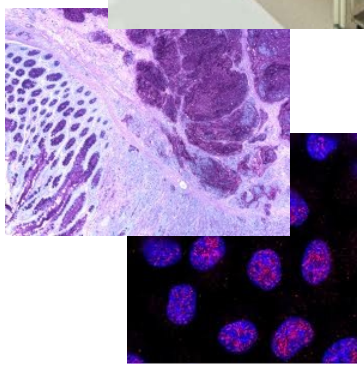
UAMS

UNIVERSITY OF ARKANSAS
FOR MEDICAL SCIENCES

UAMS College of Medicine Series

Showcase of Medical Discoveries:

A Focus on Cancer



Wednesday, September 17, 2014
4:00—5:30 p.m.

A Wine & Cheese Reception Featuring UAMS Investigators Discussing their Research and Discoveries.

Winthrop P. Rockefeller Cancer Institute
10th Floor Rotunda

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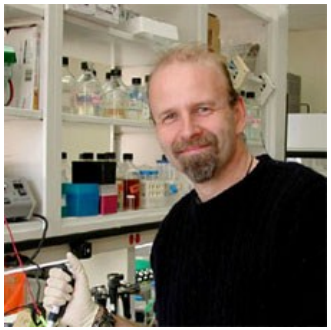
Poster #13

Improving Cancer Therapeutics - A Drug Discovery Approach Targeting Cancer Stem Cells



MacNicol, M.C., Montales, T.M., Cragle, K., Cragle, C.E., Penthala, N.R., Janganati, V., Hardy, L.L., Simmen, R.C.M., Crooks, P.A. and MacNicol, A.M.

Cancer remains one of the leading causes of death in the U.S. While many drugs target rapidly dividing cancer cells and show initial efficacy, tumors may nonetheless grow back and metastasize. Clearly, better anti-cancer medications and treatments are needed to improve patient outcome and long-term survival.

Recent findings show that tumors possess a subpopulation of cells with stem cell properties - that is the ability to self-renew (perpetuate themselves) indefinitely as well as the ability to generate the more rapidly dividing bulk cells of the tumor. These so-called "cancer stem cells" are highly resistant to current anti-cancer drugs and are responsible for tumor recurrence and metastasis. Because few therapeutic strategies are available to specifically target these cells, identification of novel reagents capable of attenuating their self-renewal and survival are critical for development of improved modalities to treat and cure this devastating disease. Our goal is to identify novel drugs that allow survival of normal healthy stem cells, but prevent growth of cancer stem cells. We have developed a novel bioassay to screen for new small molecule inhibitors that specifically target cancer stem cells. Our data indicate that several small molecules identified in our assay show attenuation of brain and breast cancer stem cell function. The successful implementation of our bioassay screen, coupled with direct evaluation of candidate molecules for attenuation of human cancer stem cell self-renewal, will significantly expedite drug discovery for next-generation smart therapeutics for the treatment of cancer.

Poster #12

Genomics and Precision Medicine

Christoph Heuck, Erich A. Peterson, Michael A. Bauer, Shweta S. Chavan, Cody Ashby, Meei Liu, Ruslana Tytarenko, Owen Stephens, and Donald J. Johann, Jr.

Rapid genome-level characterization of tumors for therapeutic guidance is now a reality. This is due to technologic advances in genomics technologies and a precipitous drop in the price of Next Generation Sequencing (NGS) modalities. The Human Genome Project took 13 years to complete the first draft at a cost of ~$3 billion. Because of the success from the 25 year public-private partnership, a whole genome can now be sequenced in a few days for a few thousand dollars and a whole exome (protein coding regions) in about one day, for under $1000. NGS and related molecular profiling approaches can be used to more accurately diagnose various cancer subtypes and identify key genetic abnormalities that can make some patient's tumors respond better than others to specific cancer-fighting drugs. This has led to the use of genetic characterization of tumors to guide therapeutic assignments, known as "precision medicine". Importantly, cancer clinical trials are now incorporating rapid genomic characterization of tumors as selection criteria for use of specific "targeted" therapies. The approach is rapidly evolving and leading to important insights as well as creating new challenges in clinical trial design as well as the analysis of large and complex data sets.

Poster #1

The Capability of Canines to Prospectively Detect Thyroid Cancer

Andrew M. Hinson, MD; Arny Ferrando, PhD; Bekka L. Wilkerson, BA; Donna Waugh; Syed Abid, MD; Brendan C. Stack Jr., MD; Donald L. Bodenner, MD, PhD

We previously showed that canines imprinted on papillary thyroid cancer are able to discriminate between malignant and benign urine samples with > 95% reliability. Dogs were imprinted with diseased tissue, and taught to discriminate between cancer and non-cancer samples utilizing instinctual enhancement training and positive reinforcement. Urine was prospectively collected from 22 subjects presenting to our thyroid clinic with > 1 thyroid nodule(s). A gloved handler presented the samples to each dog. Both the experimenter and handler were blinded to sample status (cancer/non-cancer). Samples of known status from our previous study served as controls. Unknown samples were interspersed with presentation of these known samples. The dogs' responses on known samples were relayed to the study coordinator and when correct, the dog was rewarded with a treat. The dog was not rewarded on unknown samples to avoid potential reinforcement for incorrect answers. However, known controls were quickly presented after unknowns for positive reinforcement and continued dog motivation. Correct responses were given in 21 of 22 urine samples, resulting in a sensitivity of 90.9% and specificity of 100.0%. Scent detection by trained dogs to aid in the evaluation of thyroid nodules may serve as a non-invasive adjunct to current diagnostic practices.

Poster #2

A New beginning: The UAMS Center of Excellence for Bone and Soft Tissue Cancer

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Corey O. Montgomery, Charles K. Lumpkin, Frances L. Swain, Sandra G. McLaren, Archana Kamalakar, Nisreen S. Akel, Kimo C. Stine, Jerad M. Gardner, Issam Makhoul, Rangaswamy Govindarajan, Larry J. Suva and Richard W. Nicholas

The bone and soft tissue consequences of cancer, as well as the complications from the associated skeletal fractures, are a major cause of pain and suffering leading to diminished quality of life for the population of Arkansas. Although primary bone and soft tissue sarcomas are relatively rare cancers, treatment outcomes are still fraught with high five-year mortality rates averaging 50% and 40%, respectively. These cancers are best managed by a dedicated multidisciplinary team of surgeons, pathologists, and medical and radiation oncologists with experience in the care of sarcoma patients. The UAMS Center of Excellence for Bone and Soft Tissue Cancer is a multidisciplinary clinical and research engine focused on the bone and soft tissue complications of a variety of cancers. Our nationally and internationally-recognized clinical and research expertise include osteosarcoma, Ewing's sarcoma, rhabdomyosarcoma, and liposarcoma, as well as breast cancer and other carcinomas with bone metastasis. Research to characterize bone and soft tissue tumors with gene sequencing in an effort to treat with novel investigational agents are currently underway. The UAMS Center of Excellence for Bone and Soft Tissue Cancer is a statewide network that provides comprehensive multidisciplinary care to bone and soft tissue cancer patients. In all, the Center provides a shared infrastructure for patient care, education, training and research to clinicians and investigators across all research disciplines to optimize efficiency, accelerate the pace of discovery, and facilitate the translation of important cancer research discoveries to the bedside.

Poster #11

Germline Genetic Variants in ANGPT1, ANGPT2 and FGF2 are Associated with Pathologic Complete Response to Bevacizumab in Breast Cancer Patients

Issam Makhoul, Robert Griffin, Stephen Erickson, Ishwori Dhakal, Venay R. Raj, Dorothy A. Graves, Jeannette Y. Lee, Mohammed S. Orloff, Eric R. Siegel, and Susan A. Kadlubar

Vascular endothelial growth factor (VEGF) is a central mediator of angiogenesis in cancer and may be targeted by the monoclonal antibody bevacizumab (B). We have conducted a prospective phase II study using neoadjuvant B and chemotherapy (CT) in breast cancer patients and showed improved pathologic complete response (pCR) with the combination compared to the expected pCR with CT alone (41% vs. 25%, p=0.029 one sided). We evaluated baseline serum levels of ten angiogenesis-related proteins (ANG1, ANG2, bFGF, IL-1a, MMP-9, PDGF-BB, PECAM-1, Tie-2, VEGF and VEGFR2) and found that baseline Tie-2 and bFGF serum levels were associated with pCR. The goal of the current study is to explore the association of germline single-nucleotide polymorphisms (SNPs) to pCR obtained with bevacizumab therapy.

Our original patient population consisted of 27 whites (EA) and 12 African Americans (AA) who received docetaxel/cyclophosphamide/bevacizumab and doxorubicin. Only 22 EA and 11 AA had buffy coat samples available for genotyping with the Illumina® technology. We tested 555 SNPs with a minor allele frequency (MAF) of at least 5%, located in 10 angiogenesis-related genes.

Univariate analysis revealed that 73% AA patients achieved pCR compared to 27% EA (p=0.012) and that 54% of the patients with ductal carcinomas achieved pCR compared to 11% with lobular or poorly differentiated tumors (p=0.021). We therefore included race and cancer type as covariates in a logistic regression model testing for association between pCR and each of the 555 SNPs. There were five SNPs in ANGPT1, five in ANGPT2, and four in FGF2 that were associated with pCR ($p<0.05$). Because of the modest sample size, none of these SNPs reached levels of significance after adjusting for multiple comparisons.

ANGPT1, ANGPT2 and FGF2 are promising targets for future research. SNP analysis results support our hypothesis that the interaction of the host's angiogenic profile and the type of cancer explains differences in clinical response to VEGF inhibition. Specifically, our work showed that variants of the ANGPT1, ANGPT2, and FGF2 genes or their respective proteins might render certain tumors more susceptible to targeting of VEGF.

Poster #10

A Dose-escalation Phase I Clinical Trial of a Peptide-based HPV Therapeutic Vaccine, Pepcan: an Interim Report

William Greenfield, Shawna Stratton, Rebecca Myrick, Rita Vaughn, Hannah Coleman, Maria Mercado, Horace Spencer, Nancy Andrews-Collins, Katrina Davis, W. Chuck Hitt, David Hutchins, Gordon Low, Nirvana Manning, Samantha McKelvey, Kimberly Reynolds, Dora Smith, Michael Smith, Amy Phillips, C. Matthew Quick, and Mayumi Nakagawa

The main goal was to assess safety. The vaccine recipients were women with biopsy-proven HSILs {CIN2/3}. PepCan consists of four HPV 16 E6 cGMP-grade peptides covering the entire E6 protein and Candin® (Allermed,San Diego, CA}, a *Candida abicans* skin test reagent. Four injections were administered intradermally every 3 weeks in limbs. Blood samples were drawn for immunological assessments of Thl, Th2, Treg, and myeloid-derived suppressor cells (MDSCs}, and HPV-specific CD3 T-cell responses before first injection, after second injection, and after fourth injection. Histological response was assessed by performing LEEP 3 months after the last injection. HPV-DNA testing was performed at baseline and at the time of LEEP. Each subject was given a single dose level for each of the four injections (50, 100, 250, or 500 micrograms per peptide). This abstract reports on the first 2 dose cohorts (n=12}. The most common adverse events (AEs) were injection site reactions. No AEs grade 3 were reported. HSIL regression was shown in 4 of 6 subjects {67%, 3 to no CIN and 1 to CINl) in the 50 microgram group, and in 3 of 6 subjects (50%, all to no CIN} in the 100 microgram group. Two of 5 subjects (40%) with HPV 16 at the baseline showed regression while 5 of 7 subjects (71%) with HPVs other than type 16 did. Statistically significant increases in CD3 T-cell responses to the E6 protein were shown in 5 of 10 subjects (50%} after vaccinations. The percentages of Thl cells, Tregs, and MDSCs were unchanged while those of CD4 (p=0.01} and Th2 were decreased.

PepCan appears to be safe. It may be effective in regressing HSILs with and without HPV16. A small but significant decrease in CD4 appears to be due to the decrease in Th2.

Poster #3

Effects of Dehydroepiandrosterone (DHEA) treatment on Liver Steatosis using DMBA-Induced Mammary Tumor Obese Zucker Rat Model

Reza Hakkak, Ph.D. and Soheila Korourian, M.D.

Obesity has been epidemic in the US for over two decades. Obesity has been linked with the risk of development of various cancers, including breast cancer. Dehydroepiandrosterone (DHEA) is an over-the-counter dietary supplement used as an anti-cancer agent and anti-obesity supplement. The objectives of this study were to investigate the long-term effects of obesity and DHEA treatment on body weight gain and liver steatosis using 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumor model. Forty-three six-week-old obese female Zucker rats were used. Rats were randomly assigned and had ad libitum access to water and a diet of either chow (2016) as a control diet or chow with the addition of DHEA at a concentration of 6 g/kg of chow as a DHEA diet. All rats were orally gavaged at age 50 days with 65 mg DMBA/kg body weight and were sacrificed 155 days post-DMBA treatment. Obese rats fed the DHEA diet gained significantly less weight ($P<0.001$) and less liver steatosis ($P<0.001$) than control fed rats. Fifty-five percent (55%) of the control diet group developed mammary tumors, while no tumors were detected in the DHEA diet group ($P<0.001$).

Poster #4

Circulating Cell Detection Using Photoacoustic/Photothermal Signals to Monitor Anti-vascular Nanomedicine Efficacy Against Solid Tumors

Robert J. Griffin, Mazen Juratli, Nathan A. Koonce, Dmitry Nedosekin, Joseph W. Levy and Vladimir Zharov

The utilization of minimally invasive means to monitor and predict treatment efficacy or tailor treatment is one of the more compelling strategies in the fight against cancer. Comprehensive studies have demonstrated the potential of using the circulating tumor cells (CTCs) or endothelial/stromal cells shed from a primary tumor as a marker of tumor aggressiveness and may also represent the damage, or lack thereof, being inflicted by various therapeutic approaches. However, the development of high speed multiplexed CTC assays for routine clinical management of cancer with enhanced sensitivity for rare and important cell types is challenging and remains underdeveloped. Our group has developed an integrated multicolor photothermal (PT)-based flow cytometry (PTFC) for high speed multiplex detection of viable and relatively-rare CTCs in blood samples. Specifically, the new platform is able to detect and count CTCs or CECs in animals or patients. The platform has the following advantages: (1) analysis of whole blood without processing; (2) label-free detection mode; (3) high throughput (1000 mL/h vs 1 mL/h [i.e., ~103 –fold rate increase]); (4) identification and capture of viable CTC/CECs for further analysis; (5) multicolor capability (9 colors vs 2) and multiplex analysis (simultaneous detection of 6-8 biomarkers vs 1-3); (6) high speed imaging (104 frame per second [fps] vs 102); and (7) improved lower limit of detection (1 cell /20 mL vs 1 cell/7.5 mL).

Poster #9

Fibroblast Activation Protein Cleavage of Collagen Enhances Class A Scavenger Receptor Mediated Macrophage Adhesion

Anna Mazur, Shanthi Vadali, Thomas Kelly, and Steven R. Post

The importance of interactions between cancer and stromal cells within the tumor microenvironment to tumor progression is well documented. In contrast, how interactions between different stromal cell types, such as fibroblasts and macrophages, support tumor growth is not understood. Increased infiltration and retention of tumor-associated macrophages (TAMs) in tumors is indicative of poor prognosis in cancer patients. TAMs secrete a variety a factors that activate stromal fibroblasts. Tumor associated fibroblasts (TAFs) differ from quiescent fibroblasts by upregulating the expression of type 1 collagen, the primary component of the tumor extracellular matrix, and by expressing the post-prolyl serine protease, fibroblast activation protein (FAP).

In this study, we used a quenched fluorescent substrate to show that FAP proteolytically cleaves type 1 collagen, and that the adhesion of primary macrophages to collagen-1 is substantially increased by FAP-mediated cleavage. Further, using macrophages isolated from knock-out mice and specific receptor inhibitory approaches, we show that increased macrophage adhesion to FAP-modified collagen is mediated by the Class A Scavenger Receptor (SR-A). These results suggest a novel TAF-TAM axis in which FAP can promote tumor progression by cleaving collagen and increasing the SR-A-dependent retention of TAMs.

Poster #8

Metformin Induces Progesterone Receptor (PGR) and PGR Co-regulator Kriippel-like Factor 9 (KLF9) Gene Expression In Endometrial Carcinoma Cells

John Mark P. Pabona, Alexander F. Bumett, Frank A. Simmen, Rebecca Stone, Charles M. Quick, and Rosalia C .M . Simmen

Endometrial cancer (EC) is the most commonly diagnosed gynecologic malignancy. Early stage disease has a 5-year survival rate of 90%. Besides surgical removal of the uterus, there is no current definitive treatment for EC. Impaired glucose tolerance and diabetes are risk factors. In limited epidemiological studies, the oral biguanide metformin (Met) commonly used for the treatment of Type 2 diabetes was shown to lower mortality rate in diabetic EC patients. Nevertheless, a direct effect of Met to inhibit EC development or growth has not been fully elucidated. We previously reported the loss of expression of transcription factor KLF9 in endometrial tumors relative to adjacent uninvolved endometrium of women with EC. KLF9 is a PGR co-regulator in uterine cells; we and others have reported attenuated PGR and/or KLF9 expression in a number of uterine pathologies (e.g., endometriosis, infertility, EC) associated with progesterone (P) resistance. To understand the mechanism of Met action for its potential application in EC chemoprevention and therapy, we treated human Ishikawa EC cells with Met (5 µg/ml; physiologically relevant) and evaluated the temporal expression of PGR, KLF9 and other endometrial genes (PTEN, KLF4, c-FOS, TERT, p53) shown to be dysregulated in EC. Met temporally increased KLF9 transcript levels (2.4X, 2h; 4.5X, 48 h) coincident with those of total PGR and PGR-B (4.5X and 2X, respectively; 48h), relative to untreated cells. Met similarly increased PTEN (2X, 48h), KLF4 (3.5X, 2 h), cFOS (3X, 48h) and TERT (6X, 48 h) but not p53 transcript levels. To determine if KLF9 mediates Met effects, siKLF9 targeting was used to knockdown KLF9 (by 80%) and the expression of these same genes was evaluated. Total PGR and PGR-B transcript levels were higher in cells treated with Met+siKLF9 than with Met+control siRNAs. By contrast, the expression of all other genes did not differ between the treatment groups. Our results suggest that Met administration maintains PGR expression and hence, PGR sensitivity in EC even after the loss of KLF9 expression. A randomized pilot study to test the efficacy of short course Met vs. No Therapy on uterine proliferation and P-sensitivity in the period prior to hysterectomy in non-diabetic EC patients is ongoing (NCTO l 877564). These findings may have significant implications for use of Met in chemoprevention of EC and other P-resistant uterine pathologies.

Poster #5

Impact of Race on Outcome of Stage II Colon Cancer Subjects Not Receiving Adjuvant Therapy

A. L. Cleveland, M.D., E. R. Siegel, M.S., R. Govindarajan, M.D.

The etiology of racial disparity in colorectal cancer outcome has been controversial, attributable to biology or treatment-related variables. Access to care within the Veterans Affairs [VA] health system is similar for all veterans. The outcome of subjects with stage II colon cancer treated with surgery alone in the VA system was investigated in order to evaluate the possibility of difference in tumor biology rather than treatment related variables. A retrospective study of recurrence rate (RR) and overall survival [OS] of subjects with stage II colon cancer, comparing AA vs. C was conducted using data from the VA health system's 10-institution database (VISN-16) from 10/1/1996 to 3/31/2010. None received adjuvant therapy. Patient characteristics were analyzed via chi-square tests. OS was analyzed using Kaplan-Meier methods.

RR(%) was 15 for C and 11 for C (P=0.21), median OS (Yrs.) was 9.9 in AA versus 7.9 C (log-rank p=0.22).

There was no difference in RR and OS of AA compared to C with stage II colon cancer treated with surgery alone. These results indicate that the difference in the outcome of AA and C with colon cancer may not be due to tumor biology.

Poster #6

Associations of the Fecal Microbiome with Urinary Estrogens and Estrogen Metabolites in Postmenopausal Women

Barbara J. Fuhrman, Heather Spencer Feigelson, Roberto Flores,
Mitchell H. Gail, Xia Xu, Jacques Ravel and James J. Goedert

Since gut microbial communities may influence the risk of breast cancer through effects on endogenous estrogens, we investigated whether urinary estrogens and estrogen metabolites are associated with the diversity and taxonomy of the fecal microbiome. Creatinine-standardized urinary estrogens (estrone and estradiol) and 13 estrogen metabolites were measured in spot-urines by mass spectrometry. The fecal microbiome was assessed using pyrosequencing of 16S rRNA amplicons. General linear models were used to test for associations of parent estrogen (estrone + estradiol), total estrogens and estrogen metabolites, and the ratio of estrogen metabolites to parent estrogen, with measures of diversity of the fecal microbiome. The ratio has, in previous studies, been predictive of reduced risk of postmenopausal breast cancer. The ratio of metabolites to parents was directly associated with whole-tree phylogenetic diversity (R=0.35, P=0.01). Relative abundances of the order Clostridiales (R=0.32, P=0.02) and the genus Bacteroides (R=-0.30, P=0 .03) were also correlated with the ratio of metabolites to parents. Associations were independent of age, body mass index, and study design factors.

Our data suggest that women with a more diverse gut microbiome exhibit an elevated urinary ratio of hydroxylated estrogen metabolites to parent estrogen; this finding is consistent with a protective effect of gut microbial diversity on breast cancer risk.

Poster #7

Implementation of a Tobacco Cessation Program in a Multidisciplinary Oncology Clinic

Matthew A. Steliga MD (COM), Claudia P. Barone EdD APRN (CON),
Erna L. Boone PhD (COHP), Patricia L. Franklin, APRN (COM),
and Virginia Hullihan (COM)

Active smokers constitute a large and important percentage of thoracic surgery clinic patients. Tobacco use is associated with impaired wound healing, increased respiratory and cardiovascular complications, and the development of metachronous tumors. Since unaided smoking cessation has poor success rates (<5%), the goal of this study was to use a combination of integrated services to facilitate enrollment, physician recommendation, face-to-face counseling, individualized pharmacotherapy, and follow-up to improve tobacco quit and abstinence rates.

A multidisciplinary team was formed to provide evidence-based tobacco treatment within our thoracic surgery clinic. All actively smoking patients underwent brief intervention by the thoracic surgeon. Then, patients were immediately referred to cessation services housed in the clinic. Exhaled carbon monoxide testing confirmed users and cessation. Nicotine replacement therapy was selectively used. When initial participation rates were suboptimal, integration of cessation services became part of the treatment plan. Participation of eligible patients was only 5 of 15 after the first two months. Subsequently, integration of the program and introduction to the counselors was made a part of clinic visits. This resulted in enrollment of 19 of 47 patients, a 21% improvement. Of the patients who agreed to enroll in the program, 17 of 24 (71%) quit and remained abstinent at last contact (2-26 weeks) .