

TITLE: Oral antisense oligonucleotides to mitigate GI crypt survival post-irradiation**A. SPECIFIC AIMS**

Gastrointestinal (GI) injury is a major cause and contributor of death after whole-body irradiation (Jarrett 1999; NCRP 2001), agents to mitigate the effects of acute GI radiation injury are needed. Acute radiation enteritis results from (1) loss of the epithelial stem cell population within the intestinal crypts, (2) subsequent breakdown of the epithelial barrier protection, and (3) mucosal inflammation in response to the injury. The interactions of these events accentuate the injury, e.g. mucosal breakdown allows translocation of bacteria resulting in further mucosal inflammation. Subsequently, mucosal inflammation causes regional tissue damage resulting in further mucosal injury and loss of barrier protection (Roberts, Foulcher et al. 1993; Fajardo, Berthrong et al. 2001). Apoptotic and inflammatory signaling have been identified as potential biological targets to prevent radiation injury following exposure (Coleman, Blakely et al. 2003). Antisense oligonucleotide drugs targeting the signaling events in apoptosis and inflammation are in development.

We have established a collaborative agreement with Isis Pharmaceuticals (Isis Pharmaceuticals, Carlsbad, CA), the leading manufacturer of antisense oligonucleotide drugs. Isis Pharmaceuticals was the first to secure Food and Drug Administration (FDA) approval for an antisense drug, fomivirsen (currently on the market) was approved for cytomegalovirus retinitis in 1998 (Marwick 1998). Isis Pharmaceuticals has a pipeline of antisense oligonucleotide (ASON) drugs targeting apoptosis and inflammation which may have efficacy in radiation enteritis/enteropathy. We believe oral agents with long shelf-lives that do not require refrigeration and with once daily dosing have the potential for widest distribution and impact on survival following a major radiation incident. Antisense oligonucleotide drugs meet these requirements, these agents are versatile and may be formulated for oral (Stepkowski, Chen et al. 2001; Raoof, Chiu et al. 2004), enema (Miner, Geary et al. 2006; van Deventer, Wedel et al. 2006), subcutaneous (Bennett, Kornbrust et al. 1997), or aerosol inhalation (Nyce 2002; Sandrasagra, Leonard et al. 2002) delivery. To our knowledge, this class of agents has not been previously tested for its potential as radiation mitigation agents, to ameliorate radiation injury after exposure.

We have over 35 years experience and archived microscopic slides of radiation enteritis studies in murine models using the crypt and apoptotic assays (Withers and Elkind 1970; Withers, Mason et al. 1974; Weil, Stephens et al. 1996). These assays require the manual evaluation of histological sections by an expert, which has limited their use by other laboratories. Computer-assisted image analysis has been shown to improve accuracy and throughput in evaluation of cytopathology and/or histopathology (Kok and Boon 1996; Nieminen, Kotaniemi et al. 2005). We have extensive image analysis expertise, automating the crypt and apoptotic assays will improve the speed and accuracy of these techniques and allow for a broad distribution of these assay techniques to other laboratories for future studies of GI radiation injury.

Hypothesis: Antisense oligonucleotide oral therapy, biologically targeted to inhibit apoptosis or inflammation, will preserve/restore gastrointestinal crypts after radiation exposure.

Specific Aims:

1. **Test inhibition of inflammation signaling by antisense drug therapy given orally post-irradiation in a mouse model using intestinal crypt survival assay.** We will test the ability of (antisense) ICAM-1, TGF- β 1, I κ B, and IL-6 ASON drugs to reduce the radiation enteritis using an intestinal crypt survival assay endpoint. We will quantify (versus controls) for radiation dose modification.
2. **Test inhibition of apoptotic signaling by anti-sense drug therapy given orally post-irradiation in a mouse model using intestinal crypt apoptotic assay.** We will test (antisense) p38, p53, TNF, TNFR1, fas, caspase-3, I κ B and bax ASON drugs as mitigation agents of apoptosis following whole-body irradiation. We will quantify crypt cell apoptosis versus controls for dose modification (Weil, Stephens et al. 1996).
3. **Test doublet combinations for increased survival in a mouse GI morbidity model.** We will measure the LD₅₀ (<10 days) following total abdomen irradiation with 3 sets of doublet ASON drugs in a mouse GI morbidity model. Survival free from significant GI morbidity at 10 days is the end-point.
4. **Test automated image analysis for crypt assay versus manual counting.** We will develop computer-assisted histological evaluation software to automate the evaluation of cytopathology and/or histopathology of gastrointestinal radiation injury. Computer-assisted detection and analysis of crypts, scoring of crypt survival and apoptosis will be tested against manual counting by experts.

B. BACKGROUND AND SIGNIFICANCE

Radiation Enteritis

Radiation interacts with living tissue causing direct ionization and forming free radicals, both of which result in DNA damage (Hall and Giaccia 2005). A constellation of symptoms occurs following total body irradiation in mammals above 10 Gy related to loss of the epithelial lining of the GI tract. There is the onset of early symptoms consisting of severe nausea, vomiting, and diarrhea (Hempelmann, Lisco et al. 1952). The fluid loss leads to dehydration, loss of the absorptive capacity of the GI tract occurs, and loss of the epithelial barrier protection to bacteria. Death may occur from this injury within days. The epithelial lining of the gastrointestinal tract is highly organized with a polarized distribution of function, functional contact and barrier protection with the lumen side and a distribution of focal proliferative compartments at the other pole. The small intestines consist of columnar epithelium with villi protruding into the lumen, the epithelial cells are constantly being lost into the lumen from the ends of the villi. At the base of the villi lies the intestinal crypts, where the proliferation of epithelial cells occurs, new epithelial cells are generated in the crypts and migrate to the end of the villi in a few days. This cellular kinetics explains the time course of the GI acute radiation syndrome. The stem cells present in the proliferative compartment are highly sensitive to radiation injury, when radiation damages the DNA in these cells they undergo p53-dependent apoptosis (Merritt, Potten et al. 1994). Apoptosis of intestinal crypt cells peaks within hours of exposure and may remain elevated for 24 hours (Weil, Stephens et al. 1996). Leukocyte adherence and migration into the radiation injured site begins within 2 hours and is propagated by pro-inflammatory cytokines produced at the damaged site (Panes, Anderson et al. 1995). The leukocyte response remains elevated at 14 days post-irradiation (Molla, Gironella et al. 2003). The early inflammatory response is mediated by an induction of ICAM-1, whereas the prolonged leukocyte response is mediated by VCAM-1 induction (Molla, Gironella et al. 2003). Apoptosis of the intestinal crypt stem cells and the inflammatory response to injury are central to radiation enteritis.

Antisense oligonucleotide drugs

Antisense oligonucleotide (ASON) drugs are a new class of drugs targeted to suppress expression of genes involved in disease through interactions with messenger RNA (mRNA) (Crooke 1998). ASON drugs are designed to complement and bind (hybridize) to a segment of mRNA to form a double stranded hybrid. The formation of this double stranded hybrid molecule prevents the mRNA from functioning normally and from producing the specific targeted protein. The targeted mRNA is degraded (by RNase H), while the ASON drug is not a substrate for the degradation enzymes due to backbone modifications. First-generation ASON drugs contain sulfur modification (phosphorothioate oligonucleotides) to improve their stability, however they remain susceptible to *in vivo* DNase degradation. Second generation compounds further substitute the phosphorothioates with 2'-methoxyethyl (2'MOE) modification, resulting in increased antisense oligonucleotide target binding affinity and resistance to degradation *in vivo*. ASON drugs are designed through sequence specificity to interact only with the intended mRNA target. This high degree of specificity makes ASON drugs more effective and less toxic than traditional drugs. In preclinical studies, such as this one, species specific agents (e.g. mouse specific agents) ensure biologically targeted specificity. The distribution and metabolism of ASON drugs are very similar from drug to drug, resulting in a common, predictable, safety profile across antisense drugs (Jason, Koropatnick et al. 2004).

To act as radiation mitigation agents ASON drugs need to target biological signaling events that occur through new proteins induction by radiation. For example, the inflammatory signaling that occurs through the induction of ICAM-1 on endothelial cells following radiation exposure. Alicaforsen (Isis Pharmaceuticals, Carlsbad, CA) is an antisense ICAM-1 drug developed to block ICAM-1 induction which has shown efficacy in a phase II trial for inflammatory bowel disease (van Deventer, Wedel et al. 2006). Antisense ICAM-1 oligonucleotides have shown reduction of pulmonary inflammation in an endotoxin pneumonitis rodent model (Kumasaka, Quinlan et al. 1996), hence may prevent radiation pneumonitis. They were also found to be neuroprotective in a rodent ischemia model (Vemuganti, Dempsey et al. 2004), hence may more broadly ameliorate the acute inflammatory effects of whole-body radiation. Candidate biological targets should have demonstrated reduced injury in knock-out studies. In this study, we will target (with ASON drugs) apoptotic and inflammatory signaling events that have been identified as potential biological targets to prevent radiation injury.

ICAM-1

ICAM-1 is a monomeric, unpaired, cell-surface glycoprotein of 76 to 114 kdaltons and is found on the surface of most nucleated cell types. ICAM-1 (also called CD 54) is a member of the immunoglobulin supergene family, and is composed of an extracellular domain containing five tandemly arranged immunoglobulin-like domains, a

transmembrane region, and a cytoplasmic domain. The migration of leukocytes from the blood to irradiated lung tissue requires the induction of endothelial surface adhesion molecules such as ICAM-1. Leukocyte binding to the endothelium through the ICAM-1/LFA-1 interaction is necessary for activation and transendothelial migration (Yang, Froio et al. 2005). Endothelial cells up regulate expression of ICAM-1 in response to tissue-released inflammatory mediators, which facilitates the transport of leukocytes to sites of tissue injury (Dustin, Rothlein et al. 1986; Pober, Gimbrone et al. 1986; Rothlein, Dustin et al. 1986).

The development of inflammation requires leukocyte-endothelial cell interaction via ICAM-1 / LFA-1 interaction and subsequent transmigration of leukocytes into the injury tissue. Expression of ICAM-1 in response to radiation is time- and dose-dependent in irradiated cells (Hallahan, Kuchibhotla et al. 1996). In a study of oral mucositis during radiotherapy, serial punch biopsies were obtained in 13 patients after 0, 30, and 60 Gy (Handschel, Prott et al. 1999). There was a progressive increase in endothelial expression of ICAM-1 and leukocyte infiltration at 30 and 60 Gy observed over baseline. In a murine model of radiation-induced intestinal injury, administration of anti-ICAM-1 antibodies to mice and ICAM-1 deficient mice both had decreased leukocyte adhesion at 24 h post-irradiation (Molla, Gironella et al. 2003). Intestinal epithelial regeneration was enhanced in ICAM-1 deficient mice when examined 14 days following single-exposure. There was a statistically significant increase in the number of proliferating cells (non-Paneth cells) per crypt and the number of mitotic figures within intestinal crypts in the ICAM-1 deficient mice at 14 days after abdominal irradiation. In a rat model of radiation colitis, ICAM-1 mRNA levels were found to increase markedly with subsequent ICAM-1 protein expression peaking one day after 22.5 Gy irradiation, and protein expression remained elevated up to 6 days (Ikeda, Ito et al. 2000). The number of inflammatory myeloperoxidase (MPO)-positive cells in lamina propria mucosa increased in a time-dependent fashion from 6 h to 6 days after irradiation. These data suggest that up-regulation of ICAM-1 in endothelial cells and accumulation of MPO-positive cells play important roles in the development of radiation-induced gastrointestinal injury. ICAM-1 induction is seen in other tissues acutely following radiation exposure, including the central nervous system (Hong, Chiang et al. 1995; Chiang, Hong et al. 1997), pulmonary tissue (Hallahan and Virudachalam 1997; Hallahan and Virudachalam 1997), and liver (Meineke, Moede et al. 2002). We hypothesize blocking ICAM-1 induction with antisense oligonucleotide therapy after irradiation will reduce subsequent radiation enteritis. It may also result in a systemic reduction in post-irradiation inflammation in all other tissues irradiated.

In an experimental colitis murine model, administration of antisense ICAM-1 after the onset of colitis reduced the severity of colitis with every other day dosing over a 2 weeks period (Bennett, Kornbrust et al. 1997). ICAM-1 deficient mice were found to be resistant to lethal doses of endotoxin and induced septic shock (Xu, Gonzalo et al. 1994), antisense ICAM-1 may confer similar protection. Isis Pharmaceuticals (Carlsbad, CA) have developed an antisense ICAM-1 oligonucleotide drug (alicaforsen) for inflammatory bowel disease, presently in phase III trials (van Deventer, Wedel et al. 2006).

TGF- β 1

TGF- β signaling consists of a 25 kdalton homodimer binding to a membrane receptor, the ligand causes the assembly of a receptor complex, a cytoplasmic serine/threonine kinase phosphorylates proteins of the SMAD family, the phosphorylated SMAD moves to the nucleus where a complex is formed that activates TGF- β responsive gene expression (Massague 2000). There are 9 SMAD (SMAD1-9) intermediate signaling agents that have been characterized and three TGF- β isoforms (TGF- β 1, TGF- β 2, and TGF- β 3). Though the recipient cell determines the outcome of the signaling event, TGF- β 1 is implicated mainly in the pathogenesis of fibrosis. A dose dependent increased expression of TGF- β 1 mRNA is found within irradiated tissues within hours of irradiation and may persist for months (Randall and Coggle 1995; Seong, Kim et al. 2000). Blocking TGF- β signal transduction is expected to have a beneficial effect. In one study, administration of a soluble TGF- β type II receptor (T β R-II) protein for 6 weeks was found to ameliorates intestinal radiation injury in a rodent model (Zheng, Wang et al. 2000). There was reduced structural injury, preservation of the mucosal surface area, increased crypt cell proliferation, and less intestinal wall fibrosis in the treated group at 6 weeks. ASON therapy may also prove beneficial, ASON TGF- β 1 therapy demonstrated in post-surgical ocular scarring murine model reduced infiltration of inflammatory cells, and significantly reduced post-operative scarring following a single administration of anti-sense TGF- β 1 (Cordeiro, Mead et al. 2003). In another study, anti-sense TGF- β 1 blocked interstitial renal fibrosis following ureteral obstruction (Isaka, Tsujie et al. 2000). In this study we hypothesize ASON TGF- β 1 therapy will reduce radiation enteritis and improve crypt survival post-irradiation.

I κ B

NF- κ B consists of a heterodimeric complex of p50/p65 that functions as a central mediator of the immune response. Recent work (Nenci, Becker et al. 2007) confirms the critical function of NF- κ B as a regulator

epithelial integrity and intestinal immune homeostasis, suggesting an important role in controlling the pathogenesis of bowel inflammation. The cytosolic p50/p65 complex is maintained in an inactive state by the binding of the inhibitor of NF- κ B, I κ B, to p65. In response to various stimuli, I κ B is phosphorylated and degraded, allowing the translocation of NF- κ B to the nucleus, where it then binds to its DNA and induces transcription of many genes including TNF- α and IL-6. In a murine model of intestinal inflammation, p65 ASON was shown to prevent NF- κ B activation and cytokine production, abrogating intestinal inflammation at both the physiologic and histological levels (Neurath, Pettersson et al. 1996; Neurath, Becker et al. 1998). Similarly, Lawrance et al. (Lawrance, Wu et al. 2003) demonstrated that p65-specific ASON could prevent inflammation-induced fibrosis when administered both before and following repeated intrarectal exposure to trinitrobenzene sulfonic acid. Inhibition of NF- κ B with caffeic acid phenethyl ester was found to reduce inflammatory cytokines within the GI tract after total-body irradiation in rats (Linard, Marquette et al. 2004). These findings suggest that inhibition of NF- κ B may produce a long-term benefit, reducing inflammation and subsequent fibrosis. However, in the immediate post-irradiation time-frame NF- κ B may have a protective role.

Absence of the product of the ataxia telangiectasia gene (ATM) results in defective NF- κ B activation and increased radiosensitivity (Li, Banin et al. 2001), suggesting that NF- κ B mediates part of the radioprotective role of wild-type ATM. In an *in vitro* study, a dominant negative I κ B mutation that blocks NF- κ B from entering the nucleus results in enhanced radiosensitivity (Wang, Hu et al. 2005). *In vitro* and *in vivo* studies have demonstrated that constitutive NF- κ B activation appears to inhibit the cytotoxicity of radiation (Russo, Tepper et al. 2001). Wang et al. (Wang, Meng et al. 2004) demonstrated increased intestinal epithelial cell apoptosis at 24 hours following 8 Gy total body irradiation (TBI) in p65 (RelA)/TNFR1-/- mice compared to control animals. Zhang et al. demonstrated enhanced apoptosis in vascular smooth muscle cells transfected with an NF- κ B inhibitory decoy (Zhang, Park et al. 2005). In that study, p50-/- mice were found to have increased sensitivity to TBI, with an LD50 (< 7 days) of 7.75 Gy versus 13.12 Gy for the wild-type. In another study, inactivation of NF- κ B signaling in mice resulted in increased p53 activation and increased intestinal epithelial apoptosis following TBI (Egan, Eckmann et al. 2004). In that study, TBI of wild-type mice was found to result in a 2.5-fold increase in I κ B α mRNA 4 h post-irradiation, suggesting a negative-feedback role. Finally, the Cleveland BioLabs agent CBLB502, which has demonstrated radioprotection in rhesus monkeys following TBI, is proposed to act through activation of NF- κ B (Cleveland BioLabs 2006). In this study, we hypothesize that blocking I κ B induction using ASON agents following irradiation will reduce intestinal apoptosis and improve crypt survival.

Interleukin-6 (IL-6)

IL-6 is a monomeric 184 amino acid secreted peptide produced by T lymphocytes, macrophages, and endothelial cells in response to tissue injury/trauma, infection, and immunological challenge. In addition to its direct immunomodulatory role in lymphocytes where it mediates the expansion and activation of T cells and differentiation of B cells, IL-6 also plays a key role in modulating the acute-phase inflammatory response to tissue injury. This response results in the release of proteins that mediate pleiotropic effects including phagocytosis of bacteria, activation of the complement and clotting cascades, and development of a systemic pyrogenic response (Castell, Gomez-Lechon et al. 1988). IL-6 signals through a membrane-bound receptor complex consisting of the ligand-binding IL-6 receptor and the signal-transducing component gp130 (also called CD130). gp130 is ubiquitously expressed in most tissues and serves as the common signal transducer for several cytokines. Activation of the IL-6 receptor by IL-6 binding to its extracellular domain leads to dimerization of gp130 and the recruitment of a complex of signal transduction proteins, most notably the Janus kinases (JAKs) and Signal Transducers and Activators of Transcription (STATs). JAKs phosphorylate tyrosine residues on the intracellular domain of the IL-6 receptor complex. This, in turn, leads to docking of STATs that possess phosphotyrosine residue-binding SH2 domains. Furthermore, tyrosine-phosphorylation of STATs by JAK mediates their dimerization. Activated STAT hetero- and homo-dimers translocate to the nucleus and regulate the transcription of target genes controlling inflammation and oncogenesis. STATs may also be tyrosine-phosphorylated by other non-receptor tyrosine kinases such as src or non-cytokine growth factor receptors such as the epidermal growth factor receptor.

Several lines of evidence suggest that IL-6 plays a significant role in the pro-inflammatory signaling response to radiation therapy of the intestinal tract and the consequent tissue injury. First, localized irradiation of the intestine (most of the ileum and cecum) of mice to 20 Gy has been shown to lead to an increase in plasma IL-6. Administration of an anti-IL-6 mAb to intestinal-irradiated mice abrogated the elevation of fibrinogen noted during the acute phase response (Mouthon, Vandamme et al. 2001). Second, these increases in IL-6 result from activation of normal epithelial cells, immune cells, and vascular endothelial cells. Increase in IL-6 after liver irradiation contribute to radiation-induced liver disease and reactivation of hepatitis B virus in patients

treated for hepatocellular carcinoma, suggesting a role for the normal epithelial cells and the immune cells for the resultant tissue injury (Christiansen, Sheikh et al. 2006). Radiation exposure also results in vascular endothelial cell mediated induction of IL-6, IL-8, and ICAM-1 expression resulting in an inflammatory reaction (Van Der Meeren, Squiban et al. 1999). Third, blockade of IL-6 signaling using a specific IL-6 receptor antibody abrogates the inflammatory response in a murine model of colitis. In this murine model of colitis similar to Crohn's disease, treatment with a rat antibody (MR-16-1) to mouse IL-6 receptor suppressed colitis by inducing apoptosis of T cells in the lamina propria which are responsible for continual stimulation of inflammatory signals via ICAM-1, vascular cell adhesion molecule 1 (VCAM-1), interferon- γ (IFN- γ), TNF, and IL-1 β (Atreya, Mudter et al. 2000; Yamamoto, Yoshizaki et al. 2000). Lastly, in a phase II trial, treatment with 8 mg/kg tocilizumab either every 2 weeks or every 4 weeks significantly reduced Crohn's disease activity. Treatment every 2 weeks was superior to that every 4 weeks (Ito, Takazoe et al. 2004), but the incidence of adverse events did not differ between the two groups. Taken together, this evidence suggests that IL-6 serves as a key mediator of pro-inflammatory responses contributing to tissue injury in radiation enteritis and that its suppression could protect against the gastrointestinal toxicity secondary to acute radiation exposure.

p38

p38 is one member of the family of mitogen-activated protein kinases (MAPKs) involved in signal transduction in response to various stress stimuli including oxidative stress (such as ionizing radiation) and cytokine stimulation (Pearson, Robinson et al. 2001). The stress kinase response function of p38 has been shown to be essential for viability, as demonstrated by the embryonic lethality of p38 α knockout mice (Allen, Svensson et al. 2000). p38 has been implicated in GI injury and was shown to increase rat intestinal epithelial apoptosis following hydrogen peroxide exposure (Zhou, Wang et al. 2005). Inhibition of p38 using the chemical inhibitor SB203580, was found to alter the expression of I κ B in various tissues including intestine following the pro-inflammatory effect of LPS exposure in mice, thus integrating the p38 MAPK signaling pathway with that of NF- κ B (Zhang, Ahsan et al. 2005). This p38 inhibitor was also shown to block neutrophil recruitment and intestinal villous destruction induced by *Clostridium difficile* toxin A exposure in mice (Warny, Keates et al. 2000). Similarly, it is anticipated that ASON inhibition of p38 will protect against acute oxidative-stress induced intestinal injury following radiation exposure.

p53

p53 is a widely studied tumor suppressor gene, mapped to chromosome 13 in humans, which functions to arrest the cell cycle following cytotoxic injury, eventually leading either to DNA damage repair and continued growth, or, to apoptosis and programmed cell death. The response of p53 to various genotoxic injuries, such as ionizing radiation, has been shown to be tissue specific, and thought to be due to complex molecular mechanisms, related in part to the p53 expression level in different tissues following the insult (MacCallum, Hupp et al. 1996). Various studies have found no detectable p53 prior to irradiation, but both a time- and dose-dependent increase in intestinal p53 expression following irradiation in a murine model was observed (Merritt, Allen et al. 1997; Wilson, Pritchard et al. 1998). In one study, prior to irradiation there is no detectable p53 found within the small intestinal epithelial crypt cells, however within 3 hours after irradiation p53 was strongly expressed (Merritt, Potten et al. 1994). Merritt et al. demonstrated reduced intestinal injury, as measured by crypt apoptosis at 8 and 24 hours, following irradiation of p53-null mice, but no difference in apoptotic levels at 1 week, suggesting a role for p53 in mitigating the early response of the gastrointestinal track to radiation, but that apoptosis may have a p53-independent mechanism. Komarova et al. (Komarova, Kondratov et al. 2004) similarly demonstrated that the villous epithelium of p53-deficient mice did not undergo growth arrest, unlike that of the wild-type animals, following radiation exposure, but this led to rapid villi destruction and accelerated lethality in the knockout mice. These investigators also found that the inflammatory response in the p53-null mice was suppressed at molecular, cellular and organism levels compared with the wild-type mice, and that this suppression was mediated via inhibition of NF- κ B-dependent promoters (Komarova, Krivokrysenko et al. 2005). ASON targeting p53 mRNA was found to increase radiation-induced apoptosis of non-small cell lung cancer cell lines with functional p53 at baseline, with the ASON-treated cells demonstrating less cell cycle arrest than corresponding ASON-treated cells without functional p53 at baseline (Sak, Wurm et al. 2003). This effect of p53-specific ASON inhibition of cell cycle arrest was associated with enhanced liver regeneration following partial hepatectomy in rats compared to control animals (Arora and Iversen 2000). In the former studies, p53 was inhibited or absent prior to the cytotoxic event, whereas in the last study described, p53 was inhibited following the insult. Komarov demonstrated improved survival in mice following 8 Gy TBI and treatment with pifithrin- α , a p53 inhibitor (Komarov, Komarova et al. 1999). Strom demonstrated improved mice survival following 8 to 9 Gy TBI and

pretreatment with pifithrin- μ , a small molecule p53 inhibitor (Strom, Sathe et al. 2006). We hypothesize p53 ASON following ionizing radiation exposure may lead to a reduced intestinal crypt cell apoptosis and improved intestinal crypt survival.

TNF- α /TNFR1

Tumor necrosis factor (TNF) was first discovered as an endotoxin inducible serum factor in mice that produced a tumor necrotic action on transplanted tumor (Carswell, Old et al. 1975). TNF- α is a soluble 17 kdalton 157 amino acid molecule that binds as a homotrimer to either TNFR1 or TNFR2 (Szlosarek and Balkwill 2003). TNF- α is involved in inflammation, immunity, cellular organization, and is a mediator of cachexia in mice (Oliff, Defeo-Jones et al. 1987). Several investigators have shown radiation to induce TNF expression in various cell lines, including intestinal macrophages (Hallahan, Spriggs et al. 1989; Iwamoto and McBride 1994; Akashi, Hachiya et al. 1995; McKinney, Aquilla et al. 1998). In one study, injection of neutralizing anti-TNF mAb provided a 60% reduction in radiation-induced intestinal epithelial crypt cell apoptosis (Inagaki-Ohara, Yada et al. 2001). Radiation increases liver TNFR1 transcription in a dose and time dependent manner, pre-treatment with TNFR1 ASON reduces the levels of liver enzymes at 2 hours post-irradiation (Huang, Yang et al. 2006). Hepatocyte apoptosis and micronucleus formation were similarly reduced by TNFR1 ASON pre-treatment. In this study, we hypothesize that TNF- α ASON or TNFR1 ASON will result in reduced intestinal crypt cell apoptosis.

Fas

Fas (CD95) was discovered independently by two investigators who identified monoclonal antibodies which were cytolytic to various human cell lines (Trauth, Klas et al. 1989; Yonehara, Ishii et al. 1989). Fas is a 325 amino acid type I cell membrane protein that can transduce an apoptotic signal when bound by the Fas ligand (FasL) found on cytotoxic T lymphocytes (Nagata and Golstein 1995). Fas is involved in T cell mediated killing, immune tolerance, immunoregulation, cell proliferation, and NF- κ B activation (Wajant, Pfizenmaier et al. 2003). Fas induction was found in a rat model of ischemia and reperfusion injury of small intestine (Shuca, Yoshitaka et al. 2005). Fas was initially not detectable in the uninjured rat small intestine, after 1 h of ischemia fas was found in stromal and crypt epithelial cell, and after 3 h of reperfusion fas was markedly increased. Following whole-body mouse irradiation to 1.5 Gy, thymic and spleen cells in Fas-deficient mice (lpr +/+) were found to have a significantly lower rate of apoptosis than Fas wild-type mice (Reap, Roof et al. 1997). Fas surface expression was induced by irradiation in spleen cells and competitive inhibition of Fas-FasL binding with Fas-Fc fusion protein construct reduced apoptosis. In a separate study, both fasting and ischemia-reperfusion injury significantly induced Fas expression and subsequent apoptosis in the rat small intestine (Fujise, Iwakiri et al. 2006). Fasting promoted mucosal apoptosis via a Fas-mediated type I apoptotic pathway and ischemia-reperfusion injury induced apoptosis via a mitochondria mediated Fas type II pathway. These observations support a role for Fas-FasL signaling in radiation-induced apoptosis of intestinal crypt cells.

In a mouse model of fulminant hepatitis, antisense Fas oligonucleotide (ISIS 22023) treatment reduced Fas mRNA and protein expression in the liver by 90% (Zhang, Cook et al. 2000). Pretreatment with ISIS 22023 protected mice from fulminant hepatitis caused by an agonistic Fas mAb and reduced the severity of acetaminophen induced hepatitis. We hypothesize blocking Fas induction with antisense oligonucleotide therapy after irradiation will reduce subsequent radiation induced crypt cell apoptosis.

Caspase-3

The caspases are a family of proteases that are activated during apoptosis and perform the cleavage of critical cellular substrates, precipitating the morphological changes of apoptosis (Cohen 1997). Caspase-3 is a 32 kdalton proenzyme which is cleaved at an aspartate cleavage sites upon receiving a death signal to form the 17 kdalton and 12 kdalton subunits of the active protease form. During the execution phase of apoptosis, caspase-3 cleaves a number of substrates with the motif Asp-X-X-Asp. Caspase-3 is present in intestinal epithelial crypt (IEC) cells and activated upon receiving a death signal (Inagaki-Ohara, Takamura et al. 2002). Radiation induced IEC apoptosis was significantly reduced in caspase-3 -/- mice 4 h following 5 Gy TBI. DNA fragmentation was mostly absent in caspase-3 -/- mice, however blebbing, cell shrinkage, and nuclear condensation were present in the remaining apoptotic IEC cells. In another study, total abdominal irradiation increased the level of caspase-3 activity within intestinal tissue (Abbasoglu, Erbil et al. 2006). Both octreotide treated unirradiated animals and the treated irradiated group had elevated caspase-3 activity versus the corresponding group that did not receive octreotide. Further studies to investigate the effect of caspase-3 induction are necessary. We hypothesize blocking caspase-3 expression with ASON therapy will reduce crypt cell apoptosis and subsequent radiation induced lethal small bowel syndrome.

bax

The bcl-2 associated X-protein (bax), a 21 kdalton member of the bcl-2 family, was found to form dimers with either bcl-2 or itself, with only the homodimers having pro-apoptotic activity (Oltvai, Millman et al. 1993). Bcl-2 was the first protooncogene found to block programmed cell death rather than promote proliferation, the ratio of Bcl-2/Bax appears to determine the survival or death of cells following an apoptotic stimulus (Korsmeyer, Shutter et al. 1993). *In vitro* studies show that bax cooperates with ATM in radiation-induced apoptosis in the central nervous system (Chong, Murray et al. 2000). Restoring bax in malignant cell line results in enhanced radiosensitivity (Honda, Gjertsen et al. 2001). We have found antisense bcl-2 administration to mice resulted in enhanced intestinal apoptosis, crypt depletion, villi loss, loss of barrier protection, and increased susceptibility for radiation induced lethal small bowel syndrome (Wiedenmann, Valdecanas et al. 2006). Removing bax should have the opposite effect. We hypothesize blocking bax expression with ASON therapy after irradiation will reduce crypt cell apoptosis and radiation induced lethal small bowel syndrome.

C. PRELIMINARY DATA

Inhibiting apoptosis in mice following irradiation

In this preliminary study we evaluated anti-bax and anti-bid ASON agents for their ability to mitigate apoptosis of jejunum crypt cells when administered after whole-body irradiation (WBI) in C3Hf/KamLaw mice. Groups of 3 mice received total body irradiation using 300 kVp x-rays at a dose rate of 1.84 Gy/min to 2 Gy at 9:00 hrs. The experimental groups received ASON agents after TBI as follows:

50 mg/kg of anti-bax ASON administered intravenously 15 minutes after TBI to 2 Gy.

50 mg/kg of anti-bax ASON administered subcutaneously 15 minutes after TBI to 2 Gy.

50 mg/kg of anti-bax ASON administered intraperitoneal 15 minutes after TBI to 2 Gy.

150 mg/kg of anti-bax ASON administered via gavage 15 minutes after TBI to 2 Gy.

50 mg/kg of anti-bid ASON administered subcutaneously 15 minutes after TBI to 2 Gy.

The control groups received:

50 mg/kg mis-sense oligonucleotide (MSON) intravenously 15 minutes after TBI to 2 Gy.

50 mg/kg mis-sense oligonucleotide (MSON) alone (no irradiation)

50 mg/kg anti-bax ASON alone (no irradiation)

50 mg/kg anti-bid ASON alone (no irradiation)

TBI alone (no drugs)

No treatment

The mice were sacrificed at 5 hours post-irradiation for evaluation of the apoptotic index. A 2 cm length of jejunum was removed from each animal for histological evaluation. The tissue was fixed in 10% neutral buffered formalin and transverse sections cut with a thickness of 4 μ m. The section were stained with hematoxylin and eosin (H & E) and examined microscopically at 400X magnification. A total of 500 crypt cells per mouse from 3 complete crypts cut in longitudinal plane were scored as interphase, mitotic, or apoptotic. Cell classification (e.g. apoptotic cell) was based on morphological features of crypt cells on the H & E stained slides (Mason, Milas et al. 1995). The apoptotic index (AI), expressed as the mean percentage of apoptotic cells per 1500 cells examined, was calculated by the equation:

$$AI = 100 \times \frac{\# \text{apoptotic cell}}{1500} \quad (1)$$

along with the standard error is given in Table 1 below for each group. Each experimental group was compared using a t-test with the TBI alone control group and the p-value reported in the table.

Table 1 – Apoptotic index 5 hours following 2 Gy TBI and ASON agents.

Group	Mean \pm standard error	p-value
TBI alone (no drug)	11.8 \pm 1.6	-
TBI + anti-bax iv	9.0 \pm 0.5	0.18
TBI + anti-bax sc	7.5 \pm 0.3	0.06
TBI + anti-bax ip	9.9 \pm 0.8	0.35
TBI + anti-bax gavage	7.6 \pm 1.6	0.14
TBI + anti-bid iv	9.5 \pm 0.9	0.28
TBI + mis-sense iv	7.9 \pm 1.2	0.12

A second set of experiments measured jejunal crypt survival using the same agents, doses, and delivery methods. There was no significant difference versus the control group found for either agent.

Each of the ASON groups showed a reduction in the mean AI, however only the anti-bax ASON group delivered subcutaneously approached significance ($p=0.06$). The activity of each agents and delivery mode depends on the appropriateness as a target, dose, ASON affinity/specificity, biodistribution, and temporal course. These findings suggest bax may be an appropriate target, higher doses should be explored. Specifically, the gavage dose may be increased to 500 mg/kg and the parenteral doses to 100 mg/kg with mis-sense controls. The lack of activity for the anti-bid agent may reflect the mode of delivery or biodistribution, rather than appropriateness as a target.

Computer-assisted counting of intestinal crypts

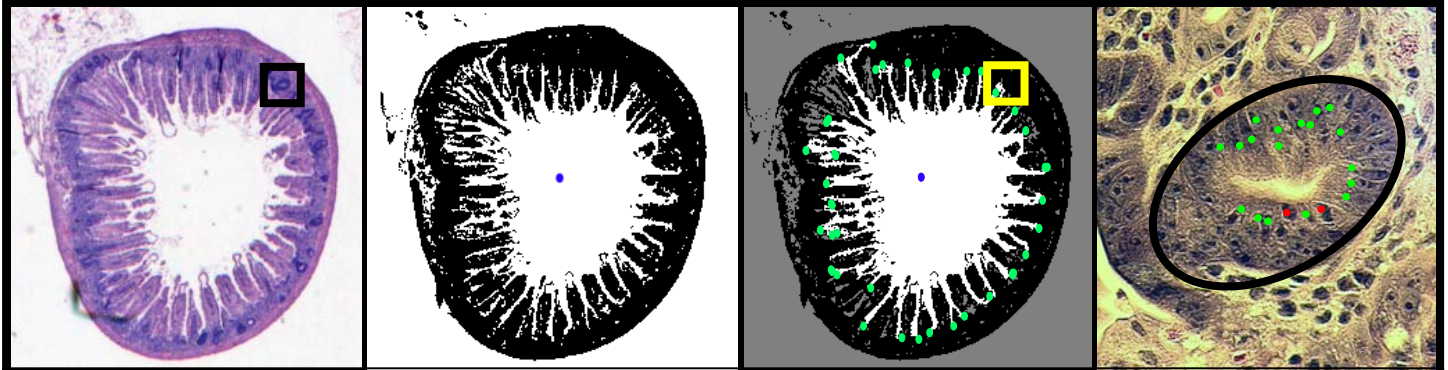


Figure 1. Illustrates identification and scoring of jejunal crypts. The first panel is a low resolution scan of the H & E stained section. A binary image is created and the intestinal structure identified (second panel). From the lumen the candidate crypt locations are identified (green dots). A higher resolution image of one candidate location is shown in the fourth panel, where the individual proliferative cells (green dots) and mitotic cells (red dots) are identified. Crypts containing greater than 10 proliferative cells are scored.

Digital images were acquired of hematoxylin and eosin (H & E) stained mouse jejunum histological sections at low magnification (Figure 1, first panel). Thresholding and erosion allows the generation of a binary image containing the silhouette of the tissue cross section (second panel). The location of individual cross sections (second panel, blue dot) are identified on the image using a morphological hit-or-miss transform (Gonzalez and Woods 2002). The lumen center is located using a centroid calculation of an image subregion around each location point. The image is then segmented into lumen (white), tissue (black), and outside (grey), as shown in panel 3 (Figure 1). Candidate crypt locations (green dots in panel 3) were identified by next computing locally maximal values of the breadth-first distance from the lumen center. High resolution images of the histological sections are then acquired at these candidate crypt locations (panel 4).

Automatic identification and classification of crypt cells in a high-resolution image of a crypt (Figure 1, fourth panel), is probably the most challenging part of the automation process. Our approach will be one based on statistical learning (Vapnik 2000). In particular, we will ask our associated experts to manually identify and classify crypt cells as proliferative, mitotic, paneth, or apoptotic cells in a set of training images. Given such a set of training images, we will investigate and identify a large set of possible features associated with the location and health of stem cells. Given the specific appearance of each class of cells, we believe that identifying such a feature set to be tractable. (For example, apoptotic cells have a distinctive appearance in which the nucleus of the stem cells forms a small dark ball.) We then intend to develop a generalized linear classifier along the lines of Support Vector Machines (Burgess 1998) to automatically evaluate and score the imaged crypts' cells. Crypts containing ≥ 10 proliferative cells are scored as viable and the total number per circumference is obtained from each jejunal section. The number of viable, apoptotic, and mitotic cells per crypt are also scored. The reported values will be a distribution of number of proliferative cells per crypt versus number of crypts, number of apoptotic cells, number of mitotic cells per circumference.

RESEARCH DESIGN AND METHODS

Specific Aim 1 Test inhibition of inflammation signaling by anti-sense drug therapy given orally post-irradiation in a mouse model using intestinal crypt survival assay.

Rationale: Acute radiation enteritis results from (1) loss of the epithelial stem cell population within the intestinal crypts, (2) subsequent breakdown of the epithelial barrier protection, and (3) mucosal inflammation in response to the injury. The interactions of these events accentuate the injury, e.g. mucosal breakdown allows translocation of bacteria resulting in further mucosal inflammation. Subsequently, mucosal inflammation causes regional tissue damage resulting in further mucosal injury and loss of barrier protection (Roberts, Foulcher et al. 1993; Fajardo, Berthrong et al. 2001). Blocking inflammatory signaling will reduce the severity of radiation enteritis and subsequent inflammation component of injury. In one study, intestinal epithelial regeneration was enhanced in ICAM-1 deficient mice when examined 14 days following single-exposure. They found a statistically significant increase in the number of proliferating cells and mitotic figures within intestinal crypts in the ICAM-1 deficient mice (Molla, Gironella et al. 2003). We hypothesize antisense oligonucleotide (ASON) drugs targeting the signaling events in inflammation (ICAM-1, TGF- β 1, p65, IL-6 induction) will reduce radiation enteritis and improve intestinal crypt survival. We will test this hypothesis in C3Hf/KamLaw mice and provide a ranking of the efficacy of each agent as radiation dose modifiers for intestinal crypt survival.

Study Overview: Inbred C3Hf/KamLaw mice bred and maintained in the defined flora specific pathogen-free colony in the Department of Experimental Radiation Oncology at the University of Texas M. D. Anderson Cancer Center will be utilized this study. An automatic 12 hour light-dark cycle is maintained in the colony. Mice at 12 – 16 weeks age, matched for weight (24 g), fed sterile feed and water ad lib will be selected. We have previously characterized the intestinal crypt survival following total-body irradiation (TBI) in C3Hf/KamLaw mice (Mason, Withers et al. 1989). To act as a mitigation agent, the ASON drugs must reach the target cell cytosol in a time frame to block induction of inflammation signaling. We will test mouse specific ICAM-1, TGF- β 1, p65, and IL-6 ASON drugs. The jejunal crypt survival will be determined for each ASON drug administered 0.5 h post-irradiation. Each mitigation agent will be delivered via gavage and a subcutaneous delivery positive control will control for gastrointestinal absorption. ASON drug dose levels of 250 and 500 mg/kg dose via gavage and 100 mg/kg intravenous administration daily will be tested. A control group will receive a mis-sense ASON agent and tested for each of the experimental conditions as a control for sequence specificity. A baseline group will receive saline gavage and a second group saline subcutaneous injection. Female (24 g and 12 – 16 weeks old) C3Hf/KamLaw mice will receive whole-body irradiation with 250 kVp x-rays at a dose rate of 1.56 Gy/min in groups of eight while loosely restrained in a well-ventilated Lucite box. A flattening filter on the x-ray source will limit the variation across the field to less than 3%. Each of 5 groups will receive between 12.5 to 16.75 Gy TBI (complete crypt survival curve). There will be an unirradiated control group for each of the experimental conditions. To avoid circadian effects (Hendry 1975), each group will be irradiated between 9 and 10 am. The mice will be harvested 3 days 14 hours following total body irradiation and a small section of the jejunum will be prepped to undergo histological evaluation (see Data Acquisition below). One histological slides will be made for each animal containing four sections of jejunum.

Study Design: There are 4 ASON drugs to evaluate, 3 drug dose levels or delivery modes, 6 radiation dose levels, 1 radiation only control, 1 nonsense ASON control, and drug plus no radiation controls. This results in

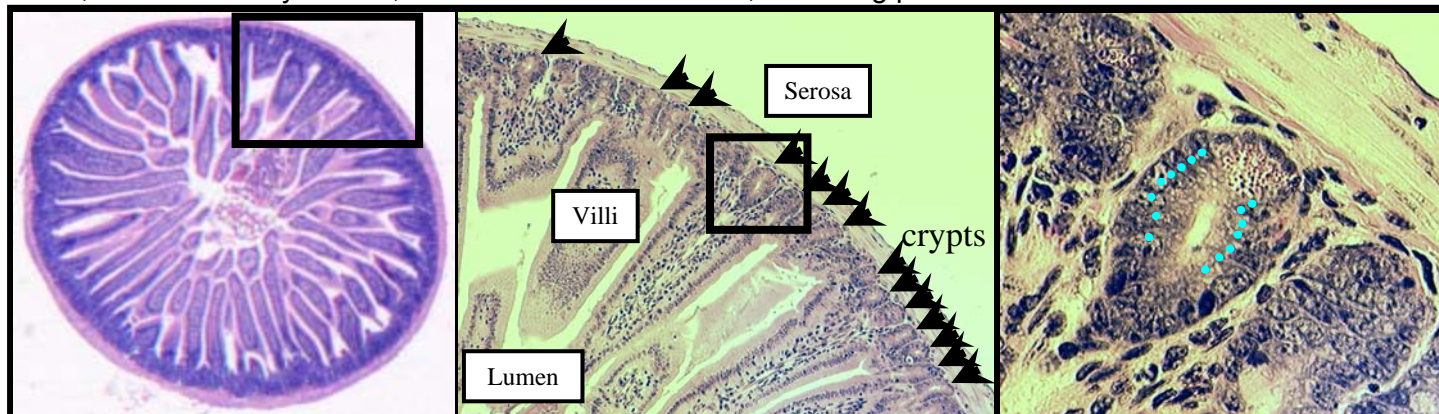


Figure 2. Illustrates crypt assay scoring. Four H & E stained jejunal complete circumferential sections per animal are evaluated. The intestinal crypts (arrows) with ≥ 10 proliferative cells (sky blue dots) per circumference are scored.

60 experimental groups and 51 controls groups. The primary analysis will be a mean comparison between the cell survival rate of an experimental group with that of a control group. From the results (Fig. 1) in Mason et al. (Mason, Withers et al. 1989) the C3Hf/KAM mice have approximately a standard deviation of 9 for the number of surviving cells per jejunum circumference in doses from approximately 12-15 Gy. Working with a mean of 30 surviving cells in a control group we find that 8 mice per group at a significance level of $\alpha=0.05$ will give 85% power to detect a 40% increase (42 surviving cells) in cell survival in an experimental group. For 60 experimental and 51 control groups, each with 8 mice, we will use a total of 888 mice for this component of the study.

Data Acquisition: (*Crypt survival assay*) We will evaluate the survival of jejunal crypts 3 days 14 hours following total body irradiation. Mice will be killed by CO₂ asphyxiation and a 2 cm length of jejunum removed for histological preparation. The tissue will be fixed in 10% neutral buffered formalin and four transverse section cut with a thickness of 4 μ m for each mouse. The sections will be stained with hematoxylin and eosin (H & E) and examined microscopically under 100X magnification. The number of surviving crypts with at least 10 viable tightly packed columnar epithelial (non-Paneth) cells located near the base of the crypt will be counted (Figure 2). The surviving crypt cells are readily identified as having chromophilic prominent nucleus, little cytoplasm, lying in close proximity appearing crowded (Withers and Elkind 1970). The mean number of surviving crypts per intestinal circumference will be determined for each group.

Data Analysis Methods: The crypt survival assay (Withers and Elkind 1970) assumes within each crypt cells survive independently and that one surviving cell is sufficient for regeneration of that crypt. These assumptions lead to a Poisson model for the number of surviving cells within a crypt; denote the Poisson parameter by λ . Let the average number of crypts per circumference in unirradiated controls be denoted by \bar{x}_c . If x_s then denotes the number of surviving crypts per circumference (of a transverse section), then the proportion of crypts destroyed by irradiation is $(\bar{x}_c - x_s)/\bar{x}_c$. Through the Poisson assumptions and model we can thus relate the fraction of surviving crypts per circumference to the number of surviving cells within a crypt. To this end, the proportion of crypts destroyed by irradiation should equal to $\exp(-\lambda)$, leading to $\lambda = -\ln[(\bar{x}_c - x_s)/\bar{x}_c]$. Since one surviving cell is sufficient for crypt regeneration, the fraction of surviving crypts per circumference, $S_{\text{circumference}}$, is thus:

$$S_{\text{circumference}} = -\ln\left(\frac{x_c - x_s}{x_c}\right) \quad (2)$$

where x_s is the number of surviving crypts per circumference and x_c is the number of crypts per circumference in the control group (Withers and Elkind 1970). A crypt survival curve versus radiation dose will be generated for each experimental condition using a modified alpha-beta dose response model (Roberts, Hendry et al. 2003). A dose-modifying factor (DMF) will be calculated for each drug then ranked in order of mitigation activity (ability to increase crypt survival) using the survival curves:

$$DMF_{\text{crypt survival}}(\text{ASON}) = \frac{D_{\% \text{ survival}}(\text{control group})}{D_{\% \text{ survival}}(\text{ASON group})} \quad (3)$$

where $D_{\% \text{ survival}}$ is the dose determined for each group that achieves a specific %survival. We will determine the average $DMF_{\text{crypt survival}}$ over the fractional survival range of [0.1, 0.5]. Agents that have a lower $DMF_{\text{crypt survival}}$ will have higher mitigation activity. To assess the rankings of the agents we will calculate 95% confidence intervals for each $DMF_{\text{crypt survival}}$ and evaluate the overlap of the intervals. The confidence intervals will be obtained by applying the bivariate Delta Method (propagation of error via Taylor series) to the ratio defining $DMF_{\text{crypt survival}}$. Alternatively, we can apply a bootstrap method to obtain confidence intervals for $DMF_{\text{crypt survival}}$.

Potential Pitfalls: Radiation injury may limit gastrointestinal drug absorption precluding oral delivery of drugs. We plan to estimate this effect by having a control group receive subcutaneous injection delivery. Another potential pitfall is that the inflammation contribution to injury may not contribute to the crypt survival measured at 3 days 14 hours post-irradiation. If we find no mitigation activity a second set of experiments can be performed to assay crypt survival at a later time, such as the 14 day delay utilized by Molla (Molla, Gironella et al. 2003). Inhibition of NF- κ B is reported to reduce inflammatory cytokines, including IL-6 and IL-6 receptor, within the GI tract (Linard, Marquette et al. 2004). However, NF- κ B p50 knock-out mice were found to have increased sensitivity to TBI, with an LD₅₀ (< 7 days) of 7.75 Gy versus 13.12 Gy for the wild-type (Wang, Meng et al. 2004). Inhibition of a radiation induction target by ASON requires adequate biodistribution, which may not occur within the post-treatment time frame. An alternative strategy is to utilize ASON agents as radioprotectors rather than mitigation agents to identify biological targets using pre-irradiation treatment.

Specific Aim 2 Test inhibition of apoptotic signaling by anti-sense drug therapy given orally post-irradiation in a mouse model using intestinal crypt apoptotic assay.

Rationale: Exposure of the intestinal epithelium to ionizing radiation results in rapid apoptotic cell death of the cells lining the crypt walls (Potten 1977). Shortening of the villi and subsequent loss of the epithelium results from the death of these regenerative crypt stem cells and potential stem cells. We hypothesize that (antisense) p38, p53, TNF, TNFR1, fas, caspase-3, bid, or bax ASON drugs may act as radiation mitigation agents to block apoptosis of intestinal crypt stems cells (or potential stem cells) allowing regeneration of the crypts. We will test this hypothesis and provide a relative ranking of the efficacy of each agent as modifiers for intestinal crypt apoptotic index.

Study Overview: Inbred C3Hf/KamLaw mice bred as described in Specific Aim 1 will be utilized in this study. We have previously characterized the radiation induced apoptosis in C3Hf/KamLaw mice (Weil, Stephens et al. 1996). Antisense oligonucleotide (ASON) drugs act in the cytosol to block new protein synthesis through hybridization with and facilitating subsequent degradation (by RNase H) of their mRNA target. To act as a mitigator, the ASON drugs must reach the target cell cytosol in a time frame to block induction of apoptotic target signals. Small intestinal crypt cell apoptosis (in mice) peaks at 4 – 6 hours post-irradiation (Weil, Stephens et al. 1996). Female (24 g and 12 – 16 weeks old) C3Hf/KamLaw mice will receive whole-body irradiation with 250 kVp x-rays (as described in Specific Aim 1) in groups of six, one control group and one experimental group, while loosely restrained in a well-ventilated Lucite box. The apoptotic index will be determined with each ASON drug administered 30 minutes post-irradiation. Each mitigator will be delivered via gavage, however a subcutaneous delivery positive control will control for gastrointestinal absorption. ASON drug dose levels of 150 and 300 mg/kg dose via gavage and 50 mg/kg subcutaneous will be tested. A control group will receive a mis-sense ASON agent at 0.5 hours delay for each administration dose and route. A baseline group will receive saline gavage and a second saline subcutaneous injection. Unirradiated controls groups will receive sham irradiation and ASON (or mis-sense) agents at each dose and administration. The mice will be killed and a 2 cm length of jejunum removed for histological evaluation at 6 hours post-irradiation.

Study Design: ASON Drug will be tested at dose levels of 250 & 500 mg/kg dose given via lavage, and 100 mg/kg given subcutaneous. The delay between irradiation and time of ASON drug administration: 0.5 hours. There are 8 ASON drugs to evaluate, 3 drug dose levels or delivery modes, 2 radiation dose levels (including 0 Gy), 1 radiation only control, 1 nonsense ASON control, and no radiation drug only controls. This yields 112 experimental groups and 273 controls groups. The primary analysis will be a mean comparison between the apoptotic index of an experimental group with that of a control group. From the results of a published study, Figure 1 in Weil et al. (Weil, Stephens et al. 1996), the C3Hf/KAM mice have approximately a mean apoptotic index of 11 (at 4 h or greater post irradiation) with an SEM \approx 2. The mean is based on 3 mice and hence the standard deviation of the apoptotic index is $\sigma=3.5$. We find that 3 mice per group at a significance level of $\alpha=0.1$ will give \approx 80% power to detect a \approx 50% decrease in apoptotic index in an experimental group. Thus the total number of mice for this component of the study is 1155.

Data Acquisition: (*Apoptotic assay*) C3Hf/KamLaw mice irradiated and treated as described above will be sacrificed at 5 hours post-irradiation for evaluation of the apoptotic index. A 2 cm length of jejunum will be removed from each animal for histological evaluation. The tissue will be fixed in 10% neutral buffered formalin and transverse sections cut with a thickness of 4 μ m. The section will be stained with hematoxylin and eosin (H & E) and examined microscopically at 400X magnification. A total of 500 crypt cells per mouse from 12 to 16 complete crypts cut in longitudinal plane will be scored as interphase, mitotic, or apoptotic. Cell classification (e.g. apoptotic cell) will be based on morphological features of crypt cells (Figure 3) on the H & E stained slides (Mason, Milas et al. 1995). The apoptotic index (AI) is expressed as the mean percentage of apoptotic cells per 1500 cells examined from the three animals in each group. The reduction of the AI for each ASON drug, delivery route, and administration delay time will be determined. The ASON drugs will be ranked in order of mitigation activity (ability to reduce the AI).

Data Analysis Methods: The apoptotic index (AI) will be calculated and compared between treatment and control groups by a two-sample t-test at the $\alpha=0.1$ one-sided significance level since our interest is in a decrease (one-sided) of the AI in an ASON drug group relative to control. Since the calculation of AI is a proportion we will repeat the analysis under an arcsine (angular) transformation of AI to stabilize the variance of proportions and bring them closer to normality; the variance of a sample proportion depends on the underlying population proportion, as well as the sample size. If there is a difference in the results between the original scale and the arcsine scale we will report that based on the latter. The apoptotic index (AI) expressed

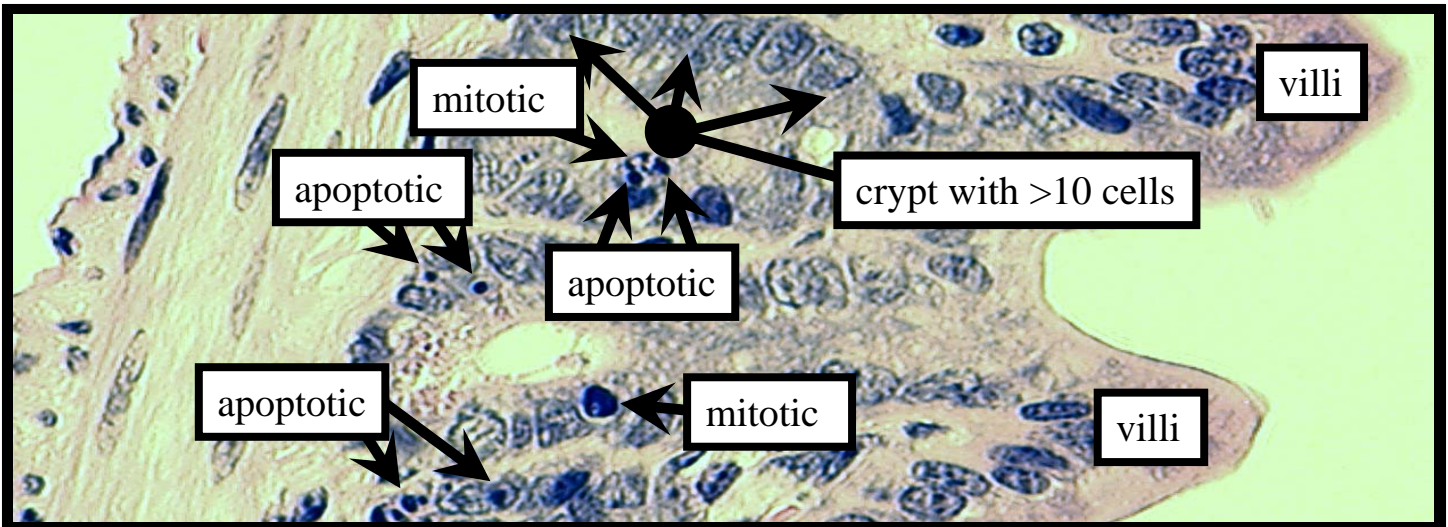


Figure 3. Scoring of crypt apoptotic cells. This high resolution (400X) micrograph of mouse jejunum 24 h after 10 Gy total body irradiation. Shortened villi, multiple apoptotic cells, and occasional mitotic cells are seen. A surviving crypt with >10 proliferative cells is shown.

as the mean percentage of apoptotic cells per 1500 cells examined, is given by the equation:

$$AI = 100 \times \frac{\# \text{apoptotic cell}}{1500} \quad (4)$$

Note that the above AI can be expressed as a weighted average of the three individual AI's, $AI = w_1 AI_1 + w_2 AI_2 + w_3 AI_3$, where $w_i = n_i/n$. In this case since the individual sample sizes are all equal the overall AI is a simple arithmetic mean of the individual AI's. The reduction of the AI for each ASON drug, delivery route, and administration delay time will be determined. The agents will be ranked based on percentage reduction in the AI:

$$\text{Percentage reduction in } AI = 100 \times \frac{AI_{\text{control}} - AI_{\text{ASON}}}{AI_{\text{control}}} \quad (5)$$

An apoptotic-mitigating factor (AMF) will be calculated for each ASON group with comparisons to the appropriate control group from the following equation:

$$AMF(ASON) = \frac{1 - \left(\frac{AI_{\text{control group}}}{100} \right)}{1 - \left(\frac{AI_{\text{ASON group}}}{100} \right)} \quad (6)$$

Agents with a lower AMF have higher mitigation activity. As in Aim 1, we can use either normal-based theory or bootstrapping to find confidence intervals and assess rankings for either the percent reduction in AI or the AMF.

Potential Pitfalls: Intestinal irradiation is known to interfere with the absorption function and as such may interfere with oral drug delivery. We plan to test subcutaneous injection delivery of the ASON drugs as an experimental control. Another potential pitfall is that apoptosis may be simply delayed rather than blocked. We plan to measure 7 time points over 24 hours to obtain a apoptotic profile similar to our previous study (Weil, Stephens et al. 1996). This will allow us to evaluate that as a potential outcome. In addition, at each time point we will measure number of mitotic cells which may provide additional information on crypt recovery. Inhibition of a radiation induction target by ASON agents requires adequate biodistribution, which may not occur within the post-treatment time frame. An alternative strategy is to utilize ASON agents radioprotectors rather than mitigation agents to identify biological targets using pre-irradiation treatment. Alternatively, the assay time point may be delayed beyond 24 h.

Specific Aim 3 Test doublet combinations for synergy in a mouse model survival assay.

Rationale: Combination therapy has been applied to anti-neoplastic chemotherapy (Frei, DeVita et al. 1966), anti-microbial therapy (McCabe 1967), anti-viral therapy (Balfour 1999), and the treatment of complex medical

conditions such as diabetes or hypertension (Holzgreve 2003). In principle, combination therapy provides maximal benefit when (1) each component agent has single-agent activity, (2) each agent targets a non-competing biological pathway, and (3) the toxicity profiles are non-overlapping (Miles, von Minckwitz et al. 2002). We hypothesize that combination therapy, using doublets from the agents in Aims 1 (anti-inflammatory) and 2 (anti-apoptotic) with the highest mitigation activity, will improve survival following whole abdomen irradiation. We will test this hypothesis by measuring the LD₅₀ (<10 days) (Mason, Withers et al. 1989) using a morbidity assay in C3Hf/KamLaw mice following whole abdomen irradiation with mitigation by the test doublets.

Study Overview: Inbred C3Hf/KamLaw mice bred and maintained in the specific pathogen-free colony described in the previous aims will be utilized this study. We have previously measured the LD₅₀ (<10 days) in C3Hf/KamLaw mice following total-body or total-abdominal irradiation (Mason, Withers et al. 1989). In this study we will test 3 doublet pairs comprised of the best mitigators from Aim 1 (ASON-1a & -1b) and 2 (ASON-2a & b) above. The doublet pairs will consist of the pairs: (ASON-1a; ASON-2a), (ASON-1b; ASON-2a), (ASON-1a; ASON-2b). Animals will receive total abdominal irradiation (TAI) to doses of 16 to 25 Gy, and will receive a doublet ASON administration daily, beginning 0.5 hours following irradiation. The animals will be meticulously followed over the ensuing 10 days for signs of GI morbidity. These signs include: 20% weight loss, debilitating diarrhea, rough hair coat, hunched posture, lethargy, labored breathing, jaundice, neurological signs, inability to feed or drink, or bleeding from any orifice. Animals exhibiting these GI morbidity signs or moribund animals will be euthanized using CO₂ asphyxiation. The LD₅₀ (<10 days) will be determined from analysis of the animal lethality for each drug doublet cohort. The ASON drug doublets will be ranked in order of mitigation activity, determined by the LD₅₀ (<10 days).

Study Design:

5 groups of 20 mice will be irradiated to doses between 16 Gy and 20 Gy for each ASON drug doublet cohort, additional groups above 20 Gy will be added if 80% lethality is not achieved. ASON drug doublets will be tested at 300 mg/kg dose given via lavage, and 100 mg/kg given subcutaneous. Delay between irradiation and time of ASON drug administration: 0.5 hours. This yields 3 experimental groups and 3 controls groups. The primary analysis will be a comparison between the LD₅₀ of the experimental arms versus the controls. Increasing the number of individuals at each dose results in a much more accurate effect than increasing the number of doses (Moermans and VanHecke 1995). To obtain an accuracy of 50% of the true value of LD₅₀, with a chance of 0.85 that this should happen, we will use 20 mice per dose.

Data Acquisition: (*GI morbidity/Survival assay*) Mice will be monitored around the clock at 6 hour intervals to score (GI morbidity) lethality over a 10 day period post-irradiation. The animals will be observed for (signs of GI morbidity): 20% weight loss, debilitating diarrhea, rough hair coat, hunched posture, lethargy, labored breathing, jaundice, neurological signs, inability to feed or drink, or bleeding from any orifice. Animals exhibiting these signs will be euthanized via CO₂ asphyxiation. Lethality will be scored based on death or GI related morbidity. The resulting number of animals per group surviving at 10 days after irradiation is the end-point.

Data Analysis Methods: statistics paragraph. Logit analysis of lethality rates at 10 days post-irradiation will be performed. We will determine the LD₅₀ (<10 days) for the control groups and for each ASON doublet treated group. The ASON drug doublets will be ranked in order of mitigation activity, determined by the LD₅₀ (<10 days). A dose-modifying factor (DMF) will be calculated from the following equation,

$$DMF_{\text{survival at 10-days}}(\text{ASON doublet}) = \frac{\text{control group } LD_{50}(<10 \text{ days})}{\text{ASON doublet group } LD_{50}(<10 \text{ days})} \quad (7)$$

for each ASON doublet group. The ASON doublets will be ranked based on DMF, agents with a lower $DMF_{\text{survival at 10-days}}$ have higher mitigation activity.

Potential Pitfalls: Activity cannot be achieved in one of the drug groups. Then the LD₅₀ (<10 days) of the top two drugs acting alone from the other category will be determined. Another possible pitfall may occur if the drugs have a significant effect, there may be insufficient events at the highest doses to establish the LD₅₀. If that proves to be the case, additional groups will be added at higher dose levels.

Specific Aim 4 Test automated image analysis for crypt assay versus manual counting.

Rationale: The crypt and apoptotic assays are the standard tools for the evaluation of radiation GI injury (Withers and Elkind 1970; Withers, Mason et al. 1974; Weil, Stephens et al. 1996). These assays require the

manual evaluation of histological sections by an expert, which has limited their use by other laboratories. Computer-assisted image analysis has been shown to improve accuracy and throughput in evaluation of cytopathology and/or histopathology (Kok and Boon 1996; Nieminen, Kotaniemi et al. 2005). Automation of the crypt and apoptotic assays will improve the speed and accuracy of these techniques and allow for a broad distribution of these assay techniques to other laboratories for future studies of gastrointestinal radiation injury.

Study Overview: We will develop computer-assisted histological evaluation software to automate the evaluation of cytopathology and/or histopathology of gastrointestinal radiation injury. Computer-assisted detection and analysis of crypts, scoring of crypt survival and apoptosis will be tested against manual counting by experts.

Study Design: Due to the tedious and delicate nature of this examination, visual evaluation by an expert is a tedious process. Given that historical assays (already in hand) and the proposed assays will generate thousands of slides, we proposed to automate this evaluation process to facilitate the testing as many drugs as possible. One of the co-PIs (Warren) has significant expertise in solving the image processing problems posed in steps 3 and 5. In particular, Warren (in conjunction with others) developed *geneatlas.org*, an on-line spatial database for comparing gene expression patterns over the mouse brain (Carson, Ju et al. 2005). The major steps of this automated process are as follows:

1. Using programmable microscope, collect low-resolution image of entire slide.
2. Identified intestinal cross-sections.
3. Identify existing villi and crypts for each cross-sections.
4. Collect high-resolution images of each crypt.
5. Automatic identification and classification of crypt cells from high-resolution images.

Steps 1 and 4 involve programming a commercially available microscope such as the Leica DM5500 (Leica Microsystems, Wetzlar, Germany) to collect appropriate images. Step 2 is a standard image processing problem and can be solved by filtering the image and determining connected sets of pixels corresponding to each distinct cross-section. Step 3 is more challenging, but still tractable. After smoothing and contouring the image associated with each cross-section, we propose to fit a simple finger-shaped deformable model to each villi (Bello, Ju et al. 2007). Given that the villi are oriented pointed towards the center of each cross-section, we believe that a reasonable initial position for the deformable modeling should be easy to compute and a dynamic positioning method based on some variant of snakes should yield a very accurate fit (Kass, Witkin et al. 1988). Positions for crypts in the cross-section will be derived from the position of the fitted villi models. Step 5, automatic identification and classification of stem cells in a high-resolution image of a crypt, is the most challenging part of the automation process. Our approach will be based on statistical learning (Vapnik 2000; Bello, Ju et al. 2007). Our expert will manually identify and classify cells in a set of training images. With a set of training images, we will investigate and identify a large set of possible features associated with the location and cells. A classifier based on Support Vector Machines to automatically evaluate the imaged crypts will be developed. A receiver-operator characteristics analysis (Metz 1978; Swets and Pickett 1982; Park, Goo et al. 2004) will be performed on a data set read by (1) an expert reader (K. Mason) as the gold standard, (2) 3 novice readers, and (3) the computer reader.

Data Acquisition: The historical slides and scores from our prior publication on mice strain apoptotic differences (Weil, Stephens et al. 1996) and from differences in crypt survival following TBI versus TAI (Mason, Withers et al. 1989) will be utilized. 3 novice readers, 1 computer reader, 1 expert reader will score the 252 apoptotic slides (2 mice strains, 5 dose levels, 7 times points, 3 mice per group, plus control) and 220 crypt survival slides (2 irradiation techniques, 10 mice per group, 5 dose levels).

Data Analysis Methods: A basic statistical analysis will be performed to compare the quantitative counts between the novice readers and the gold standard, and between the computer and gold standard. For each type slide – apoptotic and crypt - standard summary statistics (mean, SD, quantiles) will be calculated and reported for the gold standard, novices and computer. Count distributions will be compared by a Kolmogorov-Smirnov test. To determine if there is a significant difference in between novice readers and the gold standard a one-way analysis of variance will be performed, followed by a contrast between the average novice readings and the gold standard. Both fixed and random effects for readers and cases (images) will be considered (Dofrman, Berbaum et al. 1995). A two-sample paired t-test (paired by image) will be used to compare the gold standard with the computer readings. To directly determine whether the computer performs closer to the gold standard than the novice readers, consider two differences: $D(C) = \text{computer minus Gold}$ and $D(N) = \text{average novice minus Gold}$. If there is no difference in counts between the computer and the novice, then the difference

in D's should be zero. Thus, we will find a 95% confidence interval for the mean difference of the D's. Note that the variance of $D(C) - D(N)$ is a function of the individual variances of $D(C)$ and $D(N)$, across the cases. To more formally assess the accuracy of the computer and novice readings, we will also perform an ROC analysis with the expert reader as the gold standard. For comparison purposes (computer versus novice), we will use the area under the curve (AUC) method. This can be done parametrically or nonparametrically and for continuous data the two approaches tend to give similar results (Park, Goo et al. 2004). Therefore, we will assume standard binormal distributions whose parameters (a and b) will be estimated by maximum likelihood. An ROC curve will be estimated for each reader (3 novices and computer) and an average novice ROC curve will be estimated by averaging their resulting MLEs of a and b (Metz 1986). The average novice AUC will be compared to the computer AUC, and equality of curves will be assessed by evaluating the null hypothesis of equal pairs (a, b) between computer and mean novice (Park, Goo et al. 2004).

Potential Pitfalls: Computer analysis of H & E stained slides for crypt survival and apoptosis is a difficult problem and may not be tractable within this study. We will collect one digital image set along with the corresponding expert scoring for each assay and make the data set publicly available to the medical imaging scientific community at large. This will allow the problem to be addressed by a broader group of imaging scientists and provide a training set for those who wish to learn to perform these assay methods.

PHARMACOLOGIC PRODUCT DEVELOPMENT PLAN

In this study, we propose to test mouse-specific antisense oligonucleotide drugs targeted to apoptotic and inflammatory signaling targets (Table 1) as mitigation agents of radiation GI toxicity. The agents described in this proposal are in research or development for treatment of a variety of diseases and conditions unrelated to radiation injury (Table 1). One of the agents, in pre-clinical research studies, have shown a protective effect in prevention of radiation damage (radiation hepatitis or radiation induced cellular apoptosis) when administered before irradiation (Huang, Yang et al. 2006). One agent, alicaforsen, has shown promising results in phase II testing for inflammatory bowel disease (van Deventer, Wedel et al. 2006) and is currently entering phase III testing for that indication. Another agent is in phase I testing (Sewell, Geary et al. 2002) in the United Kingdom (UK). These agents will continue in their development for non-radiation indications and they may achieve FDA approval for those indications. In addition, agents found in this study to have efficacy as mitigators of radiation GI toxicity will proceed to further studies in a dog model of radiation GI toxicity. We will seek additional funding for those studies. Should those studies confirm efficacy for an agent, FDA approval will be sought through the "animal rule" (21 CFR 314.600 –650) for the specific indication of mitigation of acute radiation syndrome GI toxicity. Those agents targeting inflammation signaling may proceed to additional testing for use in preventing side effects from cancer radiation therapy. However, additional preclinical data is warranted to ensure no cancer protection occurs and the standard FDA approval process will be followed. We will pursue those studies with the most active agents found in this study.

Table 1 - A summary of the present development of each proposed agent (Isis Pharmaceuticals).

Biological Target	Stage of development	Disease site	Reference:
ICAM-1	Phase III trial	Inflammatory bowel disease	(van Deventer, Wedel et al. 2006)
TGF- β 1	Pre-clinical	Ocular scar formation	(Cordeiro, Mead et al. 2003)
I κ B	Pre-clinical	-	-
IL-6	Pre-clinical	-	-
p38	Pre-clinical	Asthma	(Duan, Chan et al. 2005)
p53	Pre-clinical	-	(Urban, Golden et al. 2003)
TNF- α	Pre-clinical Phase I (UK)	Colitis Rheumatoid arthritis, Crohn's disease	(Myers, Murthy et al. 2003) (Sewell, Geary et al. 2002)
TNFR1	Pre-clinical	Radiation hepatitis	(Huang, Yang et al. 2006)
fas	Pre-clinical	Fulminant hepatitis	(Zhang, Cook et al. 2000)
Caspase-3	Pre-clinical	-	-
bax	Pre-clinical	Apoptotic mediated disease	-
bid	Pre-clinical	Cholestatic liver injury	(Higuchi, Miyoshi et al. 2001)

- Abbasoglu, S. D., Y. Erbil, T. Eren, M. Giris, U. Barbaros, R. Yucel, V. Olgac, M. Uysal and G. Toker (2006). "The effect of heme oxygenase-1 induction by octreotide on radiation enteritis." *Peptides* **27**(6): 1570-1576.
- Akashi, M., M. Hachiya, Y. Osawa, K. Spirin, G. Suzuki and H. P. Koeffler (1995). "Irradiation Induces WAF1 Expression through a p53-independent Pathway in KG-1 Cells." *J. Biol. Chem.* **270**(32): 19181-19187.
- Allen, M., L. Svensson, M. Roach, J. Hambor, J. McNeish and C. A. Gabel (2000). "Deficiency of the Stress Kinase p38 α Results in Embryonic Lethality: Characterization of the Kinase Dependence of Stress Responses of Enzyme-deficient Embryonic Stem Cells." *J. Exp. Med.* **191**(5): 859-870.
- Arora, V. and P. L. Iversen (2000). "Antisense Oligonucleotides Targeted to the p53 Gene Modulate Liver Regeneration In Vivo." *Drug Metab Dispos* **28**(2): 131-138.
- Atreya, R., J. Mudter, S. Finotto, J. Mullberg, T. Jostock, S. Wirtz, M. Schutz, B. Bartsch, M. Holtmann, C. Becker, D. Strand, J. Czaja, J. F. Schlaak, H. A. Lehr, F. Autschbach, G. Schurmann, N. Nishimoto, K. Yoshizaki, H. Ito, T. Kishimoto, P. R. Galle, S. Rose-John and M. F. Neurath (2000). "Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis in vivo." *Nature Medicine* **6**(5): 583-8.
- Balfour, H. H. (1999). "Antiviral Drugs." *N Engl J Med* **340**(16): 1255-1268.
- Bello, M., T. Ju, J. Carson, J. L. Warren and I. Kakadiaris (2007). "Learning-based Segmentation Framework for Tissue Images Containing Gene Expression Data." *IEEE Transactions on Medical Imaging* **in press**.
- Bennett, C. F., D. Kornbrust, S. Henry, K. Stecker, R. Howard, S. Cooper, S. Dutson, W. Hall and H. I. Jacoby (1997). "An ICAM-1 Antisense Oligonucleotide Prevents and Reverses Dextran Sulfate Sodium-Induced Colitis in Mice." *J Pharmacol Exp Ther* **280**(2): 988-1000.
- Carson, J. P., T. Ju, H.-C. Lu, C. Thaller, M. Xu, S. L. Pallas, M. C. Crair, J. Warren, W. Chiu and G. Eichele (2005). "A Digital Atlas to Characterize the Mouse Brain Transcriptome." *PLoS Computational Biology* **1**(4): e41.
- Carswell, E. A., L. J. Old, R. L. Kassel, S. Green, N. Fiore and B. Williamson (1975). "An Endotoxin-Induced Serum Factor that Causes Necrosis of Tumors." *PNAS* **72**(9): 3666-3670.
- Castell, J. V., M. J. Gomez-Lechon, M. David, T. Hirano, T. Kishimoto and P. C. Heinrich (1988). "Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes." *FEBS Letters* **232**(2): 347-350.
- Chiang, C. S., J. H. Hong, A. Stalder, J. R. Sun, H. R. Withers and W. H. McBride (1997). "Delayed molecular responses to brain irradiation." *International Journal of Radiation Biology* **72**(1): 45-53.
- Chong, M. J., M. R. Murray, E. C. Gosink, H. R. C. Russell, A. Srinivasan, M. Kapsetaki, S. J. Korsmeyer and P. J. McKinnon (2000). "Atm and Bax cooperate in ionizing radiation-induced apoptosis in the central nervous system." *PNAS* **97**(2): 889-894.
- Christiansen, H., N. Sheikh, B. Saile, F. Reuter, M. Rave-Frank, R. M. Hermann, J. Dudas, A. Hille, C. F. Hess and G. Ramadori (2006). "x-Irradiation in Rat Liver: Consequent Upregulation of Hepcidin and Downregulation of Hemojuvelin and Ferroportin-1 Gene Expression." *Radiology* **242**(1): 189-197.
- Cleveland BioLabs, I. (2006). "Protectans - Cleveland BioLabs, Inc. - Official Site." from <http://www.cbiolabs.com/candidates/protectans>.
- Cohen, G. M. (1997). "Caspases: the executioners of apoptosis." *Biochem J* **326** (Pt 1): 1-16.
- Coleman, C. N., W. F. Blakely, J. R. Fike, T. J. MacVittie, N. F. Metting, J. B. Mitchell, J. E. Moulder, R. J. Preston, T. M. Seed, H. B. Stone, P. J. Tofilon and R. S. L. Wong (2003). "Molecular and Cellular Biology of Moderate-Dose (1-10 Gy) Radiation and Potential Mechanisms of Radiation Protection: Report of a Workshop at Bethesda, Maryland, December 17-18, 2001." *Radiation Research* **159**(6): 812-834.
- Cordeiro, M. F., A. Mead, R. R. Ali, R. A. Alexander, S. Murray, C. Chen, C. York-Defalco, N. M. Dean, G. S. Schultz and P. T. Khaw (2003). "Novel antisense oligonucleotides targeting TGF-beta inhibit in vivo scarring and improve surgical outcome." *Gene Ther* **10**(1): 59-71.
- Crooke, S. T. (1998). "An overview of progress in antisense therapeutics." *Antisense & Nucleic Acid Drug Development* **8**(2): 115-22.
- Dofrman, D. D., K. S. Berbaum and R. V. Lenth (1995). "Multireader, multibase receiver operating characteristic methodology: a bootstrap analysis." *Acad Radiol* **2**(7): 626-33.
- Duan, W., J. H. P. Chan, K. McKay, J. R. Crosby, H. H. Choo, B. P. Leung, J. G. Karras and W. S. F. Wong (2005). "Inhaled p38-alpha Mitogen-activated Protein Kinase Antisense Oligonucleotide Attenuates Asthma in Mice." *Am. J. Respir. Crit. Care Med.* **171**(6): 571-578.
- Dustin, M. L., R. Rothlein, A. K. Bhan, C. A. Dinarello and T. A. Springer (1986). "Induction by IL 1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1)." *J Immunol* **137**(1): 245-254.
- Egan, L. J., L. Eckmann, F. R. Greten, S. Chae, Z.-W. Li, G. M. Myhre, S. Robine, M. Karin and M. F. Kagnoff (2004). "I κ B-kinase β -dependent NF- κ B activation provides radioprotection to the intestinal epithelium." *PNAS* **101**(8): 2452-2457.
- Fajardo, L. F., M. Berthrong and R. E. Anderson (2001). *Radiation Pathology*. New York, Oxford Univ. Press.
- Frei, E., III, V. T. DeVita, J. H. Moxley, III and P. P. Carbone (1966). "Approaches to Improving the Chemotherapy of Hodgkin's Disease." *Cancer Res* **26**(6_Part_1): 1284-1289.

- Fujise, T., R. Iwakiri, B. Wu, S. Amemori, T. Kakimoto, F. Yokoyama, Y. Sakata, S. Tsunada and K. Fujimoto (2006). "Apoptotic pathway in the rat small intestinal mucosa is different between fasting and ischemia-reperfusion." Am J Physiol Gastrointest Liver Physiol **291**(1): G110-116.
- Gonzalez, R. C. and R. E. Woods (2002). Digital Image Processing. Upper Saddle River, NJ, Prentice-Hall, Inc.
- Hall, E. J. and A. Giaccia (2005). Radiobiology for the Radiologist. Philadelphia, Lippincott Williams & Wilkins.
- Hallahan, D., J. Kuchibhotla and C. Wyble (1996). "Cell Adhesion Molecules Mediate Radiation-induced Leukocyte Adhesion to the Vascular Endothelium." Cancer Res **56**(22): 5150-5155.
- Hallahan, D. E., D. R. Spriggs, M. A. Beckett, D. W. Kufe and R. R. Weichselbaum (1989). "Increased Tumor Necrosis Factor alpha mRNA after Cellular Exposure to Ionizing Radiation." PNAS **86**(24): 10104-10107.
- Hallahan, D. E. and S. Virudachalam (1997). "Intercellular adhesion molecule 1 knockout abrogates radiation induced pulmonary inflammation." Proc Natl Acad Sci U S A **94**(12): 6432-7.
- Hallahan, D. E. and S. Virudachalam (1997). "Ionizing radiation mediates expression of cell adhesion molecules in distinct histological patterns within the lung." Cancer Res **57**(11): 2096-9.
- Handscheil, J., F.-J. Prott, C. Sunderkotter, D. Metze, U. Meyer and U. Joos (1999). "Irradiation induces increase of adhesion molecules and accumulation of beta2-integrin-expressing cells in humans." International Journal of Radiation Oncology*Biophysics **45**(2): 475-481.
- Hempelmann, L. H., H. Lisco and J. G. Hoffman (1952). "The acute radiation syndrome: a study of nine cases and a review of the problem." Ann Intern Med **36**(2:1): 279-510.
- Hendry, J. H. (1975). "Diurnal variations in radiosensitivity of mouse intestine." Br J Radiol **48**(568): 312-4.
- Higuchi, H., H. Miyoshi, S. F. Bronk, H. Zhang, N. Dean and G. J. Gores (2001). "Bid Antisense Attenuates Bile Acid-Induced Apoptosis and Cholestatic Liver Injury." J Pharmacol Exp Ther **299**(3): 866-873.
- Holzgrevé, H. (2003). "Combination versus Monotherapy as Initial Treatment in Hypertension." Herz **28**(8): 725-32.
- Honda, T., B. T. Gjertsen, K. B. Spurgers, F. Briones, S. J. Lee, M. L. Hobbs, R. E. Meyn, J. A. Roth, C. Logothetis and T. J. McDonnell (2001). "Restoration of bax in prostate cancer suppresses tumor growth and augments therapeutic cell death induction." Anticancer Research **21**(5): 3141-6.
- Hong, J. H., C. S. Chiang, I. L. Campbell, J. R. Sun, H. R. Withers and W. H. McBride (1995). "Induction of acute phase gene expression by brain irradiation." International Journal of Radiation Oncology, Biology, Physics **33**(3): 619-26.
- Huang, X. W., J. Yang, A. F. Dragovic, H. Zhang, T. S. Lawrence and M. Zhang (2006). "Antisense Oligonucleotide Inhibition of Tumor Necrosis Factor Receptor 1 Protects the Liver from Radiation-Induced Apoptosis." Clin Cancer Res **12**(9): 2849-2855.
- Ikeda, Y., M. Ito, M. Matsuu, K. Shichijo, E. Fukuda, T. Nakayama, M. Nakashima, S. Naito and I. Sekine (2000). "Expression of ICAM-1 and acute inflammatory cell infiltration in the early phase of radiation colitis in rats." J Radiat Res (Tokyo) **41**(3): 279-91.
- Inagaki-Ohara, K., N. Takamura, S. Yada, Z. Alnadjim, E. Liu, X. Yu, H. Yoshida and T. Lin (2002). "Radiation-Induced Crypt Intestinal Epithelial Cell Apoptosis In Vivo Involves Both Caspase-3-Dependent and -Independent Pathways." Dig Dis Sci **47**(12): 2823-2830.
- Inagaki-Ohara, K., S. Yada, N. Takamura, M. Reaves, X. Yu, E. Liu, I. Rooney, S. Nicholas, A. Castro, C. F. Ware, D. R. Green and T. Lin (2001). "p53-dependent radiation-induced crypt intestinal epithelial cells apoptosis is mediated in part through TNF-TNFR1 system." Oncogene **20**(7): 812-8.
- Isaka, Y., M. Tsujie, Y. Ando, H. Nakamura, Y. Kaneda, E. Imai and M. Hori (2000). "Transforming growth factor- β 1 antisense oligodeoxynucleotides block interstitial fibrosis in unilateral ureteral obstruction." Kidney Int **58**(5): 1885-1892.
- Ito, H., M. Takazoe, Y. Fukuda, T. Hibi, K. Kusugami, A. Andoh, T. Matsumoto, T. Yamamura, J. Azuma, N. Nishimoto, K. Yoshizaki, T. Shimoyama and T. Kishimoto (2004). "A pilot randomized trial of a human anti-interleukin-6 receptor monoclonal antibody in active Crohn's disease." Gastroenterology **126**(4): 989-996.
- Iwamoto, K. S. and W. H. McBride (1994). "Production of 13-hydroxyoctadecadienoic acid and tumor necrosis factor-alpha by murine peritoneal macrophages in response to irradiation." Radiat Res **139**(1): 103-8.
- Jarrett, D. G. (1999). Medical Management of Radiological Casualties. Bethesda, MD, Armed Forces Radiobiology Research Institute.
- Jason, T. L., J. Koropatnick and R. W. Berg (2004). "Toxicology of antisense therapeutics." Toxicology & Applied Pharmacology **201**(1): 66-83.
- Kass, M., A. Witkin and D. Terzopoulos (1988). "Snakes: Active contour models." International Journal of Computer Vision **1**(4): 321-331.
- Kok, M. R. and M. E. Boon (1996). "Consequences of neural network technology for cervical screening: Increase in diagnostic consistency and positive scores." Cancer **78**(1): 112-117.
- Komarov, P. G., E. A. Komarova, R. V. Kondratov, K. Christov-Tselkov, J. S. Coon, M. V. Chernov and A. V. Gudkov (1999). "A Chemical Inhibitor of p53 That Protects Mice from the Side Effects of Cancer Therapy." Science **285**(5434): 1733-1737.
- Komarova, E. A., R. V. Kondratov, K. Wang, K. Christov, T. V. Golovkina, J. R. Goldblum and A. V. Gudkov (2004). "Dual effect of p53 on radiation sensitivity in vivo: p53 promotes hematopoietic injury, but protects from gastro-intestinal syndrome in mice." Oncogene **23**(19): 3265-71.

- Komarova, E. A., V. Krivokrysenko, K. Wang, N. Neznanov, M. V. Chernov, P. G. Komarov, M.-L. Brennan, T. V. Golovkina, O. Rokhlin, D. V. Kuprash, S. A. Nedospasov, S. R. Hazen, E. Feinstein and A. V. Gudkov (2005). "p53 is a suppressor of inflammatory response in mice." *FASEB J.*: 04-3213fje.
- Korsmeyer, S. J., J. R. Shutter, D. J. Veis, D. E. Merry and Z. N. Oltvai (1993). "Bcl-2/Bax: a rheostat that regulates an anti-oxidant pathway and cell death." *Semin Cancer Biol* **4**(6): 327-32.
- Kumasaka, T., W. M. Quinlan, N. A. Doyle, T. P. Condon, J. Sligh, F. Takei, A. Beaudet, C. F. Bennett and C. M. Doerschuk (1996). "Role of the intercellular adhesion molecule-1(ICAM-1) in endotoxin-induced pneumonia evaluated using ICAM-1 antisense oligonucleotides, anti-ICAM-1 monoclonal antibodies, and ICAM-1 mutant mice." *Journal of Clinical Investigation* **97**(10): 2362-9.
- Lawrance, I. C., F. Wu, A. Z. A. Leite, J. Willis, G. A. West, C. Fiocchi and S. Chakravarti (2003). "A murine model of chronic inflammation-induced intestinal fibrosis down-regulated by antisense NF-[kappa]B." *Gastroenterology* **125**(6): 1750-1761.
- Li, N., S. Banin, H. Ouyang, G. C. Li, G. Courtois, Y. Shiloh, M. Karin and G. Rotman (2001). "ATM Is Required for I κ B Kinase (IKK) Activation in Response to DNA Double Strand Breaks." *J. Biol. Chem.* **276**(12): 8898-8903.
- Linard, C., C. Marquette, J. Mathieu, A. Pennequin, D. ClarençonClarençon and D. MatheMathé (2004). "Acute induction of inflammatory cytokine expression after gamma irradiation in the rat: Effect of an NF-kappa-B inhibitor." *Int. J. Radiat. Oncol. Biol. Phys.* **58**(2): 427-434.
- MacCallum, D. E., T. R. Hupp, C. A. Midgley, D. Stuart, S. J. Campbell, A. Harper, F. S. Walsh, E. G. Wright, A. Balmain, D. P. Lane and P. A. Hall (1996). "The p53 response to ionising radiation in adult and developing murine tissues." *Oncogene* **13**(12): 2575-87.
- Marwick, C. (1998). "First "Antisense" Drug Will Treat CMV Retinitis." *JAMA* **280**(10): 871.
- Mason, K. A., L. Milas and L. J. Peters (1995). "Effect of paclitaxel (taxol) alone and in combination with radiation on the gastrointestinal mucosa." *International Journal of Radiation Oncology, Biology, Physics.* **32**(5): 1381-9.
- Mason, K. A., H. R. Withers, W. H. McBride, C. A. Davis and J. B. Smathers (1989). "Comparison of the gastrointestinal syndrome after total-body or total-abdominal irradiation." *Radiation Research* **117**(3): 480-8.
- Massague, J. (2000). "How cells read TGF-beta signals." *Nat Rev Mol Cell Biol* **1**(3): 169-78.
- McCabe, W. R. (1967). "Clinical use of combinations of antimicrobial agents." *Antimicrobial Agents Chemother (Bethesda)* **7**: 225-33.
- McKinney, L. C., E. M. Aquilla, D. Coffin, D. A. Wink and Y. Vodovotz (1998). "Ionizing radiation potentiates the induction of nitric oxide synthase by IFN-gamma and/or LPS in murine macrophage cell lines: role of TNF-alpha." *J Leukoc Biol* **64**(4): 459-466.
- Meineke, V., T. Moede, K. P. Gilbertz, A. Mayerhofer, J. Ring, F. M. KÄ¶hn and D. v. Beuningen (2002). "Protein kinase inhibitors modulate time-dependent effects of UV and ionizing irradiation on ICAM-1 expression on human hepatoma cells." *International Journal of Radiation Biology* **78**(7): 577-583.
- Merritt, A. J., T. D. Allen, C. S. Potten and J. A. Hickman (1997). "Apoptosis in small intestinal epithelial from p53-null mice: evidence for a delayed, p53-independent G2/M-associated cell death after gamma-irradiation." *Oncogene* **14**(23): 2759-66.
- Merritt, A. J., C. S. Potten, C. J. Kemp, J. A. Hickman, A. Balmain, D. P. Lane and P. A. Hall (1994). "The Role of p53 in Spontaneous and Radiation-induced Apoptosis in the Gastrointestinal Tract of Normal and p53-deficient Mice." *Cancer Res* **54**(3): 614-617.
- Metz, C. E. (1978). "Basic principles of ROC analysis." *Seminars in Nuclear Medicine* **8**(4): 283-298.
- Metz, C. E. (1986). "ROC methodology in radiologic imaging." *Invest Radiol* **21**(9): 720-33.
- Miles, D., G. von Minckwitz and A. D. Seidman (2002). "Combination versus sequential single-agent therapy in metastatic breast cancer." *Oncologist* **7 Suppl 6**: 13-9.
- Miner, P. B., Jr., R. S. Geary, J. Matson, E. Chuang, S. Xia, B. F. Baker and M. K. Wedel (2006). "Bioavailability and therapeutic activity of alicaforsen (ISIS 2302) administered as a rectal retention enema to subjects with active ulcerative colitis." *Alimentary Pharmacology & Therapeutics* **23**(10): 1427-34.
- Moermans, R. and P. VanHecke (1995). "Effect of sample size and number of doses for the determination of LD50." *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie* **119**(9): 637-640.
- Molla, M., M. Gironella, R. Miquel, V. Tovar, P. Engel, A. Biete, J. M. Pique and J. Panes (2003). "Relative roles of ICAM-1 and VCAM-1 in the pathogenesis of experimental radiation-induced intestinal inflammation." *International Journal of Radiation Oncology*Biolog*Physics* **57**(1): 264-273.
- Mouthon, M. A., M. Vandamme, A. van der Meeren, P. Gourmelon and M. H. Gaugler (2001). "Inflammatory response to abdominal irradiation stimulates hemopoiesis." *International Journal of Radiation Biology* **77**(1): 95-103.
- Myers, K. J., S. Murthy, A. Flanigan, D. R. Witchell, M. Butler, S. Murray, A. Siwkowski, D. Goodfellow, K. Madsen and B. Baker (2003). "Antisense Oligonucleotide Blockade of Tumor Necrosis Factor-alpha in Two Murine Models of Colitis." *J Pharmacol Exp Ther* **304**(1): 411-424.
- Nagata, S. and P. Golstein (1995). "The Fas death factor." *Science* **267**(5203): 1449-1456.
- NCRP (2001). Report No. 138 - Management of Terrorist Events Involving Radioactive Material. Bethesda, MD, National Council on Radiation Protection & Measurements.

- Nenci, A., C. Becker, A. Wullaert, R. Gareus, G. van Loo, S. Danese, M. Huth, A. Nikolaev, C. Neufert, B. Madison, D. Gumucio, M. F. Neurath and M. Pasparakis (2007). "Epithelial NEMO links innate immunity to chronic intestinal inflammation." *Nature* **446**(7135): 557-561.
- Neurath, M. F., C. Becker and K. Barbusescu (1998). "Role of NF-kappa B in immune and inflammatory responses in the gut." *Gut* **43**(6): 856-860.
- Neurath, M. F., S. Pettersson, K. H. Meyer zum Buschenfelde and W. Strober (1996). "Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-kappa B abrogates established experimental colitis in mice." *Nat Med* **2**(9): 998-1004.
- Nieminen, P., L. Kotaniemi, M. Hakama, J. Tarkkanen, J. Martikainen, T. Toivonen, J. Ikkala, T. Luostarinen and A. Anttila (2005). "A randomised public-health trial on automation-assisted screening for cervical cancer in Finland: Performance with 470,000 invitations." *International Journal of Cancer* **115**(2): 307-311.
- Nyce, J. (2002). "Respirable antisense oligonucleotides: a new, third drug class targeting respiratory disease." *Current Opinion in Allergy & Clinical Immunology* **2**(6): 533-6.
- Oliff, A., D. Defeo-Jones, M. Boyer, D. Martinez, D. Kiefer, G. Vuocolo, A. Wolfe and S. H. Socher (1987). "Tumors secreting human TNF/cachectin induce cachexia in mice." *Cell* **50**(4): 555-563.
- Oltvai, Z. N., C. L. Millman and S. J. Korsmeyer (1993). "Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death." *Cell* **74**(4): 609-19.
- Panes, J., D. C. Anderson, M. Miyasaka and D. Neil Granger (1995). "Role of leukocyte-endothelial cell adhesion in radiation-induced microvascular dysfunction in rats." *Gastroenterology* **108**(6): 1761-1769.
- Park, S. H., J. M. Goo and C. H. Jo (2004). "Receiver operating characteristic (ROC) curve: practical review for radiologists." *Korean J Radiol* **5**(1): 11-8.
- Pearson, G., F. Robinson, T. Beers Gibson, B.-e. Xu, M. Karandikar, K. Berman and M. H. Cobb (2001). "Mitogen-Activated Protein (MAP) Kinase Pathways: Regulation and Physiological Functions." *Endocr Rev* **22**(2): 153-183.
- Pober, J. S., M. A. Gimbrone, Jr., L. A. Lapierre, D. L. Mendrick, W. Fiers, R. Rothlein and T. A. Springer (1986). "Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon." *J Immunol* **137**(6): 1893-1896.
- Potten, C. S. (1977). "Extreme sensitivity of some intestinal crypt cells to X and gamma irradiation." *Nature* **269**(5628): 518-21.
- Randall, K. and J. E. Coggle (1995). "Expression of Transforming Growth Factor- β 1 in Mouse Skin During the Acute Phase of Radiation Damage." *Int J Radiat Biol Relat Stud Phys Chem Med* **68**(3): 301-309.
- Raoof, A. A., P. Chiu, Z. Ramtoola, I. K. Cumming, C. Teng, S. P. Weinbach, G. E. Hardee, A. A. Levin and R. S. Geary (2004). "Oral bioavailability and multiple dose tolerability of an antisense oligonucleotide tablet formulated with sodium caprate." *Journal of Pharmaceutical Sciences* **93**(6): 1431-1439.
- Reap, E. A., K. Roof, K. Maynor, M. Borrero, J. Booker and P. L. Cohen (1997). "Radiation and stress-induced apoptosis: A role for Fas/Fas ligand interactions." *PNAS* **94**(11): 5750-5755.
- Roberts, C. M., E. Foulcher, J. J. Saunders, D. H. Bryant, J. Freund, D. Cairns, R. Penny, G. W. Morgan and S. N. Breit (1993). "Radiation Pneumonitis: A Possible Lymphocyte-mediated Hypersensitivity Reaction." *Ann Intern Med* **118**(9): 696-700.
- Roberts, S. A., J. H. Hendry and C. S. Potten (2003). "Intestinal crypt clonogens: a new interpretation of radiation survival curve shape and clonogenic cell number." *Cell Proliferation* **36**(4): 215-31.
- Rothlein, R., M. L. Dustin, S. D. Marlin and T. A. Springer (1986). "A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1." *J Immunol* **137**(4): 1270-1274.
- Russo, S. M., J. E. Tepper, A. S. Baldwin, R. Liu, J. Adams, P. Elliott and J. C. Cusack (2001). "Enhancement of radiosensitivity by proteasome inhibition: Implications for a role of NF-[kappa]B." *Int. J. Radiat. Oncol. Biol. Phys.* **50**(1): 183-193.
- Sak, A., R. Wurm, B. Elo, S. Grehl, C. Pottgen, G. Stuben, B. Sinn, G. Wolf, V. Budach and M. Stuschke (2003). "Increased radiation-induced apoptosis and altered cell cycle progression of human lung cancer cell lines by antisense oligodeoxynucleotides targeting p53 and p21(WAF1/CIP1)." *Cancer Gene Ther* **10**(12): 926-34.
- Sandrasagra, A., S. A. Leonard, L. Tang, K. Teng, Y. Li, H. A. Ball, J. C. Mannion and J. W. Nyce (2002). "Discovery and development of respirable antisense therapeutics for asthma." *Antisense & Nucleic Acid Drug Development* **12**(3): 177-81.
- Seong, J., S. H. Kim, E. J. Chung, W. J. Lee and C. O. Suh (2000). "Early alteration in TGF- β mRNA expression in irradiated rat liver." *International Journal of Radiation Oncology*Biophysics* **46**(3): 639-643.
- Sewell, K. L., R. S. Geary, B. F. Baker, J. M. Glover, T. G. Mant, R. Z. Yu, J. A. Tami and F. A. Dorr (2002). "Phase I trial of ISIS 104838, a 2'-methoxyethyl modified antisense oligonucleotide targeting tumor necrosis factor- α ." *Journal of Pharmacology & Experimental Therapeutics* **303**(3): 1334-43.
- Shuca, A., H. Yoshitaka and K. Takehiko (2005). "Induction of cell death in rat small intestine by ischemia reperfusion: differential roles of Fas/Fas ligand and Bcl-2/Bax systems depending upon cell types." *Histochemistry and Cell Biology* **V123**(3): 249-261.
- Stepkowski, S. M., W. Chen, R. Geary, M. E. Wang, T. Condon, K. Stecker and C. F. Bennett (2001). "An oral formulation for intracellular adhesion molecules-1 antisense oligonucleotides." *Transplantation Proceedings* **33**(7-8): Nov-Dec.

- Strom, E., S. Sathe, P. G. Komarov, O. B. Chernova, I. Pavlovskaya, I. Shyshynova, D. A. Bosykh, L. G. Burdelya, R. M. Macklis, R. Skaliter, E. A. Komarova and A. V. Gudkov (2006). "Small-molecule inhibitor of p53 binding to mitochondria protects mice from gamma radiation." Nat Chem Biol **2**(9): 474-479.
- Swets, J. and R. Pickett (1982). Evaluation of diagnostic systems: Methods from signal detection theory. New York, Academic Press.
- Szlosarek, P. W. and F. R. Balkwill (2003). "Tumour necrosis factor-alpha: a potential target for the therapy of solid tumours." The Lancet Oncology **4**(9): 565-573.
- Trauth, B. C., C. Klas, A. M. Peters, S. Matzku, P. Moller, W. Falk, K. M. Debatin and P. H. Krammer (1989). "Monoclonal antibody-mediated tumor regression by induction of apoptosis." Science **245**(4915): 301-305.
- Urban, G., T. Golden, I. V. Aragon, L. Cowser, S. R. Cooper, N. M. Dean and R. E. Honkanen (2003). "Identification of a Functional Link for the p53 Tumor Suppressor Protein in Dexamethasone-induced Growth Suppression." J. Biol. Chem. **278**(11): 9747-9753.
- Van Der Meeren, A., C. Squiban, P. Gourmelon, H. Lafont and M.-H. Gaugler (1999). "Differential regulation by IL-4 and IL-10 of radiation-induced IL-6 and IL-8 production and ICAM-1 expression by human endothelial cells." Cytokine **11**(11): 831-838.
- van Deventer, S. J., M. K. Wedel, B. F. Baker, S. Xia, E. Chuang and P. B. Miner, Jr. (2006). "A phase II dose ranging, double-blind, placebo-controlled study of alicaforsen enema in subjects with acute exacerbation of mild to moderate left-sided ulcerative colitis." Aliment Pharmacol Ther **23**(10): 1415-25.
- van Deventer, S. J. H., M. K. Wedel, B. F. Baker, S. Xia, E. Chuang and P. B. Miner, Jr. (2006). "A phase II dose ranging, double-blind, placebo-controlled study of alicaforsen enema in subjects with acute exacerbation of mild to moderate left-sided ulcerative colitis." Alimentary Pharmacology & Therapeutics **23**(10): 1415-25.
- Vapnik, V. (2000). The Nature of Statistical Learning Theory. New York, NY, Springer-Verlag.
- Vemuganti, R., R. J. Dempsey and K. K. Bowen (2004). "Inhibition of Intercellular Adhesion Molecule-1 Protein Expression by Antisense Oligonucleotides Is Neuroprotective After Transient Middle Cerebral Artery Occlusion in Rat." Stroke **35**(1): 179-184.
- Wajant, H., K. Pfizenmaier and P. Scheurich (2003). "Non-apoptotic Fas signaling." Cytokine & Growth Factor Reviews **14**(1): 53-66.
- Wang, T., Y.-C. Hu, S. Dong, M. Fan, D. Tamae, M. Ozeki, Q. Gao, D. Gius and J. J. Li (2005). "Co-activation of ERK, NF-kB, and GADD45b in Response to Ionizing Radiation." J. Biol. Chem. **280**(13): 12593-12601.
- Wang, Y., A. Meng, H. Lang, S. A. Brown, J. L. Konopa, M. S. Kindy, R. A. Schmiedt, J. S. Thompson and D. Zhou (2004). "Activation of Nuclear Factor kappa-B In vivo Selectively Protects the Murine Small Intestine against Ionizing Radiation-Induced Damage." Cancer Res **64**(17): 6240-6246.
- Warny, M., A. C. Keates, S. Keates, I. Castagliuolo, J. K. Zacks, S. Aboudola, A. Qamar, C. Pothoulakis, J. T. LaMont and C. P. Kelly (2000). "p38 MAP kinase activation by Clostridium difficile toxin A mediates monocyte necrosis, IL-8 production, and enteritis." J. Clin. Invest. **105**(8): 1147-1156.
- Weil, M. M., L. C. Stephens, C. I. Amos, A. C. Ruifrok and K. A. Mason (1996). "Strain difference in jejunal crypt cell susceptibility to radiation induced apoptosis." International Journal of Radiation Biology **70**(5): 579 - 585.
- Wiedenmann, N. E., D. Valdecanas, N. Hunter, L. Milas and K. A. Mason (2006). Bcl-2 antisense ODN G3139 increases susceptibility for radiation induced small bowel damage. Radiation Research Society 53rd Annual Meeting. Philadelphia, Radiation Research Society.
- Wilson, J. W., D. M. Pritchard, J. A. Hickman and C. S. Potten (1998). "Radiation-induced p53 and p21WAF-1/CIP1 expression in the murine intestinal epithelium: apoptosis and cell cycle arrest." American Journal of Pathology **153**(3): 899-909.
- Withers, H. R. and M. M. Elkind (1970). "Microcolony Survival Assay for Cells of Mouse Intestinal Mucosa Exposed to Radiation." International Journal of Radiation Biology **17**(3): 261 - 267.
- Withers, H. R., K. Mason, B. O. Reid, N. Dubravsky, H. T. Barkley Jr, B. W. Brown and J. B. Smathers (1974). "Response of mouse intestine to neutrons and gamma rays in relation to dose fractionation and division cycle." Cancer **34**(1): 39-47.
- Xu, H., J. A. Gonzalo, Y. St Pierre, I. R. Williams, T. S. Kupper, R. S. Cotran, T. A. Springer and J. C. Gutierrez-Ramos (1994). "Leukocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice." J Exp Med **180**(1): 95-109.
- Yamamoto, M., K. Yoshizaki, T. Kishimoto and H. Ito (2000). "IL-6 is required for the development of Th1 cell-mediated murine colitis." Journal of Immunology **164**(9): 4878-82.
- Yang, L., R. M. Froio, T. E. Sciuto, A. M. Dvorak, R. Alon and F. W. Luscinskas (2005). "ICAM-1 regulates neutrophil adhesion and transcellular migration of TNF-alpha-activated vascular endothelium under flow." Blood **106**(2): 584-92.
- Yonehara, S., A. Ishii and M. Yonehara (1989). "A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor." J. Exp. Med. **169**(5): 1747-1756.
- Zhang, H., J. Cook, J. Nickel, R. Yu, K. Stecker, K. Myers and N. M. Dean (2000). "Reduction of liver Fas expression by an antisense oligonucleotide protects mice from fulminant hepatitis." Nat Biotech **18**(8): 862-867.
- Zhang, N., M. Ahsan, L. Zhu, L. Sambucetti, A. Purchio and D. West (2005). "Regulation of IkappaBalpha expression involves both NF-kappaB and the MAP kinase signaling pathways." Journal of Inflammation **2**(1): 10.

- Zhang, S. Y., K. W. Park, S. Oh, H. J. Cho, H. J. Cho, J. S. Park, Y. S. Cho, B. K. Koo, I. H. Chae, D. J. Choi, H. S. Kim and M. M. Lee (2005). "NF-kappaB decoy potentiates the effects of radiation on vascular smooth muscle cells by enhancing apoptosis." Exp Mol Med **37**(1): 18-26.
- Zheng, H., J. Wang, V. E. Kotliansky, P. J. Gotwals and M. Hauer-Jensen (2000). "Recombinant Soluble Transforming Growth Factor β Type II Receptor Ameliorates Radiation Enteropathy in Mice." Gastroenterology **119**(5): 1286-1296.
- Zhou, Y., Q. Wang, B. M. Evers and D. H. Chung (2005). "Signal Transduction Pathways Involved in Oxidative Stress-Induced Intestinal Epithelial Cell Apoptosis." Pediatr Res **58**(6): 1192-1197.