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POSTER ABSTRACTS
Logistical Issues in Implementing a Clinical Trial on Oral Cancer Prevention Through HPV Vaccination: Implementation of Ulacnet201 in Mexico

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This poster describes logistical issues related to implementing a randomized, double-blinded, placebo-controlled Phase III interventional trial on the nine-valent HPV vaccine (9vHPV) among cisgender men and transgender women living with HIV, at the Mexico site. The trial seeks to demonstrate that 9vHPV reduces the incidence of persistent oral HPV infection (a surrogate for HPV-associated oropharyngeal cancer) with the 9 vaccine types. Five-hundred participants will be randomized in a 1:1 allocation to receive 9vHPV or placebo, stratified based on clinical site (Brazil, Mexico, Puerto Rico) and age.

The team invites potential participants through local community organizations and public HIV clinics. People may be invited when waiting in line in the morning to get laboratory testing done, when they have an appointment for HIV care or through their treating physician or a community organizer. Initially, we worked at a single HIV clinic, although we did distribute study flyers to treating physicians at other clinics. As of mid-2022 we began enrolling at two additional clinics.

Participants are prescreened when initially invited or by phone to prevent unnecessary trips for those ineligible. Once prescreened, participants are given an appointment for their first study visit; reminders about their first or other study visits are sent by text message 2-3 times before the appointment. Participants are provided with financial compensation in cash at each visit.

We have implemented both study-wide mechanisms and additional locally-designed strategies and forms to guarantee quality control. For example, registering participant issues, study agent trail and persons invited, pre-screened and enrolled (including reasons for exclusion). Data is registered on paper forms and in a bespoke data base program (DatStat, designed at Moffitt Cancer Center). DatStat carries out the randomization (only the Mexico site pharmacist is unblinded) and requires a wireless internet connection, which can sometimes fail even though a router is installed by the study at each clinic.

Vaccine and syringe importation can be time consuming and cause enrollment delays, given the need to acquire permissions for importation or problems getting the shipment out of Customs. Making sure shipments go through an airport with better functioning Customs offices is also important.
Identification of a Potential Chemoprevention Agent for \textit{BRCA1}-mutated Cancers

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\textit{BRCA1} mutation carriers are predisposed to developing aggressive breast, ovarian, and other cancers at a relatively early age. Unfortunately, these cancers lack an effective chemoprevention option. We have found that cells containing mutant \textit{BRCA1} harbor defective repair of oxidative DNA damage, an early trigger to tumorigenesis. Therefore, we sought to target this defect and discover a novel chemoprevention agent for these cancers. High-throughput screening of a chemical library identified a novel class of small-molecules that enhance repair of oxidative DNA damage, which we termed DNA repair-activating agents. One of these agents, benserazide, increased base-excision repair of oxidative DNA damage utilizing a cell-based DNA repair assay, decreased levels of 8-oxo-g (i.e. most common oxidized lesion in human genome) using flow cytometric analysis, and decreased levels of oxidative DNA damage as determined by alkaline comet assay modified for detection of oxidized DNA lesions. These results were observed in mutant but not wild-type \textit{BRCA1} breast cancer cells. Benserazide, but not tamoxifen (current FDA-approved breast cancer chemoprevention agent), also decreased \textit{in vitro} tumorigenesis of mutant \textit{BRCA1} or \textit{BRCA1}-depleted breast cancer cells according to the soft-agar colony formation assay, and delayed tumor formation \textit{in vivo} using a xenograft mouse model. Lastly, benserazide led to elevated levels of 8-oxo-dG (a by-product of base-excision repair) in conditioned media of mutant \textit{BRCA1} breast cancer cells, and concurrently increased serum levels of 8oxodG and decreased tumor burden in mice. Taken together, benserazide is a DNA-repair activating agent that enhances repair of oxidative DNA damage and decreases tumorigenesis \textit{in vitro} and \textit{in vivo}, and thus may serve as a potential chemoprevention agent for \textit{BRCA1}-mutated cancers. Further, circulating levels of 8oxoG/8oxodG may function as a predictive biomarker for the efficacy of benserazide, which would prove useful in the clinical evaluation of benserazide for cancer chemoprevention.
Chemoprevention of Head and Neck Cancers by the Combination of Epigallocatechin Gallate (EGCG) and Resveratrol.

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Head and neck cancer (HNC) is a devastating disease and the 6\textsuperscript{th} most common cancer worldwide. Most HNC patients are diagnosed with advanced stage disease for which the 5-year survival is below 50\%, stressing the need for chemoprevention. Recently, we have reported that the combination of resveratrol and EGCG induces synergistic apoptosis and inhibits xenografted HNC growth by inhibiting AKT-mTOR pathway. In this study, we have investigated the chemopreventive efficacy of resveratrol, EGCG and their combination using 4NQO-induced oral carcinogenesis model. C57BL/6 mice were exposed to 4-NQO (50 μg/ml) via drinking water for 10 weeks, followed by treatment with vehicle (50% sweetened condense milk), resveratrol (30 mg/kg), EGCG (30 mg/kg) and their combination for 8 weeks, 5 days/week. The mice were sacrificed on week 24 and the number of visible and microscopic lesions were counted. Resveratrol alone and in combination with EGCG significantly inhibited the number of visible lesions whereas the number of microscopic lesions and lesion area were significantly inhibited only in the combination group. Furthermore, RNASeq and qPCR analysis using a HNC cell line identified GDF15, ATF3, p21, p27 and Bim as significantly upregulated genes with GDF15 being the most upregulated one. Expression of GDF15 and ATF3 proteins were confirmed by western blotting. Taken together, our data strongly demonstrate the chemopreventive potential of the combination of EGCG and resveratrol and paves the way for further clinical developments. (Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)
Identification of Lipid Pharmacodynamic Biomarkers in FAP model.

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Familial Adenomatous Polyposis (FAP) is a genetic disorder characterized by the development of numerous polyps in the colon, that unless removed by colectomy, inevitably progress to colorectal cancer (CRC). Although there is currently no FDA-approved treatment to delay colectomy, a common target for chemoprevention is cyclooxygenase-2 (COX-2). Non-steroidal anti-inflammatory drugs (NSAIDs) and omega-3 fatty acids are known to alter COX metabolism and affect downstream eicosanoids involved in CRC progression. In this study, we examined the chemopreventive efficacy of the NSAID naproxen alone, or in combination with TP-252, a novel, chemically-stable analog of the omega-3 fatty acid, eicosapentaenoic acid (EPA), in the intestinal tumorigenesis Apc\textsuperscript{am1137} PIRC rat model. Over the course of this 20-week study, rats were fed a modified AIN-93G diet supplemented with 200 ppm naproxen, alone or in combination with TP-252 (1.5%, or 3% by weight). Rats treated with 200 ppm naproxen had 66% (p < 0.0001) fewer colon tumors than controls (AIN-93G) and an 81% (p < 0.0001) reduction in tumor size. However, when Naproxen was combined with high-dose TP-252 (3%), tumor number and size were reduced by 95% (p < 0.0001) and 98% (p < 0.0001) respectively, when compared to controls. Since the primary mechanisms of action for naproxen and EPA target COX metabolic activity, we performed a comprehensive lipidomic analysis on colon tissue and plasma to determine lipidomic changes associated with drug treatment. Increased formation of several EPA-derived eicosanoids, including 9-HEPE, 12-HEPE, 18-HEPE, and resolvin E2, whose mucosal levels correlated with both reduced tumor number and volume, were observed. These metabolites were also concomitantly elevated in the plasma of treated animals, representing potential pharmacodynamic (PD) biomarkers of CRC prevention. Future studies will decipher potential mechanisms that underlie these EPA-derived PD biomarkers and their role in tumor suppression. (Supported by the NCI Prevent Program 75N91019D00019, Task Order 75N91019F00132).
Shared Gene Expression and Immune Pathway Changes Associated with Progression from Nevi to Melanoma

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There is a need to identify biomarkers of melanoma progression to assist the development of chemoprevention strategies to lower melanoma incidence. In this study, we assessed the feasibility of creating a molecular signature for melanomagenesis using three publicly available RNA sequencing and microarray expression datasets. We performed differential expression and regularized regression analyses across nevi and melanoma samples to identify consistent genes associated with melanomagenesis. The regularized regression models demonstrated that a small number of genes could successfully distinguish between nevi and melanoma, providing evidence for the feasibility of creating a molecular signature. Differential expression analysis identified consistent upregulation of C1QB, CXCL9, CXCL10, DFNA5 (GSDME), FCGR1B, and PRAME in melanoma and consistent downregulation of SCGB1D2 in melanoma compared to nevi. Additionally, each of these genes demonstrated a linear association with the progression from benign nevi to dysplastic nevi, to radial growth phase melanoma to vertical growth phase melanoma, providing additional evidence for their role in melanomagenesis. Subsequent pathway analysis demonstrated significant enrichment of immune-related pathways among the differentially expressed genes. Overall, this study 1) demonstrates the feasibility of creating a gene signature for melanomagenesis and 2) highlights genes and pathways of interest for melanoma progression. We are in the process of generating a new dataset with benign nevi, dysplastic nevi, and melanoma with which to build and validate a molecular signature of melanoma.
ASSESSING IMMUNOPREVENTION EFFICACY OF AN AGONIST OF CD137 PATHWAY IN A TOBACCO CARCINOGEN-INDUCED LUNG CANCER PRECLINICAL MODEL

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Purpose: Lung cancer is the leading cause of cancer-related deaths worldwide. Cigarette smoking is the primary risk factor for lung cancer and is linked to 80-90% of lung cancer deaths according to the Centers for Disease Control (CDC). The most carcinogenic component of tobacco smoke is NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone). SA-4-1BBL is a novel CD137 receptor agonist that has shown cancer immunoprevention efficacy in transplantable preclinical tumor models by invoking an innate immune surveillance mechanism involving CD4+CD44+ T and NK cells. As a preclinical model for high-risk populations, we evaluated the immunoprevention efficacy of SA-4-1BBL in lung tumor formation induced by NNK.

Experimental Procedures: A/J female mice were used as a preclinical model for lung cancer immunoprevention due to their susceptibility to tobacco-carcinogen-induced lung cancer. A/J mice were i.p. injected with NNK weekly for eight consecutive treatments. The mice were also treated s.c. with 100 µg SA-4-1BBL twice, two weeks apart on weeks 6 and 8 of NNK treatment. Animals were monitored for 21 weeks and euthanized to harvest the lung and lung-draining lymph nodes for histology (H&E staining and PCNA immunofluorescence staining). In separate groups, mice were treated with a depleting antibody to CD4 twice two weeks apart on weeks 6 and 8 of NNK treatment to investigate the role of CD4+ immune cells in cancer immunoprevention.

Results: NNK treatment resulted in lung tumor formation as assessed by PCNA immunofluorescence staining in all saline-injected control mice by the 21-week experimental endpoint. Treatment with SA-4-1BBL resulted in significantly fewer macroscopic and microscopic tumor numbers than the control group (p < 0.01). Animals treated with SA-4-1BBL and subjected to CD4+ cell depletion showed similar numbers of macroscopic and microscopic lung tumors to the vehicle group.

Conclusions: Prophylactic treatment with SA-4-1BBL significantly prevents NNK-induced lung tumor formation and CD4+ immune cells play a critical role in the observed immunoprevention efficacy of SA-4-1BBL.

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AI-driven image-based microbiome analysis reveals correlation of higher pre-treatment microbiome level with complete response in QUILT 3032 trial patients

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The human microbiome, both systemic and tumor microenvironment (TME)-associated, exerts a profound influence on cancer development, progression, and response to treatment. To facilitate microbiome analysis in cancer, we developed an AI-driven computational pathology system that determines relative microbiome levels in H&E-stained slides from formalin-fixed paraffin-embedded tissue specimens.

We utilized available whole-genome and whole-transcriptome sequencing data for microbiome detection in TCGA bladder cancer [Poore et al. 2020; Nature 579], for which images were available, to define microbiome-low and -high labels. The TCGA bladder cancer cohort (n = 408) was distributed into training (66.7%, n = 272), validation (8.3%, n = 34) and test (25.0%, n = 102) sets; each with equal numbers of microbiome-low and -high patients. The cohort featured an average of 1.05 diagnostic (DX) images per patient, with 429 DX images in total.

We trained a deep network using a total of 303,104 patches (of size 100 x 100 microns, equivalent to 400 x 400 pixels at 40X magnification) randomly selected from 296 DX images in the training set. Testing on untouched patient data (n = 102) gave area under the ROC curve of 0.74 and F1 score of 0.74.

The developed system was then used to determine correlations between TCGA bladder cancer patient survival and microbiome level. Treatment-naïve bladder cancer patients identified as microbiome-low by our image-based system had higher survival rates, with hazard ratio of 1.45.

Further, we assessed correlations between pathologic complete response (pCR) at 12 or 27 weeks and microbiome level in non-muscle invasive bladder cancer patients in ImmunityBio, Inc.’s QUILT-3.032 trial. Patients that achieved a pCR with treatment were found to have significantly higher probability of microbiome-high pre-treatment, as compared to those who did not achieve a pCR. Reductions in predicted microbiome levels after treatment were also observed in pCR patients. Conversely, 6 of 7 non-responders were found to have an increase in microbiome-high probability with treatment.

These findings suggest our novel AI-driven image-based computational pathology system has the potential to provide data that may not only inform clinical decision-making, but also allow further investigation into the role of the TME microbiome in cancer.
Effects of commonly prescribed β-blockers on tobacco carcinogen induced reactive oxygen species in human non-tumorigenic lung epithelial cells

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Lung cancer is a detrimental disease to humans and tobacco smoking causes more than 80% of lung cancer deaths. Chemoprevention is currently the major strategy to prevent smoking related lung cancer deaths. Key components that contribute to tobacco carcinogenicity are benzo(a)pyrene [B(a)P] and its diol epoxide metabolite BPDE. The goal of this study is to evaluate the level of reactive oxygen species (ROS), a mediator for cellular DNA damage leading to cancer, when pre-treated with the β-blockers carvedilol and nebivolol, and the antioxidant resveratrol.

B(a)P and BPDE were used to induce ROS in human lung epithelial cells (BEAS-2B). The non-fluorescent and non-polar 2’,7- dichlorofluorescein diacetate (DCFH-DA) probe was added to the cells. DCFH-DA was converted to DCFH in the cells by hydrolyzation of DCFH. ROS then induced the conversation of DCFH into 2,7-dichlorofluorescein (DCF). Florescence of DCF was measured by flow cytometry to detect ROS level.

B(a)P at concentrations 10 and 100 μM failed to induce ROS while BPDE successfully induced ROS dose-dependently during our pilot study. As a result, different concentrations of BPDE(2 μM and 4 μM) were used to induce ROS. When BEAS-2B was pre-treated with resveratrol (50 μM), ROS was reduced by 42% compared to the cells treated with 2 μM BPDE alone. Additionally, there was a decrease in ROS when pre-treated with nebivolol and carvedilol (5 μM). Nebivolol reduced 52% and 64% of the ROS induced by BPDE 2 μM and 4 μM, respectively. Carvedilol reduced 31% and 10% of the ROS induced by BPDE 2 μM and 4 μM, respectively.

These results suggest that some commonly used anti-hypertensive agents, such as the β-blockers, may block or reduce tobacco carcinogen induced ROS and thus can be used as chemopreventive agents for lung cancer.
Chemopreventive Effects of Carvedilol and Nebivolol in Benzo(a)pyrene-induced Transformation of Non-tumorous Human Bronchial Epithelial Cell

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Lung cancer is among the most common cancers worldwide. The primary risk factor for the development of lung cancer is cigarette smoking and there are a large amount of studies has confirmed the association between smoking and lung cancer. Chemopreventive drug would be beneficial among patients who are in high risk due to chronic exposure to carcinogens. In the previous study of chemopreventive activity of carvedilol, it significantly delayed and reduced skin squamous cell carcinoma development. The aim of the present study was to explore the role of carvedilol and nebivolol in benzo(a)pyrene induced transformation of the non-tumorous human bronchial epithelial cells. To evaluate the effects of carvedilol and nebivolol on chemopreventive activity against BPDE-induced lung carcinogenesis, anchorage-independent growth assay in soft agar was used to determine the degree of malignant transformation of BEAS-2b cells. Before the investigation of carvedilol and nebivolol, the appropriate drug concentration aiming minimum cytotoxicity on normal BEAS-2b cells were determined by MTT assay. The MTT assay was used to measure cellular metabolic activity as an indicator of cytotoxicity. Carvedilol at 10 µM or lower and Nebivolol at 1 µM or lower did not cause significant cytotoxicity. In non-tumorigenic human bronchial epithelial cell culture BEAS-2B vitro assays, carvedilol and nebivolol exert a protective effect against BPDE-induced transformation at non-toxic concentrations in a dose dependent manner. As shown in the figure below, Nebivolol (1 µM and 0.5 µM) and Carvedilol (1 µM and 0.5 µM) both significantly attenuated colony formation compared with BPDE control. However, the anti-transformation mechanism is unknown, further studies of carvedilol and nebivolol are needed to investigate their chemoprevention activity.

![The effect of Carvedilol and Nebivolol on BPDE-induced malignant transformation of BEAS-2B cells](image)
Effect of IL-23 knockdown on obesity driven CRC in high-fat diet fed Apc\(^{\text{min/+}}\) mice

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Colorectal cancer (CRC) is the 3rd common cancer in the United States with an estimated 151,030 new cases and 52,580 deaths in 2022. Several studies suggest that western-style diet-induced obesity contributes to CRC promotion via systemic inflammatory mediators. Recently, we have reported significant elevation of circulating IL-23 levels in obese human subjects (BMI>30). Also, TCGA gene expression data indicated IL-23A over expression in colonic tumors (CT) that correlated with tumor stage, patient obesity, nodal metastasis, and poor survival rates. Importantly, we have shown that IL-23 knockdown in Apc\(^{\text{min/+}}\) mice led to significant suppression of the intestinal tumors strongly supporting our hypothesis that pro-inflammatory cytokine IL-23 could be a possible link between obesity and CRC. Here we explored the contribution of IL-23 in obesity induce CRC *in vivo* using high-fat diet (HFD) fed Apc\(^{\text{min/+}}\) mouse model.

For this study, Apc\(^{\text{min/+}}\) and IL-23 KO (IL-23A p19\(^{-/-}\) mice; MMMRC) were cross-bred to generate Apc\(^{\text{min/+}}\) mice with IL-23 normal (IL-23\(^{+/+}\)) or KO (IL-23\(^{-/-}\)) genotypes. Six-week old Apc\(^{\text{min/+}}\) mice (N≥15/gender) were grouped by IL-23 genotypes and fed HFD (60 Kcal) until termination. Mice were euthanized at 20 weeks age, intestines were harvested and evaluated for tumors incidence and multiplicity. In Apc\(^{\text{min/+}}\) mice, IL-23 deletion had significant suppressive effect on HFD driven CT and small intestinal tumors (SIT). IL-23\(^{-/-}\) Apc\(^{\text{min/+}}\) male mice had 50% less CT (0.81±0.25 vs 1.61±0.27; p<0.05) while female mice had 40% less CT (0.40±0.16 vs 0.94±0.21; p=0.05) compared to the IL-23\(^{+/+}\) Apc\(^{\text{min/+}}\) control mice. Similarly, CT incidence was also suppressed by 36% and 48% in male and female IL-23\(^{-/-}\) Apc\(^{\text{min/+}}\) mice respectively compared to control mice. SIT multiplicity was also inhibited by 48% in male (15.00±1.13 vs 28.54±1.11; p<0.0001) and by 41% in female (14.83±1.35 vs 25.18±1.71; p<0.0005) IL23\(^{-/-}\) Apc\(^{\text{min/+}}\) compared to control mice. Tumor data was complemented by significant reduction in markers of proliferation, immune evasion, circulating proinflammatory cytokine and chemokines (IL-1, IL-17, IL-23, IL-10, CCL-3, CCL-2, CCL-5, IFN\(\gamma\), TNF\(\alpha\), etc.) in IL-23 KO compared to control mice. Our data clearly indicates that IL-23 is a promising target for CRC prevention in high-risk obese individuals and warrants further investigation. (Supported in part by P30CA225520 and Kerley-Cade Endowed Chair)
Repurposing of the macrolide antibiotic clarithromycin for the prevention of lung cancer

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Lung adenocarcinoma (LUAD), particularly K-ras mutant LUAD, is a leading cause of cancer mortality. Therefore, strategies to prevent K-ras-mutant LUAD in its earliest stages in high-risk individuals (e.g., smokers) are urgently needed to reduce the public burden of this fatal disease. We and others have shown that K-ras driven tumorigenesis in lung is intimately linked to chronic inflammation and ultimately, tumor cells immune-escape. The antibiotic clarithromycin (CAM) was identified as one of the most promising candidates for repurposing with demonstrated immunomodulatory and anticancer properties. CAM is widely used and belongs to the macrolide class of antibiotics which are among the safest broad spectrum antimicrobials available. Abundant preclinical and clinical evidence exists demonstrating the in vitro and in vivo anticancer effects of CAM. It has been shown that macrolide antibiotics exert suppression of inflammation without overt immunosuppressive effects mostly through the inhibition of proinflammatory cytokines in vitro and in vivo. In these studies, we tested the lung cancer prevention efficacy of CAM using a Kras mutant lung cancer model. In the CCSPCre;LSL-Kras-G12D (CC-LR) model, activation of the KrasG12D mutation takes place in club cells by means of removal of the lox-stop-lox genomic sequence via expression of Cre recombinase under the control of the CCSP promoter. This model is excellent for reproducing the various premalignant to malignant progression steps in the lung. CC-LR mice of both genders were randomly enrolled to four experimental arms comparing three CAM doses: 10mg/kg/day, 50mg/kg/day, and 100mg/kg/day, to vehicle control (H2O). Treatment was administered by oral gavage, 5 times per week for 10 wks., starting at 4 wks. of age. At 14 wks. of age mice were euthanized, lung surface tumors were counted, bronchial lavage fluid, as well as lung samples, were obtained for histological, immunohistochemical (IHC) and qRT-PCR analyses. Clarithromycin treatment led to significant lung cancer prevention efficacy, as determined by lung surface tumor counts. A clear dose response with CAM was observed with a mean lung surface tumor count of 30.2 tumors per mouse for vehicle (n=12 mice), 23.5 for CAM 10mg/kg (n=8), 18.8 for CAM 50mg/kg (n=8) and 13.5 tumors per mouse for the CAM 100mg/kg treatment group (n=12, p=0.0014). A significant decrease in the incidence and multiplicity of premalignant and malignant lesions was also observed in histological analyses. For profiling of the lung immune microenvironment, we analyzed by qRT-PCR the expression of 18 genes identifying various cytokines, cell surface markers and proteins characteristic of specific activation states on the various cell subtypes in the tumor microenvironment. We found significant reduction in the expression of pro-inflammatory cytokines, IL-6, TNF, and IL-1β, that are known to have a pro-tumor function in lung tumorigenesis, shown previously by us and other groups. We also found a reduction in the expression of myeloid cell-specific immunosuppressive markers, Arg1 and Fizz 1, that could be due to the reduction in the Gr1+ myeloid cell population, which was detected by means of IHC analyses on the same samples, or due to reprogramming of pro-tumor M2 type macrophages toward an anti-tumor M1 phenotype. We also see a trend toward reduction of IL-17 cytokine, which we have previously shown to have an essential role in the promotion of K-ras mutant lung tumors. This was associated with an increase, although not significant, in the expression of anti-tumor Th1-specific transcription factor. However, we see an increase in the expression of PD-1, which could be due to the potential induction of an exhausted T cell phenotype. Taken together, we see a reprogramming of the lung microenvironment from a tumor-promoting immunosuppressive phenotype toward an anti-tumor phenotype. This work was supported by NCI PREVENT TORFP75N91019F00131 (TORFP 2019 E-05).
Metabolomics of acute vs. chronic spinach intake in an Apc-mutant genetic background: linoleate and butanoate metabolites targeting HDAC activity and IFN-γ signaling

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Abstract

There is growing interest in the crosstalk between the gut microbiome, metabolomic features, and disease pathogenesis. Colorectal cancer is a major health burden worldwide, linked in part to modifiable risk factors associated with diet and lifestyle (1). The differential roles of the metabolites in targeting Wnt/b-catenin signaling were recently reported (2). The current investigation compared long-term (26 week) and acute (3 day) dietary spinach intake in a genetic model of colorectal cancer. Metabolomic analyses in the polyposis in rat colon (Pirc) model and in wildtype animals corroborated key contributions to anticancer outcomes by spinach-derived linoleate bioactives and a butanoate metabolite linked to increased a-diversity of the gut microbiome (3). Combining linoleate and butanoate metabolites in human colon cancer cells revealed enhanced apoptosis and reduced cell viability, paralleling the apoptosis induction observed in colon tumors from rats given long-term spinach treatment. Mechanistic studies in cell-based assays and in vivo implicated the linoleate and butanoate metabolites in targeting histone deacetylase (HDAC) activity and the interferon-γ (IFN-γ) signaling axis. Clinical translation of the findings from this investigation to at-risk patients might provide valuable quality-of-life benefits by delaying surgical interventions and drug therapies with adverse side effects (4,5).

References

Targeting TAK1 for intercepting PanIN progression to ductal adenocarcinoma in an inducible KC mouse model

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Despite advances in our knowledge of human pancreatic ductal adenocarcinoma (PDAC), targeted therapies have not yet significantly translated to an improved overall survival for patients. Pancreatic tumor microenvironment enriched by infiltrating immune cells and consisting of dense fibrotic stroma is characterized by desmoplasia, the major contributors of tumor-associated inflammation. Transforming growth factor-β (TGF-β) activated kinase 1 (TAK1) is widely accepted as a key player in the TNF-α and TGF-β-induced signaling, and principal contributor of tumor fibrosis, inflammation and cell proliferation. Here, we show that pancreatic tumors over-express TAK1, and are associated with fibrotic-stroma and infiltrating immune cells. Also, p-TAK1(ser412) expression is correlated with progression of pancreatic intraepithelial neoplasia (PanIN) lesions to PDAC. Thus, we hypothesized TAK1 as an important target for inhibition of the tumor-associated fibrosis, inflammation and PanIN progression to PDAC.

To prove above hypothesis, we tested a selective TAK1 inhibitor, Takinib for its toxicity and efficacy against PanIN progression in the inducible Ptf1CreERT.LSL-KrasG12D (KC) mouse model. Takinib was synthesized and fed to wild type C57BL/6J mice (n=6) at 250 ppm and 500ppm in diet for 6 weeks to determine toxicity. Bodyweight gain, organ weights and serum enzyme analysis did not indicate any toxicity at the tested doses. For efficacy study, LSL-KrasG12D mice were bred with Ptf1CreERT mice. Pups were genotyped and randomized to groups (n=12). PanINs- PDAC was induced in the KC mice by tamoxifen (oral gavage) followed by 250ppm Takinib administration in diet for 20 weeks. After termination, pancreas tissue sections were evaluated for PanIN multiplicity and PDAC incidence/spread. Administration of Takinib led to significant reduction in PanIN 1 by 54% (p<0.02), PanIN 2 by 77% (p<0.001) and PanIN 3 by 80% (p<0.02) in the KC mice compared to untreated mice. PDAC incidence was also reduced with an increase in normal pancreas in Takinib administered KC mice. Taken together our results suggest that TAK1 is a valuable target for PDAC, and Takinib possesses efficacy against the PanIN and PDAC progression in the KC mouse model warranting further studies.

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The β-Blocker Carvedilol Prevents DMBA-Induced Mammary Gland Tumors

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Breast cancer is the most diagnosed cancer globally and is the fifth leading cause of cancer related mortality worldwide. Chemoprevention is a promising method of reducing cancer mortality. However, there are no drugs that are used routinely as breast cancer preventative agents. Carvedilol (CAR), a beta-adrenergic receptor blocker, use in humans reduces breast cancer incidence; therefore, we hypothesized that carvedilol acts as a breast cancer preventative agent. Two protocols were used to evaluate the ability of carvedilol to prevent 15 mg 7,12-Dimethylbenz(a)anthracene (DMBA)-induced mammary tumors. In protocol 1, two doses of carvedilol (2 and 10 mg/kg) were provided as a 7-day pretreatment and throughout the study. In protocol 2, 10 mg/kg tamoxifen and 10 mg/kg carvedilol were provided as a 7-day pretreatment and treatment was halted after six weeks. In all studies carvedilol and tamoxifen were administered in the drinking water, animals were palpated weekly beginning at week 5 to detect the presence and location of mammary tumors, tumors were measured via calipers, and the experiments terminated at week 13. In protocol 1, 10 mg/kg, but not 2 mg/kg carvedilol was effective in delaying the tumor occurrence ($P = 0.0002$); the first appearance of tumors and median tumor appearance were separated by 6 and 4 weeks, respectively, compared to rats receiving DMBA alone. However, the tumors that appeared, grew at similar rate as those in the DMBA group. In protocol 2, tamoxifen prevented all but 1 rat developing a tumor and was similar to the negative control; however, 10 mg/kg carvedilol was indistinguishable from the DMBA group. Although tamoxifen prevented DMBA-induced breast cancer, the rats drank significantly less water resulting in statistically lower body weight than all other groups. The rats also drank significantly less of the carvedilol-laced water, but not to a degree that affected their body weight. Therefore, continuous carvedilol treatment is essential to achieve the observed chemopreventive effects. Our data demonstrates the chemopreventive activity of carvedilol in a rat model of mammary gland carcinogenesis. The results provide significant implications in breast cancer chemoprevention using carvedilol, which is a safe FDA-approved medicine.

Figure: Tumor incidence
Successful Design and Execution of Two Gastric Cancer Chemoprevention Trials in Central America and Puerto Rico

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Gastric adenocarcinoma (GAC) is the third leading global cause of cancer mortality and leading infection-associated cancer. The high incidence regions are Latin America, East Asia, and Eastern Europe. In the U.S., GAC represents a major cancer disparity, double the incidence rates in all non-white populations, the opposite of Barrett’s Esophagus and EAC. Immigrants from high incidence regions maintain the risk profile of their nations of origin. In a paradigm shift, recent guidelines now recommend surveillance endoscopy (eg, 3 years) for patients with high-risk gastric premalignant conditions (GPMCs). Clinical trials of chemoprevention agents for patients with GPMCs are lacking. We conducted two independent, NCI DCP funded, phase II placebo-controlled chemoprevention trials in patients with GPMCs (intestinal metaplasia, atrophic gastritis). The oral agents were curcumin and eflornithine (DFMO). A highly bioavailable preparation of curcumin was used. The RCTs were conducted in Puerto Rico and rural Honduras, with important characteristics: (1) representative of Caribbean and Mesoamerican populations and linked to large U.S. immigrant populations; (2) high prevalence of *H. pylori* infection and GPMCs; (3) absence of turmeric and curcuminoids in the local diets; (4) proven bidirectional collaboration with academic institutions in the U.S. In the curcumin trial (NCT02782949) *H. pylori* negative patients were randomized to study drug or placebo for 6 months. In the eflornithine study (NCT02794428), *H. pylori* positive and negative subjects were randomized to study drug or placebo for 18 months, with endoscopy at baseline, and 6, 18, and 24 months. The primary outcomes were based upon changes in histologic parameters at 6 months. Principal study challenges included: (1) International and bilingual regulatory environment; (2) Strengthening of the research infrastructure, particularly in Central America; (3) Participant recruitment, eg, in the curcumin RCT in Honduras wherein only 10-15% are *H. pylori* negative; (4) The Covid-19 pandemic; (5) Natural disasters (3 hurricanes). In conclusion, eflornithine and curcumin RCTs have been successfully completed, despite important challenges in implementation and execution. No losses to follow-up were encountered related to the pandemic or natural disasters. The south-south partnership may provide a model for chemoprevention and translational studies in Latino populations with prevalent cancers such as GAC.
Lung cancer chemoprevention with the β-blocker carvedilol

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ABSTRACT

Chronic exposure to carcinogens that are present in tobacco smoke is a causative factor for lung carcinogenesis. Although smoking cessation successfully reduced the prevalence of cigarette use in the US, lung cancer risk for current and former smokers remains high, for whom no effective chemopreventive agent currently exists. In the present study, carvedilol, an FDA-approved β-blocker was examined on lung carcinogenesis induced by the tobacco carcinogen benzo(a)pyrene [B(a)P] in vitro and in vivo. In non-tumorigenic human bronchial epithelial cell culture BEAS-2B, carvedilol inhibited benzo(a)pyrene diol epoxide (BPDE)-induced malignant transformation at non-toxic concentrations in a dose-dependent manner. Although β-adrenergic receptors (β-ARs) are expressed in BEAS-2B cells, the anticancer activity of carvedilol is independent of β-blockade since the non-β-blocking R-carvedilol enantiomer also blocked transformation while the β-blockers atenolol (β1-AR selective blocker), ICI-118,551 (β2-AR selective blocker), and propranolol (nonselective blocker) had no effect. Carvedilol’s anti-transformation activity is possibly mediated by aryl hydrocarbon receptor signaling because carvedilol, R- and S-carvedilol all inhibited B(a)P-activated xenobiotic responsive element (XRE) promoter and CYP1A1 mRNA expression. In a B(a)P-induced acute lung toxicity model in CD-1 mice, pretreatment with carvedilol, R- or S-carvedilol (20 mg/kg/day) for 7 days significantly attenuated increased plasma levels of lactate dehydrogenase and malondialdehyde, inflammatory cell infiltration, histopathologic changes, and overexpression of COX-2 in the lung. In a B(a)P-induced lung carcinogenesis model in A/J mice, carvedilol at 3.2 and 20 mg/kg significantly attenuated tumor multiplicity and burden, to a similar degree as the EGFR inhibitor gefitinib. Our study reveals a previously unexplored role for the FDA approved cardiovascular drug carvedilol in the prevention of tobacco carcinogen-associated lung cancer.
Targeting STAT3 for bladder cancer prevention – in vitro studies using spheroid and organoid models

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Signal Transducer and Activator of Transcription 3 (STAT3) is tightly regulated in normal cells to maintain a transiently active state. In contrast, persistent STAT3 activation is frequently observed in bladder cancer (BC) and is associated with poor prognosis and chemoresistance. Hence, developing small molecule inhibitors targeting STAT3 may be helpful for preventing BC progression and improving the survival rate of patients with metastatic BC. Recently, the use of three-dimensional in vitro models in drug development has gained popularity as they closely resemble, to an extent, the in vivo environment in heterogeneity and physiological conditions. Here we established spheroid and organoid models for bladder cancer and evaluated STAT3 inhibitors (C188-9 and SH5-07) for their anticancer activity in vitro.

Initially, we optimized the spheroid growth from human, rat, and mouse BC cell lines (J82, NBT-II, MB49, respectively) and tumoroid growth from the BBN-rat bladder cancer model. The anticancer efficacy of C188-9 and SH5-07 was evaluated in vitro at various doses (0-50 µM) in the 3D models of BC. Assays were performed to determine spheroid viability (calcein AM (CA) and EtBr staining), ATP and ROS production (MitoSOX™). Protein isolated from control and drug treated spheroids/tumoroids was used to evaluate pharmacodynamic biomarkers of cell proliferation, apoptosis, and STAT3 signaling. We demonstrate that treatment with C188-9 and SH5-07 significantly decreased the spheroids size (39-45% smaller compared to untreated, p<0.0001) along with decreased ATP (20%-40%, p<0.05), and pSTAT3 protein expression in spheroids derived from BC cell lines and rat BC organoids. Further, MitoSOX™ staining showed that STAT3 inhibitor treatment induced mitochondrial mediated ROS generation in BC spheroids. CA and EtBr staining showed that C188-9 and SH5-07 treatment induced cell death in BC spheroids that was also associated with caspase-3 cleavage.

These findings indicate that C188-9 and SH5-07 could suppress the activation of the STAT3 pathway and inhibit the bladder cancer spheroid growth by inducing ROS production and thus warrants further evaluation in vivo. Furthermore, our study provided valuable spheroid and organoid models for evaluating therapeutic candidates in an in vivo-mimic microenvironment, thereby providing great potential for drug testing. (Partly supported by P30CA225520 and Kerley-Cade Endowed Chair)
Targeting STAT3 for Lung Cancer Prevention

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Former smokers have an elevated risk of lung cancer and account for a large proportion of newly diagnosed lung cancer. Former smokers with airway dysplasia have received modest benefit in randomized chemoprevention trials, while active smokers have not. More effective chemoprevention agents for former smokers at risk for lung cancer are needed. We previously reported that a cyclic double-stranded DNA oligonucleotide STAT3 decoy (CS3D) administered intravenously (IV) had strong anti-tumor effects in lung cancer xenograft models and was also effective as a chemoprevention agent using a tobacco carcinogen (NNK)-induced lung cancer mouse model that mimics “ex-smokers”. CS3D prevents the binding of activated STAT3 dimers to the promoter of target genes and induces p-STAT3 degradation. Our goal was to develop a clinically applicable direct delivery method of CS3D to the lungs. Using fluorescently tagged CS3D, we evaluated the feasibility and determined the optimal dosing schedule of CS3D administered intratracheally (IT, a mimic of human inhalation) compared to IV in mice. IT delivery 3 times/week provided a high initial drug level that was cleared from the lungs within a few hours, while IV dosing provided lower initial drug levels but persisted up to 8 hrs, with CS3D accumulation over time in the lungs. While the pharmacodynamic effect was also greater with systemic dosing, IT delivery produced inhibition of the STAT3 pathway. Phosphorylated STAT3, VEGF, IL6 and Ki67 expression in NNK-induced lung preneoplasias was downmodulated by IT CS3D delivery after 4 weeks of treatment compared to the mutant oligonucleotide (CS3M), confirming our previous findings with IV delivery. We also demonstrated that CS3D delivered IT altered the pulmonary environment by promoting a proinflammatory, anti-tumor response in myeloid cells of the lung directly, or by acting on tumor and stromal components to establish an immune-reactive tumor-microenvironment. No toxicities were found during IT or IV treatment and no organ abnormalities were detected by either regimen. A long-term chemoprevention study of CS3D delivered IT in the ex-smoker lung cancer murine model is underway. Our findings suggest that blocking STAT3 may be a useful strategy for lung cancer prevention, and may involve both inhibition of oncogenic signaling and enhanced anti-tumor immunity.
Exploring the Effects of Resveratrol on the Gut Microbiome in High Fat Diet-Fed BRAF<sup>V600E</sup> Mutant Mice

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The polyphenol resveratrol, widely known for its cancer protective activities has been shown to protect against the pro-tumorigenic effects induced by high-fat diets (HFD) in mouse models of colorectal cancer (CRC). Although, the mechanisms of actions of this compound in humans still remain elusive, recent studies suggest its effects may be mediated partly through its interaction with the gut microbiota. Here we sought to determine the changes induced by HFD on the intestinal microbiome and the impact of resveratrol supplementation on these alterations in a mouse model of BRAF<sup>V600E</sup> mutant CRC.

*BRAF<sup>V600E</sup>; BRAF<sup>WT</sup>* (*wild-type*) mice were randomised into 8 groups and fed either a standard diet or HFD supplemented with low or high-dose of resveratrol (0.7mg/kg/day and 14mg/kg/day, respectively) for 6-weeks. The mouse faecal microbial profile was then assessed by targeting the V3-V4 area of the bacterial 16S rRNA gene.

Consumption of HFD was associated with significant alternations in the gut microbiome composition in both *BRAF<sup>V600E</sup>* and *BRAF<sup>WT</sup>* based on beta-diversity analysis (*P*<sub>ADONIS</sub>< 0.05). HFD increased the *Firmicutes* and *Clostridia* abundance in *BRAF<sup>WT</sup>* compared to animals on a standard diet (*P*< 0.01, *P*< 0.008 respectively), while in *BRAF<sup>V600E</sup>* mice, alterations were observed in lower taxonomic levels including the enrichment of *Faecalibaculum* (*P*< 0.02) and growth inhibition of *Lactobacillus johnsonhii* (*P*< 0.05). High-dose resveratrol resulted in significant differences in the microbial composition compared to HFD-treated group in the *BRAF<sup>V600E</sup>* mice (*P*<sub>ADONIS</sub>< 0.05) but not in *BRAF<sup>WT</sup>*. Resveratrol administration did not counteract the HF-induced taxonomic changes but high-dose resveratrol changed *Muribaculum*, and *UBA181* genera abundances in *BRAF<sup>V600E</sup>* mice (*P*< 0.001 and *P*< 0.05 respectively) compared to HF-treated mice. Low dose resveratrol had no significant effects on bacterial abundance in both *BRAF<sup>V600E</sup>* and *BRAF<sup>WT</sup>* mice. It was also observed that *BRAF<sup>V600E</sup>* mutation influenced the gut microbiome independently of diet based on beta and alpha-diversity (*P*<sub>ADONIS</sub>< 0.05, *P*<sub>Chao1</sub>< 0.05 respectively).

Resveratrol administration modified the gut microbiota composition of *BRAF<sup>V600E</sup>* mice but did not completely reverse the HFD-induced changes indicating that alternative mechanisms may contribute to the protective effects of resveratrol in this mouse model.
Development of a novel MEK inhibitor, NFX-179, as a chemoprevention agent for squamous cell carcinoma

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Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer comprising at least 20% of all non-melanoma skin cancers. While cSCC contributes to significant morbidity and mortality in high-risk individuals, deployment of otherwise effective chemoprevention of cSCC is limited by toxicities. To help address this, we conducted a systematic computational drug repositioning screen which predicted that selumetinib, an FDA-approved MEK inhibitor (MEKi), would reverse transcriptional signatures associated with cSCC development. This is consistent with our genomic analysis implicating ETS2, a transcription factor in the canonical RAS/RAF/MEK/ERK Mitogen-Activated Protein Kinase (MAPK) pathway, as an upstream regulator of cSCC development. Therefore, as a key regulator of the MAPK pathway, we reasoned MEK would be a viable chemopreventive target. Although systemic MEK inhibition suppresses the formation of cSCC in mice, systemic MEKi administration causes significant adverse effects, including diarrhea, peripheral edema, cardiomyopathy and retinal toxicity.

Here, we report the development of a topically formulated, metabolically labile, novel MEKi, NFX-179, designed to potently and selectively suppress the MAPK pathway in the skin prior to rapid metabolism in the systemic circulation. NFX-179 was identified from a targeted drug discovery effort based on its biochemical and cellular potency, selectivity, and rapid metabolism upon systemic absorption. In our UV-induced cSCC mouse model, topical application of NFX-179 gel reduced the formation of new cSCCs by an average of 60% at doses of 0.1% and greater at 28 days. No systemic or skin toxicities were observed in this model. Furthermore, we conducted a second split-mouse randomized controlled study in which NFX-179 0.5% gel was applied to one half of the back and vehicle was applied to the opposite half of each of the UV-irradiated mice. Near complete suppression of cSCC was observed only in the drug-treated area, demonstrating the targeted and dermal effect of the intervention. NFX-179 inhibits the growth of human SCC cell lines in a dose-dependent manner and topical NFX-179 application penetrates human skin and inhibits MAPK signaling in human cSCC explants. Together our data provide compelling rationale for using topical MEK inhibition through application of NFX-179 gel as an effective strategy for cSCC chemoprevention.
Limonene as a vehicle in topical delivery of carvedilol for skin cancer chemoprevention

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Skin cancer is currently one of the most common cancers and is defined as an abnormal growth of the skin cells. The goal of this study is to develop a topical delivery system of carvedilol with limonene as the vehicle. Limonene is a major component found in the rinds of citrus fruits and other herbs. It is one of many natural compounds that have demonstrated inhibitory activity in different types of cancers such as breast, lung, and stomach cancer. Furthermore, limonene has shown to increase skin permeation of drugs including carvedilol. The soft agar colony formation assay was used to evaluate anchorage-independent growth of JB6 mouse epidermal cells. This assay tested concentrations of carvedilol (0.1, 1.0 and 10 μM); limonene (1.0, 10, 100 and 1000 μM); and a combination of carvedilol (1.0 μM) and limonene (1.0, 10 or 100 μM). Results demonstrated that carvedilol and limonene as single treatment inhibited colony formation. The combination of carvedilol and limonene showed higher colony inhibition than single treatment, although not statistically significant. Franz diffusion cell was used to test for permeation of the drug. The device has two chambers separated by a membrane, which in this case is rat skin. The drugs, in vehicles of 40% polyethylene glycol (PEG) 400 in PBS or 40% PEG400 in PBS plus 5% limonene were applied into the donor chamber while the samples are collected through the receptor chamber as the drug permeates through the skin. The samples were collected at 16 hours, 20 hours, and 24 hours. Results showed that limonene significantly increased permeation. Therefore, our data indicate that limonene was able to increase skin permeation and the chemopreventive activity of carvedilol. This new formulation will be further examined in animal models for its efficacy against UV-induced skin cancer.