TRANSLATIONAL ADVANCES IN CANCER PREVENTION AGENT DEVELOPMENT ABSTRACTS

PLENARY SESSIONS

Inflammation and inflammatory mediators as targets for cancer prevention/interception

Raymond N. DuBois

The Medical University of South Carolina, 96 Jonathan Lucas Street, Charleston, SC 29425

Evidence for the link between inflammation and cancer comes from epidemiologic and clinical studies showing that use of nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, reduces the relative risk for developing colorectal cancer (CRC) by 40-50% and in some cases reduces cancer incidence. NSAIDs exert some of their anti-inflammatory and anti-tumor effects by targeting cyclooxygenase enzymes (COX-1 and COX-2). Metabolism of arachidonic acid, a major ingredient in animal fats, by cyclooxygenase enzymes provides one mechanism for the contribution of dietary fats and chronic inflammation to carcinogenesis. COX-2-derived prostaglandin E_2 (PGE_2) is a pro-inflammatory mediator that promotes tumor progression. Our new preliminary data show that a COX-2 selective inhibitor (COXIB) or an EP4 antagonist reduces intestinal adenoma burden accompanied with decreased PD-1 (an immune checkpoint receptor) expression in tumor-infiltrating $CD8^+$ T cells and tumor-associated macrophages (TAMs). EP4 is one of four PGE_2 receptors. Our *in vitro* data also show that PGE_2 induces PD-1 expression in $CD8^+$ T cells and macrophages. These novel findings prompted us to postulate that PGE_2 produced locally in the tumor microenvironment promotes tumor immune evasion by suppressing functions of tumor-infiltrating $CD8^+$ T cells and TMAs via induction of PD-1.

Considering the importance of PGE_2 signaling in inflammation and colorectal carcinogenesis, we have determined some of the mechanisms by which the pro-inflammatory PGE_2 mediates the connection of chronic inflammation to colorectal carcinogenesis. Collectively, these findings uncover a previously unrecognized role for PGE_2 promotion of tumor formation and progression.

Novel Cancer Prevention Strategies in the Molecular Era

Powel Brown

Department of Clinical Cancer Prevention, MD Anderson Cancer Center, Houston, Texas.

Multiple clinical trials have demonstrated that it is possible to prevent cancer using medications and vaccines. However, most drugs that have been shown to successfully prevent cancer in Phase III trials have not been accepted for general use because of concerns about toxicity. Results of several Phase III cancer prevention trials will be discussed, and the results of preclinical studies and early phase cancer prevention trials testing novel cancer prevention approaches with a focus on reducing toxicity will be presented. Studies striving to prevent breast cancer, colorectal cancer, and lung cancer will be discussed. Novel interventions include low-dose tamoxifen therapy, topically applied tamoxifen, molecular targeted drugs inhibiting COX-2, mTOR, or PARP pathways, vaccines, checkpoint inhibitors, and cytokine inhibitors. These studies demonstrate that it is possible to prevent breast, colon, and lung cancer in animal models, and early-phase clinical trial results show promising results. However, toxicity remains a major issue that must be overcome before these novel prevention strategies are generally accepted. A challenge for the future will be to develop minimally toxic prevention strategies that will be acceptable to individuals at high-risk of cancer. Despite this challenge, significant advances in targeting oncogenic driver molecules and in harnessing the immune system offer great promise to safely prevent many life-threatening cancers in the near future.

SESSION 1

Estrogen Metabolism: A Target for Intervention in Non-small Cell Lung Cancer

Margie L. Clapper[1], Jing Peng1, Lisa Vanderveer1, J. Nicholas Bodor[2], Daniel Krzizike[3], Andrew Andrews[3], Joseph Treat[2]

[1]Cancer Prevention and Control Program; [2]Department of Hematology/Oncology; [3]Cancer Epigenetic Program; Fox Chase Cancer Center, Philadelphia, PA

Lung cancer remains the leading cause of cancer-related death in both men and women in the US. While smoking is the primary risk factor for disease, 15-20% of males and >50% of females diagnosed with lung cancer worldwide have never smoked. Based on its unique clinical and pathological features, NSCLC among never-smokers appears to be a disease entity distinct from that attributed to smoking. The predominance of women among NSCLC patients who have never smoked prompted an investigation of the contribution of estrogen to lung tumorigenesis. This research group is the first to demonstrate that the mouse and human lung can extensively metabolize estrogen. In general, 17β estradiol (E2) and estrone (E1) are metabolized by cytochrome P450 (CYP)1A1 and CYP1B1 to form 2- and 4-hydroxyestrogen (2- and 4-OHE), respectively; metabolites that can retain their estrogenic activity or induce DNA damage. In the absence of efficient detoxication, catechol estrogens undergo oxidation to form reactive quinones and semiquinones. 4-OHE has been reported to be genotoxic, while methoxyestradiol exhibits anti-proliferative and pro-apoptotic properties. Three parent estrogens and 5 metabolites, including the putative carcinogen 4-OHE, were detected within the perfused mouse lung of both males and females by mass spectrometry. A similar profile was observed in human lung tissue, where the level of each parent estrogen (E1, E2, E3) and estrogen metabolite was significantly higher in females vs. males. Both 4-OHE as a percentage of total estrogen, as well as the ratio of 4-OHE/2-OHE was elevated significantly in tumors vs. adjacent normal tissue from patients with NSCLC. Exposure to tobacco smoke induced the production of 4-OHE in both normal lung tissue and the urine of NSCLC lung cancer patients, suggesting urinary levels may serve as a noninvasive surrogate biomarker of those within the lung. The mechanism by which CYP1B1 contributes to lung tumorigenesis was investigated using oral leukoplakia cells, a precancerous cell line of the aerodigestive tract. Knockdown of CYP1B1 using shRNA reduced both cell motility and cell proliferation by approximately 50%, as compared to vector controls. Likewise, use of a chemical CYP1B1 inhibitor caused dose-dependent reductions in cell proliferation and cell motility, confirming this pathway as a viable target for intervention. Recent translational efforts have focused on defining those populations that could potentially benefit from a targeted therapeutic intervention. The high proportion of never-smokers among female lung cancer cases in Southeast Asia (60-80%) prompted an analysis of the ability of Asian women to produce 4-OHE. Urinary levels of 4-OHE (% total) were significantly higher and 2-OHE levels lower in Chinese American vs. Caucasian American women matched for age and body mass index, suggesting healthy Chinese women are high producers of 4-OHE. A UPLC-MS-MS assay has been developed that detects parent estrogens, catechol estrogens and methoxy derivatives in biofluids with high resolution and with much shorter run times than analyses reported previously. This assay has been used to profile urinary estrogen metabolites in NSCLC patients from the Never-Smoker Lung Cancer Clinic at FCCC. A significantly higher level of urinary 4-OHE (percent of total estrogen) was found in lung cancer patients with EGFR-mutated tumors as compared to cancer-free controls, indicating 4-hydroxylation of estrogen may be enhanced in this subset of patients. Further elucidation of the regulatory mechanisms and clinical factors that impact the activity of the estrogen metabolizing enzymes will allow identification of those individuals with detrimental profiles who would benefit most from targeted therapy. When combined, these data suggest that estrogen metabolism represents a promising target for intervention in NSCLC. (Supported by R01 CA217161, a FCCC Pilot Award, and generous donors).

Targeting the immune system for the prevention of KRAS-driven cancers

Karen Liby; Di Zhang; Jessica Moerland; Lyndsey Reich; and Ana Leal

Michigan State University, East Lansing, MI

More effective drugs are needed to prevent lung and pancreatic cancer, as more than 285,000 new cases of these cancers will be diagnosed in the United States each year. These two cancers account for 30% of all cancer deaths in the U.S. *KRAS* mutations are among the most frequently mutated genes in cancer, including 35% of lung cancers and 95% of pancreatic cancers. Tumors driven by *KRAS* mutations are considered “undruggable” and are largely resistant to standard therapies. Rexinoids, selective agonists for retinoid X receptors, are attractive drugs for preventing lung and pancreatic cancer because they target a number of processes that drive cancer. Activators of the cytoprotective Nrf2 pathway, including the synthetic oleanane triterpenoid bardoxolone methyl, inhibit carcinogenesis in a variety of animal models. Both rexinoids and triterpenoids are active in preclinical models of lung and pancreatic cancer driven by *Kras* mutations.

Because activation of the Ras pathway recruits and activates immune cells that drive tumor progression, we studied the ability of triterpenoids and rexionoids to modulate immune cells in clinically relevant mouse models. Both classes of drugs favorably altered immune cells populations, including reducing the number of immunosuppressive Gr1+ myeloid derived suppressor cells (MDSCs) in the pancreas of the LSL-$Kras^{G12D/+}$;Pdx-1-Cre (KC) or LSL-$Kras^{G12D/+}$;LSL-$Trp53^{R127H/+}$;Pdx-1-Cre (KPC) mice. These autochthonous models of pancreatic cancer closely mimic the genetic lesions, clinical symptoms, and histopathology found in human pancreatic cancer. When tested for prevention of lung cancer, new rexinoids reduced both MDSCs and macrophages around lung tumors without elevating triglycerides, a known side effect of many rexinoids.

Although numerous activators of Nrf2, such as triterpenoids, inhibit carcinogenesis, genetic alterations resulting in constitutive Nrf2 activation in human lung tumors promotes tumor growth. Surprisingly, we found a higher tumor burden and a novel immune signature in the lungs and tumors of Nrf2 knockout (KO) mice compared to wildtype (WT) mice with lung cancer. The numbers of tumor-promoting macrophages and MDSCs were elevated in the Nrf2 KO mice, while beneficial cytotoxic CD8+ T cells were decreased. Over 30 immune response genes were significantly upregulated in the lung tumors of Nrf2 KO mice, especially a series of cytokines that promote tumor growth. These findings are consistent with data from lung cancer patients found in The Cancer Genome Atlas. Taken together, these studies suggest that activation of the Nrf2 pathway in immune cells can be used to prevent lung cancer. Future studies will determine if depletion of immune cells reverses the ability of triterpenoids and rexinoids to prevent *Kras*-driven lung and pancreatic cancer.

Cancer and Aging Prevention by mTOR Inhibition

Zelton Dave Sharp[1,2,3], Manish Parihar[1], Sherry G. Dodds[1], Gene Hubbard[2], Marty Javors[2,5,] Randy Strong[4,6,] Paul Hasty[1,2,3]

[1]Department of Molecular Medicine and Institute of Biotechnology, [2]Barshop Institute for Longevity and Aging Studies, [3]Mays Cancer Center, [4]Department of Pharmacology, [5]Department of Psychiatry, [6]San Antonio Geriatric Research, Education and Clinical Center, University of Texas Health, San Antonio, Texas.

Apc$^{Min/+}$ mice model familial adenomatous polyposis (FAP), a disease that causes numerous colon polyps leading to colorectal cancer. We previously showed that chronic treatment of Apc$^{Min/+}$ females with an enteric formulation of the anti-aging drug, rapamycin (eRapa), restored a normal lifespan through reduced polyposis and anemia prevention. Lifespan extension by chronic rapamycin in wildtype UM-HET3 mice is sex-dependent with females gaining the most benefit. Whether Apc$^{Min/+}$ mice have a similar sex-dependent response to chronic mTOR inhibition is not known. To address this knowledge gap and gain deeper insight into how chronic mTOR inhibition prevents polyposis, we compared male and female Apc$^{Min/+}$ mouse responses to chronic treatment with an eRapa-containing diet. Surprisingly, we found that survival of males is greater than females in this setting. Immunohistochemistry assays of rpS6 phosphorylation showed that rapamycin reduction of mTORC1 activity is most prominent in small intestine crypt Paneth cells. Chronic rapamycin also reduced crypt depths equally in both male and female Apc$^{Min/+}$ mice, consistent with reduced crypt epithelial cell proliferation. Apc$^{Min/+}$ mice develop colon tumors with a low frequency but can be converted to a human relevant model of colon cancer by dextran sodium sulfate (DSS) treatments (Apc$^{Min/+}$-DSS model). We next asked what effect pretreatment of Apc$^{Min/+}$ mice with chronic rapamycin prior to DSS exposure has on survival and colonic neoplasia? Chronic eRapa diet significantly extended lifespan of Apc$^{Min/+}$-DSS mice (both sexes) by reductions in colon neoplasia and prevention of anemia. Immunoblot assays showed the expected inhibition of mTORC1 and effectors (S6K$\rightarrow$rpS6) in colon by eRapa. To determine the cell types affected by chronic enteric rapamycin treatment in colon, IHC analyses demonstrated that cells at the base of crypts had a prominent reduction in rpS6 phosphorylation relative to controls. Collectively, our data indicate that enteric rapamycin prevented polyposis in *Apc*$^{Min/+}$ mice (both sexes) and delayed or prevented colon neoplasia in Apc$^{Min/+}$-DSS mice (both sexes) through inhibition of mTORC1 in small intestine Paneth cells and cells at the base of colon crypts, respectively. Consistent with cancer and anemia prevention effects in both models, chronic eRapa significantly extended their life and health span.

Bringing Broccoli Sprouts (Sulforaphane) to Clinical Trials: Dose Matters

Thomas W. Kensler[1,2,3] for the Qidong Clinical Trials Team

[1]Translational Research Program, Fred Hutchinson Cancer Research Center, Seattle, WA,

[2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD;[3]Qidong Liver Cancer Institute, Qidong People's Hospital, Qidong, Jiangsu, China.

Broccoli sprouts are a convenient and rich source of the glucosinolate, glucoraphanin, which can generate the chemopreventive agent sulforaphane through the catalytic actions of plant myrosinase or β-thioglucosidases in the gut microflora. Sulforaphane, in turn, is an inducer of glutathione S-transferases and other cytoprotective enzymes through activation of Nrf2 signaling, and a potent inhibitor of carcinogenesis in multiple murine models. Translating this efficacy into the design and implementation of clinical chemoprevention trials, especially food-based trials, faces numerous challenges including the selection of the source, formulation, placebo, and dose of the intervention material. Notable in the case of sulforaphane, selection of doses has been ill-informed by most pre-clinical studies. Whilst sulforaphane has been employed in hundreds of pre-clinical studies in the aggregate over a 5-log dose range, most even after allometric scaling, exceeded tolerable levels for oral use in humans. We have conducted a series of randomized clinical trials to evaluate the effects of composition (glucoraphanin-rich versus sulforaphane-rich or mixture beverages), formulation (beverage versus tablet) and dose on the efficacy of these broccoli sprout-based preparations to enhance the detoxication of aldehydes, benzene and polycyclic aromatic hydrocarbons in residents of Qidong, China who are exposed to moderately high ambient levels of air pollution during the winter months. Urinary excretion of the mercapturic acids of these pollutants were measured before and during the interventions using liquid chromatography tandem mass spectrometry. Rapid and sustained, statistically significant increases in the levels of excretion of the glutathione-derived detoxication conjugates of benzene and acrolein were found in those receiving broccoli sprout beverages compared with placebo beverages. These pharmacodynamic effects persisted throughout the intervention period and likely reflected, at least in part, responses to activation of the NRF2 signaling pathway. A subsequent randomized, placebo-controlled, multi-dose trial of broccoli sprout beverage determined the lowest effective level to enhance benzene detoxication adjudged by enhanced excretion of the urinary biomarker, S-phenyl mercapturic acid (SPMA). A dose/formulation that evoked a urinary elimination of ~25 μmol sulforaphane metabolites per day [a measure of "internal dose"] significantly increased the rate of urinary excretion of SPMA; lower doses were not effective. Interestingly, the dynamic range for enhancement of SPMA excretion was quite limited, perhaps reflecting a limited capacity to modulate NRF2 in humans. In these trials, there is substantial inter-individual variation in internal dose and excretion levels of sulforaphane, especially when only the biogenic precursor glucoraphanin is administered. Improved and more consistent bioavailability is achieved with tablets containing glucoraphanin and myrosinase. Thus, interventions with broccoli sprout-based preparations enhance the detoxication of some airborne pollutants and may provide a frugal means to attenuate their associated long-term health risks. Supported by NIH grants R01 CA190610 and R35 CA197222 and the Washington State Andy Hill CARE Fund.

SESSION 2

STAT3 Decoy for Preventing Lung Cancer

Jill M. Siegfried, Christian Njatcha, Mariya Farooqui, and Abdulaziz Almotlak
Department of Pharmacology, University of Minnesota, Minneapolis, MN.

Former smokers are at elevated lung cancer risk, and account for a large proportion of newly diagnosed lung cancer. Former smokers with airway dysplasia have received some benefit in randomized trials of chemoprevention, while active smokers have not. To date, prevention trials in lung cancer have only shown modest effects. There is a great need for more effective chemoprevention agents for the millions of former smokers at risk for lung cancer. CS3D is a novel inhibitor of the STAT3 pathway. It is a cyclic double-stranded DNA oligonucleotide decoy that mimics the STAT3 response element. CS3D competitively blocks the binding of activated STAT3 dimers to the promoter regions of STAT3 target genes, and also induces p-STAT3 degradation. We previously showed that CS3D, compared to a mutant inactive version termed CS3M, reduced expression of STAT3 target genes and had strong anti-tumor effects in xenograft models of non-small cell lung cancer (NSCLC). We next determined the chemoprevention efficacy of CS3D using a tobacco carcinogen-induced mouse model of lung cancer that mimics an “ex-smoker,” in which NNK was given for 4 weeks to induce airway dysplasia. Following a 1-week rest period to mimic smoking cessation, CS3D or CS3M was given as a short-term intermittent therapy via IV injection (5 mg/kg three times per week for 8 weeks). Compared to CS3M, CS3D blocked formation and progression of airway preneoplasia. CS3D also reduced both incidence and size of lung tumors that arose over time, and induced apoptosis in the airways, while showing no discernable toxicity. Efficacy was associated with reduction of p-STAT3 protein detected by immunohistochemistry in dysplastic airway lesions, lung adenomas, and lung adenocarcinomas from these mice. Phospho-STAT3 protein was reduced both during and after treatment with CS3D, remaining suppressed 8 weeks after the end of the treatment course. Total STAT3 expression was unchanged, during or after therapy. Other pathways downregulated by CS3D were NFκB, COX2, IL6, and VEGF. CS3D also produced a less immunosuppressive microenvironment in the lungs, with fewer M2 macrophages and MDSC cells. No signs of toxicity were detected during therapy and no organ abnormalities were detected at necropsy. These results suggest that a short course of intermittent therapy with CS3D has persistent chemoprevention effects against lung cancer in a model of tobacco carcinogenesis. Blocking STAT3 may be a useful strategy for lung cancer prevention, and may involve both inhibition of oncogenic signaling and enhanced anti-tumor immunity.

Mediation analysis to elucidate the role of inflammation reduction in cancer prevention: exploratory findings from the CANTOS trial

Robert J. Glynn

Department of Biostatistics, Brigham & Women's Hospital, Harvard Medical School, Boston, MA

Background: The Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) found lower rates of incident lung cancer and overall cancer death in subjects randomized to active canakinumab versus placebo. We used mediation analysis to quantify the extent to which these effects were achieved through changes in inflammatory markers, and explored the sensitivity of estimated mediation effects to alternative mediator specifications.

Methods: CANTOS randomized 10,061 individuals with prior myocardial infarction, who were free of previously diagnosed cancer and had concentrations of high-sensitivity C-reactive protein (hsCRP) $\geq$ 2 mg/L, to either placebo or one of three active doses of canakinumab (50 mg, 150 mg, or 300 mg subcutaneously every 3 months). An oncology endpoint committee, masked to treatment allocation, adjudicated incident cancer diagnoses. We evaluated achieved levels of hsCRP and interleukin-6 (IL6) at 3 months post randomization, as well as percent changes in these biomarkers, as mediators of observed treatment effects on cancer outcomes. Analyses adjusted for baseline biomarker levels and patient characteristics potentially related to cancer risk and mediator changes.

Results: During median follow-up of 3.7 years, incident lung cancer occurred in 129 and fatal cancer in 196 subjects, with relative hazards of 0.55 (95% CI: 0.39-0.78) and 0.71 (95% CI: 0.53-0.94) in those receiving active canakinumab versus placebo, respectively. For both outcomes, each of six measures of achieved level or change in an inflammatory biomarker showed evidence of significant mediation, ranging from 75% of the observed effect of canakinumab on fatal cancer mediated through continuous level of log(hsCRP) at 3 months to 33% of the observed effect of canakinumab on incident lung cancer mediated through achieving a level of hsCRP at 3 months <2 mg/L. For incident lung cancer, change in IL6 (mediating 51% of the observed canakinumab effect) had the strongest mediation effect. We saw little evidence for a direct effect of canakinumab outside the pathway of measured inflammation reduction.

Conclusions: Measured changes in inflammatory markers strongly mediated reductions in incident lung cancer and fatal cancer associated with canakinumab treatment. Identification of powerful mediating variables can help target the most likely responders soon after treatment initiation.

CD137 immune checkpoint pathway as an effective target for cancer immunoprevention

Haval Shirwan

Department of Child Health and Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia, MO

Cancer immunotherapy has shown remarkable clinical efficacy and is becoming the mainstay of treatment for cancer. Given the demonstrated efficacy of immune checkpoint blockade for multiple types of tumors, we focused on immune checkpoint stimulators, CD137 pathway in particular, for cancer treatment as the corollary. The 4-1BB pathway plays a paramount role in the expansion of CD8+ T cells, acquisition of effector function, survival, and establishment of long-term memory that are important to cancer eradication and control of recurrence. The natural CD137 ligand lacks the costimulatory function as a soluble protein; thus, we generated an oligomeric form of the ligand, SA-4-1BBL, and demonstrated that it has better costimulatory activity and safety profile than agonistic CD137 Abs, which are presently being evaluated in cancer immunotherapy trials. Treatment with SA-4-1BBL as a single agent conferred protection against subsequent challenge with multiple tumor types, including melanoma, lung, lymphoma, triple-negative breast cancer, a surprising finding that has not previously been reported in the scientific literature. Importantly, the immunoprevention was a bona fide feature of SA-4-1BBL as agonistic Abs to CD137 receptor had no impact on the tumor growth. The cancer immunoprevention effect of SA-4-1BBL operated through an innate immune surveillance mechanism that evolved within three weeks of treatment, lasted for months, and did not involve CD8+ T cells. SA-4-1BBL was also effective in controlling post-surgical recurrence against various tumors. The ability of an immune checkpoint stimulator as a single agent to train the immune system for long-lasting broad protection against many tumor types is exciting conceptually and sets the stage for a cancer immunoprevention modality that does not require tumor-specific antigens.

Epithelium-Derived Alarmins Role in Breast Cancer Immunoprevention

Shadmehr Demehri

Center for Cancer Immunology and Cutaneous Biology, Massachusetts General Hospital, Boston, MA

Advances in cancer immunology has led to the successful use of patients' immune cells to combat metastatic cancers. However, the therapeutic potential of the immune system in eliminating premalignant cells and preventing their progression to invasive cancers is unclear. To determine the benefit of activating the immune system to prevent cancer development and recurrence, we study the immune pathways that lead to effective immune activation against early phases of breast cancer development. Breast cancer is the most common internal cancer and second cause of cancer deaths among women in the United States. Importantly, the individuals at high risk of developing breast cancer due to underlying genetic mutations and those with premalignant lesions can be clinically identified and treated. Therefore, discovering an effective approach to activate the patients' own immune system against early breast precursor lesions may yield a lasting memory that can prevent breast cancer development and recurrence in this high-risk population. Here, we demonstrate that a skin-derived alarmin cytokine, thymic stromal lymphopoietin (TSLP), suppresses the early stages of breast cancer development. In order to determine the precise mechanism of TSLP-induced immune response against early premalignant cells in the breast, we have studied immune cells and signals that target breast premalignant cells in response to TSLP. We have found that $CD4^+$ T helper 2 (Th2) are required and sufficient to deliver the tumor-protective effect of TSLP on breast cancer. Further, this antigen-specific immunity is mediated through terminal differentiation of breast tumor cells, which lasts long after TSLP induction is stopped. Our findings establish a foundation for the use of the alarmin cytokines in blocking breast cancer development and provide novel therapeutic targets for breast cancer immunoprevention.

SESSION 3

Chemoprevention of aerosolized let-7 microRNA mimic in mouse lung cancer model

Qi Zhang[1], Jing Pan[1], Yian Wang[1], Mark S Miller[2], Alberto Izzotti[3], and Ming You[1].

[1]Center for Disease Prevention Research and Department of Pharmacology & Toxicology, Medical College of Wisconsin, Milwaukee, WI,.

[2]Chemopreventive Agent Development Research Group, Division of Cancer Prevention, National Cancer Institute, Bethesda, MD, USA.

[3]Department of Health Sciences, University of Genoa, 16139 Genoa, Italy

The delivery of microRNA (miRNA) via inhalation is a potential strategy for lung cancer prevention in high risk individuals such as current and former smokers. Previous studies have shown that intratracheal or intranasal exposure of genetically engineered mice to viral vectors expressing let-7 miRNA resulted in a reduced lung tumor burden. However, these studies were done in genetically engineered mouse models that developed highly aggressive tumors more relevant for a treatment regimen. In this study, we investigated the efficacy of let-7b in lung cancer prevention. An aerosolized let-7b miRNA formulation was tested in the benzo(a)pyrene (B[a]P) mouse model. The particle size of the let-7b miRNA aerosol has been systematically characterized as particles with sizes between 1.9 to 35 nm (average size of 29.0 nm). Following the aerosol formulation evaluation, the biodistribution of let-7b in the lung by tail vein injection and aerosolized delivery were compared. The distribution of the aerosolized let-7b produced higher levels of the miRNA in the lung compared with systematic delivery. The aerosol delivery of let-7b had no effect on animal body weight nor were there any other signs of toxicity. The development of an aerosolized let-7b formulation inhibited B[a]P - induced lung adenoma volume by 72% in A/J mice. Preliminary scRNA-seq analyses showed that let-7b targets both tumor cells and immune cells in the tumor microenvironment. Collectively, these findings show that aerosolized miRNA is a promising approach for lung cancer prevention.

Oral Cancer Chemoprevention by Local Delivery

Susan R. Mallery
Division of Oral and Maxillofacial Pathology and Radiology
Ohio State University College of Dentistry

There are numerous routes to administer chemoprevention agents including oral formulations, injections (either intramuscularly or intravenous), mucosal application for systemic uptake or direct application to the treatment site. First pass metabolism, which can inactivate the bioactive components, negatively impacts oral formulations for systemic delivery. Furthermore, systemically delivered agents often fail to deliver therapeutic levels and can result in adverse systemic effects. While injections avoid first pass metabolism, they are cumbersome and generally administered in a health care setting. For a disease such as oral squamous cell carcinoma, which arises from surface epithelium in a visibly accessible site, our team has found local chemoprevention to be advantageous. By direct application to the treatment site, local delivery formulations demonstrate a pharmacologic advantage i.e. achieve therapeutic levels at the target with negligible systemic adverse effects. For a variety of reasons including reduced toxicity and diverse mechanisms of action, we have focused on natural products or derivatives thereof. Direct application of a bioadhesive gel that contained 10% w/w of freeze dried black raspberries (BRB) to microscopically confirmed premalignant oral epithelial lesions (0.5 gm x q.i.d. for 3 months) provided positive clinical data that included significant reduction in histologic grade, lesional size and loss of heterozygosity at putative tumor suppressor gene loci. While these results were positive, there were areas for improvement. Not all patients responded uniformly, which was determined to reflect at least in part the capacity for local enteric recycling of BRB chemopreventives like anthocyanins. As a natural product, BRB cannot be standardized for sustained, high level production. Finally, while use of a gel as a vehicle was reasonable, inter-patient variations in gel-dispersing salivary flow and local adherence could not be regulated. For these reasons, other chemopreventive agents and delivery formulations were investigated. Due to its well-confirmed capacities to regulate aberrant growth of premalignant cells via induction of terminal differentiation and apoptosis, the synthetic derivative of vitamin A fenretinide (4HPR) was selected. Previous OSCC chemopreventive trials that employed systemic oral delivery of 4HPR capsules were unsuccessful, findings that reflected appreciable first-pass inactivation and failure to achieve therapeutic levels. As a highly hydrophic drug, 4HPR delivery to a saliva-rich site like the mouth is challenging. A Tegaderm-backed patch that incorporated 4HPR solubility enhancing agents (sodium deoxycholate and Tween-80) and permeation enhancers (menthol, propylene glycol) successfully delivered therapeutically-relevant 4HPR levels to rabbit oral mucosa. Furthermore, no 4HPR was detected in the sera (LC-MS/MS), and no clinical adverse events such as a contact mucositis were noted. Consistent with 4HPR's growth modulatory effects, treated mucosa demonstrated a slight increase in the outer cornified layer (lower 4HPR levels) and increased apoptosis (higher 4HPR levels). An additional positive effect was the increase in surface epithelial levels of the Phase II detoxification enzyme UGT1A1. These promising data, along with additional 4HPR chemopreventive mechanistic studies, were the foundation of our NCI-supported Phase Ib fenretinide patch secondary chemoprevention study. Work is ongoing to refine the clinical patch formulation to be used in our upcoming clinical trial.

Safer Chemopreventive Approaches to Colonic Adenoma Prevention

Altaf Mohammed[2], Naveena B Janakiram[1,3], Venkateshwar Madka[1], Yuting Zhang[1], Anil Singh[1], Laura Biddick[1], Qian Li[1], Stan Lightfoot[1], Vernon E. Steele[2], Ronald Lubet[2], Mark S. Miller[2], Chen S. Suen[2], Shizuko Sei, Chinthalapally V. Rao[1,3]

[1]Center for Cancer prevention and Drug Development, Department of Medicine, Hem-Onc Section, Stephenson Cancer Center and University of Oklahoma Health Sciences Center, Oklahoma City, OK, and [2]Division of Cancer Prevention, Chemopreventive Agent Development Research Group, National Cancer Institute, Rockville, MD; [3]VA Medical Center, Oklahoma City, OK.

Colorectal Cancer (CRC) is a major public health issue with an estimated 880,000 deaths annually, worldwide. Current trends show an increase of CRC incidence and mortality worldwide. Colonic adenomas are very common in ages 50 and over. Though, nonsteroidal antiinflammatory agents like Aspirin and Naproxen are useful to prevent the polyp progression and reduce CRC burden. Continuous/chronic usage of both drugs is limited by GI toxicity and unwanted side effects. Thus, the rationale to establish intermittent dosing regimens of Naproxen and Aspirin may provide efficacy without GI toxicity. Male F344 rats were used to establish Naproxen and Aspirin pharmacodynamic efficacy and dose-response effects. Rat (36 animals/group) colon cancers were induced by azoxymethane (AOM). At the adenoma stage, rats were fed diets containing Naproxen (200 and 400 ppm) or Aspirin (700 and 1,400 ppm) either continuously, 1 week on/1 week off, or 3 weeks on/3 weeks off, or Aspirin (2,800 ppm) 3 weeks on/3 weeks off. All rats were euthanized 48 weeks after AOM treatment and assessed for efficacy, dose-response effects and biomarkers in tumor tissues. Dietary administration of Naproxen and Aspirin did not show any overt toxicities. Administration of 200 and 400 ppm of Naproxen inhibited colon adenocarcinoma multiplied by 54.5% and 70.5% ($p<0.0001$) (continuous treatment); 53.3% and 68.4% ($p<0.0001$) (1 week on/1 week off); and 22.5% ($p<0.03$) and 61.5% ($p<0.0001$) (3 weeks on/3 weeks off), respectively. Importantly, inhibition of invasive colon carcinoma was reduced by 53% ($p<0.0009$) - >88% ($p<0.0001$) with different treatment regimens of Naproxen. Based on the biomarkers of proliferation and apoptosis, both agents showed significant modulation of proliferative (PCNA, p21) and apoptotic markers (p53, Casp3) in colonic tumors. Transcriptomic data revealed that proinflammatory cytokines, particularly interleukins and metalloproteases, were significantly reduced in tumors of rats exposed to Aspirin and Naproxen. Additional approaches tested, including targeting COX/5-LOX by Licofelone, showed superior efficacy compared to COX-2 inhibitor (celecoxib) alone in suppressing adenoma progression to adenocarcinoma. An additional safer approach tested targeting mPGES-1/5-LOX by LFA-9 which would provide sparing of PGI_2 and suppression of colonic adenocarcinoma in both mice and rat colon cancer models. Overall, our results suggest that intermittent dosing with Naproxen or Aspirin; targeting COX/5-LOX and mPGES-1/5-LOX are considered to be safer approaches to colon adenoma progression adenocarcinoma. {This work was supported by NCI-N01-CN-250026 and R01 CA213987}

A dual AKT/PDPK1 inhibitor for actinic keratosis and skin cancer prevention in immunocompromised individuals

Emmanuelle J Meuillet, Garth Powis and Lynn D. Kirkpatrick

Phusis Therapeutics, CA

Epicutaneous (topical) therapy with molecularly targeted agents is an attractive modality for treatment of basal cell carcinoma (BCC), early UVB induced squamous cell carcinoma (SCC) and cutaneous metastatic disease (CMD) associated with metastatic breast cancer and Actinic Keratoses, especially in immunocompromised subjects (organ transplants, HIV, other cancers etc.) . There is evidence that Akt and PDKP1 play complimentary yet independent roles in PI3K pathway signaling associated with driving the growth of BCC, UVB induced SCC, and breast CMD. Both Akt and PDPK1 possess a pleckstrin homology (PH) domain, a highly conserved three-dimensional superfold with a high affinity for binding phosphatidylinositol-3-phosphates, causing Akt and PDPK1 to translocation to the plasma membrane where Akt is activated. Through reiterative molecular docking and structure refinement using a proprietary computational platform, we have identified PHT-427 as an agent that binds to the PH domains of Akt and PDPK1, inhibiting their activity. PHT-427 has antitumor activity when administered orally but importantly also following epicutaneous administration. We evaluated the antitumor potential of topical PHT-427 against CMD in an intradermal breast xenograft model and in an early UVB induced skin cancer model in mice. We show promising effects of topical PHT-427 in cancers with skin involvement. Thus, topical application of PHT-427 can deliver active drug to skin and tumor, inhibiting AKT and PDPK1, both of which drive the PI3K pathway important in UVB induced SCC, breast CMD, and AKT in immunocompromised subjects, with significant inhibition of tumor growth without adverse effects on normal skin.

Optimization of erlotinib plus sulindac dosing regimens for intestinal cancer prevention in an Apc-mutant model of Familial Adenomatous Polyposis (FAP)

Ahmet M. Ulusan[1], Praveen Rajendran[1], W. Mohaiza Dashwood[1], Omer F. Yavuz[1], Sabeeta Kapoor[1], Trace Gustafson[1], Michelle I. Savage[2], Powel H. Brown[2], Shizuko Sei[3], Altaf Mohammed[3], Eduardo Vilar[2], Roderick H. Dashwood[1,2]

[1]Center for Epigenetics & Disease Prevention, Texas A&M Health Science Center, Houston, TX;

[2]Department of Clinical Cancer Prevention, The University of Texas MD Anderson Cancer Center, Houston, TX; [3]Chemopreventive Agent Development Research Group, Division of Cancer Prevention, National Cancer Institute, Rockville, MD

A randomized clinical trial in Familial Adenomatous Polyposis (FAP) patients demonstrated that sulindac plus erlotinib (SUL+ERL) had good efficacy in the duodenum and colon; however, toxicity issues raised concerns for long-term prevention. We performed a short-term biomarker study in the polyposis in rat colon (Pirc) model, observing phosphorylated extracellular signal-related kinase (pErk) inhibition in colon polyps for up to 10 days after discontinuing ERL+SUL administration. In a follow-up study lasting 16 weeks, significant reduction of colon and small intestine tumor burden was detected, especially in rats given 250 ppm SUL in the diet plus once-a-week intragastric dosing of ERL at 21 or 42 mg/kg body weight (BW). A long-term study further demonstrated antitumor efficacy in the colon and small intestine at 52 weeks, when 250 ppm SUL was combined with once-a-week intragastric administration of ERL at 10, 21 or 42 mg/kg BW. Tumor-associated *matrix metalloproteinase-7* (*Mmp7*), *tumor necrosis factor* (*Tnf*) and *early growth response 1* (*Egr1*) were decreased at 16 weeks by ERL+SUL, and this was sustained in the long-term study for *Mmp7* and *Tnf*. The optimal dose combination of ERL 10 mg/kg BW plus 250 ppm SUL lacked toxicity, normalized hematocrit/organ weights, inhibited tumor-associated molecular biomarkers, and exhibited effective antitumor activity. We conclude that switching from continuous to once-per-week ERL, given at one-quarter of the current therapeutic dose, will exert good efficacy with standard of care SUL against adenomatous polyps in the colon and in the small intestine, with clinical relevance for FAP patients before or after colectomy.

SESSION 4

Frameshift neoantigen vaccination prevent Lynch syndrome mouse model intestinal cancer

Özkan Gelincik[4] , Matthias Kloor[1], Mine Özcan[1], Aysel Ahadova[1], Yan Yuan[2], Hamza Ibrahim[4], Shizuko Sei[3], Robert Shoemaker[3], Johannes Gebert[1], Magnus von Knebel Doeberitz[1] , Steven M Lipkin[4]

[1]Department of Applied Tumour Biology, Institute of Pathology, University of Heidelberg, Heidelberg, Germany and Collaboration Unit Applied Tumor Biology, German Cancer Research Center (DKFZ), Heidelberg, Germany, [2] Structural and Computational Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany, [3] Division of Cancer Prevention, National Cancer Institute, Maryland, USA, [4] Weill Cornell Medical College, New York, USA

Microsatellite-unstable (MSI) cancers occurring in the context of Lynch syndrome elicit pronounced tumor-specific immune responses directed against frameshift peptide (FSP) neoantigens, which result from mismatch repair (MMR) deficiency-induced insertion/deletion mutations in coding microsatellites (cMS). We have recently completed a clinical phase I/IIa trial that successfully demonstrated safety and immunogenicity of an FSP neoantigen-based vaccine in MSI colorectal cancer patients (Clinical trial number: NCT01461148). The vaccine was safe and induced robust cellular and humoral immune responses in all vaccinated patients. To further develop a cancer preventive vaccine against MSI cancers in Lynch syndrome, we aimed to establish a preclinical mouse model. A systematic database search was performed to identify cMS sequences in the murine genome. Subsequently, intestinal tumors obtained from Lynch syndrome mice (Msh2flox/flox VpC+/+) were evaluated for mutations affecting these candidate microsatellites. Thirteen candidate cMS were detected that presented with a mutation frequency of 15% or higher. Epitope prediction using the netMHC4.0 algorithm was performed, and ten most promising FSP neoantigens were synthesized. Immunogenicity was evaluated after vaccination of C57BL/6 mice using IFN-gamma ELISpot. Four FSP neoantigens derived from cMS mutations in the genes Nacad, Maz, Xirp1, and Senp6 elicited strong antigen-specific cellular immune responses. CD4-specific T cell responses were detected for Maz, Nacad, and Senp6 and CD8-positive T cells were detected for Xirp1 and Nacad. Vaccination with peptides encoding these four intestinal cancer FSP neoantigens promoted anti-neoantigen immunity, reduced intestinal tumorigenicity and prolonged overall survival ($P<0.01$). Additionally, NSAIDs, which have chemopreventive efficacy for Lynch syndrome, increase T cell immunity against neoantigens. Mechanistic tumor mutation burden and adaptive immune response studies will be shown. In summary, these data support the further development of vaccination strategies for preventing cancers associated with Lynch syndrome.

Randomized, Double-Blind, Placebo-Controlled Trial of MUC1 Vaccine in Patients with Newly Diagnosed Advanced Adenoma of the Colon

Robert E. Schoen[1], Lisa A. Boardman[2], Marcia Cruz-Correa[3], Ajay Bansal[4], David Kastenberg[5], Chin Hur[6], Lynda Dzubinski[1], Sharon F. Kaufman[2], Luz M. Rodriguez[7], Ellen Richmond[7], Asad Umar[7], Eva Szabo[7], Andres Salazar[8], John McKolanis[9], Pamela Beatty[9], Reet Pai[10], Ryan McMurray[2], Nathan R. Foster[2], David M. Zahrieh[2], Paul J. Limburg[2], Olivera J. Finn[9]

[1]Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh, Pittsburgh, PA; [2]Mayo Clinic Cancer Center, Rochester, MN; [3]University of Puerto Rico, San Juan, PR; [4]Kansas City VA Hospital, Kansas City, KS; [5]Thomas Jefferson University, Philadelphia, PA; [6]Massachusetts General Hospital, Boston, MA (now at Columbia University, NYC); [7]Division of Cancer Prevention, National Cancer Institute, Bethesda MD; [8]Oncovir, Inc., Washington, DC; [9]Department of Immunology, University of Pittsburgh, Pittsburgh, PA; [10]Department of Pathology, University of Pittsburgh, Pittsburgh, PA

Background: Immunoprevention via targeting antigens aberrantly expressed on colorectal cancer (CRC) and its precursor, adenomas, could provide a less invasive approach to preventing CRC than repeated endoscopic surveillance. **Methods**: We performed a double blind, randomized trial of a 100aa peptide MUC1 vaccine admixed with an adjuvant TLR-3 agonist, polyICLC, vs. placebo in individuals with newly diagnosed advanced adenomas. Immunogenicity, measured by anti-MUC1 IgG at 12 weeks and immune memory at 1 year, and adenoma recurrence were assessed. Following vaccine or placebo administration at 0, 2, 10 and 52 weeks, an anti-MUC1 IgG ratio of ≥2.0 at 12 weeks (week 12/week 0) and at 55 weeks (week 55/week52) was defined as a positive immune response. Adenoma recurrence was determined at colonoscopy ≥1 year from initial vaccination. A pre-specified secondary outcome examined adenoma recurrence in immune responders. **Results**: 103 subjects were randomized at 6 centers. 102 (52 MUC1 and 50 placebo) completed the protocol to week 12, 95 (51 MUC1 and 44 placebo) completed testing for immune memory at 1 year, and 95 (48 MUC1 and 47 placebo) had an endpoint colonoscopy. Subjects had a mean age of 59.4±7.0 years, 62.1% were male, 88.3% were white, and 18.4% Hispanic, with no significant difference by arm. 13/52 (25.0%) MUC1 vaccine recipients had a week12/week0 ratio ≥2.0, (range 2.9-17.3) vs. 0/50 in the placebo group (1-sided Fisher's exact P<0.0001). 17/51 (33.3%) MUC1 vaccine recipients had a week 55/week 52 ratio ≥2.0 vs. 2/44 (4.5%) placebo (1-sided Fisher's exact P=0.0003). 11 of the 13 (84.6%) responders at week 12 responded at week 55/week 52 and were classified as immune responders. The mean time (SD) to follow up colonoscopy from initial vaccination was 886.1 days (248.9) for MUC1 vs. 923.0 (258.7) in the Placebo group (P=0.36). In those receiving MUC1 vaccine, adenoma recurrence was observed in 27/48 (56.3%) vs. 31/47 (66.0%) receiving placebo (P=0.22). In immune responders, adenoma recurrence was 3/11 (27.3%) vs. 51/78 (65.4%) in non-responders (p=0.02). Grade 1+ and Grade 2+ adverse events (AE) were more common in vaccine recipients: 51/53 (96.2%) vaccine vs. 39/50 (78.0%) placebo (P= 0.005) and 46/53 (86.8%) vaccine vs. 27/50 (54.0%) placebo (P=.0003), respectively, due primarily to injection site skin reactions. There was no difference in grade 3+ AE's: 11/53 (20.8%) vaccine vs. 10/50 (20.0%) placebo, (P=0.92). **Summary and Conclusion**: Only vaccine recipients developed a positive anti-MUC1 IgG immune response. MUC1 vaccine recipients had a reduced, but non-statistically significant 10% lower adenoma recurrence rate. In vaccine recipients who developed a positive anti-MUC1 IgG immune response, there was a statistically significant 38% reduction in adenoma recurrence. There was no significant toxicity to the MUC1 vaccine compared to placebo, other than increased injection site reactions. MUC1 vaccine is a promising approach to CRC prevention. Further efforts at optimizing the vaccine to maximize the immune response are planned.

Development of vaccines for broad protection against, and elimination of, HPV infection

Richard B.S. Roden

Department of Pathology, Johns Hopkins University, Baltimore, MD 21287 USA

HPV-associated cancer incidence is significantly elevated in cervical and at other sites in HIV+ patients. HIV+ patients acquire more frequent multi-type infections, including many genotypes infrequently seen in healthy individuals, and not targeted by the current HPV preventive vaccines. We previously developed a candidate therapeutic and preventive HPV vaccine, pNGVL4a-CRTE6E7L2 (CRTE6E7L2), which comprises a DNA vector encoding the heat shock protein calreticulin fused genetically with HPV16 E6 and E7 (that are obligately expressed in HPV malignancies) as well as the L2 capsid protein (a broadly protective antigen). We showed that fusion with calreticulin (CRT) profoundly enhances the potency of DNA vaccines in generating HPV antigen-specific CD8+ T cell mediated immune responses even in CD4-depleted animals. In addition, vaccination with the CRTE6E7L2 DNA vaccine induces both L2-specific neutralizing antibodies and protection from experimental vaginal challenge. These features make the CRTE6E7L2 DNA vaccine particularly promising for use in HIV+ patients, a challenging group to treat, and to prevent multiple types of HPV infections. With these data we produced the DNA for clinical study and performed GLP preclinical tresting with the support of the NCI PREVENT program. Although DNA vaccines are relatively safe and well suited for multiple administrations, they generally exhibit suboptimal immunogenicity when administered by conventional intramuscular needle injection, likely reflecting inefficient host cell transduction. We have previously shown that electroporation is a much more effective DNA vaccine administration method to generate HPV-specific CD8+ T cell immune responses as compared to conventional intramuscular injection or epidermal delivery via gene gun. Thus, the goal of our recently opened NCI SPORE-funded Phase I clinical study (NCT04131413) is to use the Ichor TriGrid™ Delivery System Electroporation Device, which has been used in multiple clinical trials, for intramuscular administration of the CRTE6E7L2 DNA vaccine at escalating doses in both HIV– and HIV+ patients with HPV16-associated high-grade cervical intraepithelial neoplasia grades 2 and 3 (CIN2/3), and to examine the safety, virologic, and disease outcomes. This study will (1) evaluate the safety and toxicity of CRTE6E7L2 administered via electroporation in HIV– and HIV+ patients with HPV16+ CIN2/3; (2) characterize the HPV16 E6/E7-specific cell-mediated and humoral immune responses in HIV– and HIV+ patients with HPV16+ CIN2/3 vaccinated with CRTE6E7L2 via electroporation; (3) characterize L2-specific humoral immune responses in HIV– and HIV+ patients with HPV16-associated CIN2/3 upon vaccination with CRTE6E7L2 DNA vaccine via electroporation; and (4) determine the HPV load and histopathological changes in the lesion and its microenvironment in HIV– and HIV+ patients with HPV16-associated CIN2/3 upon treatment with CRTE6E7L2 DNA vaccine via electroporation. In sum, our studies aim to provide a new immunotherapy for the treatment of HPV-associated high-grade squamous intraepithelial lesions in both HIV– and HIV+ patients that also broadly prevents acquisition of new HPV infections.

Development of a Kras Preventive Vaccine

Bryon D. Johnson

Department of Microbiology & Immunology, Medical College of Wisconsin, Milwaukee, WI

Lung cancer continues to be a major cause of cancer mortality worldwide, and KRAS mutations can occur in up to one third of lung adenocarcinomas. To date, targeted therapies to mutant KRAS have been challenging. Ongoing studies are examining whether vaccines can be used to target mutant KRAS by mobilizing the immune system. Unfortunately, treatment of active malignancies poses many challenges to vaccines including the potent immune suppression that typically occurs. Due to these challenges, using vaccines to prevent the development of cancer could prove to be more efficacious. We developed a multi-peptide KRAS vaccine formulated with four peptides that had 100% homology between human and mouse KRAS. The vaccine was designed using MHC Class II binding algorithms, and peptides were screened for promiscuity across multiple MHC alleles. Using an inducible KRAS mouse model, we demonstrated that the multi-peptide vaccine was able to significantly reduce tumor burden when administered prior to induction of mutant KRAS expression. Anti-tumor efficacy correlated with induction of a T cell immune response to the individual KRAS peptides. More recent data from our laboratory suggests that combining the KRAS vaccine with other immune-modulating therapies could further improve vaccine efficacy. With its direct translatability to humans, our preclinical data makes a compelling case for testing the multi-peptide KRAS vaccine in clinical trials as an immune-preventive agent for lung cancer.

SESSION 5

Chemoprevention for Pancreatic Cancer

Peter J. Allen, Department of Surgical Oncology, Duke University, Durham, NC

Pancreatic cancer is essentially incurable. This outcome is secondary to the late stage at diagnosis, and the ineffectiveness of current systemic therapies. Even when patients are diagnosed prior to the development of identifiable metastatic disease (as occurs in <15% of all pancreatic cancer patients) and are treated with resection and adjuvant systemic therapy, the probability of long-term survival is quite low. These patients, with the earliest lesions that can be identified by either cross-sectional imaging or endoscopy, experience dismal five-year survival rates of between 10-25%. These survival outcomes suggest that we are currently unable to identify pancreatic cancer at a curable stage**.**

For these reasons, our group has focused on understanding and managing high-risk precursor lesions of pancreatic cancer. Intraductal papillary mucinous neoplasms (IPMN) of the pancreas represent the only radiographically identifiable precursor lesion of pancreatic cancer. This pathway of progression to pancreatic cancer is believed to represent between 20–30% of pancreatic cancer and currently represents the only identifiable lesion for which intervention can lead to cure. Our multi-institutional research group has focused on three broad areas of IPMN research: 1. Serum and cyst fluid diagnostic markers (mutational profiling, fluid protein markers); 2. Serum and cyst fluid markers of malignant progression (cell surface proteins, cyst fluid inflammatory proteins); and 3. Clinical and radiographic modeling of high-risk disease. Based on our findings, we believe that IPMN represents an ideal opportunity for the evaluation of chemoprevention strategies in pancreatic cancer.

IPMN is considered a “whole-gland” process. It is known that patients who undergo partial pancreatectomy for IPMN have an increased risk of developing cancer in the pancreatic remnant. In addition, patients who are found to have high-grade dysplasia (high-risk IPMN) following partial pancreatectomy are likely to have high-grade dysplasia in the remnant pancreas. These patients represent a very high-risk group for progression to pancreatic cancer. We have shown that 7–15% of these patients will develop pancreatic cancer in their pancreatic remnant over a 4–5 year time period. In addition, we have recently found that up to 25% of these patients will develop radiographic signs of progression over the same time period. Between our four institutions, we are currently monitoring over 450 patients who have undergone partial pancreatectomy for non-invasive IPMN. These patients are ideally suited for a chemoprevention trial.

Anti-inflammatory agents have been extensively studied as chemoprevention agents. The non-steroidal anti-inflammatory (NSAID) agent sulindac has been demonstrated to be effective at causing polyp regression in patients with familial adenomatous polyposis. The exact mechanism of its NSAID properties is unknown, but it is thought to act on both COX-1 and COX-2 enzymes, inhibiting prostaglandin synthesis. Pre-clinical models of pancreatic cancer have also found these agents to be effective at decreasing progression to pancreatic cancer[10-14]. Data generated within our group has found a distinct association between inflammation and IPMN progression, and thus we believe that anti-inflammatory strategies are applicable and promising for prevention of progression in IPMN patients. Indeed, there is preliminary human evidence that sulindac may have efficacy in preventing radiographic progression in IPMN. Because of these findings, our group has just launched the first randomized placebo controlled trial to evaluate the ability of sulindac to prevent progression in patients with IPMN.

Local transdermal drug delivery to the breast for cancer prevention

Seema A. Khan

Department of Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL

Poor acceptance of oral endocrine therapy for breast cancer prevention necessitates the development of alternative approaches. The reluctance of eligible high-risk women to accept oral endocrine agents is based, to a large extent, on concerns about systemic toxicity. Any proposed alternatives must address these concerns. One such alternative is the use of transdermal formulations of effective drugs, such as 4-hydroxytamoxifen (4-OHT) an active metabolite of oral tamoxifen. This has been shown to penetrate breast skin, with good retention in the breast and low systemic exposure; and to be as effective as oral tamoxifen in reducing cell proliferation of invasive and in-situ malignancy. However, several questions remain regarding the distribution of drug through the breast, the kinetics of clearance though the breast, and optimal frequency of dosing. Our recent data show that orally and transdermally administered drug is distributed similarly though the breast, but further optimization of doses, schedules, and transdermal formulations is needed. Ongoing randomized Phase II trials using 4-OHT gel will add weight to existing data, and position us to develop a Phase III trial of this agent, comparing it to oral endocrine therapy of choice, with the major endpoints being reduction in risk of new breast events, and reduction in breast density. Other agents are also under development, notably topical endoxifen, which may be more effective than topical 4-OHT. And topical bexarotene, which is already in use for cutaneous T-cell lymphoma, but safety and efficacy in the breast remains to be demonstrated. Other possibilities include topical NSAIDs (diclofenac is already available and approved for analgesia) and progesterone receptor antagonists, as well as small molecules such as lapatinib. For these, it may be necessary to involve nanoscience approaches, which are the next step in transdermal delivery development.

Novel Strategies for Aspirin Prevention of Colorectal Cancer

Andrew T. Chan

Massachusetts General Hospital and Harvard Medical School., Boston, MA

Remarkably consistent experimental and epidemiologic evidence demonstrates that aspirin is associated with a lower risk of colorectal cancer. In 2016, the U.S. Preventive Services Task Force updated its primary prevention guidelines to recommend low-dose aspirin (81 mg/day) for chronic disease prophylaxis, including colorectal cancer prevention, among U.S. adults between ages 50-59, and possibly ages 60-69, with a greater than 10% ten-year risk of cardiovascular events. Despite this advance, there remains uncertainty about aspirin's risk-benefit profile in many populations, especially older adults. Our group has led several studies into the mechanistic basis of aspirin's anti-cancer effect that culminated in the successful completion of the NCI-supported ASPirin for the REDuction of Colorectal Cancer Risk (ASPIRED) randomized placebo-controlled trial (RCT) which showed that aspirin reduces urinary prostaglandin metabolites, a validated biomarker of colorectal neoplasia (Drew *et al*…Chan, *Can Prev Res* 2020). We have also led an analysis of the Aspirin to Prevent Events in the Elderly (ASPREE), an RCT of aspirin in 19,114 apparently healthy older adults aged 65+. Surprisingly, aspirin was associated with *increased* all-cause mortality that was driven primarily by cancer deaths that was *not* accompanied by an increase in cancer incidence over 4.7 years (McNeil *et al*…Chan, *JNCI* 2020). These results underscore our limited understanding of the influence of age on the biology of cancer and the context-dependent mechanisms by which aspirin may influence initiation, growth, and spread of cancer. Further investigation into potential age-dependent mechanisms of aspirin may lead to mechanistic biomarkers to improve risk-stratification for chemoprevention.