strong staining of cancer tissues with an antibody to the receptor of the downstream 5-LOX metabolite, leukotriene B(4). The current study demonstrated marked expression of 5-LOX and the leukotriene B(4) receptor in human pancreatic cancer tissues. These findings provide further evidence of up-regulation of this pathway in pancreatic cancer and that LOX inhibitors are likely to be valuable in the treatment of this dreadful disease

Ferry DR, Deakin M, Baddeley J et al. A phase II study of the 5-lipoxygenase inhibitor, CV6504, in advanced pancreatic cancer: correlation of clinical data with pharmacokinetic and pharmacodynamic endpoints. *Ann Oncol* 2000 September;11(9):1165-70.
Abstract: PURPOSE: Primary objective was to determine response rate of patients with advanced pancreatic cancer to a novel lipoxygenase and thromboxane A2 synthetase inhibitor (CV6504); secondary objectives included estimation of pharmacokinetics of CV6504, target-enzyme inhibition, safety and tolerance, quality of life and survival. PATIENTS AND METHODS: Thirty-one patients with advanced pancreatic cancer were planned to receive CV6504, 100 mg TDS, orally for three months, at which point CT scans were performed to assess therapeutic response rates. Steady state concentrations of CV6504 and thromboxane B2 (an indirect measure of thromboxane A2 synthetase (TA2S) inhibition) were made. Of the 31 patients entered into the study, 23 were considered fully evaluable for response. RESULTS: The drug was well tolerated with few side effects; no partial or complete responses were seen, but 10 patients had stable disease at 3 months; quality of life was maintained during therapy; mean CV6504 steady state plasma concentrations of 14 +/- 6 ng/ml resulting in 75 +/- 18% inhibition of TA2S were achieved; median-survival time for all patients considered eligible for assessment of efficacy was 36.6 weeks after the initial dose of study medication. The actuarial one-year survival was approximately 25%. CONCLUSION: CV6504 inhibits its target enzyme in vivo, maintains stable disease in 32% of evaluable patients and is well tolerated

Ding XZ, Iversen P, Cluck MW, Knezetic JA, Adrian TE. Lipoxygenase inhibitors abolish proliferation of human pancreatic cancer cells. *Biochem Biophys Res Commun* 1999 July 22;261(1):218-23.
Abstract: Epidemiologic and animal studies have linked pancreatic cancer growth with fat intake, especially unsaturated fats. Arachidonic acid release from membrane phospholipids is essential for tumor cell proliferation. Lipoxygenases (LOX) constitute one pathway for arachidonate metabolism, but their role in pancreatic cancer growth is unknown. The expression of 5-LOX and 12-LOX as well as their effects on cell proliferation was investigated in four human pancreatic cancer cell lines (PANC-1, MiaPaca2, Capan2, and ASPC-1). Expression of 5-LOX and 12-LOX mRNA was measured by nested RT-PCR. Effects of LOX inhibitors and specific LOX antisense oligonucleotides on pancreatic cancer cell proliferation were measured by (3)H-thymidine incorporation. Our results

showed that (1) 5-LOX and 12-LOX were expressed in all pancreatic cancer cell lines tested, while they were not detectable in normal human pancreatic ductal cells; (2) both LOX inhibitors and LOX antisense markedly inhibited cell proliferation in a concentration-dependent and time-dependent manner; (3) the 5-LOX and 12-LOX metabolites 5-HETE and 12-HETE as well as arachidonic and linoleic acids directly stimulated pancreatic cancer cell proliferation; (4) LOX inhibitor-induced growth inhibition was reversed by 5-HETE and 12-HETE. The current studies indicate that both 5-LOX and 12-LOX expression is upregulated in human pancreatic cancer cells and LOX plays a critical role in pancreatic cancer cell proliferation. LOX inhibitors may be valuable for the treatment of pancreatic cancer

Wenger FA, Kilian M, Achucarro P et al. Effects of Celebrex and Zyflo on BOP-induced pancreatic cancer in Syrian hamsters. *Pancreatology* 2002;2(1):54-60.
Abstract: BACKGROUND/AIMS: Selective inhibition of eicosanoid synthesis decreases inflammation, however, it is still unknown whether oxidative stress and carcinogenesis might be influenced in ductal pancreatic ductal cancer as well. METHODS: 120 male hamsters were randomized into 8 groups (n = 15). While control group 1-4 received 0.5 ml normal saline s.c. weekly for 16 weeks, groups 5-8 were injected 10 mg BOP/kg body weight to induce pancreatic cancer. After establishment of pancreatic cancer, groups 1 and 5 received no therapy, groups 2 and 6 were fed 7 mg Celebrex daily, groups 3 and 7 were given 28 mg Zyflo and groups 4 and 8 received Celebrex and Zyflo orally daily in weeks 17-32. In week 33, all animals were sacrificed, macroscopic size of pancreatic carcinomas was measured, incidence of pancreatic cancer was analyzed histopathologically and activities of antioxidative enzymes and concentration of products of lipid peroxidation in tumor-free and pancreatic intratumoral tissue were determined. RESULTS: Incidence and size of macroscopic pancreatic carcinomas were decreased by single therapy with Zyflo as well as combined therapy (Zyflo + Celebrex). Activities of antioxidative enzymes were increased and the concentration of products of lipid peroxidation was decreased in tumor-free pancreas. On the other hand, lipid peroxidation was increased in pancreatic tumors. CONCLUSION: Zyflo alone or in combination with Celebrex reduce tumor growth in pancreatic cancer and thus might be a new therapeutic option in advanced pancreatic cancer

Nelfinavir

Brunner TB, Geiger M, Grabenbauer GG et al. Phase I trial of the human immunodeficiency virus protease inhibitor nelfinavir and chemoradiation for locally advanced pancreatic cancer. *J Clin Oncol* 2008 June 1;26(16):2699-706.
Abstract: PURPOSE: Preclinically, HIV protease inhibitors radiosensitize tumors with activated PI3-kinase/Akt pathway. We determined the toxicity of nelfinavir chemoradiotherapy in borderline resectable and unresectable pancreatic cancer. PATIENTS AND METHODS: Oral nelfinavir (2 x 1,250 mg) was started 3 days before and continued throughout chemoradiotherapy to 50.4 Gy (boost, 59.4 Gy) in 12 patients. Two gemcitabine dose levels (DL) were tested (200 mg/m(2) and 300 mg/m(2) on days 1, 8, 22, and 29). Cisplatin was administered on the same days at 30 mg/m(2). Phospho-Akt downregulation by nelfinavir was monitored by immunoblotting in patient leukocytes. Restaging positron emission tomography (PET)/computed tomography (CT) and CA19-9 levels served to assess response, and responding tumors were resected. RESULTS: At each DL, five of six patients completed chemoradiotherapy, and two of 12 patients had incomplete chemoradiotherapy because of clinical depression (DL1) and peritoneal metastasis (DL2). Grade 4 toxicities were a transaminase elevation (DL2) as a result of biliary stent occlusion and acute cholecystitis as a result of peritoneal metastasis (DL2). Stent occlusions led to dose-limiting toxicities of grade 3 liver enzyme and bilirubin elevations

(two patients at DL1, one patient at DL2). Grade 3 nausea and vomiting occurred in a DL2 patient, and weight loss occurred in a DL1 patient who refused supportive feeding. Secondary complete resection was possible in six of 10 patients with complete chemoradiotherapy, including one tumor with pathologic sterilization. Partial CT responses were observed in five of 10 patients who completed chemoradiotherapy. Of nine patients assessable by PET,responses were complete in five patients and partial patients, and stable disease was observed in two patients. CONCLUSION: The combination of nelfinavir and chemoradiotherapy showed acceptable toxicity and promising activity in patients with pancreatic cancer

Kimple RJ, Vaseva AV, Cox AD et al. Radiosensitization of epidermal growth factor receptor/HER2-positive pancreatic cancer is mediated by inhibition of Akt independent of ras mutational status. *Clin Cancer Res* 2010 February 1;16(3):912-23.
Abstract: PURPOSE: Epidermal growth factor receptor (EGFR) family members (e.g., EGFR, HER2, HER3, and HER4) are commonly overexpressed in pancreatic cancer. We investigated the effects of inhibition of EGFR/HER2 signaling on pancreatic cancer to elucidate the role(s) of EGFR/HER2 in radiosensitization and to provide evidence in support of further clinical investigations. EXPERIMENTAL DESIGN: Expression of EGFR family members in pancreatic cancer lines was assessed by quantitative reverse transcription-PCR. Cell growth inhibition was determined by MTS assay. The effects of inhibition of EGFR family receptors and downstream signaling pathways on in vitro radiosensitivity were evaluated using clonogenic assays. Growth delay was used to evaluate the effects of nelfinavir on in vivo tumor radiosensitivity. RESULTS: Lapatinib inhibited cell growth in four pancreatic cancer cell lines, but radiosensitized only wild-type K-ras-expressing T3M4 cells. Akt activation was blocked in a wild-type K-ras cell line, whereas constitutive phosphorylation of Akt and extracellular signal-regulated kinase (ERK) was seen in lines expressing mutant K-ras. Overexpression of constitutively active K-ras (G12V) abrogated lapatinib-mediated inhibition of both Akt phosphorylation and radiosensitization. Inhibition of MAP/ERK kinase/ERK signaling with U0126 had no effect on radiosensitization, whereas inhibition of activated Akt with LY294002 (enhancement ratio, 1.2-1.8) or nelfinavir (enhancement ratio, 1.2-1.4) radiosensitized cells regardless of K-ras mutation status. Oral nelfinavir administration to mice bearing mutant K-ras-containing Capan-2 xenografts resulted in a greater than additive increase in radiation-mediated tumor growth delay (synergy assessment ratio of 1.5). CONCLUSIONS: Inhibition of EGFR/HER2 enhances radiosensitivity in wild-type K-ras pancreatic cancer. Nelfinavir, and other phosphoinositide 3-kinase/Akt inhibitors, are effective pancreatic radiosensitizers regardless of K-ras mutation status

Bernstein WB, Dennis PA. Repositioning HIV protease inhibitors as cancer therapeutics. *Curr Opin HIV AIDS* 2008 November;3(6):666-75.
Abstract: PURPOSE OF REVIEW: Although designed to target only the HIV protease, HIV protease inhibitors induce toxicities in patients such as insulin resistance and lipodystrophy that suggest that protease inhibitors have other targets in mammalian cells. Akt controls insulin signaling and is an important target in cancer, but no Akt inhibitors are approved as cancer therapeutics. These observations have prompted the study of HIV protease inhibitors as inhibitors of Akt and possible cancer therapeutics. This review will highlight the latest advances in repositioning HIV protease inhibitors as cancer therapeutics. RECENT FINDINGS: Although protease inhibitors can inhibit Akt activation and the proliferation of over 60 cancer cell lines, as well as improve sensitivity to radiation or chemotherapy, these effects do not always correlate with Akt inhibition. Other important processes, such as the induction of endoplasmic reticulum stress, appear critical to the biological activity of protease inhibitors. These impressive and surprising preclinical data have prompted clinical testing of nelfinavir as a lead HIV protease inhibitor in cancer patients. SUMMARY: Although mechanisms of action for the antitumor effects of HIV protease inhibitors are complex, their broad spectrum of activity, minimal toxicity, and wide availability make protease inhibitors ideal candidates for repositioning as cancer therapeutics

Chloroquine/Hydroxychloroquine

Kim J, Yip ML, Shen X et al. Identification of anti-malarial compounds as novel antagonists to chemokine receptor CXCR4 in pancreatic cancer cells. *PLoS ONE* 2012;7(2):e31004.
Abstract: Despite recent advances in targeted therapies, patients with pancreatic adenocarcinoma continue to have poor survival highlighting the urgency to identify novel therapeutic targets. Our previous investigations have implicated chemokine receptor CXCR4 and its selective ligand CXCL12 in the pathogenesis and progression of pancreatic intraepithelial neoplasia and invasive pancreatic cancer; hence, CXCR4 is a promising target for suppression of pancreatic cancer growth. Here, we combined in silico structural modeling of CXCR4 to screen for candidate anti-CXCR4 compounds with in vitro cell line assays and identified NSC56612 from the National Cancer Institute's (NCI) Open Chemical Repository Collection as an inhibitor of activated CXCR4. Next, we identified that NSC56612 is structurally similar to the established anti-malarial drugs chloroquine and hydroxychloroquine. We evaluated these compounds in pancreatic cancer cells in vitro and observed specific antagonism of CXCR4-mediated signaling and cell proliferation. Recent in vivo therapeutic applications of chloroquine in pancreatic cancer mouse models have demonstrated decreased tumor growth and improved survival. Our results thus provide a molecular target and basis for further evaluation of chloroquine and hydroxychloroquine in pancreatic cancer. Historically safe in humans, chloroquine and hydroxychloroquine appear to be promising agents to safely and effectively target CXCR4 in patients with pancreatic cancer

Yang S, Wang X, Contino G et al. Pancreatic cancers require autophagy for tumor growth. *Genes Dev* 2011 April 1;25(7):717-29.
Abstract: Macroautophagy (autophagy) is a regulated catabolic pathway to degrade cellular organelles and macromolecules. The role of autophagy in cancer is complex and may differ depending on tumor type or context. Here we show that pancreatic cancers have a distinct dependence on autophagy. Pancreatic cancer primary tumors and cell lines show elevated autophagy under basal conditions. Genetic or pharmacologic inhibition of autophagy leads to increased reactive oxygen species, elevated DNA damage, and a metabolic defect leading to decreased mitochondrial oxidative phosphorylation. Together, these ultimately result in significant growth suppression of pancreatic cancer cells in vitro. Most importantly, inhibition of autophagy by genetic means or chloroquine treatment leads to robust tumor regression and prolonged survival in pancreatic cancer xenografts and genetic mouse models. These results suggest that, unlike in other cancers where autophagy inhibition may synergize with chemotherapy or targeted agents by preventing the up-regulation of autophagy as a reactive survival mechanism, autophagy is actually required for tumorigenic growth of pancreatic cancers de novo, and drugs that inactivate this process may have a unique clinical utility in treating pancreatic cancers and other malignancies with a similar dependence on autophagy. As chloroquine and its derivatives are potent inhibitors of autophagy and have been used safely in human patients for decades for a variety of purposes, these results are immediately translatable to the treatment of pancreatic cancer patients, and provide a much needed, novel vantage point of attack

Zeilhofer HU, Mollenhauer J, Brune K. Selective growth inhibition of ductal pancreatic adenocarcinoma cells by the lysosomotropic agent chloroquine. *Cancer Lett* 1989 January;44(1):61-6.
Abstract: Pancreatic adenocarcinoma cells show characteristics of macrophages as phagocytosis and exocytosis. These phagocytic activities can be inhibited in macrophages by antirheumatic drugs, e.g. gold compounds and antimalarials. We have now tested the activity of auranofin and chloroquine to inhibit growth of a pancreatic tumor cell line (PaTu II) and of human foreskin fibroblasts (HFF) as a control. We found that auranofin (greater than or equal to 2 microM) inhibits both PaTu II cell and HFF

growth. In contrast, chloroquine (4-18 microM) selectively interferes with the growth of PaTu II cells. This specific vulnerability of PaTu II cells was confirmed in cocultures of tumor cells and fibroblasts

Itraconazole (Inhibitor of Hedgehog Pathway)

Kim J, Tang JY, Gong R et al. Itraconazole, a commonly used antifungal that inhibits Hedgehog pathway activity and cancer growth. *Cancer Cell* 2010 April 13;17(4):388-99.
Abstract: In a screen of drugs previously tested in humans we identified itraconazole, a systemic antifungal, as a potent antagonist of the Hedgehog (Hh) signaling pathway that acts by a mechanism distinct from its inhibitory effect on fungal sterol biosynthesis. Systemically administered itraconazole, like other Hh pathway antagonists, can suppress Hh pathway activity and the growth of medulloblastoma in a mouse allograft model and does so at serum levels comparable to those in patients undergoing antifungal therapy. Mechanistically, itraconazole appears to act on the essential Hh pathway component Smoothened (SMO) by a mechanism distinct from that of cyclopamine and other known SMO antagonists, and prevents the ciliary accumulation of SMO normally caused by Hh stimulation

Huang FT, Zhuan-Sun YX, Zhuang YY et al. Inhibition of hedgehog signaling depresses self-renewal of pancreatic cancer stem cells and reverses chemoresistance. *Int J Oncol* 2012 November;41(5):1707-14.
Abstract: Pancreatic cancer stem cells play a crucial role in tumorigenesis and chemoresistance. The Hedgehog signaling pathway is a key regulator in pancreatic tumorigenesis and drug resistance. To identify pancreatic cancer stem cells, tumorspheres derived from the PANC-1 pancreatic cancer cell line were cultured under a floating-culture system. PANC-1 tumorspheres possessed properties of self-renewal, differentiation, higher tumorigenesis and chemoresistance. It was observed that Hedgehog pathway is active in PANC-1 tumorspheres as shown by expression of hedgehog components Smo, Gil 1 and Gli 2, detected by quantitative RT-PCR and western blotting. After cyclopamine-mediated blockade of hedgehog, a decrease in proliferation of PANC-1 tumorspheres and G0/G1 transition were observed, as well as a decreased expression of Bmi-1 in PANC-1 tumorspheres. Cyclopamine reversed chemoresistance to gemcitabine, resulting in decreased expression of ABCG2 in PANC-1 tumorspheres. Taken together, our data indicate that PANC-1 tumorspheres have 'stemness' potential, and hedgehog signaling pathway plays an important role in the regulation of self-renewal and reversal of chemoresistance in cancer stem cells in pancreatic adenocarcinoma

Hwang RF, Moore TT, Hattersley MM et al. Inhibition of the hedgehog pathway targets the tumor-associated stroma in pancreatic cancer. *Mol Cancer Res* 2012 September;10(9):1147-57.
Abstract: Purpose: The Hedgehog (Hh) pathway has emerged as an important pathway in multiple tumor types and is thought to be dependent on a paracrine signaling mechanism. The purpose of this study was to determine the role of pancreatic cancer-associated fibroblasts (human pancreatic stellate cells, HPSCs) in Hh signaling. In addition, we evaluated the efficacy of a novel Hh antagonist, AZD8542, on tumor progression with an emphasis on the role of the stroma compartment. Experimental Design: Expression of Hh pathway members and activation of the Hh pathway were analyzed in both HPSCs and pancreatic cancer cells. We tested the effects of Smoothened (SMO) inhibition with AZD8542 on tumor growth in vivo using an orthotopic model of pancreatic cancer containing varying amounts of stroma. Results: HPSCs expressed high levels of SMO receptor and low levels of Hh ligands, whereas cancer cells showed the converse expression pattern. HPSC proliferation was stimulated by Sonic Hedgehog with upregulation of downstream GLI1 mRNA. These effects were abrogated by AZD8542 treatment. In an orthotopic model of pancreatic cancer,

AZD8542 inhibited tumor growth only when HPSCs were present, implicating a paracrine signaling mechanism dependent on stroma. Further evidence of paracrine signaling of the Hh pathway in prostate and colon cancer models is provided, demonstrating the broader applicability of our findings. Conclusion: Based on the use of our novel human-derived pancreatic cancer stellate cells, our results suggest that Hh-targeted therapies primarily affect the tumor-associated stroma, rather than the epithelial compartment. Mol Cancer Res; 10(9); 1147-57. (c)2012 AACR

Onishi H, Morifuji Y, Kai M, Suyama K, Iwasaki H, Katano M. Hedgehog inhibitor decreases chemosensitivity to 5-fluorouracil and gemcitabine under hypoxic conditions in pancreatic cancer. *Cancer Sci* 2012 April 6.
Abstract: Pancreatic cancer is one of the deadliest types of cancer. Previously, we showed that hypoxia increases invasiveness through upregulation of Smoothened (Smo) transcription in pancreatic ductal adenocarcinoma (PDAC) cells. Here, we first evaluated whether hypoxia-induced increase in Smo contributes to the proliferation of PDAC cells. We showed that Smo, but not Gli1, inhibition decreases proliferation significantly under hypoxic conditions. To further investigate the effects of Smo on PDAC growth, cell cycle analysis was carried out. Inhibition of Smo under hypoxia led to G(0) /G(1) arrest and decreased S phase. As 5-fluorouracil (5-FU) and gemcitabine, which are first-line drugs for pancreatic cancer, are sensitive to S phase, we then evaluated whether cyclopamine-induced decreased S phase under hypoxia affected the chemosensitivity of 5-FU and gemcitabine in PDAC cells. Cyclopamine treatment under hypoxia significantly decreased chemosensitivity to 5-FU and gemcitabine under hypoxia in both in vitro and in vivo models. In contrast, cis-diamminedichloroplatinum, which is cell cycle-independent, showed significant synergistic effects. These results suggest that hypoxia-induced increase of Smo directly contributes to the proliferation of PDAC cells through a hedgehog/Gli1-independent pathway, and that decreased S phase due to the use of Smo inhibitor under hypoxia leads to chemoresistance in S phase-sensitive anticancer drugs. Our results could be very important clinically because a clinical trial using Smo inhibitors and chemotherapy drugs will begin in the near future. (Cancer Sci, doi: 10.1111/j.1349-7006.2012.02297.x, 2012)

Singh BN, Fu J, Srivastava RK, Shankar S. Hedgehog signaling antagonist GDC-0449 (Vismodegib) inhibits pancreatic cancer stem cell characteristics: molecular mechanisms. *PLoS ONE* 2011;6(11):e27306.
Abstract: BACKGROUND: Recent evidence from in vitro and in vivo studies has demonstrated that aberrant reactivation of the Sonic Hedgehog (SHH) signaling pathway regulates genes that promote cellular proliferation in various human cancer stem cells (CSCs). Therefore, the chemotherapeutic agents that inhibit activation of Gli transcription factors have emerged as promising novel therapeutic drugs for pancreatic cancer. GDC-0449 (Vismodegib), orally administrable molecule belonging to the 2-arylpyridine class, inhibits SHH signaling pathway by blocking the activities of Smoothened. The objectives of this study were to examine the molecular mechanisms by which GDC-0449 regulates human pancreatic CSC characteristics in vitro. METHODOLOGY/PRINCIPAL FINDINGS: GDC-0499 inhibited cell viability and induced apoptosis in three pancreatic cancer cell lines and pancreatic CSCs. This inhibitor also suppressed cell viability, Gli-DNA binding and transcriptional activities, and induced apoptosis through caspase-3 activation and PARP cleavage in pancreatic CSCs. GDC-0449-induced apoptosis in CSCs showed increased Fas expression and decreased expression of PDGFRalpha. Furthermore, Bcl-2 was down-regulated whereas TRAIL-R1/DR4 and TRAIL-R2/DR5 expression was increased following the treatment of CSCs with GDC-0449. Suppression of both Gli1 plus Gli2 by shRNA mimicked the changes in cell viability, spheroid formation, apoptosis and gene expression observed in GDC-0449-treated pancreatic CSCs. Thus, activated Gli genes repress DRs and Fas expressions, up-regulate the expressions of Bcl-2 and PDGFRalpha and facilitate cell survival. CONCLUSIONS/SIGNIFICANCE: These data suggest that GDC-0499 can be used for the management of pancreatic cancer by targeting pancreatic CSCs

Bahra M, Kamphues C, Boas-Knoop S et al. Combination of hedgehog signaling blockage and chemotherapy leads to tumor reduction in pancreatic adenocarcinomas. *Pancreas* 2012 March;41(2):222-9.
Abstract: OBJECTIVES: Activation of the hedgehog signal transduction pathway, triggered by hedgehog binding to the transmembrane receptor patched 1 (PTCH1) or by mutations in the PTCH1 gene, plays an important role in the development of various tumors. METHODS: To investigate whether the Hedgehog signaling pathway is also active in human pancreatic adenocarcinomas, we determined the expression levels of the known Hedgehog target genes PTCH1 and GLI-1 in pancreatic tumors. To determine whether alterations in the PTCH1 gene are responsible for this pathway activation, we screened pancreatic carcinomas for mutations in PTCH. To investigate the contribution of hedgehog signaling to the tumorigenicity of pancreatic tumor cells, we blocked the Hedgehog pathway in cultured tumor cells and xenografts using the steroidal alkaloid cyclopamine and the small-molecule Hedgehog inhibitor Hh-Antag. RESULTS: We identified single nucleotide polymorphisms (SNPs) within the PTCH1 gene but no somatic PTCH1 mutations. Pathway-blockage resulted in a significant dose-dependent reduction of tumor cell growth in vitro and in vivo. Moreover, combined treatment with cyclopamine and the conventional antimetabolite gemcitabine revealed a synergistic effect on the reduction of tumor growth in pancreatic adenocarcinoma xenografts. CONCLUSIONS: Inhibition of Hedgehog signaling could be a promising approach for the treatment of pancreatic adenocarcinomas

Tang SN, Fu J, Nall D, Rodova M, Shankar S, Srivastava RK. Inhibition of sonic hedgehog pathway and pluripotency maintaining factors regulate human pancreatic cancer stem cell characteristics. *Int J Cancer* 2012 July 1;131(1):30-40.
Abstract: Activation of the sonic hedgehog (SHh) pathway is required for the growth of numerous tissues and organs and recent evidence indicates that this pathway is often recruited to stimulate growth of cancer stem cells (CSCs) and to orchestrate the reprogramming of cancer cells via epithelial mesenchymal transition (EMT). The objectives of this study were to examine the molecular mechanisms by which (-)-epigallocatechin-3-gallate (EGCG), an active compound in green tea, inhibits self-renewal capacity of pancreatic CSCs and synergizes with quercetin, a major polyphenol and flavonoid commonly detected in many fruits and vegetables. Our data demonstrated that EGCG inhibited the expression of pluripotency maintaining transcription factors (Nanog, c-Myc and Oct-4) and self-renewal capacity of pancreatic CSCs. Inhibition of Nanog by shRNA enhanced the inhibitory effects of EGCG on self-renewal capacity of CSCs. EGCG inhibited cell proliferation and induced apoptosis by inhibiting the expression of Bcl-2 and XIAP and activating caspase-3. Interestingly, EGCG also inhibited the components of SHh pathway (smoothened, patched, Gli1 and Gli2) and Gli transcriptional activity. Furthermore, EGCG inhibited EMT by inhibiting the expression of Snail, Slug and ZEB1, and TCF/LEF transcriptional activity, which correlated with significantly reduced CSC's migration and invasion, suggesting the blockade of signaling involved in early metastasis. Furthermore, combination of quercetin with EGCG had synergistic inhibitory effects on self-renewal capacity of CSCs through attenuation of TCF/LEF and Gli activities. Since aberrant SHh signaling occurs in pancreatic tumorigenesis, therapeutics that target SHh pathway may improve the outcomes of patients with pancreatic cancer by targeting CSCs

Kelleher FC. Hedgehog signaling and therapeutics in pancreatic cancer. *Carcinogenesis* 2011 April;32(4):445-51.
Abstract: OBJECTIVE: To conduct a systematic review of the role that the hedgehog signaling pathway has in pancreatic cancer tumorigenesis. METHOD: PubMed search (2000-2010) and literature based references. RESULTS: Firstly, in 2009 a genetic analysis of pancreatic cancers found that a core set of 12 cellular signaling pathways including hedgehog were genetically altered in 67-100% of cases. Secondly, in vitro and in vivo studies of treatment with cyclopamine (a naturally occurring antagonist of the hedgehog signaling pathway component; Smoothened) has shown that

inhibition of hedgehog can abrogate pancreatic cancer metastasis. Thirdly, experimental evidence has demonstrated that sonic hedgehog (Shh) is correlated with desmoplasia in pancreatic cancer. This is important because targeting the Shh pathway potentially may facilitate chemotherapeutic drug delivery as pancreatic cancers tend to have a dense fibrotic stroma that extrinsically compresses the tumor vasculature leading to a hypoperfusing intratumoral circulation. It is probable that patients with locally advanced pancreatic cancer will derive the greatest benefit from treatment with Smoothened antagonists. Fourthly, it has been found that ligand dependent activation by hedgehog occurs in the tumor stromal microenvironment in pancreatic cancer, a paracrine effect on tumorigenesis. Finally, in pancreatic cancer, cells with the CD44+CD24+ESA+ immunophenotype select a population enriched for cancer initiating stem cells. Shh is increased 46-fold in CD44+CD24+ESA+ cells compared with normal pancreatic epithelial cells. Medications that destruct pancreatic cancer initiating stem cells are a potentially novel strategy in cancer treatment. CONCLUSIONS: Aberrant hedgehog signaling occurs in pancreatic cancer tumorigenesis and therapeutics that target the transmembrane receptor Smoothened abrogate hedgehog signaling and may improve the outcomes of patients with pancreatic cancer

Bisht S, Brossart P, Maitra A, Feldmann G. Agents targeting the Hedgehog pathway for pancreatic cancer treatment. *Curr Opin Investig Drugs* 2010 December;11(12):1387-98.
Abstract: Recent evidence has demonstrated that aberrant reactivation of the Hedgehog signaling pathway contributes to tumor initiation and progression in various human malignancies, including pancreatic cancer; therefore, the Hedgehog pathway has emerged as a promising novel therapeutic target. Initial translational studies conducted using cyclopamine, a small-molecule inhibitor of the Smoothened (SMO) component of the Hedgehog pathway, demonstrated that pharmacological blockade of aberrant Hedgehog signaling has the potential to inhibit tumor initiation, progression and metastatic spread. This concept has been corroborated using different compounds in various preclinical models of pancreatic cancer and other malignancies; several of these studies suggest possible therapeutic synergisms of Hedgehog inhibitors with established antineoplastic agents. This review provides a concise overview of translational studies assessing the use of Hedgehog inhibitors as novel therapeutic strategy for cancer, particularly pancreatic cancer

Inaguma S, Kasai K, Ikeda H. GLI1 facilitates the migration and invasion of pancreatic cancer cells through MUC5AC-mediated attenuation of E-cadherin. *Oncogene* 2011 February 10;30(6):714-23.
Abstract: The Kruppel-like zinc-finger protein GLI1 functions as a downstream transcription factor of Hedgehog signaling and plays a pivotal role in the cellular proliferation of many types of tumors, including pancreatic ductal adenocarcinoma (PDA). PDA develops from dysplastic lesions called pancreatic intraepithelial neoplasia (PanIN) through a multistep carcinogenesis process that changes its cellular characteristics, including a mucin expression profile. Increased expression of a gel-forming mucin, MUC5AC, was previously revealed as a major biomarker for the poor prognosis of PDA patients, but the molecular mechanisms responsible for its expression and correlation with poor prognosis are not fully understood. Here we show that MUC5AC is a direct transcriptional target of GLI1 in PDA cells. Overexpression of GLI1 enhanced MUC5AC expression, and a double knockdown of GLI1 and GLI2 suppressed endogenous MUC5AC expression in PDA cells. Luciferase reporter assays revealed that GLI1 and GLI2 can activate the MUC5AC promoter through its conserved CACCC-box-like cis-regulatory elements. We also found that GLI1-upregulated MUC5AC was expressed in the intercellular junction between cultured PDA cells and interfered with the membrane localization of E-cadherin, leading to decreased E-cadherin-dependent cell-cell adhesion and promoting the migration and invasion of PDA cells. Consistently, GLI1 induced the nuclear accumulation and target gene expression of beta-catenin in a MUC5AC-dependent manner. Finally, immunohistochemical analysis revealed that GLI1 expression statistically correlated with MUC5AC expression and also with altered subcellular localization of E-cadherin and beta-catenin in

PanIN lesions and PDA. This evidence revealed a new aspect of GLI1 function in modulating E-cadherin/beta-catenin-regulated cancer cell properties through the expression of a gel-forming mucin

Dai J, Ai K, Du Y, Chen G. Sonic hedgehog expression correlates with distant metastasis in pancreatic adenocarcinoma. *Pancreas* 2011 March;40(2):233-6.
Abstract: OBJECTIVES: To investigate the expression and clinical significance of Sonic hedgehog (Shh) in pancreatic adenocarcinoma. METHODS: The expression of Shh protein was examined in 34 surgical specimens of primary pancreatic adenocarcinoma, 21 nonmalignant specimens of the pancreas by immunohistochemistry streptavidin-perosidase (SP) method. In addition, semiquantitative reverse transcriptase polymerase chain reaction was carried out to analyze Shh mRNA expression in 22 pairs of freshly resected pancreatic adenocarcinoma tissues and their adjacent nontumorous tissues. RESULTS: The positive expression rate of Shh protein was 64.7% (22/34) in 34 surgical specimens of primary pancreatic adenocarcinoma and 0% (0/21) in 21 nonmalignant specimens of the pancreas. The expression rate of Shh was higher in pancreatic adenocarcinoma tissues than that of nonmalignant pancreatic tissues (chi2 = 22.647, P = 0.000). Sonic hedgehog protein expression correlated with TNM stages and distant metastasis. Moreover, the expression levels of Shh mRNA were higher in pancreatic adenocarcinoma tissues than that of the matched adjacent nontumorous tissues. CONCLUSIONS: Sonic hedgehog might play a pivotal role during tumorigenesis of pancreatic adenocarcinoma, and high Shh expression might be associated with the malignant potential of pancreatic cancer

Olive KP, Jacobetz MA, Davidson CJ et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 2009 June 12;324(5933):1457-61.
Abstract: Pancreatic ductal adenocarcinoma (PDA) is among the most lethal human cancers in part because it is insensitive to many chemotherapeutic drugs. Studying a mouse model of PDA that is refractory to the clinically used drug gemcitabine, we found that the tumors in this model were poorly perfused and poorly vascularized, properties that are shared with human PDA. We tested whether the delivery and efficacy of gemcitabine in the mice could be improved by coadministration of IPI-926, a drug that depletes tumor-associated stromal tissue by inhibition of the Hedgehog cellular signaling pathway. The combination therapy produced a transient increase in intratumoral vascular density and intratumoral concentration of gemcitabine, leading to transient stabilization of disease. Thus, inefficient drug delivery may be an important contributor to chemoresistance in pancreatic cancer

Chun SG, Zhou W, Yee NS. Combined targeting of histone deacetylases and hedgehog signaling enhances cytoxicity in pancreatic cancer. *Cancer Biol Ther* 2009 July;8(14):1328-39.
Abstract: Combined targeting of distinct cellular signaling mechanisms may improve the efficacy and reduce the toxicity of therapy in pancreatic cancer. Histone deacetylases (HDACs) control cellular functions through epigenetic modulation, and HDACs inhibitors suppress cell growth in pancreatic adenocarcinoma. The Hedgehog (Hh) pathway regulates the development of the pancreas, and aberrant Hh signaling promotes the initiation and progression of pancreatic neoplasia. We hypothesize that HDACs and the Hh pathway cooperatively interact to regulate cellular proliferation of the exocrine pancreas. A combination of the HDAC inhibitor SAHA and the Smoothened antagonist SANT-1 was evaluated for their ability to suppress growth of the Gemcitabine-resistant pancreatic adenocarcinoma cell lines Panc-1 and BxPC-3. The combination of SAHA and SANT-1 supra-additively suppressed cellular proliferation and colony formation. Flow cytometric and immunohistochemical analyses indicated that enhanced induction of apoptotic cell death, cell cycle arrest in G(0)/G(1) phase, and ductal epithelial differentiation are involved. Cell death was associated with nuclear localization of survivin, increased bax expression, and activation of caspases 3 and 7. Consistent with the cell cycle arrest and cytodifferentiation, the cyclin-dependent kinase inhibitors p21(waf) and p27(kip1) were upregulated, and cyclin D1 downregulated. The potentiated anti-proliferative effect by the combination of SAHA and SANT-1 may involve cooperative suppression

of the Hh pathway activity, as shown by the upregulation of HHIP by SAHA, and enhanced repression of of Ptc-1 mRNA expression. In summary, we have developed a molecular target-based therapeutic approach that overcomes chemoresistance in pancreatic cancer cells by chemically inhibiting HDACs and Hh signaling in combination

Cengel KA. Targeting Sonic Hedgehog: a new way to mow down pancreatic cancer? *Cancer Biol Ther* 2004 February;3(2):165-6.
Abstract: Despite continuing development of new therapies, the prognosis for patients with pancreatic cancer remains extremely poor. In part, this may relate to molecular abnormalities that stimulate pancreatic tumorigenesis and also contribute to reduced sensitivity to standard treatments such as chemotherapy and radiotherapy. Two recent reports in Nature suggest that Sonic Hedgehog (Shh) overexpression may contribute to pancreatic tumorigenesis and that cyclopamine, a specific inhibitor of Shh signaling, can reduce pancreatic cancer cell growth and viability. This discovery is exciting and suggests that targeting Shh signaling may be an effective novel approach to therapy in patients with this devastating disease

Metronomic Cyclophosphamide

Bojko P, Schimmel G, Bosse D, Abenhardt W. Metronomic oral cyclophosphamide in patients with advanced solid tumors. *Onkologie* 2012;35(1-2):35-8.

Abstract: BACKGROUND: Cure is rarely achieved in patients with advanced metastatic solid tumors, and quality of life including times without burdening therapies is an important endpoint. Metronomic oral cyclophosphamide (Cy) has been studied before and is a reasonable option. PATIENTS AND METHODS: 24 patients with a mean age of 64.4 years (range 36-82 years) were studied. 18 patients had breast cancer, 4 prostate cancer, 1 uterine carcinoma, and 1 carcinoma of unknown primary. RESULTS: All patients had advanced disease with a mean of 2 metastatic sites. Cy was given at a mean dosage of 52 mg daily. Time from diagnosis to start of Cy was 108.6 +/- 7.6 months, and from occurrence of metastatic disease to Cy 45.8 +/- 45.6 months. Patients had received a mean of 4.2 +/- 2.1 prior regimens for metastatic disease. The mean time to treatment failure was 6.4 +/- 5.4 months, and mean overall survival was 12.7 +/- 7.3 months. Patients received 2.1 +/- 1.4 further treatments upon progression. Main toxicities were grade 1 and 2 (n = 25); 3 patients had grade 3 nausea, leucopenia, and elevated gamma glutamyl transferase, respectively. CONCLUSION: Low-dose oral Cy is a reasonable, generally well tolerated, and inexpensive option for patients with advanced solid tumors

Lasalvia-Prisco E, Goldschmidt P, Galmarini F et al. Addition of an induction regimen of antiangiogenesis and antitumor immunity to standard chemotherapy improves survival in advanced malignancies. *Med Oncol* 2012 July 19.
Abstract: Studies have shown that cancer requires two conditions for tumor progression: cancer cell proliferation and an environment permissive to and conditioned by malignancy. Chemotherapy aims to control the number and proliferation of cancer cells, but it does not effectively control the two best-known conditions of the tumor-permissive environment: neoangiogenesis and tolerogenic immunity. Many malignant diseases exhibit poor outcomes after treatment with chemotherapy. Therefore, we investigated the potential benefits of adding an induction regimen of antiangiogenesis and antitumor immunity to chemotherapy in poor outcome disease. In a prospective, randomized trial, we included patients with advanced, unresectable pancreatic adenocarcinomas, non-small cell lung cancer, or

prostate cancer. Two groups of each primary condition were compared: group 1 (G1), n = 30, was treated with the standard chemotherapy and used as a control, and group 2 (G2), n = 30, was treated with chemotherapy plus an induction regimen of antiangiogenesis and antitumor immunity. This induction regimen included a low dose of metronomic cyclophosphamide, a high dose of Cox-2 inhibitor, granulocyte colony-stimulating factor, a sulfhydryl (SH) donor, and a hemoderivative that contained autologous tumor antigens released from patient tumors into the blood. After treatment, the G2 group demonstrated significantly longer survival, lower blood level of neoangiogenesis and immune-tolerance mediators, and higher blood levels of antiangiogenesis and antitumor immunity mediators compared with the G1 group. Toxicity and quality of life were not significantly different between the groups. In conclusion, in several advanced malignancies of different primary localizations, an increase in survival was observed by adding an induction regimen of antiangiogenesis and antitumor immunity to standard chemotherapy

Blansfield JA, Caragacianu D, Alexander HR, III et al. Combining agents that target the tumor microenvironment improves the efficacy of anticancer therapy. *Clin Cancer Res* 2008 January 1;14(1):270-80.
Abstract: PURPOSE: Over the past 60 years, cytotoxic chemotherapy has targeted the cancer cell. Despite this, there have been few cancer cures. A new approach to cancer therapy is to target the multicellular biological entity of the tumor microenvironment. EXPERIMENTAL DESIGN: Lenalidomide, an immunomodulatory drug, sunitinib, a tyrosine kinase inhibitor, and low-dose metronomic cyclophosphamide, were tested alone and in combination for their abilities to inhibit endothelial cell tube formation, rat aortic ring outgrowth, tumor growth, and metastatic development in mice. In addition, ectopic tumor lysates were evaluated for the presence of proangiogenic proteins. RESULTS: The three agents alone were shown to significantly inhibit endothelial cells' ability to form tubes and significantly inhibit the multicellular microenvironment in the rat aortic ring assay ($P < 0.01$ and $P < 0.001$). This effect was also significantly augmented when the agents were combined. Furthermore, the three-drug combination was able halt the progression of tumor growth almost completely in xenograft models of ocular melanoma, colon cancer, pancreatic cancer, and cutaneous melanoma. These agents significantly decrease the number of proliferating cells in tumors, significantly increase the number of cells undergoing active cell death in tumors, and significantly decrease the number of blood vessels in treated tumors ($P < 0.05$). Combination therapy shows a decrease in the compensatory up-regulation of proangiogenic proteins after treatment when compared with single-agent therapy. CONCLUSIONS: This combination of agents causes an inhospitable microenvironment for tumor cells and shows great promise for use in the clinic

Man S, Bocci G, Francia G et al. Antitumor effects in mice of low-dose (metronomic) cyclophosphamide administered continuously through the drinking water. *Cancer Res* 2002 May 15;62(10):2731-5.
Abstract: A number of recent preclinical studies have sparked interest in the concept of exploiting conventional chemotherapeutic drugs as antiangiogenics. Such antiangiogenic activity is achieved or optimized by metronomic-dosing protocols in which the drug is given at comparatively low doses using a frequent schedule of administration (e.g., once to three times per week) with no breaks, particularly when combined with an endothelial cell-specific antiangiogenic drug. The use of p.o. chemotherapeutic drugs is particularly suitable for this type of treatment strategy. We tested one such drug, cyclophosphamide (CTX), in a protocol wherein the drug was administered to mice at low doses, of approximately 10-40 mg/kg on a daily basis through the drinking water. CTX is typically given p.o. to patients, but it has almost always been injected when treating preclinical mouse tumor models. We found p.o. CTX to be a safe and convenient treatment with significant antitumor efficacy. Growth delays were observed for human orthotopic breast or ectopic colon cancer xenografts in nude or SCID mice. Established PC3 human prostate tumor xenografts could be induced to almost fully regress, remaining virtually nonpalpable for > or =2 months of continuous therapy, after which tumors began to

grow progressively. These re-emergent tumors were not found to be drug resistant when tested in new hosts, using the same treatment protocol. Regression of spontaneously arising, late-stage pancreatic islet cell carcinomas in Rip Tag transgenic mice was also observed. The effects of continuous p.o. CTX treatment were enhanced significantly in an orthotopic, metastatic breast cancer xenograft model when used in combination with an antivascular endothelial growth factor receptor-2 blocking antibody. Maximum tolerated dose levels established for other mouse strains proved highly toxic to SCID mice, whereas daily p.o. low-dose regimens of CTX were well tolerated. Taken together, the results demonstrate the feasibility of delivering CTX in a p.o. metronomic chemotherapy regimen, which proved safe, reasonably efficacious, and potentially applicable to chronic treatment. Such a regimen may be particularly well suited for integration with antiangiogenic drugs

Hanahan D, Bergers G, Bergsland E. Less is more, regularly: metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. *J Clin Invest* 2000 April;105(8):1045-7.