The University of Arizona Cancer Center

Scientific Retreat

APRIL 10, 2015
Westward Look Resort • 245 East Ina Road
THE UNIVERSITY OF ARIZONA CANCER CENTER MISSION IS TO PREVENT AND CURE CANCER.

The University of Arizona Cancer Center’s vision is to be the preeminent leader in achieving freedom from cancer by extending and enhancing the lives of individuals regionally, nationally, and throughout the world. We achieve this vision through creative collaborations, excellence in research, and research driven, multi-specialty cancer prevention and patient care programs. The creation and dissemination of our knowledge is achieved through translational research, technology development, and novel programs in education and training. Our priority is to assure that all those at risk for and affected by cancer have access to the highest quality care.

Organizing Committee
Amanda Baker, Sherry Chow, Anne Cress, Bob Dorr, Nathan Ellis, Art Gmitro, Marty Pagel

Administrative Support
Kayla Coe, Sara Hammond, Deboragh McDonnell, Meredith Mullins, Nick Prevenas

Supported in part by the American Cancer Society Institutional Research Grant 74-001-34 from the American Cancer Society.
Thank you very much for attending the University of Arizona Cancer Center Scientific Retreat.

Through exchange of ideas and new laboratory and clinical results we have the chance to impact on cancer outcomes. It is only with novel scientific observations that we will prevent, control, diagnose and treat this disease.

This scientific retreat, which we will organize annually, serves as a focal point for excellence in research at the Cancer Center. We honor today our best young scientists, conferring travel awards on the winners of the speaker and poster presentation competitions.

As we move toward the submission of our Cancer Center Support Grant (CCSG), it is important that we increase our focus on the scientific and clinical interactions that will build our programs and our Cancer Center. It is essential that we develop exceptional shared resources that support our programmatic research efforts. These cores should be driven by strong science and translational expertise that has the chance to change patient outcomes.

I look forward to working with every Cancer Center member to enhance his or her research efforts. Working together as an interactive Cancer Center will bring us all success in 2015.

Sincerely,
Andrew S. Kraft, MD
Director, University of Arizona Cancer Center
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Scientific Retreat Agenda

April 10, 2015 • Westward Look Resort • 245 East Ina Road

7:30 Check-in and continental breakfast • Sonoran Ballroom
8:20 - 8:30 Welcome and introduction • Andrew Kraft, MD, UACC Director

Session 1: Oral Presentations from the Cancer Center Programs
Session Chair: Robert Dorr, PhD

8:30 - 8:55 Novel TIAM1 inhibitors target the PH domain for prostate cancer bone metastasis inhibition • Emmanuelle Meuillet, PhD
8:55 - 9:20 Polarity-driven tumor suppression • Joyce Schroeder, PhD
9:20 - 9:45 ModPET imaging for cancer research • Lars Furenlid, PhD
9:45 - 10:10 Application of oxylipin profiling to a sulindac intervention of aromatase inhibitor-induced pain • Jessica Miller Martinez, PhD
10:10 - 10:30 Break • Refreshments on the Sonoran Terrace

Session 2: Oral Presentations from the Proffered Abstracts
Session Chair: Amanda Baker, PharmD, PhD, and Nathan Ellis, PhD

10:30–10:45 A functional role for the MYC i-motif in transcription • Caleb Sutherland
10:45 – 11:00 Loss of A3B1 expression promotes A6B1 integrin internalization to Rab4 vesicles and migration of human prostate cancer cells • Lipsa Das
11:00 – 11:15 Role of hypoxia and PERK signaling in the regulation of CD49f expression and HNSCC plasticity • Saravano Kumar
11:15-11:30 Response assessment of cerebral metastases after high-dose stereotactic radiation: using combined diffusion and perfusion MRI • James Knitter
11:30-11:45 Detection of enzyme activities with diamagnetic catalyCEST MRI contrast agents • Sanhita Sinharay
11:45 -12:00 Combining low dose microtubule targeting agents with belinostat potentiates cytotoxic response in HDACi resistant DLBCL • Aaron Havas
12:00-12:15 Targeting tumor associated immunosuppression by lenalidomide in combination with metronomic cyclophosphamide in patients with metastatic, castration-resistant prostate cancer • Jue Wang, MD
12:15-12:30 Association of calcium and vitamin D intake and vitamin D receptor genotypes with prostate cancer in multiethnic samples • Ken Batai, PhD

Session 3: Poster Session

12:30-3:30 Poster Session • Coyote Room in the Sonoran Ballroom
12:30-2:00 Buffet lunch available • Sonoran Terrace

Session 4: Keynote Address
Session Chair: Andrew Kraft, MD

3:30-4:30 Deconstructing breast cancer using a developmental perspective and single cell approaches Geoffrey M. Wahl, PhD • Professor, Gene Expression Laboratory, Salk Institute
4:30-5:00 Closing remarks • Andrew Kraft, MD, UACC Director • Award announcements
5:00-6:30 Happy Hour Reception with Geoffrey M. Wahl, PhD • Canyon Mesa Terrace
Dr. Wahl has made many seminal contributions on the p53 tumor suppressor and its role in the preservation of genetic integrity.

His lab has recently investigated how activation of p53 in stromal cells can limit or reverse the fibrosis that contributes to drug resistance and aggressiveness in pancreatic cancer.

Geoff has also become fascinated with mammary stem cells and their potential relationship to breast cancer. His lab has identified stem cells in the embryonic mammary anlage that are progenitors of the stem cells in adult mammary gland. These early stem cells share gene expression profiles with those present in some of the most aggressive forms of human breast cancer. Geoff is looking for the critical genes that can be targeted to prevent cells from inappropriately reprogramming into an early stem-like state in the face of oncogenic challenges—an approach that has significant therapeutic potential.
KEYNOTE ABSTRACT

Deconstructing breast cancer using a developmental perspective and single cell approaches

Geoffrey M Wahl, Christopher Dravis, Benjamin T Spike, Claire Johns, Rose Rodewald, Cristy Trejo
Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla, California

Parallels between embryonic development, stem cells, and cancer have long been recognized. We have identified, isolated, and characterized stem cells that first become committed to a mammary fate during embryogenesis, which we refer to as fetal mammary stem cells (fMaSCs). Interestingly, 50-60% of the basal-like intrinsic subgroup of breast cancers are highly enriched for genes that are significantly and differentially expressed in fMaSCs. As P53 is frequently inactivated in basal-like tumors, and p53 inactivation also contributes to cellular reprogramming, we speculate that basal-like breast cancers arise either as the result of a p53 mutation in a mammary stem cell population, or that breast epithelial cells that suffer p53 mutation may reprogram to resemble fMaSCs.

We used population based and single cell RNA-sequencing and bioinformatics to suggest growth factor-ligand interactions of potential functional significance. In vitro studies then demonstrated the relevance FGF, EGF, and HGF pathways. Interestingly, other have shown that there is a feedback loop between FGF signaling and induction of Sox9, and that Sox9 and Slug can elicit stem like properties in mammary epithelial cells. Furthermore, the TCGA showed that increased Sox9 and Sox10 expression are highly correlated with basal-like breast cancers. Studies to be discussed reveal that Sox10 also serves as a marker for stemness in the fetal mammary gland, and enables isolation of highly enriched fMaSC populations in both females and males. Studies to be discussed demonstrate that Sox10 is also functionally relevant for generating the mammary stem cell state, and that excessive expression can induce stem cells to acquire a mesenchymal phenotype that might enable metastatic spread.

(Supported by Breast Cancer Research Foundation; Susan G. Komen for the Cure; Department of Defense; Helmsely Trust; National Institutes of Health, Salk Cancer Center Support Grant CA014195)
ABSTRACTS FOR SESSION 1 SPEAKERS
Background: TIAM1 (T-lymphoma invasion and metastasis-inducing protein-1) is a highly conserved guanine nucleotide exchange factor and contains a Dbl Homology (DH)/C-terminal Pleckstrin Homology (PH) domain. The crystal structure of DH-PH of TIAM1 in complex with Rac-1 has been reported. TIAM1 has been found to be over-expressed in cancers such as breast, colon, and prostate cancers. An increase in TIAM1 expression has been shown to be associated with increased metastatic potential of breast cancer cell lines and is also correlated with poor prognosis of patients with prostate cancer. TIAM1 is thus a novel PH domain-containing drug target directly related to cancer progression, metastasis, and patient survival.

Results: We have identified and characterized novel small molecule inhibitors targeting the phosphoinositide lipid binding PH domain of TIAM1 in order to selectively inhibit cell migration, invasion and survival. An in silico screen of our internal and Maybridge library using the crystal structure of TIAM1 has identified 10 compounds that bind to the PH domain. In vitro assays revealed that compounds TPH-3 and TPH-10 significantly reduced the amount of active Rac1 in PC-3 prostate cancer cells (EC50 of 2.73±0.13 and 2.38±0.98 µM) by binding to PH-TIAM1 with high affinity (KD in the micromolar range) using surface plasmon resonance (SPR) spectrometry. Both compounds displaced PtdIns-3,4,5-P3 in a SPR competitive binding assay. TPH-3 and TPH-10 inhibited cell proliferation with EC50 of 19.8±3.2 and 28.7±1.7 µM in prostate cancer cells LnCaP and EC50 of 18.6±1.8 and 33.3±6.7 µM in PC-3. In comparison, compounds such as Enzalutamide and Bicalutamide had EC50s of 11 µM and 30 µM, respectively, in LnCaP cells. Wound healing assays and lamellipodia formation were both inhibited by the compounds. TPH-10 and TPH-3 inhibited ~70% and ~ 80% respectively of PC-3 invasion using a matrigel invasion assay. Novel analogues of TPH-3 are being synthesized and will be tested in similar cellular assays. Finally, TPH-10 exhibits anti-tumor properties in a PC-3 mouse xenograft study (%T/C~38.8) with good pharmacokinetic properties (T1/2~6 hours). Cardio-injection of prostate PC-3 cells in SCID mice was used to test the effects of TPH-10 in the prevention of bone metastasis. Mice were treated with 75 mg/kg ip of TPH-10 once a day for 5 days. TPH-10-treated mice developed significantly less pelvic bone lesions than the untreated group and TPH-10 treated mice lived longer, on average, than the untreated group.

Conclusion: Overall, we have identified novel compounds that exhibit the ability to reduce prostate cancer bone metastasis by binding to the PH domain of TIAM1, an important GEF in the process of metastasis.
**SPEAKER ABSTRACT 2**

**Polarity-driven tumor suppression**

**Joyce Schroeder, Erin Greenwood, Sabrina Maisel, David Ebertz, Tony Fabiano, Atlantis Russ**  
**Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ**

**Background:** Virtually all solid cancers are associated with the deregulation of Receptor Tyrosine Kinases (RTKs). RTKs are potent drivers of transformation, regulating proliferation, survival, migration and even cancer stem cell identity. Negative regulation of RTKs is largely mediated by ligand-induced receptor degradation via trafficking to lysosomes, and this negative regulation is frequently lost in cancer. Thus, many RTKs are frequently overexpressed at the protein level in cancer, without concomitant increases in transcription. Similarly, many epithelial cancers have lost key components of apicobasal polarity, changes that also induce proliferation, survival and migration. For example, over 75% of breast cancers have lost the apicobasal polarity driver, HUGL1. We have shown that this loss results in tissue overgrowth, epithelial to mesenchymal transition and mixing of apical and basolateral membrane domains. The Epidermal Growth Factor Receptor (EGFR) is a canonical RTK, and we have shown that EGFR loses basolateral restriction and negative regulation during breast cancer progression. In addition, its ability to drive breast cancer in mouse models is dependent upon its association with the apical adapter protein MUC1. It is unknown whether loss of apicobasal polarity alone is required for this loss of negative regulation or whether adapter proteins such as MUC1 are required.

**Results:** Our long-term goal is to understand how loss of polarity drives RTK activation and to design effective targeted therapies that re-establish polarity and receptor degradation. Our central hypothesis is that loss of apicobasal polarity alters the normal trafficking of oncogenic RTKs (such as EGFR), prolonging signaling and enhancing both recycling and nuclear localization. Our hypothesis is based on data demonstrating that EGFR degradation is lost upon either MUC1 co-localization or through HUGL1 knockdown. Further, EGFR undergoes altered intracellular trafficking upon ligand binding, resulting in nuclear localization of EGFR where it can directly interact with transcriptional complexes. Co-localization of EGFR and MUC1 at the apical domain also results in increased cell migration and loss of contact inhibition. We have found that immortalized breast epithelial cells lacking HUGL1 undergo a transition to a stem cell like state in the presence of EGFR activation. Furthermore, driving EGFR to the apical domain by either mutation of the basolateral targeting domain, or by knockdown of HUGL1, results in the activation of the HIPPO/TAZ stem cell program. Together, these results point to apicobasal polarity as a global inhibitor of the cancer stem cell phenotype by suppression of RTK-mediated transformation.

**Conclusions:** There are dozens of RTKs that drive cancer progression, all of which are negatively regulated through lysosomal degradation in normal cells. Discovering a global mechanism by which negative regulation is lost (colocalization with apical membrane machinery) may uncover a targetable process that would effectively target cancerous, but not normal, cells. Furthermore, we may discover an essential link between the establishment and maintenance of epithelial polarity and suppression of the cancer stem cell state.
Small-animal (or pre-clinical) positron emission tomography (PET) is a powerful tool for carrying out research on cancer in mouse and rat models, and most major cancer-research institutions that have small-animal imaging facilities have PET or PET/CT alongside MRI, CT, ultrasound, bioluminescence and SPECT instrumentation.

As part of our technology development in the Center for Gamma-Ray Imaging, we have developed a low-cost alternative to the geometry and detectors used in commercial pre-clinical PET systems. This approach revisits the origins of PET imaging – which originally comprised pairs of gamma cameras and coincidence electronics. Instead of solving the question of how to achieve adequate angular sampling by building expensive rings of detectors, we employ planar gamma cameras, but make them very large in comparison to the imaging subject.

The straightforward geometry is complemented by careful calibration and statistically-rigorous data-processing and reconstruction methods. Much to our delight, this simple system produces images easily comparable to commercial offerings at more than ten times the cost. We are in the process of producing a second copy of the instrument that will be dedicated to cancer imaging and provide full access to preclinical PET to the Arizona Cancer Center community.
Application of oxylipin profiling to a sulindac intervention of aromatase inhibitor-induced pain

Jessica A. Martinez, Betsy Werthiem, Jun Yang, Bruce Hammock, Denise Roe, Alison Stopeck, Patricia A. Thompson

University of Arizona Cancer Center, Tucson, AZ; Department of Nutritional Sciences, University of Arizona, Tucson, AZ; University of California, Davis, CA; Stony Brook University, Stony Brook, NY

Introduction: The regular use of non-steroidal anti-inflammatory drugs (NSAIDs) has been associated with a lower risk for epithelial cancers including breast. NSAIDs block cyclooxygenase (COX)-1 and -2 enzyme metabolism of ω-6 polyunsaturated fatty acids (PUFA) to prostaglandins (PGs). Additionally, ω-6 and ω-3 PUFA are metabolized by lipoxygenases (LOX) and cytochrome P450 (CYP450) enzyme families to produce over 100 metabolites known as ‘oxylipins’. Oxylipins exhibit a wide spectrum of biological activity including mediators of pain and inflammation. The overarching objective of the work is to determine if sulindac (an NSAID) intervention changes oxylipin profiles in breast cancer patients, and if individual oxylipins are related to pain caused by aromatase inhibitors (AI).

Methods: This work takes place within the context of an R01-funded single arm, open-label clinical trial of sulindac (N=75). Breast cancer patients that are stable on AIs complete pain and quality of life questionnaires, and provide blood and urine samples after a 4-week NSAID washout, after a 3 month observation period, at baseline and 3, 6 and 12 months on sulindac intervention (150 mg bid). Oxylipins were quantified in blood and urine (n=10) at the end of washout, baseline, and after 3 months on sulindac using liquid chromatography (LC) mass spectrometry (MS)-based methods.

Results: We quantified 62 total oxylipins in plasma and 87 in urine. Thus far, our analysis has focused on plasma oxylipins. Wilcoxon signed-rank tests were used to compare oxylipin levels at baseline and after 3 months of sulindac. COX metabolites of both ω-6 and ω-3 PUFA were non-significantly decreased. In terms of ω-6 metabolites, 7 LOX products were significantly decreased 11-HETE (P=0.028), 15-HETE (P=0.037), 9,12,13-TriHOME (P=0.017), 9,10,13-TriHOME (P=0.012), 8-HETE (P=0.028), 9-HETE (P=0.028), and 12-HETE (P=0.047). Significantly decreased ω-6 CYP450 metabolites were 15(S)-HETrE (P=0.028), and 5,6-DiHETrE (P=0.028). The ω-3 metabolites that significantly decreased were the LOX-derived 5-HEPE (P=0.022), and 15-HEPE (P=0.028), and CYP450-derived 5,15-DiHETE (P=0.047), 5,6-DiHETE (P=0.009), and 15,16-DiHODE (P=0.012). The ω-6 CYP450 metabolite 11(12)-EpETrE was significantly correlated with both “worst pain” (ρ=-0.669; P=0.034) and severity (ρ=-0.778; P=0.008). The ω-6 CYP450 metabolite 20-HETE was significantly correlated with severity (ρ=-0.714; P=0.047).

Conclusions: Our preliminary data indicate that oxylipin profiles change in response to sulindac and may be related to pain. Success in shifting plasma oxylipin profiles toward an anti-inflammatory/anti-thrombotic cardioprotective profile would maximize efficacy and to reduce potential toxicities of NSAIDs within the context of for prevention of breast and other cancers.
ABSTRACTS FOR SESSION 2 SPEAKERS
SPEAKER ABSTRACT 5

A functional role for the MYC i-motif in transcription regulation

Caleb Sutherland, Laurence Hurley
Department of Pharmacology, University of Arizona

Background: MYC is overexpressed in most types of tumors, but a means to selectively decrease its expression is yet to be found. Our recent findings on modulation of bcl-2 gene expression through protein interactions with the bcl-2 i-motif have provided a basis for further investigation of MYC gene control. It is proposed that the MYC i-motif could function by a similar molecular switch mechanism as in bcl-2.

Results: Binding sites for heterogeneous nuclear ribonucleoprotein K (hnRNP K) within the MYC promoter also exist in the i-motif-forming sequence. Circular dichroism and bromine footprinting confirmed that this DNA sequence is able to form an i-motif, and systematic mutation of the cytosine residues in this sequence has revealed a 5:5:5 loop configuration. Indeed, all loops of the i-motif, when folded into a 5:5:5 loop configuration, contain the hnRNP K consensus sequence (CCCT). Previous studies show that hnRNP K binds to this i-motif-forming sequence, but it was assumed to be single-stranded. Binding studies revealed that hnRNP K has more binding affinity to its consensus sequence in the i-motif compared to a mutant sequence where the i-motif cannot form. Further investigation of the MYC promoter revealed an additional two runs of cytosine seven bases downstream of the MYC i-motif. Biophysical studies showed that the additional two runs were not involved in i-motif formation, however recent studies describe their importance for transcriptional activation. We found that hnRNP K preferred the longer 5CT sequence compared to the i-motif forming 4CT sequence when using a competitive binding assay. Utilizing luciferase reporters containing either the 4CT or 5CT sequence validated that hnRNP K required both the i-motif and 5th CT element for maximum transcriptional activation. Competition binding studies and bromine footprinting showed that hnRNP K bound to the downstream 5th CT element and the central and lateral loops of the i-motif.

Additionally, we found that co-overexpression of Sp1 and hnRNP K induced a 10-fold increase in luciferase activity in the 5CT reporter only. We hypothesize that Sp1 continuously primes the promoter to initiate transcription inducing more negative superhelicity and increasing the melting of duplex DNA. This increased melting grants hnRNP K’s three KH domains access to the i-motif loops and the 5th CT element. Confirmation by ChIP analysis validated that Sp1 overexpression causes an increase in hnRNP K occupancy at the MYC promoter.

Conclusions: These findings provide new insight into the mechanisms of MYC transcriptional control by the i-motif and G-quadruplex. We are employing drug discovery efforts that can target this molecular switch and inhibit MYC from being transcribed. The use of such interactive compounds is the first step into the development of new innovative approaches to treat cancers that have MYC overexpression.
Loss of A3 Integrin Expression Promotes Increased A6 Integrin Internalization to Rab4 Vesicles and Increased Migration of Human Prostate Cancer Cells

Lipsa Das¹, Todd A Anderson⁷, Jaime MC Gard⁶, Isis C Sroka³, Stephanie R Strautman⁵, Raymond B Nagle⁴⁺⁶, Colm Morrissey⁸, Beatrice S. Knudsen⁹, Anne E Cress³⁵⁶
Departments of ¹Cancer Biology, ²Pharmacology, ³Cellular and Molecular Medicine, ⁴Pathology, ⁵Molecular and Cellular Biology, ⁶University of Arizona Cancer Center, University of Arizona, Tucson, AZ; ⁷University of California Irvine School of Medicine, Irvine, CA; ⁸University of Washington, Seattle WA; ⁹Cedars Sinai Medical Center, Los Angeles, CA

Background: Eighty percent of metastatic prostate cancer express the laminin binding integrins, A3B1 and A6B1, while the variety of integrin expression in normal glands is not observed. In this study, we quantitated the internalization of integrins A3 and A6 and whether their expression influences respective internalization and subsequent cancer cell migration.

Results: Metastatic prostate cancers (185 samples from 45 patients) expressed laminin-binding A6 integrin (CD49f) on the plasma membrane and/or within the cytoplasm consistent with active membrane receptor internalization. In human xenograft tissues, increased expression of transmembrane trafficking proteins correlated to loss of A6 integrin expression from the cell surface and expression in the cytoplasm. Cell surface labelled A6 and A6p integrin heterodimers were internalized within 10 minutes at 37°C and achieved maximal internalization within 30 minutes. We quantitated the internalization rates of A6 and A3 integrins, using fluorophore conjugated antibody based internalization assays and flow cytometry. Integrin A6 was internalized with first order kinetics and a rate constant (kactual) of 3.25 min⁻¹ which was 3 fold faster than A3 integrin (ITGA3, CD49c) (1.0 min⁻¹), 1.5 fold faster than the vitronectin binding Av integrin (ITGAv, CD51) (2.2 min⁻¹) but significantly slower than the unrelated transferrin receptor (15 min⁻¹). The A6 integrin distributed to early endosome antigen 1 (EEA1) containing early endosomes and Rab4 containing recycling vesicles. Silencing A3 integrin expression led to 1.8 fold increase in migration which was dependent on A6B1 integrin and redistributed A6 integrin to a cell-cell staining pattern similar to that observed in human tissue specimens. Silencing integrin A6 expression, however, did not affect the internalization of A3 integrin indicating a unidirectional regulation of integrin internalization.

Conclusions: The changes in the integrin internalization could be clearly ascribed to alpha subunits, as the obtained internalization kinetics of A6 and A3 integrins was independent of the antibody used or integrin engagement by ligand mimetic peptides. A6B1 and A3B1 integrins have different internalization kinetics and coordination exists such that the presence of A3B1 integrin can influence the function of A6 integrin by a decreased internalization rate and cell migration. These data are consistent with previous observations in normal systems suggesting that A3B1 provides a provisional matrix during early stages of wound healing, requiring subsequent stable adhesion complexes and integrin A6 function. In cancer, the loss of A3 integrin and the increased internalization of A6 integrin may account for the “wounds that won’t heal” phenotype and provide a strategy for biological intervention to block tumor metastasis.

(Supported in part by NIH Grants CA23074 and CA159406)
Role of hypoxia and PERK signaling in the regulation of Integrin α6 expression and HNSCC plasticity

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Background: Tumor recurrences and metastatic spread are the common causes of mortality in patients with head and neck squamous cell carcinoma (HNSCC) whose survival rates have not improved in over thirty years. Therefore improvement of survival rates requires a deeper understanding of the cellular processes that mediate the tumorigenicity of these tumor cells.

Using an in vivo experimental model of HNSCC (HEp3) as well as other HNSCC cell lines, we have shown that a tumor cell sub-population known as tumor initiating cells (TICs), characterized by CD49f as integrin α6-high/ALDH1A1-high (denoted as CD49f-high) cells, have the capacity to drive tumor growth, which is functionally dependent on CD49f expression. Additionally, we found that TIC potential is dynamic and reversible as even pure non-TICs, characterized as CD49f-low/ALDH-low (denoted as CD49f-low), subpopulations can eventually give rise to CD49f-high TICs. Such dynamic regulation of tumor initiating properties was ascribed to microenvironmental and/or epigenetic factors. We therefore hypothesize that adaptive signals modulated by tumor hypoxia play a critical role in regulating the TIC behavior of HEp3 tumors.

Results: Tumor hypoxia, which not only activates adaptive signals that promote therapy resistance in HNSCC, can also induce the expression of factors, which promote “stem”-like properties and drive the generation and expansion of TICs. We have found that these G0-G1-arrested CD49f-high TICs are predominantly localized within hypoxic niches of HEp3 tumors. Most importantly using qPCR and FACs analysis we show that when placed in hypoxic environments CD49f-low non-TICs display a significant increase in CD49f mRNA and surface expression comparable to the levels in CD49f-high TICs. Furthermore we also show here that CD49f-high cells were also associated with increased activation of hypoxia and ER stress activated pathway PERK-eIF2α. Analysis of the role of PERK signaling on expression of CD49f/integrin α6, showed that downregulation of PERK signaling resulted in a significant decrease in basal CD49f mRNA and surface protein levels. In addition, knockdown of PERK regulated transcription factor ATF4 also resulted in a 2-fold decrease in CD49f mRNA levels in parental T-HEp3 cells as well as CD49f-high and CD49f-low cells. Moreover, we observed a significant decrease in the mRNA levels of CD49f/integrin α6 interacting partners β1 and β4 following PERK inhibition, while the expression of other integrins namely αV, α5 subunits were unaffected.

Conclusions: These findings suggest that PERK signaling is critical for the regulation of α6 containing integrins and may play a functional role in the hypoxic induction of CD49f in CD49f low cells and their subsequent reprogramming to tumorigenic phenotype. Future work is focused on providing important mechanistic insight into the role of hypoxia and PERK signaling in the stochastic transition of non-TICs to TICs in HNSCC. We believe that identifying this functional interplay in regulating the TIC plasticity will not only allow us to understand how tumor cells adapt to microenvironments such as hypoxia but could also lead to interventions that can effectively block these adaptive mechanisms and eliminate tumor recurrences.

(Supported in part by the American Cancer Society Institutional Research Grant 74-001-34 from the American Cancer Society)
Response Assessment of Cerebral Metastases After High-Dose Stereotactic Radiation: Using Combined Diffusion and Perfusion MRI

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Purpose: Accurate assessment of treatment response vs. pseudoprogession or radiation necrosis can be challenging on conventional imaging when evaluating cerebral metastases treated with radiation. The purpose of this study was to determine whether multiparametric MR biomarkers can predict response to radiation treatment in cerebral metastases.

Materials & Methods: Inclusion criteria for this retrospective study were: cerebral metastases treated with radiation and available multiparametric MRI studies including MR diffusion and dynamic susceptibility contrast (DSC) prior to radiation and on at least two post-treatment follow-up exams. Image analysis was performed using FDA approved software (Olea Medical). Volumetric analysis of lesions was performed based on the signal intensity subsuming the entire region of enhancement on T1 post-contrast images. DSC studies were processed using Bayesian probabilistic method to generate CBV maps. Using coregistered images, voxel-based apparent diffusion coefficient (ADC) and regional cerebral blood volume (rCBV) values were obtained from enhancing lesions. Disease progression was defined as an increase in lesion volume >40% over baseline pretreatment volume. Follow-up rCBV and ADC values were scored to assess fit with expected patterns of favorable response (for rCBV: steady decrease or increase followed by decrease; for ADC: increase or stable) or non-favorable response (for rCBV: steady increase or decrease followed by increase; for ADC: decrease). Pretreatment and follow-up values were assessed for significant differences using t-test and scores were assessed for diagnostic correlation using Fisher’s exact test.

Results: Fifteen cerebral metastases were included with a total of 60 MRI scans evaluated in this longitudinal study. The mean follow-up was 7.8 months after initial scan (range, 2.4 to 13.9 months). Twelve lesions (80%) remained stable or regressed after radiation as determined by lesion volume. The mean ± SD of imaging biomarkers in pretreatment scans vs. sequential follow-up studies were: 1070 ± 325 vs. 1227 ± 316 (10-6 mm2/s) for ADC (p=0.34); and 3.9 ± 5.1 vs. 2.4 ± 2.0 for rCBV (p=0.37). Longitudinal follow-up MRI analysis demonstrated a progressive increase in ADC and a progressive decline in rCBV values compared to pretreatment scans. Using sequential ADC and rCBV scored values, expected response patterns matched volume-based response assessment in eleven (92%, p=0.37) and ten (83%, p=0.52) responding lesions, respectively. In sub-analysis of the group with favorable biomarker response, 5 lesions (50%) showed an interesting trend of early rCBV increase followed by a gradual decline. It is plausible that the initial increase in perfusion can further accentuate the effect of radiation in this group.

Conclusions: Multiparametric MR diffusion and perfusion can be used in the characterization of post-radiation changes in patients with cerebral metastases, independent of morphological changes on conventional imaging.
Detection of enzyme activities with diamagnetic catalyCEST MRI contrast agents

Sanhita Sinharay¹, Dina Hingorani², Amanda Baker³, Julio Cardenas-Rodriguez⁴, Mark D Pagel⁴
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Background: Optical surgical navigation has strong potential to improve cancer surgery. Exposed tissue can be painted with an optical imaging agent, causing the tumor to “light up” and be easily visualized during surgery. Optical imaging agents are often used that are activated by enzymes in tumors. For example, an optical imaging agent has been developed that can detect gamma glutamyl transpeptidase (GGT) that is overexpressed in the extracellular environment of some types of metastasized ovarian tumors and in glioblastoma. However, not all tumors overexpress GGT. Therefore, a pre-screening method is required to identify patients who have high GGT expression in tumors, and therefore can benefit from optical navigation during surgery.

Results: To address this need for a pre-screening method, we have developed a CEST MRI contrast agent that can detect GGT activity. This agent generates two CEST signals, and the ratiometric comparison of the enzyme-responsive and "control" signals improves the detection of GGT activity. We have measured the Michaelis-Menten kinetics of GGT for our CEST agent substrate to validate that we are detecting enzyme activity. We have optimized our CEST MRI protocol for this agent. We are currently performing in vivo CEST MRI studies that detect GGT activity within orthotopic OVCAR8 tumor models that have high GGT activity, and orthotopic OVCAR3 tumor models that have no detectable GGT activity, to demonstrate that CEST MRI can eventually pre-screen patients and identify patients who are candidates for optical navigation during surgery. We have also performed fluorescence imaging studies to simulate optical surgical navigation with these tumor models.

Conclusions: These pre-clinical in vivo studies are establishing our “catalyCEST MRI” method as a platform technology for detecting the activities of many enzymes within in vivo models. For example, we have developed CEST MRI contrast agents that detect kallikrien-6 (KLK-6), which is overexpressed in ovarian and breast cancer; urokinase plasminogen activator (PLAU) activity, which is overexpressed in pancreatic cancer; and many types of reductase enzymes, which are overexpressed in hypoxic tumors. Therefore, catalyCEST MRI may be a useful tool for many collaborations within the University of Arizona Cancer Center.
Combining low dose microtubule targeting agents with belinostat potentiates cytotoxic response in HDACi resistant DLBCL

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**Background:** Patients diagnosed with Diffuse Large B-cell lymphoma have an overall 60% five year survival rate. New therapeutic approaches are needed to effectively treat aggressive forms of DLBCL that are refractory to the standard treatment or that relapse within two years of treatment. Histone deacetylase inhibitors (HDACi) are novel therapeutics that are well-tolerated in humans and are being extensively evaluated in combination with other therapeutics against hematologic malignancies. However, because their cell type-specific mechanisms of action are only vaguely understood, rational selection of companion therapeutics is difficult.

**Results:** We have developed a pre-clinical model system of sensitivity and resistance to HDACi in DLBCL cell lines that share characteristics with aggressive DLBCL tumors. We previously reported that HDACi resistance is associated with reversible arrest in G1 that involves sustained up-regulation of cyclin-dependent kinase inhibitors. In the current study we demonstrate that HDACi-sensitive cell lines undergo mitotic arrest prior to anaphase in response to treatment with the approved HDACi, belinostat, consistent with activation of the spindle assembly checkpoint (SAC). In contrast, HDACi-resistant cell lines are capable of completing mitosis in the presence of belinostat. To force SAC activation in resistant cell lines, we used low doses of the microtubule targeting agent (MTA) vincristine. The combination of vincristine and belinostat efficiently caused SAC failure, mitotic slippage, and apoptosis. Cytotoxicity was also efficiently induced by belinostat when combined with paclitaxel.

**Conclusion:** Our study identifies the use of low dose MTA/HDACi combination as a potential therapeutic strategy for treatment of relapsed or refractory DLBCL.
Targeting tumor associated immunosuppression by lenalidomide in combination with metronomic cyclophosphamide in patients with metastatic, castration-resistant prostate cancer

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Purpose: Angiogenesis and inhibition of host immunity contribute to the progression of metastatic castration-resistant prostate cancer (mCRPC). Both lenalidomide (LEN) and metronomic cyclophosphamide (CTX) have known anti-angiogenic and immunomodulatory activities and reduced toxicity relative to standard chemotherapeutic protocols.

Experimental Design: A phase-I/II, dose-escalation trial of LEN plus oral CTX was conducted to establish the maximum tolerated dose (MTD) and potential clinical and immunoregulatory efficacy in patients with previously treated mCRPC. In the phase-I study, CTX was given 50 mg PO QD (day 1-28) and LEN was given 10-25 mg PO QD (day 1-21) on a 28-day cycle. A “3+3” study design was used. In phase II, patients received LEN 25 mg PO QD (day 1-21) with CTX 50 mg PO QD (day 1-28) on a 28-day cycle. Quantification of circulating tumor cells (CTCs) and prostate-specific antigen (PSA) levels were performed, as well as measurement of peripheral T-regulatory (Treg) cells, myeloid-derived suppressor cells (MDSCs) and cytokine plasma concentrations.

Results: In phase I, 19 patients with mCRPC were enrolled, and all patients were evaluable for toxicity. The MTD was not observed at any of the dose levels (DLs) tested. Six patients received treatment on phase II before the trial was closed (sponsors withdrew support for drug). Overall, 7 of 22 evaluable patients (31.8%) experienced a ≥ 50% reduction in PSA. One patient had a partial response, and stable disease was documented by bone scan in 15 of 22 (68%) evaluable patients after two cycles of therapy.

Decreases in CTCs were documented in 5 of 22 (22.7%) and remained stable in 7 of 22 (31.8%) patients. The number of peripheral MDSCs at baseline was significantly increased relative to normal donors and directly correlated with CTC numbers (but not PSA levels) and inversely with T- and B-cell frequency supporting immunosuppression. Therapeutic intervention significantly reduced the frequency and absolute number of MDSCs that was associated with a stabilization of T-, but not B-cell frequency; further, IL-6, but not IL-8, significantly correlates with PSA and CTC levels.

Conclusions: The combination of LEN and metronomic CTX can be safely administered, resulting in a recommended phase-II dose of LEN of 25 mg PO QD (day 1-21) with CTX given 50 mg PO QD (day 1-28) on a 28-day cycle. The preliminary findings of the reversal of cellular suppressors by this novel combination are encouraging; yet, further research is required to refine the efficacy of this regimen, as well as validation of the preliminarily biomarkers of clinical response.
Association of calcium and vitamin D intake and vitamin D receptor genotypes with prostate cancer in multiethnic samples

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Background: Prostate cancer (PCa) is the most common cancer among men in the U.S., and African American (AA) men have higher incidence and mortality rate compared to European American (EA) men. Social and behavioral factors affect stage and tumor grade at diagnosis, treatment choice, and mortality. However, the cause for PCa disparities is still unclear. Several roles have been proposed for calcium, vitamin D, and the vitamin D receptor (VDR) in PCa pathogenesis and progression, but epidemiologic studies, mainly conducted in European descent populations, often show inconsistent evidence of associations. Here, we investigated the association of calcium and vitamin D intake and VDR genotypes with prostate cancer incidence and aggressiveness in multiethnic samples.

Methods: The total of 1,403 individuals was included in this study (751 AAs, 481 EAs, 126 Hispanic Americans, and 45 others). Study participants were recruited from six hospitals in Chicago, IL and Washington, D.C. Calcium and vitamin D intake was evaluated using the Block calcium and vitamin D screener. Seven single-nucleotide polymorphisms (SNPs) in and around the VDR gene and 105 ancestry informative markers were genotyped. STRUCTURE was used to estimate genetic ancestry. We performed logistic regression analyses adjusting for relevant variables using SPSS.

Results: In the pooled data set, calcium and vitamin D intake was not associated with PCa risk (P>0.05), but high total calcium intake (≥800 mg/day) was significantly associated with aggressive PCa (Gleason Score ≥4+3, P=0.002, OR=2.05, 95% C.I.: 1.29-3.26). In the race/ethnicity stratified analyses, we confirmed the statistically significant associations of calcium intake with aggressive PCa in AAs. High total vitamin D intake (≥600IU/day), on the other hand, revealed a protective effect against aggressive PCa (OR=0.47, 95% C.I.: 0.24-0.92, P=0.026). In EAs, total calcium intake was significantly associated with PCa aggressiveness, only when we compared PCa cases with Gleason Score ≥4+3 to cases with Gleason Score <4+3 (P=0.047, OR=2.52, 95% C.I.: 1.01-6.27). In AAs, the G allele of rs11568820 (Cdx2) had a protective effect, and AG/GG genotype was strongly associated with high risk PCa (P=0.014, OR=0.35, 95% C.I.: 0.15-0.81). In EAs, the odds ratio for BsmI AG/GG genotypes was 0.33 (95% C.I.: 0.14-0.76, P=0.009) in EAs. We also observed evidence of interactions between Cdx2 genotypes and total calcium intake and between TaqI genotype and total calcium intake in AAs. Significant interactions were also observed between TaqI genotype and total calcium intake in EAs.

Conclusion: In this study, we showed that high calcium intake increases the risk of prostate cancer aggressiveness, while high vitamin D intake has protective effects. Although a larger sample size is necessary to confirm the observation, we demonstrated that VDR genotypes may modify the effect of calcium intake. The findings from this study may help develop better PCa management plans.
Schedule dependence of topical rapamycin to prevent UV-induced non-melanoma skin cancer in vivo

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Background: The PI3Kinase/Akt/mTOR pathway has important roles in cancer development for multiple tumor types. However, little is known about the role of this pathway in non-melanoma skin cancer (NMSC) caused by solar-simulated UV light (SSL).

Results: Acute treatment of SKH-1 hairless mice with SSL (105 kJ/m2 UVA/6.4 kJ/m2 UVB) caused immediate phosphorylation of Akt (S473), mTOR (S2448) and p70S6 Kinase (T389) in the epidermis, which peaked at 30 min and resolved after 4 hr. Reverse-phase protein microarray analysis of these samples revealed statistically significant expression of multiple phosphorylated proteins of interest as a function of time after SSL exposure. We therefore hypothesized that inhibition of this pathway in the context of SSL exposure would lead to reduced tumorigenesis in mice. To test this hypothesis we used SSL to induce skin carcinogenesis in female SKH-1 hairless mice treated with topical rapamycin to inhibit mTOR activity. Rapamycin is currently FDA-approved for prevention of organ transplant rejection and has been shown to lessen the incidence of skin squamous cell carcinomas in transplant patients requiring immunosuppressants vs. calcineurin inhibitors. Acute exposure to SSL of rapamycin-treated mouse skin resulted in efficient inhibition of mTOR signaling in the epidermis. The skin tumorigenesis experiment consisted of three treatment arms. All mice were exposed to SSL (93% UVA, 7% UVB) three times a week for 15 weeks (cumulative dose of 427 kJ/m2 UVA/33 kJ/m2 UVB). SSL treatment was then stopped but mice continued drug treatment and observation until sacrifice at week 28.

The treatment arms included: 1) Vehicle control (application of 200ul/back of acetone for weeks 1-28); 2) Rapamycin Prevention (50 nmol of rapamycin/back for weeks 1-28); and 3) Rapamycin Intervention (vehicle during weeks 1-15, but switched to 50 nmol of rapamycin for weeks 16-28). All vehicle/drug treatments were performed topically 1 hr prior to each light exposure and continued 3 times a week after light stopped (weeks 16-28). At the conclusion of the experiment, the Rapamycin Intervention protocol displayed reduced multiplicity and tumor burden compared to the vehicle control arm. However, mice in the Rapamycin Prevention group experienced increased tumor multiplicity and burden compared to vehicle control. Although the absolute measurements in groups 2 and 3 were not significantly different from the vehicle control, mixed model analysis indicates that the slopes of the multiplicity and burden curves for the treatment groups were significantly different from the acetone group (p < 0.001).

Conclusions: Together these data indicate that carcinogenic effects of rapamycin co-exposure with SSL may be schedule-dependent and may warrant additional warnings for sun exposure during clinical use. This work was supported in part by the following NIH grants: P01CA27502 and P30CA23074.

(Supported in part by the American Cancer Society Institutional Research Grant 74-001-34 from the American Cancer Society)
Developing selective hMC1R RadTag-Multimodal Bio-imaging for the Earlier Diagnosis and Treatment of Melanoma

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This work introduces the most sensitive and specific agents for targeted delivery of imaging and therapy to metastatic melanoma. These agents contain two molecules as conjugates: the most selective ligands for the human melanocortin 1 receptor, which is the highest expressed protein on the melanoma cell surface (MC1R) and the RadTag-Antigen/antibody-Melanoma. By applying these two molecules simultaneously, one can obtain the most sensitive and specific imaging of melanoma and also, trigger the apoptosis of the metastatic melanoma. The novel conjugated agents will provide a most sensitive platform that can be labeled and activated as needed for the early and accurate diagnosis and treatment of melanoma.
Histopathological Identification of an Aggressive Nuclear Phenotype in Thin Melanomas and Skin SCCs

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Introduction: Thin cutaneous melanomas (<1 mm thick) are quite common, but it is unclear which of them will metastasize. Karyometry uses nuclear chromatin patterns to differentiate nuclei by morphologic characteristics. In a previous publication, we used karyometry to identify an aggressive nuclear phenotype associated with recurrence or metastasis of squamous cell carcinomas of the skin (SCCs) (Glazer, Bartels, Alberts, et al. Cancer Prevention Research, 4:1770-1777, 2011). We hypothesized that the percent of aggressive nuclei (classification score [CS], as measured by nuclear karyometry, can distinguish between thin melanomas that have metastasized and those that have not.

Methods: We reviewed the records of patients with thin melanomas who underwent resection and who had no metastasis at diagnosis. In a blinded fashion, we used nuclear karyometry to determine the CS in each primary tumor, according to statistically distinct nuclear features obtained from 115 nuclei from each lesion. We collected clinicopathologic data and compared melanomas that metastasized with those that did not.

Results: Our study group included a total of 18 patients without metastatic disease and 10 patients with metastatic disease. In both subgroups, the median follow-up was 4.6 years. The average age in the nonmetastatic subgroup was 62 ± 15 years; in the metastatic subgroup, 50 ± 16 years. Most patients in each subgroup were male. No melanomas had ulceration; only 1 patient in the metastatic subgroup had a melanoma with a high mitotic index. The mean tumor thickness in the nonmetastatic subgroup was 0.53 ± 0.25 mm; in the metastatic subgroup, 0.68 ± 0.26 mm (P = 0.2). We found that 4 karyometric nuclear features distinguished between aggressive and nonaggressive melanomas (P < 0.001). The mean CS in the nonmetastatic subgroup was 34% ± 5%; in the metastatic subgroup, 69% ± 8% (P < 0.001). The sensitivity of our analytical technique was 0.80; the specificity, 0.72; and the accuracy, 75%.

Conclusion: In our study we documented quantitative nuclear chromatin phenotypes associated with thin melanomas that metastasized and those that did not. Thus, nuclear karyometry could prove a useful tool to identify patients with thin melanomas that are likely to metastasize. Prospective, blinded validation studies are needed to compare karyometric analysis to other clinicopathological characteristics and putative biomarkers of thin melanoma metastatic potential. A large, validation study is under design and in search of funding.
POSTER ABSTRACT 16

Telephone Counseling and Education with Latinas with Breast Cancer

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Introduction: Latinas with breast cancer experience significant health disparities in cancer treatment. The purpose of this study was to compare a telephone delivered 8-week supportive health education intervention (SHE) with an 8-week telephone interpersonal counseling intervention (TIP-C) to improve QOL with 106 Latinas with breast cancer. These data are from a larger RCT still in progress.

Method: This study used an RCT design with Latinas and their supportive partners randomly assigned to either SHE or TIP-C. Measurement occurred 4 times over 6 months, baseline (T1), immediately post intervention (T2), and at 4 and 6 months post-T1. All study related materials, assessments and sessions were conducted in English or Spanish, depending on patient preference. Average session time ranged from 21.11-28.63. TIP-C sessions were longer by about 5 minutes.

Results: Latinas in the SHE (n=44) and TIP-C (n=62) were not significantly different for demographic characteristics, except for education, employment and income. Women in the SHE had lower incomes and education, with women in TIP-C more likely to be unemployed. For illness characteristics, no significant differences were found for stage, type of treatment, other chronic illnesses or current medications. Significant differences were found for women in TIP-C for recurrence, anxiety and anxiety treatment. There were significant differences found between groups for depression, anxiety, perceived stress, symptom number and symptom distress. Latinas in the TIP-C demonstrated greater improvement than the SHE group in these outcomes over time.

Implications: Telephone delivered counseling interventions may be a cost-effective method to provide psychosocial interventions with global populations.
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**Wearable sensor-based balance training in older adult cancer patients with chemotherapy-induced neuropathy: A randomized controlled trial**

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**Background:** Chemotherapy-induced peripheral neuropathy (CIPN) can affect lower extremity joint proprioception leading to balance deficits and increased fall risk. This study was aimed to evaluate the effect of a sensor-based exercise program on improving postural control in older adult patients with CIPN.

**Methods:** Twenty two patients (Age 70.3±8.7 years) with objectively confirmed CIPN (vibration perception threshold test score >25 Volt, VPT: 49.6 ± 26.7V) were randomized to 4 weeks (twice a week) of sensor-based training including weight shifting and virtual obstacle crossing with real-time visual feedback of lower-extremities through wearable wireless sensors (Intervention Group (IG), n=11)) or no intervention (control group (CG), n=11)). Outcome measures included changes in sway of ankle, hip, and center of mass (CoM) in both mediolateral (ML) and anterior/posterior (AP) directions during standing in feet closed (FC) position (both feet next to each other) and in semi-tandem position (big toe of one by arch of the other foot), at baseline and post-intervention. These measures have been related to balance deficits and increased risk of fall [1,2]. Additionally, gait speed and gait variability were assessed. All assessments were made using wearable sensors.

**Results:** Post intervention, sway of hip, ankle and CoM (ML) were significantly reduced in the IG compared to the CG during FC position (p=.010-.022). During the more challenging position of semi-tandem, all sway parameters except ankle were reduced significantly (p=.008-.039). Effect sizes were moderate-large (eta squared=.255-.388). No effects were found for gait parameters.

**Conclusions:** To our knowledge, this is the first randomized controlled study demonstrating improvements in balance among CIPN patients from wearable sensor-based training. We speculate that the sensor-based training with real-time visual joint position feedback provided participants with enhanced information about joint movements and motor error in order to compensate for deteriorated/lost lower extremity joint proprioception from CIPN. We envisage that the proposed sensor-based balance training can be easily translated to be able to be performed with minimum training in a home-setting, thus will decrease fall risk and improve cancer patients’ quality of life.

**References cited**
Evaluation of pH in a lung fibrosis mouse model using respiration gated acidoCEST MRI

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Introduction: During the development of idiopathic pulmonary fibrosis, it is hypothesized that there is an acidosis-dependent activation of transforming growth factor beta (TGF-β) resulting in activation of fibroblasts, thus producing scar tissue and causing impaired lung function that often results in death. Directly measuring pH of fibrotic lesions in a mouse model may significantly strengthen the hypothesis that fibrosis is associated with acidosis. This study uses a novel respiration gated acidoCEST-MRI method to noninvasively measure the pH of lung fibrotic lesions to correlate pH with fibrotic lesion volume.

Methods: 15 C57BL/6 mice were exposed to 2 units/kg of bleomycin by inhalation to create the fibrotic model. 7 mice were imaged on day 14, day 21 and day 28 post exposure and the remaining 8 mice were imaged on day 18, day 25 and day 32 post exposure. Prior to the imaging scan, a bolus of 200 μL iopamidol at 300 mgI/mL was injected i.v. via the catheter. Another bolus of 500 μL iopamidol was injected into the i.p. cavity at 150 μL/hr. A novel respiration gated CEST-FISP pulse sequence with a 5 second saturation period consisting of 2.8 mT power, 90 Hz bandwidth and 54 saturation frequencies between +10 and -10 ppm was used to obtain an acidoCEST MRI result in 5.8 minutes on a 7T Bruker MRI scanner. This acidoCEST MRI scan was repeated six times. To generate pixel-wise pH maps of the lesions, the six CEST spectra for a pixel were averaged, Gaussian filtering was used to smooth the CEST spectrum, each CEST spectrum was fitted to a single function with a sum of three Lorentzian line shapes using Matlab and only CEST effects greater than 2√2*noise were retained (which represents a 95% probability that the CEST effect is real), and the pH was determined form a CEST-pH calibration performed using an identical acidoCEST MRI protocol. In addition, the percent of lesion volume that showed at least one CEST effect was used to calculate percent uptake of the agent, which was used as a biomarker to estimate vascular perfusion. A T2-weighted image was used to measure lesion volume, using a basic RARE8 sequence.

Results: Using a respiration gated sequence significantly reduces motion artifacts in CEST-FISP MR images. The pH and percent uptake values show a significant increase at Day 28 and 32 relative to Day 14, while the lesion volumes showed a significant decrease during this time period.

Conclusions: An inverse relationship between pH and lesion volume implies that as the disease is progressing or growing in size, there is an increase in acid content, which supports the hypothesis that acidosis is associated with the development of idiopathic pulmonary fibrosis. More generally, this study shows that respiration gated acidoCEST MRI can measure pH of lung tissue thereby providing opportunities to investigate other pathologies and biological processes that require noninvasive measurements of lung pH.
Nipple sparing mastectomy: Risks of wound complication in the setting of neo-adjuvant chemotherapy

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Background: Surgical care of breast cancer has evolved significantly over the past 40 years. Nipple sparing mastectomy (NSM) is increasingly used for women with malignant breast disease. There are limited recommendations about the role of NSM in patients receiving aggressive neo-adjuvant chemotherapy. The purpose of this investigation is to determine whether NSM in the setting of neo-adjuvant chemotherapy increased the risks for wound complications.

Methods: A retrospective chart review of nipple sparing mastectomy procedures from 2007 to 2014 for breast cancer at a single institution was performed. Data was examined from our surgical database with appropriate IRB approval. 116 breasts in 64 patients were identified as meeting criteria for evaluation. Data were collected on complications, pre-operative exposure to chemotherapy, smoking history, prior radiation, presence and type of reconstruction.

Results: Complications were classified as minor- seroma, delayed wound healing, epidermolysis; major -implant infection, nipple necrosis, nipple loss, skin necrosis, implant loss, implant rotation; surgical - deep venous thrombosis (DVT). 30 of the 116 (26%) breasts had a complication; 12 minor, 16 major and 2 surgical. 22 of the 116 breasts were exposed to neo-adjuvant chemotherapy. Of the 22 breasts in the neo-adjuvant group, a total of 5 complications were identified (22%); 1 minor (seroma) and 4 major (implant infection, nipple necrosis with nipple loss, implant rotation and implant loss). Of the 69 breasts in the non neo-adjuvant group, there were a total of 25 complications (36%); 11 minor (seroma, minor areolar loss, delayed wound healing and epidermolysis) and 12 major complications (nipple necrosis with nipple loss, skin necrosis) and 2 surgical complications (DVTs) were identified. Of the 45 breasts without complications, two breasts received immediate implant reconstruction with one breast having minimal implant rotation.

Conclusions: Nipple sparing mastectomy is emerging as a safe and adequate option for the management of malignant breast disease. In the neo-adjuvant chemotherapy group, although the rate of complication was lower than in the non-chemotherapy group, 22% vs 36%, the complications were mostly major complications versus in the non-chemotherapy group where the incidence of major and minor complications were similar in number. Our study is limited by small sample size, and our overall complication rate is higher than that reported by other groups. Further studies are planned to evaluate the utilization of nipple delay prior to nipple sparing mastectomy in a wide range of patients to reduce the rate of complications such as nipple necrosis and nipple loss.
Cardiac Vagal Control and Coping Strategies as Predictors of Depression in Breast Cancer

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Background: Depression is related to poorer outcomes in breast cancer patients. Cardiac vagal control, indicating increased efferent parasympathetic activity, is an important physiological variable involved in affect regulation, coping with stressors, and depression. This study examined whether cardiac vagal control, as measured by respiratory sinus arrhythmia (RSA), and coping strategies were associated with depressive symptoms over 12 months following breast cancer diagnosis.

Methods: We studied a consecutive sample of 171 women with Stage 1-4 breast cancer (months since diagnosis = 2.02±0.78; range .5 to 4.2), taking no medications that affect cardiac function. At study entry, a 5-minute electrocardiogram segment was recorded. Participants completed the Center for Epidemiologic Studies Depression (CES-D) scale and a measure of cancer-related coping (COPE) at study entry and at 6, 12, 18, 24, and 36 weeks. Different coping strategies were examined separately in multilevel models along with RSA to predict CES-D. Mean levels of coping responses across study assessments were used in the analyses. Age, education, employment and treatment variables were included.

Results: Women with lower RSA exhibited higher depressive symptoms at study entry (b = (-) 2.40, p < 0.01). Depression decreased more rapidly over time in those with low RSA, and no significant difference in depressive symptoms between high and low RSA subjects remained at 9 months after study entry. There were no associations between RSA and coping strategies (rs <0 .11|, ns). Higher mean emotion-expression coping was associated with higher initial depressive symptoms (b = 3.60, p <0 .05), and a faster decline in depression (b= (-) 0.90, p<0.05).

Conclusion: Cardiac vagal control was associated with lower depression during the first year after diagnosis of breast cancer. Over time, most women returned to a relatively low level of depression, and coping and RSA play a role in determining initial depressive symptoms and the rate of recovery.

*This work was supported by a Del Webb Basic-Clinical Partnership Grant to Drs. Weihs and Allen.
Imaging breast cancer risk biomarkers using quantitative MRI

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Introduction: Breast cancer is the most common cancer in the United States. About 1 in 8 (12%) of American women will develop invasive breast cancer during their lifetime. One of the known biomarkers for breast cancer risk is the increase in breast density (BD). BD is routinely measured in mammography by comparing the ratio of stromal tissue to fatty tissue. Since increased BD is associated with a higher risk for breast cancer, therapies that reduce BD have been proposed as cancer preemptive treatments. Studies that assess the effect of BD reduction therapies require following changes in BD longitudinally. Unfortunately, the radiation exposure in mammography makes the technique impractical for serial studies of BD. Moreover, in quantitative studies of BD, mammography has been shown to have poor reproducibility.

Magnetic resonance imaging (MRI) is a non-invasive three-dimensional imaging modality that uses non ionizing radiation. MRI is sensitive to the presence of fat and water in tissues and thus, provides an alternative method to measure BD. MRI is also sensitive to the diffusion of water molecules in tissues, a property that has been linked to an increase in cellularity in tissue.

Results: As part of a collaborative effort between the Cancer Imaging and Cancer Prevention and Control programs at the Arizona Cancer Center, we have developed unique MRI techniques to measure biomarkers of BD. One technique is based on a radial gradient- and spin-echo technique developed and the University of Arizona, which provides a quantitative measure of the fat fraction (FF) in the breast. Another technique uses a diffusion-weighted MRI method to estimate the apparent diffusion coefficient of water (ADCw) in the breast. Both FF and ADCw can be used as surrogates of BD. To improve the efficiency and accuracy of FF and ADCw we developed, respectively, (1) a new algorithm for the automated segmentation of the breast region-of-interest (ROI) and (2) a new multi-component signal model for the accurate estimation of ADCw that accounts for the presence of adipose tissue in the breast. The automated breast ROI segmentation algorithm was evaluated against the manual tracing of experts. The performance of the new algorithm was excellent showing a mutual overlap greater than 0.88. The multi-component signal model for the estimation of ADCw was evaluated using computer simulations, phantoms, and in vivo. The results show that the model yields accurate values for ADCw (error <5%) and is insensitive to the presence of fat throughout a wide range of FF (0-0.45). The multi-component model outperformed the conventional single-component model where the error in ADCw ranged from 5-50% increasing with the amount fat present.

Conclusions: Novel non-invasive MRI techniques for measuring and following BD without exposing subjects to non-ionizing radiation have been developed. The new techniques can be safely used in subjects enabling studies related to changes in BD such as the molecular and cellular bases for breast cancer prevention and efficacy of chemoprevention treatment.
Use of multiple wire localization for breast conservation surgery

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Background: Mastectomy is often indicated for larger sized breast cancers, breast cancers with extensive calcifications or patients with higher tumor: breast ratios. Recently there has been a marked increase in the rate of mastectomies versus only a slight increase in breast conserving therapy (BCT). In 2004, the rate of mastectomies was 35% and by 2006, had jumped to 60%. This has prompted newer studies to evaluate the long-term outcomes of both mastectomies and BCT which have noted a higher overall survival and a breast cancer specific survival in patients undergoing BCT. There is a need to find reliable, accurate breast cancer localizing techniques to allow larger masses to be excised using BCT without affecting re-excision rates or local re-occurrence. One method is to perform wire bracketing of larger, more complex breast cancers to better outline a border for surgical resection, allowing for BCT with clear margins. A recent study states the re-excision rate for positive margins for BCT was 21.6%.

Method: For our study, we compared breast cancer tumor size and the rate of re-excision for positive margin in BCT using three or more wires to bracket breast lesions versus two wires. A single institution retrospective review of 71 female subjects with non-invasive or invasive breast cancer who underwent a partial mastectomy with two or more localization wires from 2007 to 2013 was performed. Inclusion criteria include patients over the age of 18 who have a diagnosis of breast cancer, either non-invasive or invasive who underwent a partial mastectomy with multiple wire localization. All patients had biopsy proven carcinoma in situ or invasive carcinoma. Comparisons were made between partial mastectomies performed with two or less wires and three or more wires and the rate of return to the operating room for re-excision of positive or close margins noted on finalized tissue pathology results.

Results: In our study, 16 patients had three or more wires and 55 patients had two or less wires. For the lesions localized with three or more wires, the average size of the lesion was 3.8cm (range 1.13cm-6.47cm) and for the lesions localized with two or less wires 1.7cm (range 0.38cm-3.0cm). Two out of the 16 patients (12.5%) in the three or more wire group required additional surgery for re-excision due to positive or close margins on final pathology results versus five (11.4%) of the 55 patients (9.1%) in the two or less wire group, however, was not found to be statistically significant. Overall, our re-excision rate was found to be 9.86%.

Conclusion: Our study demonstrates there is no statistically significant increased risk for re-excisions based on the number of wires used. However, it was observed that larger breast lesions were localized with three or more wires for excision. We can conclude that localizing larger areas is feasible and can lead to further breast conservation. Further study of long-term outcomes is warranted.
Evaluation of Real-time Multi-modal Image Processing to Detect Oral Neoplasia

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Introduction: Oral cancer is treatable if detected early; however, a majority of cases are diagnosed in more advanced stages, leading to a high rate of morbidity and mortality. Currently, the standard of care is white light examination and palpation. However, with this method there is poor contrast between normal and abnormal tissue. Wide-field autofluorescence imaging (AFI) is a technique that can rapidly screen a macroscopic region of tissue and identify areas of high risk. In the clinic, it has been observed that neoplastic tissue shows a loss of autofluorescence due to the breakdown of collagen cross-links. By implementing automated image analysis, the loss of autofluorescence can be quantified over an entire image. This would enable quantitative real-time analysis, allowing this method to serve as an objective diagnostic aid to clinicians.

Results: In order to implement AFI in the clinic, an autofluorescence image processing Matlab program was optimized and validated. In addition, an automated image registration algorithm was developed to eliminate offsets due to movements between the white light and autofluorescence images. While using this algorithm, an outline of an area of interest in the white light image is overlaid on the registered autofluorescence image. The algorithm goes through a series of steps quantifying the autofluorescence intensity within the area of interest. The end result of the program is a white light image highlighting areas with loss of fluorescence, indicating potential cancer sites. The image-processing algorithm will then help guide the biopsies. In a clinical study at MD Anderson Cancer Center in Houston, Texas, 41 images from 30 patients were compared using the automated registration algorithm versus a manual selection method. The automated method proved to be statistically different compared to the manual selection method in quantifying and normalizing the loss of fluorescence. Both methods proved to have similar diagnostic performance. The benefit of using the automated algorithm is to decrease the dependency of the operator processing the images.

Conclusion: These algorithms are promising approaches for improved image analysis that will aid in diagnostics and better patient outcome.
**POSTER ABSTRACT 24**

**Measuring pH by MRI with only one CEST signal of clinically approved agents**

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**Background:** Low Extracellular pH (pHe) is a hallmark of the tumor microenvironment [1]. A non-invasive MRI method, term “acidoCEST MRI”, has been used to accurately measure pHe and assess tumor acidosis in murine models of cancer [2]. AcidoCEST MRI is performed using Ultravist®, a contrast agent originally approved for CT. We have translated acidoCEST MRI to the clinic, and a clinical trial is under way to assess the potential of acidoCEST to measure pHe in patients with breast cancer and correlate it with their response to therapy. However, in vivo acidoCEST MRI is still a challenging new technique because it requires a relative high concentration (5-10 mM) of the contrast agent, and the generation of at least two CEST MRI signals by the contrast agent. Ultravist fulfills these requirements, but few clinically approved agents do, which limits the discovery and applications of new clinical CEST MRI contrast agents. On the other hand, it is not possible to measure pH higher than 7.0 units with the current method. We hypothesize that CEST MRI acquired at different radiofrequency saturation powers can be used to measure pH with a single CEST signals and at pH > 7.0 units.

**Methods:** Six samples of Ultravist ® and Hexabrix ® (20 mM) were prepared in PBS and adjusted to a final pH of 5.5, 6.2, 6.4, 6.8, 7.0, 7.2, and 7.4. The sample at pH of 5.4 units was also prepared at 5, 10, 20, and 50 mM. MRI Acquisition: A CEST-FISP pulse sequence (1.0 μT, 4.7 sec, Rect. Pulse), with 61 saturation frequencies (+10 to -10 ppm) was used to acquire an acidoCEST image in 4.8 min on 7T MRI scanner. The same experiment was repeated 9 times increasing the saturation power by 0.5 μT (10 experiments, from 1.0 to 5.5 μT). MRI Processing and Analysis: Each CEST spectrum was fitted to a single function that consisted of a sum of three Lorentzian line shapes for Hexabrix, and four Lorentzian line shapes for Ultravist (Matlab R2013B). The amplitude, width, and offset of each Lorentzian curve were calculated.

**Results:** Ultravist and Hexabrix present CEST signals at 4.2 and 4.6 ppm relative to water; we used the relative amplitudes of these signal at 9 different saturation powers relative to 1.0 μT to construct the model \( Y = mX + b \), where \( Y = \text{pH} \), and \( X = \text{CEST amplitude at power N / CEST amplitude at 1.0 μT} \). This model is valid from 5.5 to 7.4 pH units with \( r^2 > 0.98 \).

**Clinical Significance:** These results make it possible to image pH within the physiological range. Requiring only one CEST signal open the possibility to test several X-ray contrast agents that have been approved by the FDA as pH contrast agents for MRI.

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Disparities in the Incidence of Colorectal Cancer in the State of Arizona; Race, Gender and Socioeconomic Status

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Background: Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer related deaths in the US with more than 150,000 of new cases and approximately 50,000 deaths each year. Arizona is the 15th largest state by population and the 8th fastest growing state in the US. It was estimated that in 2014 there will be 2,560 of new CRC cases in Arizona with 990 deaths from this disease. Disparities have been shown in CRC incidence amongst different races. African Americans (AA) have been shown to have a higher overall incidence and mortality rates. In 2012, CRC was the second most commonly diagnosed cancer among White Hispanics (WH) who comprises 30% of population in Arizona. Moreover, there is significant representation of American Indians (AI) in Arizona who usually manifests more advanced stage disease compared to other races. Furthermore, gender disparities in mortality rates and cancer incidence are often reported. The association between socioeconomic status (SES) and cancer incidence and survival has been well established. Since Arizona does not participate in the SEER national database, the purpose of our study was to determine inequalities in the incidence and mortality rates of CRC in Arizona with special attention to race, gender and SES.

Results: A retrospective study of 40,314 CRC cases from the Arizona Cancer Registry between 1995 and 2011 was conducted. Differences in categorical variables were assessed by the Fisher exact test or $\chi^2$ test. Logistic regression analysis was performed to identify the association between socioeconomic factors and changes in CRC incidence. The overall CRC incidence in Arizona decreased by 17% what mirrors the national trend. From all CRC cases reported in Arizona, 47% accounted for females and 53% for males. Majority of them (85%) were reported in White non-Hispanics (WNH), 9% in WHs, 3% in AAs and 1% in Asian/Pacific Islanders (A/PI). The highest incidence rates were seen in WNHs and AAs while AIs and A/PIs showed the lowest rates. However, while WNHs and AAs had mostly right-sided tumors and showed significant decrease in incidence (17.6% and 13%), an increasing trend was observed in AIs with the majority of rectal tumors and A/PIs with the majority of left-sided tumors. With regard to gender, females were more likely to develop right-sided tumors while males developed rectal tumors that might be an explanation for higher mortality rates observed in males. From all CRC tumors, 4% were stage 0, 34% stage I, 13% stage II, 21% stage III and 16% stage IV, which mirrors a stage distribution within all races with AAs having the highest occurrence of more advanced disease. There was a negative correlation between changes in CRC incidence and SES, such as education and household income were found.

Conclusion: AAs presented with a higher stage disease; males had higher mortality rates and more educated people and those with higher household incomes had lower decrease in CRC incidence.
Inherited breast cancer risk in Mexican and Mexican American women with invasive breast cancer

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Background: Breast cancer occurs earlier in Hispanic women than in non-Hispanic white women and risk factors, including parity and obesity, affect risk differently between these populations. In order to understand the genetic landscape underlying risk, we are sequencing breast cancer risk genes in germline DNA from 1222 Mexican American with breast cancer who volunteered for the ELLA study.

Results: Forty-six percent of participants in the ELLA cohort were under age 50 at diagnosis, suggesting the presence of inherited cancer risk. In the first 186 samples analyzed, we identified 14 women whose BRCA1 and BRCA2 genes encode an incomplete protein. These deleterious mutations clearly increase breast cancer risk. Seven additional women have clearly deleterious mutations in other breast cancer risk genes. In addition, more than 10% of participants carry sequence variants that are either novel or very rare and that have unclear effects on breast cancer risk. The problem of novel and difficult-to-characterize genetic variation is not unique to Mexican Americans, but it does disproportionately affect people from traditionally understudied ethnic groups. Sequencing of breast cancer risk genes from germline (non-tumor) samples of the women in the ELLA cohort will be completed in March 2015. Analysis of novel and rare variants of uncertain significance will continue.
MUC1 and polarity loss alter EGFR trafficking in EGFR-dependent breast cancer

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Background: MUC1 is an oncogenic protein overexpressed by cancers derived from ductal epithelium, including pancreatic, lung, and breast. MUC1 drives tumor progression by serving as an adapter protein, through which its cytoplasmic tail interacts with a variety of oncogenes and alters their localization and activity. One such oncogene, the Epidermal Growth Factor Receptor (EGFR), is normally highly regulated via ligand-mediated endocytosis, ubiquitination, and lysosomal degradation, although this regulation is lost in breast cancer.

Results: Our research has focused on understanding the effect of MUC1 expression on EGFR-dependent transformation and EGFR trafficking and activity in the breast. We have found that MUC1 and EGFR interactions, while missing in normal ductal epithelium, are highly enriched in metastatic breast cancer patient samples, cell lines, and mouse models of breast cancer. We have discovered that upon interaction with MUC1, EGFR trafficking is altered, promoting both its cellular internalization (without degradation) and its kinase-independent function as a transcription factor. It is unclear whether the EGFR-MUC1 interaction is a driver or consequence of apical-basolateral polarity loss. Based on our data, we hypothesize that EGFR-dependent breast cancer relies upon alterations in EGFR trafficking that can be initiated by its interactions with MUC1. We plan to investigate this hypothesis by determining the mechanism by which MUC1 alters EGFR localization and function. This will be studied using epithelial cells in which EGFR is colocalized to the apical side where MUC1 resides. Further, we will analyze these interactions in human breast cancer cell lines and patient samples to determine how their co-expression affects anti-EGFR therapy and explore the mechanism of our MUC1/EGFR interaction blocking decoy peptide.

Conclusion: Together, we expect these aims to define specific mechanistic targets of EGFR and MUC1 interactions that will be effective in treating metastatic breast cancer.
The Hugl1 polarity protein is a regulator of breast cancer stem cells

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Background: Maintenance of epithelial polarity is essential to the prevention of neoplasia. Polarity complexes regulate diverse cellular activities including growth, survival, migration and invasion, and tissue organization and size. There are 3 distinct polarity complexes, including the Crumbs complex, the Par complex and the SCRIBBLE/LGL/DLG complex. Of these, the SCRIBBLE/LGL/DLG complex is the only one whose loss results in the development of a tumor-like phenotype in model organisms. LGL loss drives proliferation, tissue overgrowth and invasion, key components of transformation and metastasis.

Results: We have shown that a loss of HUGL1 (human ortholog of drosophila LGL) expression results in a failure of growth control, gain of mesenchymal phenotypes and the acquisition of stem cell characteristics. While the exact mechanism by which HUGL1 regulates these phenotypes is unknown, the YAP/TAZ pathway regulates many of these same phenotypes, and is a known LGL effector. In addition, the Epidermal Growth Factor Receptor (EGFR) also regulates TAZ and is essential for its oncogenic phenotypes. It is unknown how HUGL1 regulates the transition between differentiation and stemness in mammary epithelium, how it regulates EGFR or the TAZ pathways, or whether it’s expression is essential to the suppression of breast cancer progression. We are working to understand how HUGL1 expression promotes polarity and differentiation in normal breast epithelium, how expression is lost in metastatic breast cancer, and whether we can re-activate the polarity program to treat metastatic breast cancer. We are working on the hypothesis that HUGL1 functions as a tumor suppressor through two key mechanisms – downregulation of growth factor receptors and inhibiting the activity of stem cell-specific transcription factors. Our hypothesis is based on preliminary data demonstrating that HUGL1 is expressed in normal differentiated mammary epithelium and is lost in primary breast cancers. Knockdown of HUGL1 in breast epithelium results in a mesenchymal phenotype that is promoted by EGFR activity. Furthermore, epithelial cells without HUGL1 expression have an enhanced ability to form mammospheres and display cancer stem cell markers.

Conclusions: We believe that an understanding of how loss of HUGL1 promotes a loss of differentiation will allow us to understand a regulator of cell fate and cancer progression that can be therapeutically targeted.
HCMV modulation of EGFR signaling

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Background: Human cytomegalovirus (HCMV) is a clinically important herpes virus that is ubiquitously found within the worldwide population and is associated with a number of major health concerns. HCMV persists in the human host through a latent infection where genomes are maintained in a reversibly quiescent state. Upon receiving cellular cues, such as through changes in cellular signaling and differentiation, HCMV can reactivate virion production to maintain a persistent infection. Reactivation occurs subclinically in the immunocompetent host, but can result in life-threatening disease in the immunocompromised. HCMV disease is a major cause of morbidity and mortality in leukemia and lymphoma patients following bone marrow transplantation. Further, viral disease can also manifest in cancer patients undergoing intensive chemotherapy regimens and can result in hearing loss, blindness, pneumonia, or gastroenteritis. Lastly, HCMV infection has been reported to have oncomodulation activities, promoting malignancy in cancerous cells. Little is known about the mechanisms involved in HCMV latency and reactivation. No vaccine exists for HCMV and antivirals do not target latently infected cells.

Results: To address this gap, our lab works to define the viral genes important to latency and reactivation. We have identified two viral proteins, pUL135 and pUL138, that antagonize one another to promote latency in the case of pUL138 or to overcome pUL138-mediated suppression for reactivation in the case of pUL135. To define the mechanisms by which pUL135 and pUL138 function, we screened for cellular interacting proteins. Intriguingly, both pUL135 and pUL138 target the epidermal growth factor receptor (EGFR), but with opposing effects. pUL135 decreases EGFR levels, while pUL138 increases EGFR levels at the cell surface by manipulating EGFR protein trafficking pathways. Based on the roles of pUL135 and pUL138 in mediating the latent infection, we hypothesize that pUL138 maintains EGFR signaling to promote latency and that pUL135 down-regulates EGFR from the cell surface to attenuate signaling to promote reactivation. Consistent with this hypothesis, EGFR inhibitors promote virus replication and reactivation from a latent state. Further, we have found that virus infection, and specifically pUL135 and pUL138, alter signaling pathways downstream of EGFR, including MAP kinase and PI3 kinase pathways. EGFR is involved in a multitude of homeostatic cellular processes.

Conclusion: Sustained manipulation of EGFR signaling by a persistent virus could promote an oncogenic environment within the cell. Understanding how pUL135 and pUL138 modulate EGFR signaling to control viral replication will provide a target for controlling HCMV reactivation in cancer patients and the immunocompromised.
POSTER ABSTRACT 30

Monitoring extracellular pH, spatial heterogeneity and contrast agent uptake in lymphoma tumor models with acidoCEST MRI

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Background: Extracellular pH (pHe) is a hallmark for tumor microenvironment. We have developed a non-invasive MRI method, termed “acidoCEST MRI”, that can accurately measure pHe to assess tumor acidosis.

Method: This method measures a ratio of the Chemical Exchange Saturation Transfer (CEST) effects of Iopromide (Ultravist®) using a CEST-FISP MRI protocol. Lopromide has two amides that generate different CEST effects, and a ratio of these CEST effects is correlated with pH over a range of 6.0-7.0 pH units in a manner that is independent of the agent’s concentration and the sample’s T1 relaxation time. The pixel-wise pHe mapping allows us to access spatial heterogeneity and also contrast agent uptake.

Results: We have applied acidoCEST MRI to investigate the differences in pHe between Raji, Granta519 and Ramos lymphoma tumor cell lines, the temporal relationship between tumor growth and acidosis, and the spatial heterogeneity of tumor acidosis. Our results showed mildly acidic pHe (6.74-6.85) in all 3 tumor models. For Granta519 xenografts, the pHe decreased significantly over the course of 3 weeks (ph 6.82-6.74, p-value = 0.02). There was no significant trend in spatial heterogeneity and growth rate for any of the three xenografts models. The Ramos tumors were statistically less spatially heterogeneous than Raji and Granta519 tumors (p-value < 0.006). Granta519 and Ramos xenografts did not show correlation of contrast agent uptake with tumor growth. However, as the Raji tumors became larger, the % contrast uptake increased. The % contrast agent uptake correlated with ex vivo VEGF-A score (r = 0.822).
Post-Transplant Bendamustine Effectively Reduces GvHD while preserving GvL in Experimental T-replete Murine Haploidentical Hematopoietic Cell Transplantation

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Background: Advances in haploidentical bone marrow transplantation (h-BMT) have drastically increased the treatment options for patients needing BMT. The possibility of significantly reducing the complications inherent to GvHD with the administration of post-transplant cyclophosphamide (PT-CY) has substantially improved the efficacy and applicability of T-replete h-BMT.

Objective: As higher frequency of disease recurrence remains one of the major challenges in reduced intensity conditioned h-BMT with PT-CY, we investigated bendamustine as a novel post-haploidentical transplant agent that can mitigate GvHD while sparing GvL.

Design/Method: We established clinically relevant murine experimental h-BMT models following myeloablative (MAC) or reduced intensity conditioning (RIC) (1000 cGy or 600 cGy total body irradiation, respectively). Conditioned BALB/c × A/J F1 (CAF1; H-2d/a) recipient mice bearing A20 B cell leukemia received h-BM with or without haploidentical splenocytes (h-SC) or purified T cells from BALB/c × C57Bl/6 F1 (CB6F1; H-2d/b) donor mice. The bifunctional mechlorethamine derivative bendamustine (BEN), an alkylating agent and purine analog, was given in a single dose on day +3 following h-BMT.

Results: We have reproducibly demonstrated that post-transplant bendamustine (PT-BEN) alleviates GvHD, significantly improving survival, while preserving engraftment and graft-versus-leukemia effects. PT-BEN was less myelosuppressive than PT-CY, significantly increasing the number and proportion of suppressive CD11b+Gr-1hi cells, while decreasing lymphoid cells. We further documented that PT-BEN can diminish GvHD even in the absence of T regulatory cells.

Conclusion: PT-BEN warrants further investigation as a new therapeutic platform for the clinical implementation in h-BMT.
Pathogen identification by DNA sequencing for neutropenic fever oncology patients

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Background: Neutropenic fever is a common side effect of chemotherapy. Neutropenic fever in oncology patients often indicates infection as a result of impaired immune function. Proper treatment requires diagnosis of the causative pathogen, typically by blood culture. Blood cultures involve growing colonies of bacteria on agar plates with specific nutrients followed by microscopy and biochemical assays to identify bacteria that grew. Bacterial identification by culture has many drawbacks including bias for the bacteria that will grow, bias for fast growing bacteria, no information about antibiotic susceptibility, and no ability to detect fungal or other types of pathogens. In the case of neutropenic fever in oncology patients the drawbacks to blood culture result in a diagnosis in only one half of patients. The lack of specific information about the pathogen causing fever leaves physicians making empirical treatment decisions that result in ineffective treatment of the infection (e.g. antibiotics prescribed for a fungal infection) and contribute to the development of drug resistance.

Results: With the advent of next-generation DNA sequencing, it became possible to use the sequence of portions (16s rRNA in the case of bacteria), or the entire genome to identify pathogens – essentially a “DNA fingerprint” unique to each species of pathogen. Pathogens are isolated from whole blood and DNA sequence libraries prepared for whole genome shotgun sequencing or gene-specific sequencing by polymerase chain reaction. The sequences generated can then be compared to a reference library to identify the pathogen(s) present in the sample. Sequencing-based approaches to identification of pathogens do not have the inherent bias that culture-based methods possess, and can be faster, cheaper, and more accurate. One problem with the approach, however, is the data analysis of high-throughput sequencing runs, including computational resources required and lack of appropriate reference sequences.

Conclusions: We have developed a method for using short DNA fragments for identification of pathogens on the Ion Torrent Personal Genome Machine, along with fast computational methods for data analysis. Combined, our methods could allow same day diagnosis of pathogens that cause neutropenic fever, along with drug susceptibility. These methods have the potential to improve patient outcomes by allowing physicians to make evidence based decisions how to treat their patients’ infections. Here we present our initial studies and proof of principle demonstrating our workflow for diagnosing infections in oncology patients with neutropenic fever.
Role of Transmembrane Protein CD47 in Tumor Evasion and Normal Tissue Survival In Cancer Treatment

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Background: We are pursuing a new anticancer therapeutic target, CD47, for drug discovery. Our team includes the laboratories of William Montfort (University of Arizona), David Roberts (NCI) and Thomas Miller (Paradigm Shift Therapeutics, PSTx). We seek to discover new compounds targeted to CD47, a transmembrane protein implicated in cancer cell evasion of innate immunity and poor prognosis for healing of normal tissue damaged during cancer treatment. In addition, we seek to characterize the regulation of downstream targets to CD47 that play a role in the progression of tumorigenesis, with an emphasis on the regulation of soluble guanylate cyclase (sGC).

Results: CD47 is a widely expressed transmembrane protein that has emerged as key for self-recognition by the immune system. Cells expressing CD47 are recognized as part of the home team and not engulfed by immune cells. Thrombospondin 1 (TSP1) was the first anti-angiogenic protein to be discovered and functions in part through binding to CD47. SIRPα, a central factor in immune recognition of same-self cells, also binds to CD47. Moreover, binding of CD47 by TSP1 and SIRPα convey key information about the status of the extracellular microenvironment and serve to limit cell and tissue survival in response to multiple types of stress. Because of this, CD47, TSP1, and SIRPα are increasingly targeted for drug discovery to treat diverse injuries and diseases, including surgery, transplantation, cardiovascular disease, diabetes, radiation injuries, and cancer. The Roberts laboratory also discovered that TSP1 binding to CD47 inhibits nitric oxide-dependent vasorelaxation, while the Montfort laboratory discovered that signal transduction involves a Ca2+-dependent phosphorylation of the nitric oxide receptor, soluble guanylyl cyclase (sGC). sGC is the primary nitric oxide effector, and has been implicated in the promotion of cell proliferation, survival, and angiogenesis. The mechanism of sGC inhibition through CD47 is currently under investigation.

Here, we focus on the role of CD47 in cancer and in response to radiotherapy. Over 60% of cancer patients receive radiation therapy during treatment, but success with this widely used approach is limited by the low tolerance of nearby normal tissue to radiation damage. Recent studies by our team and others have demonstrated that overexpression of CD47 by tumor cells interferes with the immune response for cancer cell removal while also inhibiting normal tissue healing after radiation damage. CD47’s dual role in protecting tumor cells while inhibiting survival of damaged normal tissue makes it an outstanding target for drug discovery and a novel new potential cancer treatment. CD47 has recently been targeted by immunotherapy, siRNA, morpholinos, and mouse knockout studies to confirm its importance as a target for cancer therapy. The Roberts laboratory has been a leader in discovering the role for CD47 in radiation recovery, where it inhibits survival of damaged tissue.

Conclusions: This combination of CD47 assisting in tumor cell escape from the immune system with suppression of radiation-damaged tissue recovery provides for an outstanding new target in cancer therapeutics. Our team is pursuing CD47 structure, function and inhibition (Montfort lab), regulation of CD47 downstream targets including sGC (Montfort lab), matricellular protein signaling (Roberts lab), and translational drug development (PSTx).
Combined proteasome and histone deacetylase inhibition promotes ER stress in non-small cell lung cancer cell lines

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Background: Non-small cell lung cancer (NSCLC) is a molecularly heterogeneous disease resulting in disappointing efficacy of many targeted therapies. Targeting the endoplasmic reticulum (ER) is one strategy that has shown selectivity for cancer vs normal cells and has activity across broad molecular phenotypes including lung cancer.

Objective: To investigate the combined synergy of carfilzomib CFZ), a second generation proteasome inhibitor (PI), and vorinostat (SAHA), a histone deacetylase (HDAC) inhibitor, in NSCLC cell lines and measure associated expression of ER stress response proteins.

Methods: NSCLC cell lines (H520, A549, H1993, H460, and H1299) were treated with CFZ, SAHA, or the combination and cell proliferation measured using the MTT assay. To determine synergism, the median effect method of Chou and Talalay was used and quantitated using Calcusyn software. Cell death was measured using trypan blue exclusion and assay of dead cell protease activity. Western blot analysis of whole cell lysates was used to measure markers of apoptosis, including cleaved caspase 3 and PARP, and to measure ER stress related protein expression. Reactive oxygen species (ROS) production was measured using the fluorophore H2DCFDA.

Results: Synergistic activity was observed for all five cell lines following 72 hours of combined treatment. The H520 and A549 cell lines were used to assess cell viability and apoptosis following treatment with each drug alone and in combination. Increased cell death was observed following combination treatment compared to monotherapies. An increase in cleaved caspase 3 was observed with combination therapy in both cell lines. CFZ treated resulted in similar changes to those observed with bortezomib (BTZ), a first generation proteasome inhibitor. Combination therapy was associated with increased ER stress regulated proteins including heat shock protein 70 (HSP70), activating transcription factor 4 (ATF4), GRP78/BiP, and C/EBP homologous protein (CHOP) and increased reactive oxygen species (ROS) in both H520 and A549 cell lines.

Conclusions: The combination of proteasome inhibition and HDAC inhibition results in ER stress which may contribute to the anti-cancer of this combination in lung cancer cell line models. Given CFZ’s favorable clinical tolerability and efficacy profile compared to BTZ, further pre-clinical and clinical studies of these two drug classes should include CFZ.
Pharmacological Modulation of Proteotoxic Stress Targeting Malignant Melanoma

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Background: It is now widely accepted that tumor cells are exposed to high levels of endogenous proteotoxic stress originating from mutation-driven expression of misfolded proteins and adverse conditions associated with the tumor microenvironment including hypoxia, energy crisis, and redox dysregulation. Pharmacological modulation of proteotoxic stress (by targeting the cellular heat shock or unfolded protein responses, the ubiquitin-proteasome system, or the autophagic-lysosomal proteolytic machinery) may trigger preferential cytotoxicity in cancer cells without compromising viability of normal cells displaying lower constitutive levels of endogenous proteotoxic stress. Dysregulation of proteotoxic stress has been observed in human melanoma tissue contributing to the notorious chemoresistance of metastatic melanoma cells. Cumulative evidence suggests the involvement of autophagic dysregulation in melanomagenesis, and the emerging role of autophagy as a prognostic factor and therapeutic target in melanoma has been substantiated recently. Moreover, pathological alterations affecting expression and function of heat shock proteins (including Hsp27, Hsp70, Hsp90, and GRP78) have been documented in human melanoma tissue. Specifically, Hsp90 serves as an essential factor stabilizing oncogenic V600EBraf in malignant melanoma cells, and its inhibition has emerged as a promising strategy for antimalanoma intervention. Based on this rational, our laboratory pursues molecular strategies that aim at increasing proteotoxic stress through pharmacological modulation of proteasomal, autophagic-lysosomal, or heat shock response functions for experimental and investigational chemotherapeutic intervention targeting malignant melanoma.

Results: Screening a focused library of compounds containing redox-directed electrophilic pharmacophores employing the Stress & Toxicity PathwayFinderTM PCR-array technology as a discovery tool, a drug-like triphenylmethane-derivative [aurin; 4-[bis(p-hydroxyphenyl)methylene]-2,5-cyclohexadien-1-one] was identified as an experimental cell stress modulator that causes (i) heat shock factor transcriptional activation, (ii) upregulation of heat shock response gene expression (HSPA6, HSPA1A, DNAJB4, HMOX1), (iii) early unfolded protein response (UPR) signaling (phospho-PERK, phospho-eIF2α, CHOP), (iv) proteasome impairment with increased protein-ubiquitination, and (v) oxidative stress with glutathione depletion. Fluorescence polarization-based experiments revealed that aurin displays activity as a geldanamycin-competitive Hsp90a-antagonist, a finding further substantiated by molecular docking and ATPase inhibition analysis. Aurin exposure caused caspase-dependent cell death in a panel of human malignant melanoma cells (A375, G361, LOX-IMVI), but not in non-malignant human skin cells (Hs27 fibroblasts, HaCaT keratinocytes, primary melanocytes) undergoing the aurin-induced heat shock response without impairment of viability. Aurin-induced melanoma cell apoptosis depends on Noxa upregulation as confirmed by siRNA rescue experiments demonstrating that siPMAIP1-based target downregulation suppresses aurin-induced cell death.

Conclusion: Taken together, our data suggest feasibility of apoptotic elimination of malignant melanoma cells using the quinone methide-derived heat shock response inducer aurin.
Resveratrol and SIRT1 are Novel Positive Modulators of Vitamin D Signaling via Apparent Deacetylation of VDR

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Background: Vitamin D, acquired via dietary sources/supplements or synthesized upon UVB exposure, is endogenously converted to the physiologically active secosteroid, 1,25-dihydroxyvitamin D (1,25D). 1,25D initiates its bioeffects by binding directly to the nuclear vitamin D receptor (VDR) and driving heterodimerization of VDR with the retinoid X receptor (RXR). The liganded VDR-RXR complex modulates gene transcription in 1,25D target tissues, including the small intestine, colon, kidney, bone, brain and skin; thus the chemopreventive properties of vitamin D stem from the regulation of cellular proliferation, differentiation, metabolism, and apoptosis. The significance of vitamin D in numerous facets of health stresses the importance of elucidating the molecular mechanism of 1,25D-VDR signaling modulators.

Results: Resveratrol (Res), a natural polyphenolic phytochemical and antioxidant, is one such putative VDR modulator. Res functions as a potent activator of NAD-dependent deacetylase sirtuin-1 (SIRT1), an enzyme highly associated with longevity in animal models. The current study employed mammalian-two-hybrid (M2H) and vitamin D responsive element (VDRE)-based transcriptional assays to investigate the potential effects of Res and SIRT1 on VDR signal transduction. Results from VDRE-based assays indicated that Res and SIRT1 potentiate 1,25D-VDR activity via cell and promoter-specific pathways. Transcriptional assays conducted in human colon cancer (HCT116), embryonic kidney (HEK293), osteosarcoma (TE85), and glioblastoma (U87) cell lines indicated that SIRT1 enhanced 1,25D-VDR activity in HEK293 and TE85 cells; however, no significant stimulation was observed in HCT116 or U87 cells. In addition, 1,25D displacement experiments revealed an increase in VDR-bound radiolabeled 1,25D in the presence of Res, suggesting that Res may potentiate VDR transactivation by promoting 1,25D binding. M2H assays in HEK293 cells were utilized to assess levels of interaction between VDR and VDR comodulators, including RXR, SRC-1, and DRIP-205. Both Res and SIRT1 increased the ability of VDR to associate with RXR; however, SRC-1 and DRIP-205 interactions were not enhanced. To analyze the acetylation status of VDR, the activity of a novel, non-acetylatable VDR mutant, K413R, was probed in VDRE-luciferase assays, revealing that K413R possesses amplified transactivation capacity in comparison to WT VDR in the presence of 1,25D. A specific SIRT1 inhibitor, EX-527, was also used to suppress endogenous SIRT1 levels, resulting in significantly decreased VDR transactivation. Finally, qRT-PCR in HEK293 cells was employed to examine how the 1,25D-dependent expression of an endogenous VDR target gene, CYP24A1 is affected by Res, SIRT1, and the combination of Res and SIRT1. Results revealed that 1,25D induction of CYP24A1 expression was further enhanced (185%) by co-expression of SIRT1, while Res increased 1,25D-dependent CYP24A1 expression by 394%. In the presence of 1,25D, SIRT1, and Res, CYP24A1 expression increased by 426% over 1,25D alone.

Conclusion: We conclude that acetylation of VDR comprises a negative feedback loop that attenuates 1,25D-VDR signaling. This negative loop is suppressed by resveratrol/SIRT1-catalyzed deacetylation of VDR, restoring full receptor activity and corresponding chemopreventive properties associated with 1,25D-VDR. In summary, this study illuminates a coordinated interaction between two nutritionally-derived anticancer lipids, vitamin D and resveratrol, thereby identifying a link between 1,25D-VDR signaling and SIRT1 function.
POSTER ABSTRACT 37

Gemcitabine resistant pancreatic cancer cell lines acquire an invasive phenotype with collateral hypersensitivity to histone deacetylase

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Background: Pancreatic cancer is the fourth leading cause of cancer death in the United States. One of the major reasons for the poor prognosis of pancreatic cancer is its high resistance to gemcitabine and other currently available chemotherapeutic agents. In the present study, we have developed gemcitabine-resistant cell lines to investigate the mechanisms by which adaptive resistance occurs during pancreatic tumor progression.

Results: Gemcitabine-resistant (GR) cell lines were established from the MiaPaCa2 cell line. Increasing drug selection pressure resulted in the establishment of populations that proliferate in the continuous presence of 300 nM (GR300), 800 nM (GR800), or 2µM (GR2000) gemcitabine. Initial drug exposure selected for an early resistant phenotype that was independent of drug metabolic pathways. Using a SCID mouse xenograft mouse model, we demonstrate that gemcitabine resistance is maintained in vivo, and is stable in the absence of drug selection pressure. Cross resistance profiles demonstrate approximately 100-fold cross resistance to pyrimidine nucleoside cytarabine, but no resistance to the same in class agents, azacytidine and decitabine, and no resistance to DNA damaging agents or microtubule inhibitors. Most strikingly, the gemcitabine resistant subclones demonstrated a dose dependent hypersensitivity to the Class I histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid(SAHA/Vorinostat) (IC50: MP 1.6µM; GR300 1.0µM; GR800 0.2µM; GR2000 0.08µM).

This collateral hypersensitivity extended to additional Class I HDAC inhibitors including MS-275 (Entinostat) and Trichostatin A, but not to the Class IIa selective agent MC1568. There was no significant difference in basal levels of histone acetylation, but the gemcitabine resistant clones demonstrated a greater increase in histone acetylation in response to HDAC treatment. Additionally, the GR cells were found to have decreased expression of H4K20me3, H3K9me3 and H3K27me3, suggesting a global change in chromatin structure. Cell morphology of the drug resistant cell lines demonstrated a fibroblastic type appearance with loss of cell-cell junctions and an altered microarray expression pattern, using Gene Ontology (GO) annotation, consistent with progression to an invasive phenotype. Of particular note, the gemcitabine resistant cell lines displayed up to a 15 fold increase in invasive potential that directly correlates with the level of gemcitabine resistance.

Conclusions: These findings suggest a mechanistic relationship between chemoresistance and metastatic potential in pancreatic carcinoma. In addition, the resistance phenotype that emerges under drug pressure is associated with a phenotypic reprogramming that can be exploited to devise new therapeutic options for patients with advanced pancreatic adenocarcinoma.
Functional coupling of activating transcription factor-6α to osteosarcoma pathogenesis and chemoresistance

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Background: Despite advances in therapy, survival for patients with metastatic osteosarcoma (OS) at diagnosis or with relapsed disease continue to be dismal (~20%) and has not improved in the past 20-30 years. Identification and improved understanding of the molecular pathways involved in OS pathogenesis and response to chemotherapy is urgently needed to impact overall patient survival. We show here that sensors of the unfolded protein response (UPR)/endoplasmic reticulum stress response (ER stress), a pathway known to promote tumor cell survival and therapy resistance, were induced in human OS cell lines (SaOS-2 and 143b cells).

Results: Western blot (WB) analysis following treatment with the glycosylation inhibitor tunicamycin showed an increase in PERK-eIF2α signaling and XBP-1 splicing (marker of IRE-1 activation). Similarly, using immunofluorescence and WB analysis we show that ATF6α was also activated in OS cells albeit at different levels. This correlated with increased transcriptional activation of 5XATF6-GL3 luciferase reporter in response to hypoxic stress as well as Tm treatment. In addition the levels of BiP protein, a downstream effector of ATF6α activation was also induced in response to hypoxic as well as Tm induced ER stress. Together these results suggest that both the OS cells lines activate the UPR. Using SRB assay we observed that SaOS-2 and 143b cells have significantly different sensitivity to both cisplatin and irinotecan with an IC50 of ~100 μM (SaOS-2) and 10 μM (143b) respectively for both drugs. We next tested if upregulation of UPR markers served as a survival factor for OS cells. While downregulation of ATF6α did not affect basal survival or proliferation of the OS cells, we observed a statistically significant increase in sensitivity to cisplatin (10-12.5μM, for 18-20h hours) as well as irinotecan (10μM for 18h) induced cell death. This occurred in part via enhanced Bax activation. Interestingly inhibition of PERK or IRE-1α signaling pathways did not affect the sensitivity of OS cells to cisplatin treatment. Furthermore, following ATF6α knockdown, OS cells were also more susceptible to combinatorial treatment with cisplatin and rapamycin, an inhibitor of mTOR, which is a pathway that propagates strong cell survival signals. This was accompanied by a decrease in the mRNA levels of RAS homolog enriched in brain (RHEB) and a decrease in phosphorylation of S6-ribosomal protein levels. Next we performed a preliminary retrospective immunohistochemistry analysis of 12 banked and de-identified OS patient samples for ATF6α expression. We found that 66.6% of primary tumor samples displayed high expression of the nuclear active form of ATF6α (i.e. ≥50% of the tumors cells). In contrast, ~60% of metastases had low (i.e. <10% of tumor cells) nuclear ATF6α levels. These results suggest that a vast majority of OS primary tumors are associated with high levels of active ATF6α expression.

Conclusion: Our findings emphasize a previously uncharacterized role for the UPR induced transcription factor ATF6α in the therapy resistance of OS and could potentially be used as a new combinatorial treatment to eliminate chemoresistant tumor cells as well as programs that drive tumor recurrences.
**Genetic and Epigenetic Contributions in Human Rhabdomyosarcomas**

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**Background:** An important characteristic of many human cancers is the high rate of copy number variations (CNV) and structural variations (SV). However, recent data from whole genome sequencing of cancer genomes has identified subsets of tumors with few CNVs and SVs suggesting that other mechanisms may be contributing to tumor progression.

**Results:** We previously characterized the genomic and epigenetic landscape of human retinoblastomas and discovered that these tumors have few genetic lesions but have epigenetic deregulation of multiple cancer and developmental pathways. Much like retinoblastomas, human alveolar rhabdomyosarcomas have few CNVs and SVs and we hypothesize that these tumors will likely have epigenetic deregulation of cancer pathways. To test this, we performed DNA methylation, RNAseq, proteomics and ChIP-seq in patient derived human rhabdomyosarcoma orthotopic xenografts. Preliminary integrative data analysis reveals epigenetic deregulation of a subset of developmental genes, some of which are reported to be aberrantly expressed in other cancer types, including ovarian and leukemia cancers.

**Conclusion:** Data from our analysis will shed light on the relative genetic and epigenetic contributions during development and tumorigenesis and provide novel therapeutic targets for rhabdomyosarcomas.

*(Supported in part by the American Cancer Society Institutional Research Grant 74-001-34 from the American Cancer Society)*
The SUMO-targeted ubiquitin ligase RNF4 regulates BLM helicase’s function in dormant origin firing

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Background: Regulation of dormant origin firing under conditions of replication stress is poorly understood. The gene mutated in Bloom’s syndrome BLM is a DNA helicase that functions in maintenance of replication fork stability, and BLM normally suppresses dormant origin firing. We previously found that BLM is modified by the Small Ubiquitin-Related Modifier 2 (SUMO-2) and that SUMO-2 modification regulates BLM’s function in stabilization of stalled replication forks. We hypothesize that BLM’s function at stalled forks is further regulated by the SUMO-targeted ubiquitin ligase RNF4. To test this hypothesis, we investigated the effects on BLM modification and function of RNF4 depletion by transfection with RNF4-specific siRNAs.

Results: We find that BLM sumoylation levels increase in response to replication stalling by hydroxyurea (HU) and to proteasome inhibition; depletion of RNF4 causes increased levels of BLM sumoylation. RNF4 directly interacts with BLM and can ubiquitylate sumoylated BLM in vitro. These data indicate that sumoylated BLM is an RNF4 substrate. Consistent with a role in regulation of BLM at stalled forks, RNF4 is recruited to DNA repair foci in response to replication stalling. Indeed, RNF4 depletion causes excess accumulation of BLM at HU-induced repair foci; however, RPA, RAD51, and gamma-H2AX accumulations are normal at stalled forks in RNF4-depleted cells, suggesting that homologous recombination repair is normal. Consistent with these data, sister chromatid exchanges are normal in RNF4-depleted cells.

Although RNF4 does not affect homologous recombination repair in HU-treated cells, RNF4 depletion reduces cell proliferation and survival of untreated cells, and it confers hypersensitivity to replication stalling by HU. Studies of cell-cycle progression using bromodeoxyuridine incorporation and flow cytometry show that RNF4 depletion causes a delay of re-entry into S phase in HU-treated cells and the replication delay is partially rescued by BLM mutation. The delay is not caused by RNF4-mediated modification of checkpoint kinase activities. Analysis of replication dynamics using the DNA fiber assay show that RNF4 depletion causes an increase of permanently stalled replication forks and a decrease in activation of dormant origins following recovery from HU-induced replication stress. Co-depletion of RNF4 and BLM partially rescues these defects. Replication dynamics is unaffected by RNF4 depletion in untreated cells.

Conclusions: These data indicate that RNF4 regulates BLM’s functions in dormant origin firing, without affecting BLM’s roles in homologous recombination. We propose that BLM inhibits the firing of dormant origins at stalled forks. Sumoylation of BLM at stalled forks is required for homologous recombination repair and modification thereby maintains replication-fork integrity. However, if the fork collapses, then RNF4-mediated ubiquitylation leads to proteasome-dependent degradation of BLM and BLM clearance from the stalled fork. BLM clearance from the stalled fork allows firing at a nearby dormant origin, which ensures that replication is properly completed distal to the collapsed fork.
Aberrant upregulation of 14-3-3 gamma promotes mononucleated polyploidization in human lung cancers

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Background: Advanced non-small cell lung cancers (NSCLC), frequently display increased expression of 14-3-3 gamma (14-3-3γ)—a scaffolding protein shown to have oncogenic capacity. This aberrant increase in expression has also been correlated with poorer survival, indicating that up-regulation of 14-3-3γ results in a more aggressive tumor phenotype. Our lab is in pursuit of characterizing the mechanisms driving this more advanced cancer phenotype.

Results: One such mechanism currently being investigated is 14-3-3γ's ability to promote polyploidization in lung cancer cells. Polyploidization is a known mechanism observed to increase tumorigenicity, resistance to conventional therapies, and theorized to promote chromosomal instability. Our data show that overexpression of 14-3-3γ results in a subpopulation of cells that harbor mono-nucleated tetraploid DNA content. This suggests that elevated expression of 14-3-3γ promotes aberrant cell cycle progression, either by mechanisms of an abortive mitosis known as endomitosis, or by means of endoreplication, a re-replication event occurring in the absence of mitosis. Further analyses have confirmed that these cells, in the absence of tumor suppressor p53, remain viable and undergo cellular division; supporting our hypothesis that upregulation of 14-3-3γ is resulting in an unstable, tumorigenic tetraploid cell state.

Conclusion: Taken together, our data elucidates one role by which 14-3-3γ may be contributing to a more aggressive tumor phenotype in patients with advanced NSCLC.

*Poster presenter
Elucidating the mechanism of bile acid-induced activation of estrogen receptor β in colorectal adenocarcinoma

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Background: Ursodeoxycholic Acid (UDCA) is a putative chemopreventive agent that was shown to suppress azoxymethane-induced colorectal tumors in rodent models. A double-blinded placebo controlled Phase III study conducted here at the University of Arizona showed that UDCA also suppressed the recurrence of highly dysplastic polyps in test subjects. Further analysis of the data revealed that UDCA’s efficacy as a preventive agent was gender specific. UDCA significantly lowered the odds of the recurrence of advanced lesions in men, but had little or no effect in women. Moreover, UDCA caused a significant increase in the recurrence of advanced lesions in women.

Results: We found similar gender-specific preventive activity in mice treated with azoxymethane; UDCA suppressed polyp formation in males but not females. Hence, we sought to determine whether UDCA’s preventive activity might involve hormonal receptors. Because estrogen receptor β (ERβ) is the predominant estrogen receptor in both normal and malignant human colon tissue, we sought to determine whether UDCA or Deoxycholic Acid (DCA), a cytotoxic secondary bile acid, had any effect on this receptor. Through western blot and qRT-PCR analysis, we showed that UDCA and DCA-treated HT-29 colorectal adenocarcinoma cells induced the phosphorylation of ERβ, as well as increased gene expression of estrogen response elements (EREs) indicating stimulated activity of this hormonal receptor. Moreover, we found that pre-treating the cells with the MEK1/2 inhibitor, PD98059, UDCA and DCA-induced phosphorylation of ERβ, along with ERE gene up-regulation, was significantly reduced.

Conclusion: Collectively, this data suggests that UDCA and DCA can stimulate ERβ signaling through a noncannonical mechanism via the MAPK signaling cascade. Hence, UDCA’s gender-specific effects may be due, in part, to signaling through hormone receptors.
Functional Repertoire of the Gut Microbiome in a Mouse Colon Cancer Model

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Nearly 20% of human colorectal cancers (CRC) has mutations in Transforming Growth Factor-β (TGFβ) signaling genes, but the underlying mechanisms are not clear. Mouse CRC models with mutations in this pathway require the presence of Helicobacter spp-induced inflammatory stress. Because the altered gut microbiome's interaction with a TGFβ-deficient mucosal epithelium is critical for progression to cancer, we have analyzed the functional alterations of the Helicobacter-positive gut microbiome in the presence and absence of SMAD3. Although there were no large-scale functional differences between these groups, removal of all metabolic pathways shared by the groups revealed a small number of pathways specific to the group with tumorigenic potential.
Epigenetic Regulation of the Bile Acid/Farnesoid X Receptor by High Fat Diet and APC in Colon Cells

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Background: The farnesoid-X-receptor (FXR) regulates hepatic and intestinal bile acid (BA) homeostasis. In intestinal cells carrying defective adenomatous polyposis coli (Apc) gene the expression of FXR is reduced through yet unknown mechanisms.

Results: To investigate if Western high-fat diet (HFD) and Apc status influence epigenetic regulation of the Fxr in colonic mucosa, weaned C57BL/6J male mice were fed a HFD containing 44% energy (44%E) from safflower oil (22% SO by weight) or control 13%E low fat diet (LFD) from SO (5% by weight) for 6 wks. We examined the effects of HFD on CpG methylation of Fxr, and expression of FXR, peroxisome-proliferator activated receptor-γ 1 (PPARγ1), and cyclooxygenase-2 (COX-2) mRNA. Also, we studied the influence of Apc mutation status on CpG methylation of the Fxr gene, and expression of FXR, ileal bile acid-binding protein (IBABP), small heterodimer partner (SHP), and COX-2 mRNA in normal colonic mucosa and colon tumors from ApcMin/+ mice. Mice fed the HFD had reduced (60%) Fxr promoter methylation and increased (2-3-fold) FXR, COX-2, and PPARγ1 mRNA levels. Conversely, Apc-deficiency was associated with constitutive hypermethylation of the Fxr gene, elevation of COX-2, and reduced (60-90%) baseline FXR, IBABP, and SHP mRNA. In human HCT-116 colon cells, siRNA knock-down of APC expression reduced (50%) basal FXR mRNA levels, which were further reduced to 80% by the secondary deoxycholic acid (DCA).

Conclusion: We conclude that APC deficiency leads to constitutive epigenetic silencing of Fxr increasing the risk of inflammation and colon cancer associated with a Western HFD.
Specific microRNA-mRNA regulatory network of colon cancer invasion mediated by tissue kallikrein-related peptidase 6

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Background: Growing evidence indicates that serine proteases kallikreins are associated with malignancy and may have potential diagnostic/prognostic applications in cancer. Kallikrein 6 (KLK6) is a member of the family of 15 highly conserved secreted trypsin- or chemotrypsin-like serine proteases. Overexpression of KLK6 has been observed in different pathophysiological states such as neurodegenerative diseases, inflammation and cancer. Activating K-RAS mutations are common in colon cancer progression and contribute to many cancerous phenotypes including increased proliferation, evasion of apoptosis, increased migration and invasion. We have shown that acquisition of K-RAS mutations in colon cancer cells leads to overexpression of membrane protein caveolin 1 and results in an increase of KLK6 expression and secretion. MicroRNAs (miRNAs) are 21-23 nucleotide long endogenous, non-coding RNA molecules that target different mRNAs and lead to gene silencing through inhibition of translation and mRNA destabilization. The aim of this study was to elucidate the miRNA-based mechanism of regulation of invasion in metastatic CRC overexpressing KLK6.

Results: To address this question, we developed HCT116 colon cells with knockdown of KLK6 expression using short-hairpin RNA (shRNA) technology (SureSilencing shRNAs, Qiagen, Inc.). The HCT116 isogenic clones stably expressing shRNA for KLK6 gene (shKLK6 clones) had decreased expression and secretion of KLK6 protein with the minimal effect on the cell growth and viability in cell culture. SCID mice injected with shKLK6 cells exhibited a statistically significant increase in the survival rates (p=0.005), decrease in the incidence of distant metastases and the shift in the location of the metastatic foci closer to the cells injection site. Levels of KLK6 protein secreted into the bloodstream were significantly lower in animals injected with shKLK6 clone compared to the HCT116 control clone (p<0.04). We performed mRNAs and miRNAs expression profiling of HCT116 and shKLK6 cell lines using the Affymetrix Human Gene 1.0ST (28,869 well-annotated genes) and Affymetrix GeneChip miRNA 2.0 arrays (1,105 human mature miRNAs), respectively. A bioinformatics analysis was then applied to identify miRNAs-mRNA target interactions involved in KLK6-mediated metastasis formation. Thirty miRNAs and their 55 target mRNAs were identified by LIMMA analysis (p<0.01) and prediction by miRanda and mirSVR microRNA databases. Three upregulated miRNAs (miR-183, miR-182, miR-203) and three downregulated miRNAs (miR-181-d, miR-214, miR-330-5p), as well as their predicted target mRNAs (upregulated PAM, BAMBI, FOS) and down regulated (EHF, TGF-beta2, RIT1, GNG11), which are involved in K-Ras signaling cascade, were selected and validated by quantitative Real Time PCR. The miR181-d was found to be the most significantly downregulated miRNA in shKLK6 cells. The functional analysis using 3’UTR luciferase reporter plasmids showed that miRNA 181-d interacts strongly with 3’UTR regions of PAM, EHF and FOS mRNAs.

Conclusion: The data reported here underscore the significance of KLK6 overexpression for distant metastasis formation in CRC and present the opportunity for therapeutic interventions using miRNA mimics approach.
Identification of microRNA target genes involved in colon cancer progression and liver metastasis

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Background: Colon cancer patients with liver metastasis present a high rate of mortality and enhanced resistance to conventional chemotherapy. It is therefore critical to identify key molecular pathways in these lesions to develop new target-directed therapies.

Results: Our results thus far support the hypothesis that the S100P/RAGE inflammatory signaling pathway contributes to colon cancer hepatic metastasis by elevating the expression of the oncogenic microRNA, miR-155. Our group demonstrated for the first time that activation of RAGE by S100P regulates miR-155 expression through Activator Protein-1 (AP-1) in human colon cancer cells. Stimulation of the S100P/RAGE signaling pathway up-regulated miR-155 levels while miR-155 inhibition attenuated colon cancer cell growth, motility, and invasion in vitro. Although, we have been able to establish that S100P/RAGE signaling can regulate the expression of miR-155, miR-155 targets important in colon cancer progression and metastasis remain undefined. To determine potential miR-155 target genes, transcriptome analysis by microarray of colon cancer cells expressing S100P was performed. Bio-informatics identified seven genes differentially expressed and containing the miR-155 target sequence. microRNAs negatively regulate gene expression in most instances thus, our investigations centered on the two identified differentially down-regulated genes: WNK lysine deficient protein kinase 1 (WNK1) and zinc finger protein 493 (ZNF493). We discovered an inverse correlation between S100P and ZNF493 expression, but not WNK1, in colon cancer cases derived from The Cancer Genome Atlas cohort. We corroborated the inverse correlation between ZNF493 and S100P by quantitative PCR analysis of paired normal and tumor specimens from the University of Arizona tissue bank. ZNF493 expression was elevated in two cases of appendiceal cancer. In addition, ectopic expression of S100P in colon cancer cell lines reduced ZNF493 mRNA levels. Furthermore, attenuation of miR-155 expression in colon cancer cells via transfection of a microRNA sponge increased ZNF493 mRNA levels.

Conclusion: Taken together, these data suggest that the zinc finger protein ZNF493 may be a downstream target of the S100P/RAGE/miR-155 signaling axis. Further investigation of ZNF493’s biological function and role within colon cancer progression and metastasis could further implicate its use as a therapeutic target or biomarker for the disease.
Vigilin forms a translational repressor loop to suppress c-fms mRNA by interacting with deadenylation/translation initiation complexes

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Background: Proto-oncogene c-fms encodes a cell surface receptor tyrosine kinase and the sole receptor for colony stimulating factor-1 (CSF-1). Overexpression of c-fms is strongly correlated with lymph node metastasis in human breast cancer. Although significant efforts have been devoted, the precise mechanism of the regulation of abnormal expression of c-fms in breast cancer is not well elucidated.

Results: Our lab previously demonstrated that vigilin, a RNA binding protein (RBP) also known as high density lipoprotein binding protein (HDLBP), represses c-fms expression at both the RNA and protein levels by binding the pyrimidine-rich sequence in the 3'untralslated region (3'UTR) of c-fms mRNA, where HuR, another RBP, binds and increases c-fms expression. The in vitro competition assay indicates that vigilin competes with HuR for the binding on the pyrimidine-rich sequence in the c-fms mRNA 3'UTR. Furthermore, the overexpression of vigilin decreased c-fms mRNA stability. These results suggest that vigilin may function as a tumor repressor by suppressing c-fms expression through its ability to compete against HuR. We studied further to understand the role of vigilin in regulatory mechanism of c-fms mRNA expression and to identify vigilin interacting proteins. The co-immunoprecipitation assay revealed that vigilin interacts with eIF4G and eIF4E in the eIF4F translation initiation complex as well as CNOT7 and Pan3 in the deadenylation complex. The results indicate that vigilin may induce the formation of a translational repressor loop by sequestering eIF4E and eIF4G in the eIF4F translation initiation complex formed in the mRNA 5'UTR, as well as by binding a pyrimidine-rich linear sequence in the 3'UTR. Due to the role of vigilin as a translational repressor on c-fms mRNA expression, we compared the expression pattern of vigilin in vivo in a limited number of normal, precancer and breast cancer tissues. Preliminary immunohistochemistry analysis showed that there may be high expression of vigilin in normal breast samples, with a decline in expression during the transition to carcinoma.

Conclusion: Overall, we propose that vigilin may have a repressor function to c-fms mRNA translation by forming a translational repressor loop.
Differentiation of colorectal cancer lesions from inflammatory tissues with a novel molecular imaging probe

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Background: The development of colorectal cancer (CRC) is a slow process involving chronic inflammatory reactions, angiogenesis and malignant transition. Patients with inflammatory bowel disease (IBD), especially those with longstanding colitis, have an increased risk of CRC. Early detection of IBD-associated CRC is a major focus of current research to reduce CRC progression and mortality. Periodic endoscopic surveillance with biopsy is the standard method for early CRC diagnosis. However, in approximately 5-10% of cases, visualization of the mucosa of the entire colon is not achieved. In addition, visible findings at colonoscopy lack morphologic detail and molecular specificity, and a dysplastic lesion can be difficult to distinguish from epithelial regeneration due to inflammation. Average dysplasia miss-rates have been reported as high as 22% with conventional colonoscopy. Thus, new imaging approaches with specific molecular probes capable of differentiating CRC lesions from inflammatory non-tumor sites are critically needed for early CRC diagnosis.

Methods and Results: A small peptide with the amino-acid sequence CTPSPFSHC (TCP-1) was conjugated with succinimidyl-6-hydrazino-nicotinamide (SHYNIC) and radiolabeled with technetium-99m (99mTc) to produce a molecular imaging probe, 99mTc-SHYNIC-TCP-1, for specific CRC imaging. TCP-1 was originally identified using CRC phage library selection. We hypothesized that 99mTc-labeled analogues of TCP-1 peptide might have a fundamentally distinct targeting mechanism with high selectivity for CRC cells within the inflammatory colorectal microenvironment. Unlike most molecular imaging probes that bind to receptors on the tumor cell surface, 99mTc-SHYNIC-TCP-1 can undergo endocytosis and distribute in the nucleus, with the result that the probe is not easily removed from the target and is retained longer than cell-surface probes. 99mTc-SHYNIC-TCP-1 images were acquired in living mice carrying orthotopic CRC (mouse CT26 colon cancer cells and human HCT116 cancer cells, n=2 each) and control mice with dextran sulfate sodium (DSS)-induced colitis (n=2) using a high-resolution SPECT imager, FastSPECT II. Images acquired two hours after intravenous injection showed that 99mTc-SHYNIC-TCP-1 selectively localized in early CRC lesions and distinguished small CRC lesions from non-tumor inflammatory tissues. In subcutaneous cancer xenografts, 99mTc-SHYNIC-TCP-1 selectively accumulated in colon cancer (HCT116 cell line; 1.21±0.27 %ID/g, n=4) but not in prostate cancer (PC3 cell line; 0.15±0.01 %ID/g, n=4, P<0.01).

Conclusion: The results of our preliminary studies showed that 99mTc-SHYNIC-TCP-1 could differentiate malignant colorectal lesions from inflammatory sites without malignancy. We expect that the uptake of 99mTc-SHYNIC-TCP1 peptide is highly selective not only in orthotopic CRC but also in spontaneous early CRC lesions. We are currently preparing to acquire 99mTc-SHYNIC-TCP-1 imaging data in a spontaneous CRC mouse model that produces chronic inflammation resembling ulcerative colitis and provides a better platform for clinical translation. Characterization of the 99mTc-SHYNIC-TCP-1 probe in the spontaneous CRC mouse model will also provide useful information for further study of the TCP-1 CRC-binding mechanism.
Background: Triple-negative breast cancer (TNBC) is a fatal disease that is highly aggressive accounting for a large number of the metastatic disease cases and breast cancer related deaths and for which there are no targeted treatments. Upregulated Hedgehog (Hh) signaling is common in human cancers and is a feature of TNBC. The Hh pathway has emerged as a promising target for treatment of TNBC based on exciting in vivo studies demonstrating that inhibition of Hh activity in TNBC reduces both tumor growth and metastasis. Primary cilia are microtubule-based organelles that protrude from some vertebrate cells. In mammals, Hh-signaling proteins including Ptch, Smo, Gli transcription factors and other associated proteins, localize to primary cilia for ligand-dependent activation or repression of the Hh pathway. Importantly, we have shown that ciliogenesis is inhibited in TNBC. It is therefore unclear how TNBC activate Hh signaling in the absence of cilia. The mechanisms by which cancer cells activate Hh signaling via non-canonical pathways are emerging and we hypothesize that inhibition of ciliogenesis (loss of repressor function) is required. Furthermore, we hypothesize that inhibition of ciliogenesis will promote mammary tumorigenesis. To test this, we utilized a mammary transplantation model to inhibit ciliogenesis (by deleting intraflagellar transport genes) in the context of oncogenic PYMT expression and measured changes in tumor growth and Hh signaling.

Result: We observed an enhancement of PYMT-induced tumor growth, with earlier tumor formation and a faster tumor growth rate when ciliogenesis was inhibited. Tumors from transplants with inhibited ciliogenesis were also of higher grade. Inhibiting ciliogenesis decreased apoptosis levels in premalignant and malignant glands. Hh target genes (Gli2, Ptch1, Slug) were found to be upregulated by real time PCR, suggesting activation of the pathway. Slug protein was investigated by immunohistochemistry, and Slug-positive cancers were more frequently observed in tumors with inhibited ciliogenesis. Consistent with increased Hh signaling and increased expression of Slug, we also observed that inhibition of ciliogenesis increased the incidence of metastasis to the lung.

Conclusion: The data supports the hypothesis that inhibition of ciliogenesis plays a role in promoting breast cancer growth and metastasis by increasing Hh signaling. Future studies are focused on understanding the mechanisms by which primary cilia inhibit non-canonical Hh signaling in TNBC. Our long-term goal is to develop therapeutic strategies that mimic the inhibitory role of primary cilia to prevent activation of the Hh pathway to improve survival in patients with TNBC.

(Supported in part by the American Cancer Society Institutional Research Grant 74-001-34 from the American Cancer Society)

*Poster presenter
Metformin-induced Metabolic Changes are K-RAS-dependent in Animal Models of Pancreatic Cancer

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Background: Pancreatic ductal adenocarcinoma (PDA) is an aggressive malignancy, often diagnosed late in the course of the disease, and very difficult to treat. To date, systemic treatment for PDA has shown only modest benefits. Numerous epidemiological studies have reported that metformin (MET), a widely used anti-diabetic drug, may provide protective benefits in reducing PDA risk among the diabetic population.

Results: Using a stable isotope glucose (GLUC) tracer for dynamic metabolic profiling (SiDMAP), we recently reported that high cholesterol (CHOL) alters the cellular metabolism of MiaPaCa2 (a PDA cell line harboring mutant K-Ras) cells by redirecting glucose-derived acetyl-CoA toward fatty acid (FA) synthesis. We further showed that this response to MET depended on the level of intracellular CHOL synthesis. We recently performed a SiDMAP study in the LSL-K-RasG12D/+, LSL-Trp53R172H/+, Pdx-1-Cre (KPC) and the LSL-K-RasG12D/+, Pdx-1-Cre (KC) mouse models for PDA, and their wild-type littersmates (C57Bl6.129). The mice were treated (or not) with MET (250 mg/kg, i.p. Q5D) and subjected to an Intra Peritoneal GLUC Tolerance Test (IPGTT) pre- and post-MET treatment. The KPC mice with the average tumor volume of 80.83±8.57 mm³ and the KC mice with PanIN lesions (aver. 9 mo old) were put on study. K-Ras mutation, with the presence of the tumor (KPC mice), induced an increase in plasma GLUC production via de novo synthesis by the liver using futile cycling of GLUC derived lactate and pyruvate. MET treatment decreased this flux in mutated (KC) and tumor-bearing animals (KPC), but increased this flux in liver of control mice. The presence of the tumor decreased, yet MET treatment increased the complete GLUC oxidation into 13CO2 in the pancreas of KC and KPC animals. This indicates that the pancreas in MET-treated animals use less GLUC for RNA, DNA and FA synthesis. For 13CO2 production, we found that tumor growth has to be established; the mutation is not enough to induce changes in this flux. As in plasma, tumor growth increases pancreas lactate production from GLUC. These results are consistent with data from the DMBA-induced PDA model. MET treatment dramatically decreased the Warburg effect in the pancreas of mutated (KC) and mutated/tumor-bearing (KPC) mice. Finally, MET decreased GLUC-derived acetyl-CoA delivery to FAS and palmitate in control, mutated and tumor-bearing mice. Immunohistochemical analysis of the tumors from the KPC mice revealed that treatment with MET decreased the staining of phospho-Ser79-ACC by ~54%, total FAS staining (mainly in the tumor stroma) by ~43%, and the growth-associated transcription factor HMGA2 by ~36.7%. These decreases correlated with a decrease in Ki67 staining by almost 80%, indicative of an inhibitory effect of MET on PDA growth.

Conclusions: These results suggest that mutant K-Ras is responsible for the metabolic adaptation in both the tumor- (KPC) and non-tumor-bearing animals (KC). Indeed, it has been reported that K-Ras drives the production of glutamate, which is also important for FA synthesis. Our findings provide a strong rationale for targeting the metabolic changes induced by activated K-RAS in PDA patients which harbor mutations in K-RAS gene in >95% of cases.
A New type of responsive MRI contrast agent that modulates T2ex relaxation: Detection of nitric oxide

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Background: The rate of water exchange (or proton exchange) in lanthanide complexes is one of the key parameters in the design of responsive MR imaging agents. Very fast to relatively fast water exchange rate is the main characteristic of T1-based MR agents while relatively slow water exchange is observed in CEST (Chemical Exchange Saturation Transfer) agents. A class of agents called T2 exchange (T2ex) is placed in between in term of the rate of water exchange where the exchange rate is not as fast as those in T1-based molecules or as slow as CEST agents. PARACEST (Paramagnetic Chemical Exchange Saturation Transfer) MRI contrast agents with a proton that has a large chemical shift and a fast chemical exchange rate can generate T2ex relaxation, which negatively impacts the sensitivity of CEST detection.

Results: We sought to use this disadvantage as an advantage to create a new class of molecular imaging agents, known as responsive T2ex MRI contrast agents. Specifically, Yb(III)-(1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid)-orthoaminoanilide (Yb-DO3A-oAA) has been shown to be responsive to nitric oxide, but in vivo CEST detection of this agent is difficult due in part to T2ex relaxation caused by the agent. We designed a similar agent, Tm-DO3A-oAA, which has a larger chemical shift and therefore should have a stronger T2ex relaxation effect. A chemical reaction with nitric oxide should modify the chemical structure of the agent and consequently a change in the water exchange rate of the agent should be observed. Our results demonstrate that Tm-DO3A-oAA (as the first example of a responsive T2ex agent) can detect nitric oxide via T2-weighted MRI by a 6-fold increase in T2ex relaxivity. Importantly, the T1 relaxivity does not change after treatment with nitric oxide, and the ratio of T2 and T1 relaxivities is independent of concentration, so that this ratio can detect nitric oxide in a concentration-independent manner. This ratio of T2 and T1 relaxivities is only mildly dependent on temperature, which improves the specificity of the nitric oxide detection.

Conclusion: These results demonstrate that a new class of responsive MRI contrast agents can be developed based on changing the chemical exchange rate of an agent and obtaining T2- weighted and T1-weighted MR images.
Pim protein kinase regulates oncogenic metabolism

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**Background:** Pim is oncogenic kinase that contributes to cancer development. However, mechanism by which Pim promotes oncogenic growth is not clear.

**Results:** We found that mouse embryo fibroblasts (MEFs) lacking all three isoforms of Pim protein kinases, triple knockout (TKO), cannot tolerate the expression of activated K-Ras (K-RasG12V) and undergo cell death. Similarly, cancer cells expressing mutant K-Ras were sensitive to a Pim kinase inhibitor. A markedly increased the level of cellular reactive oxygen species (ROS) was considered as a mechanism for cell death because the addition of N-acetyl cysteine attenuated ROS production and reversed the cytotoxic effects of K-RasG12V in the TKO MEFs. The altered cellular redox state caused by the loss of Pim occurred as a result of lower levels of metabolic intermediates in the glycolytic and pentose phosphate pathways as well as abnormal mitochondrial oxidative phosphorylation.

**Conclusion:** These results demonstrate that the Pim protein kinases have an important role in regulating cellular redox, metabolism, and K-Ras-stimulated cell growth.
Towards the non-invasion detection of hypoxic tumors by monitoring reductase activity with catalyCEST MRI

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Background: The detection of hypoxic tumors has the potential of improving precision medicine by impacting the choice of radiation therapy or hypoxia-activated chemotherapies. Within the microenvironment of hypoxic tumors, there is often an overexpression of reductase enzymes with high catalytic activities which serves as a biomarker and provides a target for novel imaging strategies. The development of non-invasive detection of reductase enzyme activity has the potential to improve tumor diagnoses.

Results: We have developed an innovative imaging method, catalyCEST MRI, to detect the activity of reductase enzymes within in vivo mouse tumor models. This methodology creates Chemical Exchange Saturation Transfer (CEST) by selectively saturating the magnetic resonance of a proton on the agent which transfers the saturation effect to water through a chemical exchange. With catalyCEST MRI, the enzyme catalyzes a change in the chemical structure of the agent and the change in CEST is monitored. Additionally, a control CEST signal unresponsive to enzymatic catalysis is also monitored to improve quantitative imaging. We have synthesized several catalyCEST agents designed to detect nitro- and quinone reductase activities. Derived from salicylic acid, these agents have the potential to be translated for clinical studies.

Conclusion: This report will present the catalyCEST MRI mechanism for the detection of reductase enzyme activity and progression towards in vivo studies with mouse tumor models.
Mutated in Colorectal Cancer (MCC) interacts with Keap1 and activates Nrf2 signaling

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Background: Mutated in colorectal cancer (MCC) is a protein that was originally thought to be the cause of familial adenomatous polyposis (FAP) since it is frequently methylated in colorectal tumors and is in close proximity to the adenomatous polyposis coli (APC) gene. Nevertheless, discovery of APC as the main contributor to APC deviated attention from MCC and its function and regulation remain largely unknown. Previous studies have characterized MCC as a putative tumor suppressor since its overexpression blocks cell cycle progression and negatively regulates the Wnt-β catenin and NFκB pathways. Additionally, MCC is frequently methylated in colorectal, lung and other epithelial tumors. Therefore, identification of the mechanisms of action and the signaling associated to MCC will be of great importance in understanding its role in cancer initiation and progression.

Results: Here we describe MCC as a novel participant of the Nrf2 signaling pathway. We identified an STGE motif near the N terminus of MCC and tested if this protein could be a Keap1-binding partner. In HEK293 cells, overexpressed MCC immunoprecipitates with overexpressed Keap1. In addition, MCC upregulates Nrf2 protein levels, as well as of its downstream targets, indicating that MCC can activate Nrf2 signaling. Moreover, overexpressed mutant MCC (STGE→SAGE) does not immunoprecipitate with Keap1 or upregulate Nrf2 protein levels.

Conclusion: MCC is a new possible non-canonical Nrf2 inducer. Further characterization of the role of MCC in Nrf2 signaling regulation will reveal new insights into MCC function and alternative Nrf2 regulatory mechanisms.
Tuning the withanolides to target the AAA+ chaperone p97

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Background: Understanding the mode of action of natural products can be puzzling with mechanistic clues that seem to lack a common thread. One such puzzle lies in the evaluation of the antitumor properties of the natural product withaferin A (WA). WA has a storied history being the primary bioactive constituent of the Ashwaganda tree, which has been used in traditional medicines for thousands of years. A variety of seemingly unrelated pathways have been identified to explain its activity, suggesting a lack of selectivity.

Results: We now show that WA acts as a moderate reversible inhibitor of the chaperone, p97, in addition to inhibiting the core particle of the proteasome. Then through medicinal chemistry, using both synthetic chemistry and epigenetic manipulation of cultures, we have isolated 18 analogues of withaferin A and tested each against p97. Using this procedure, we have tuned the activity towards p97 and away from the proteasome, with the best molecule, a previously unknown azide derivative, showing no activity against the proteasome both \textit{in vitro} (using purified proteasomal core-particles) and in cell-based assays. We have also evaluated each of the molecules against a panel of other ATP-utilizing enzymes and demonstrated selectivity as well as using a variety of p97 mutants to gain an understanding of the mechanism of inhibition. To further confirm p97 as a target of the withanolides, we looked at the unfolded protein response (UPR), which was activated, and autophagy, which was inhibited. The tuned withanolides also showed potent activity against a variety of lymphoma cell lines. Because of the complicated MOA history of withanolides, each of the compounds was tested in a series of cellular assays to look at other described pathways, including proteasome inhibition, and in our hands we saw no effect at the levels that showed modulation of p97 pathways and cellular toxicity.

Conclusion: This collective of data suggests that the MOAs reported for the withanolides are connected by modulation of the ubiquitin proteasome system. Through this endeavor, this study highlights how the parallel integration of medicinal chemistry with chemical biology offers a potent solution to some of natures’ more intriguing molecular puzzles.
Pan-PIM Kinase Inhibitors Show Anti-Cancer Activity in a Subset of T-cell Acute Lymphoblastic Leukemia cells

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**Background:** The proviral insertion in murine (PIM) kinase is a small family of serine/threonine/tyrosine kinases, composed of 3 isoforms PIM1, PIM2, and PIM3 which are a closely related and constitutively active proto-oncogenic kinases. PIM kinases are known to phosphorylate broad range of cellular substrates that regulate transcription, translation, cell cycle, and survival pathways. Increased expression and activity of PIM kinases has been observed in a number of human cancers and frequently associated with poor prognosis in most hematopoietic malignancies including acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia, and non-Hodgkin lymphoma.

**Results:** Our study demonstrated that AZD-1208, a pan-PIM inhibitor is active in a subset of T-cell Acute Lymphoblastic Leukemia (T-ALL) cell lines by inhibiting proliferation and mTORC1 signaling. In addition, incubation of these T-ALL cells with AZD-1208 blocked cell cycle progression by inducing a partial G1 phase cell-cycle arrest and dose dependent increase in p27. Results from microarray expression profiling showed that, PIM inhibitor sensitive T-ALL cells have high levels of PIM1, Jak/STAT, NF-kB and low c-MYC expression, whereas T-ALL cells resistant to PIM inhibitor have low levels of PIM1 but have high levels of c-MYC and NOTCH expression. These results were validated by western blot and quantitative Real Time-PCR.

**Conclusions:** Taken together, our results suggested that a significant percentage of T-ALL cell population is driven by the PIM kinase pathways in parallel with Jak/STAT. Additionally, NF-kB, NOTCH and c-MYC may play a critical role in the determination of cell sensitivity to PIM inhibitor. Further studies are required to confirm that Pan-PIM inhibitors will have significant activity in blocking T-ALL growth in pre-clinical setting consequently having an important impact towards the treatment of hematological malignancies.
Pak2 regulates hematopoietic progenitor cell proliferation, survival and differentiation

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Introduction: p21-activated kinase 2 (Pak2), a serine/threonine kinase, has been previously shown to be essential for hematopoietic stem cell (HSC) engraftment. However, Pak2 modulation of long-term hematopoiesis and lineage commitment remain unreported.

Results: Utilizing a conditional Pak2 knock out (KO) mouse model, we found that disruption of Pak2 in HSCs induced profound leukopenia and a mild macrocytic anemia. Although loss of Pak2 in HSCs leads to less efficient short- and long-term competitive hematopoiesis than wild type (WT) cells, it does not affect HSC self-renewal per se. Pak2 disruption decreased the survival and proliferation of multi-cytokine stimulated immature progenitors. Loss of Pak2 skewed lineage differentiation toward granulocytopoiesis and monocytopoiesis in mice as evidenced by 1) a three to six-fold increase in the percentage of peripheral blood granulocytes and a significant increase in the percentage of granulocyte-monocyte progenitors (GMPs) in mice transplanted with Pak2-disrupted BM; 2) Pak2-disrupted BM and c-kit+ cells yielded higher numbers of more mature subsets of granulocyte-monocyte colonies and polymophonuclear neutrophils (PMNs), respectively, when cultured in the presence of granulocyte-macrophage colony stimulating factor (GM-CSF). Pak2 disruption resulted respectively in decreased and increased gene expression of transcription factors JunB and c-Myc, which may suggest underlying mechanisms by which Pak2 regulates granulocyte-monocyte lineage commitment. Furthermore, Pak2 disruption led to 1) higher percentage of CD4+CD8+ double positive T cells and lower percentages of CD4+CD8- or CD4-CD8+ single positive T cells in thymus and 2) decreased numbers of mature B cells and increased numbers of Pre-Pro B cells in BM, suggesting defects in lymphopoiesis.

Conclusions: Pak2 displays different functions at different points in hematopoiesis. Pak2 negatively regulates granulocyte-monocyte lineage commitment while positively regulating survival and proliferation of HPCs that are not committed to granulocyte-monocyte lineage. Furthermore, Pak2 may act at other aspects of hematopoiesis including PMN segmentation and circulation, as well as T and B cell differentiation/maturation in thymus and BM.

(Supported in part by the American Cancer Society Institutional Research Grant 74-001-34 from the American Cancer Society)
Monitoring Early Therapeutic Response by Measuring Extracellular pH in a Tumor Model with acidoCEST MRI

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Introduction: The purpose of the present study is to examine whether tumor extracellular pH (pHe) is a sensitive and specific biomarker for measuring immediate, short term drug response to the mTOR inhibiting agent everolimus by a novel, non-invasive MRI-based pH imaging method acidoCEST MRI.

Methods: Two groups of SCID mice with Granta 519 human mantle cell lymphoma flank xenograft models were used in the study. First, to investigate if RAD001 has anti-tumor activity in the Granta 519 model, mice were injected IP with 5 mg/kg per day with RAD001 or vehicle (DMSO) (N=12 per group) every day until tumors reached 2000 mm³. Survival analysis was conducted using GraphPad software. In a separate correlative imaging study mice with tumors averaging 400 mm³ (N=8) were scanned with acidoCEST MRI pretreatment, one day post initial treatment, and one week after continuous treatment. To prepare for each acidoCEST MRI scan session, a mouse was anesthetized with 1.5% isoflurane, respiration rate and body temperature were monitored, and body temperature was maintained at 37.0°C with warm air. A bolus of 200 mL of 370 mgI/mL iopamidol was injected IV prior to scanning, followed by continuous infusion of iopamidol at 150 µL/hour during scanning. A CEST-FISP MRI protocol was performed with a Bruker Biospec 7T MRI scanner with a 72 mm volume coil using previously published procedures. The results were analyzed using Matlab, following previously published procedures. The average pHe was determined for each tumor. Pixels which registered a pHe of 6.00 to 6.99 were grouped together as the “acidic fraction,” and those pixels with pHb≥7 were collectively grouped together as the “neutral fraction.” The percent uptake of the contrast agent in the tumor area was measured to assess vascular perfusion in the tumor microenvironment.

Results: RAD001 treatment results in a significant survival advantage in Granta 519 flank xenograft tumors. AcidoCEST imaging of tumors showed pretreatment an average pHe value of 6.82, demonstrating that the tumor model was moderately acidic. The standard deviation of pixelwise pHe values was 0.24, indicating moderate heterogeneity of pHe in the measured tumor area. Post initial treatment, the pHe had a statistically significant increase to 6.92 with a standard deviation of 0.14, indicating a decrease in average acidosis and a decrease in heterogeneity of acidosis. After continuous treatment, the pHe dropped to 6.74 with a standard deviation of 0.26, suggesting a return to a more normal metabolic rate. In keeping with the trend, the acidic fraction of the tumor decreased from 33.1% to 19.6%, before increasing to 49% during this study. The tumor growth study showed a temporary growth delay before returning to a more normal growth rate, which paralleled the acidoCEST MRI results.

Conclusion: Tumor pHe measured with acidoCEST MRI can detect early response to RAD001. Additional pre-clinical studies are warranted to determine if this biomarker is a robust measure of early therapeutic response. In addition, translation of acidoCEST MRI to the clinic should be pursued to provide this biomarker for clinical studies.
Modulating Growth Pathways in Prostate Cancer: Improving Diagnostics via 2+2 Fixation and Pentoxifylline as an Adjunct Therapy

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Background: Environmental exposure to carcinogens causes oncogene activation in many cancers and upregulation of growth signaling pathways. Targeting these pathways has proven to be an effective therapeutic strategy in many cancers, however metastatic prostate cancer prognosis remains poor. Combination treatment with amuvatinib, a receptor tyrosine kinase inhibitor, and erlotinib, an epidermal growth factor inhibitor, effectively decreases cyclin D1 protein levels, via different pathways, in LNCaP (PTEN-) and DU145 (PTEN+) human prostate cancer cell models. Current studies investigated 2+2 formalin fixation (2h 4˚C, 2h 45˚C) for the preservation of growth signaling proteins in response to amuvatinib and erlotinib treatment in LNCaP and DU145 mouse xenograft models. Additional studies seek to assess pentoxifylline as a potential anticancer therapy.

Results: 2+2 fixation significantly improves immunohistochemical (IHC) staining for cyclin D1, showing reduction due to drug treatment compared to traditional 24h (25˚C) fixation. However, 2+2 fixation slightly decreased effective IHC for 4EBP1 pThr70 and pERK. Mechanistic studies to understand the modulation of cyclin D1 expression as a potential anticancer therapy revealed that pentoxifylline (PT, 1 mg/ml), a phosphodiesterase inhibitor, decreased cyclin D1 protein levels and decreased cell proliferation in LNCaP cells. Moreover PT reduced pAKT as well as downstream phospho-4EBP1, as indicated by western analysis. Interestingly, pAKT levels returned to baseline by 24h while phospho-4EBP1 and cyclin D1 remain decreased at 48h. In combination with first line therapy docetaxel, PTX effectively decreases the necessary dose of docetaxel for appreciable loss cell viability. Combination dosing also produces faster and more prominent induction of apoptotic markers as shown by caspase-3 and -7 as well as PARP cleavage.

Conclusion: In summary, 2+2 fixation may be an effective technique to improve diagnostic IHC in prostate cancer. PT effectively inhibits the AKT pathway in prostate cancer cells although additional studies are required to determine its utility as an adjunct therapy in prostate cancer.

(T32ES016652, T32ES007091, P30ES006694, AstraZeneca Studentship, Ventana-Roche)
Analyzing oncogenesis at the level of single cells

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We have developed a Genetically-Engineered Mouse Model (GEMM) specifically to analyze the initial stages of oncogenesis within single cells. The GEMM comprises a floxed stop separating the constitutive CAGGS promoter from a histone 2B-GFP fusion protein. Expression of the Cre recombinase within cells carrying this integrated transgene results in the appearance of green-fluorescent nuclei. These nuclei are then individually isolated from tissue homogenates via fluorescence-activated sorting. We have devised RNA-seq methods to characterize the transcriptional activities of the individual sorted nuclei. Charting the initial transcriptomic responses of cells to oncogenesis involves genetic combination of additional GEMMs with our reporter line. These GEMMs recapitulate oncogenesis in response to Cre-based activation of oncogenes or inactivation of tumor-suppressors. Application of these technologies will be described with respect to models of prostate and pancreatic cancer. Potential implementations for analysis of normal growth and development will also be provided.
Synergistic drug interaction between morphine and a cannabinoid receptor 2 agonist in a model of neuropathic pain

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Background: Neuropathic pain is a complex pain disorder that is difficult to manage. Current therapeutic options not only lack efficacy, but are also associated with adverse events. Therapeutics such as Vicodin® and Percocet® that combine opioids with NSAIDs for a synergistic analgesic effect are limited by their association with hepatotoxicity, constipation, and the potential for abuse. Cannabinoid 2 receptors (CB2) are not known to possess psychotropic, hepatotoxic or GI effects and have been shown to be active against inflammatory pain. Therefore, we investigated whether a drug interaction existed between morphine and the CB2 agonist, JWH015 in a rodent model of post-surgical and neuropathic pain. We hypothesized that the co-administration of morphine and JWH015 would synergistically attenuate mechanical and thermal sensitivities.

Results: Spared nerve injury (SNI) and post-surgical incision surgeries were performed on Sprague Dawley rats. One week following SNI and 24-hours following incision, animals were treated with morphine, JWH015, or their combination. Animals were tested for mechanical and thermal responsiveness over a 2-hour time course. SNI produced robust alterations in pain behaviors in the ipsilateral sural area of the hindpaw. Isobolographic analyses revealed a synergistic relationship for mechanical sensitivity and an additive relationship for thermal hyperalgesia between morphine and JWH015 when co-administered at a 1:1 (morphine:JWH015) dose ratio. Similarly, an additive drug interaction was uncovered in animals exposed to post-surgical incision when animals were concomitantly treated with morphine and JWH015 (1:3 morphine:JWH015). Moreover, the combination also decreased morphine’s rewarding effects in conditioned place preference (CPP) and significantly reduced opioid-induced dopamine release in the striatum.

Conclusions: In conclusion, we uncovered a synergistic drug interaction between morphine and JWH015 in a model of neuropathic pain. These findings reveal the potential for morphine and CB2 agonist combination therapy as adjunct to current treatments for neuropathic pain while reducing side effects.
Introduction: Skeletal metastases may result in pain, structural instability and hypercalcemia. Radiation therapy (RT) is known to provide palliative pain benefits. It has also been utilized in a protracted (1-3 week fractionation) to prevent the formation of fractures without surgical stabilization in patients with a low Mirel’s bone score. This is particularly true in weight-bearing bones of the axial skeleton. Little data exists to confirm the ability of single fraction RT to prevent fractures in patients with metastatic cancer. Bisphosphonates/RANK ligand inhibitors may also be used in this patient subset to strength/rebuild the bone. In a review of patients with metastases to the axial skeleton, we assessed the results of single fraction RT in preventing fractures as well as eliciting a decreased pain response.

Methods: A retrospective chart review was conducted of patients treated from July 2012 through June 2014 at the University Medical Center. Inclusion criteria were: axial skeleton metastases treated with 8Gy in 1 fraction. All primary cancer sites and histology were reviewed. kV or MV imaging was conducted at the time of treatment delivery to confirm accurate targeting. Patients were seen for consultation, during treatment, at one-month, and further RT follow-up as needed. Pain scores were collected per the standard Basic Pain Score as well as per standard CTCAE v4.0 toxicity criteria. Follow up imaging was reviewed for bony stability.

Results: In reviewing 150 charts, 9 patients were eligible for review. These patients had primary gastrointestinal cancers (n=4), lung (n=2), skin (n=2), and prostate (n=1) tumors. The median follow up was 3 months. The patients’ median pre-treatment pain level was 4 (range:0-10) with improvement to a median level of 0 (range:0-10) post RT. Median overall survival was 13 months from the time of consultation, with 56% of patients still alive at the time of analysis. The remaining 44% all died pain-free (grade 0). Bisphosphonates or rank ligand inhibitors were administered to a third of patients during therapy or follow-up. A pathologic fracture was seen in 1 patient prior to RT while the remaining patients had no evidence of disruption or instability (n=8). After RT, the patient with a fracture showed no progression of disease and/or bone instability at the treated site. Thirty-three percent of patients (n=3) underwent consultation with orthopedic surgery and 22% (n=2) proceeded to surgical intervention with arthroplasty. Follow up imaging varied, including CT (n=5), bone scan (n=4), and MRI (n=3). No new fractures occurred in irradiated areas.

Conclusion: The use of 8Gy in a single fraction palliative RT to weight bearing bones for pain reduction and fracture prevention is a viable treatment method to prevent fracture in patients with a low Mirel’s score. In patients with a high Mirel’s score single fraction RT with surgical intervention should be considered. Single fractionation provides both preventative care and pain control in patients who have bone metastases in all sites, including the axial skeleton.
New approaches to precision characterization of drug delivery to the tumor bed

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In current practice chemotherapy is personalized by attempting to match the drug to the patient’s genomic profile, but little or no attention is given to quantifying and optimizing the amount of drug that actually reaches the tumor cells. Factors that impede drug delivery are the complicated, tortuous nature of tumor vasculature; the biochemical signals that control the vessel permeability; the collagenous stroma that results from desmoplastic reaction; and the ‘end-game’ processes of receptor binding, internalization, catabolism and residualization. There is a profound need for new methods of quantifying these processes and developing patient-specific interventions to facilitate drug delivery. Standard compartmental pharmacokinetic (CPK) analysis is poorly suited to this endeavor. A compartment is a defined volume of tissue within which there are no spatial gradients (there are no spatial derivatives in the CPK equations) and where equilibrium concentrations within the compartment and with the plasma are reached essentially instantaneously. Moreover, it is assumed that the plasma concentration of the drug is the same at all points in the subject. Departures from the CPK assumptions, including nonequilibrium processes and spatial gradients, can result in heterogeneity of the drug distribution and reduced efficacy of the therapy. There is considerable current interest in non-compartmental mechanistic pharmacokinetic (MPK) models for studying drug delivery, but the realism and validity of the models are often questionable.

We are now beginning a project to use radiolabeled chemotherapeutic agents and small-animal imaging systems developed in the Center for Gamma-Ray Imaging to study drug distributions with high spatial and temporal resolution in mouse models. Both PET and SPECT systems are available for this project. SPECT has the advantage that it can use isotopes with longer half-lives than those used in PET, but commercial clinical and preclinical SPECT systems have poor temporal resolution because of the need for mechanical motion of the subject or the detector. CGRI systems such as FastSPECT II and AdaptiSPECT do not require any motion and can be optimized for drug-delivery studies and for the specific subject being imaged. These capabilities will allow us to use bolus injections and study the distributions on multiple time scales. The hypothesis is that the various processes that impede drug delivery can be separately quantified from these data without restrictive mathematical models.

A status report on this project will be presented, and the prospects for applying the methods clinically will be discussed.
Development and validation of a predictive model for cancer clinical trial accrual

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Background: It takes $2.6B and 128 months from preclinical drug development through FDA approval, 76% is clinical testing (DiMasi, 2014). This highlights the need to streamline clinical pipeline processes. London et al. stated “the trick is... getting [researchers] to recognize the uncertainty inherent in the accrual rates and quantify it in terms of a prior distribution.” We sought to create and validate a predictive model with the outcome of anticipated accrual to be used when considering a prospective clinical trial.

Methods: This retrospective cohort study used 5.8 years of registry data from treatment and supportive care interventional studies at our center for model construction. A negative binomial regression model was employed using variables known pre-study abstracted from the OnCore clinical trial management system (Forte Systems, Madison, WI) and clinicaltrials.gov. Statistical significance was set a priori to 0.05. Normality and collinearity of independent variables and model fit were assessed. Accrual was predicted for studies used to build the model. Validation studies included 14.5 months of registry data using criteria from the original model. Studies were run through the prediction model and actual accrual plotted against predicted accrual. Actual, team- and model-predicted subjects accrued; percent of trials meeting cut-off values; and model sensitivity and specificity were calculated.

Results: The model included 207 trials with complete information. Mean accrual was 7.3 per trial (±18.4); 55 (26.6%) trials accrued zero subjects locally. In univariate analysis, use of an investigational drug, DMT, number of national sites, use of local IRB, number of total months open nationally, months of accrual already completed, and overall proposed national enrollment were significantly associated with accrual. In multivariate analysis, DMT, proposed national enrollment, number of sites, use of local IRB, number of total months open, and number of months already opened were significantly and independently associated with accrual. The full model was significant (P<0.001) and predicted accrual at 94% of actual, maintaining predictive value at multiple cutoff values. For the validation study, 61 trials met the inclusion criteria. Total accrual was 373 subjects (mean: 6.1±17.2); 16 (26.2%) had zero accrual, 23 (37.7%) accrued 88.7% of the total subjects. The model predicted accrual of 513 subjects (138% of actual) versus the DMT predicted accrual of 1111 subjects (298% of actual). The model correctly predicted whether a study would accrue 4+ subjects 75.4% of the time. Twenty-seven studies (44.3%) correlated perfectly at the category level. Model sensitivity is 70.0%; specificity is 78.1%. For the 17 studies not correctly categorized using a cutoff of four, nine (60%) would have been wrongly opened (predicted 4+, <4 accrued) and six (40%) would have incorrectly not opened (predicted <4, 4+ accrued).

Conclusions: We identified key factors, both nationally and locally, associated with subject trial accrual at our site. This model can aid in deciding whether a study is likely to accrue a desirable number of subjects. The validation of this model shows it to be an accurate, quick and valuable metric in assessing trial success as well as planning resource allocation and clinical trial costs.
The Cystine-Glutamate Antiporter System xc- Drives Tumor Cell Glutamate Release and Cancer-Induced Bone Pain

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Background: Many common cancers, including breast, prostate, and lung cancers, have a propensity to metastasize to bone. Although these cancers go undetected in their native tissues, bone metastases often result in excruciating pain, the etiology of which is poorly understood.

Cancer-induced bone pain (CIBP) is not well-controlled with existing medications, severely compromising patient quality of life. While CIBP is multifaceted, increased levels of the excitatory neurotransmitter glutamate in the bone-tumor microenvironment may contribute to the pain state. Here, we demonstrate for the first time a relationship among reactive oxygen/nitrogen species, glutamate in the bone-tumor microenvironment and pain behaviors.

Results: The murine mammary adenocarcinoma cell line 66.1 is found to release glutamate via the cysteine-glutamate antiporter system xc-. In a syngeneic model of breast CIBP in which 66.1 cells are inoculated into the femur intramedullary space, administration of sulfasalazine, an established system xc- inhibitor and anti-inflammatory agent, reduces femur glutamate level and attenuates CIBP-related behaviors. Peroxynitrite, a reactive nitrogen species known to be generated in breast tumors, is shown to drive 66.1 system xc- functional expression and tumor cell glutamate release. The peroxynitrite decomposition catalyst FeTMPyP not only modulates tumor cell system xc- functional expression in vitro and in vivo, significantly altering glutamate levels, but also assuages CIBP. In sum, we demonstrate that pharmacological inhibition of system xc- transport attenuates CIBP-related behaviors.

Conclusions: These data support a role for tumor-derived glutamate in CIBP and validate system xc- an analgesic target in this pain state. Further research is warranted to evaluate the potential repurposing of sulfasalazine as an adjunct therapy for patients with CIBP.
Background: Native Americans are the most underrepresented racial/ethnic group among physicians and scientists. According to the CDC, the number one cause of death among Native Americans is cancer. This is in contrast to the majority population for which heart disease is the number one killer. With the large number of tribes in Arizona, the state’s universities are ideally positioned to train a greater number of Native Americans for biomedical careers. It is anticipated that this can be an effective approach to addressing cancer health disparities in Native American communities.

The Partnership for Native American Cancer Prevention (NACP) began in 2002 as an NCI-funded collaboration between the University of Arizona Cancer Center and Northern Arizona University. NACP investigators partner with tribal communities in Arizona to develop research, outreach, and training activities aimed at defining and eliminating obstacles to health equity in areas of Native American cancer incidence, mortality, and survivorship. The NACP Training Core provides Native American students with mentoring and research experiences of importance to their own communities to help them achieve their training goals for a career in the health sciences.

Educational objectives: Activities in which NACP trainees engage include: 1) summer programs to transition from high school or community college to the university; 2) mentored projects with investigators conducting cancer-related research; 3) summer internships at other institutions; 4) development of an individualized career plan; 5) attendance at national meetings focused on health research in Native American communities; 6) mentoring sessions with Native American researchers as role models; and 7) a graduate programs primer that provides guidance on applying for post-baccalaureate degree programs.

Results: Evaluation data shows that the NACP Training Core is making an impact. Between 2009 and 2014, the 6-year baccalaureate graduation rate for Native American students in the US was 38%. In contrast, the NACP Native American students had a substantially higher graduation rate of 68%. A total of 179 students have participated in NACP training activities at UA. Forty students have obtained a Bachelor’s degree, 17 have received a Master’s degree and 15 have been awarded a doctorate degree.

Summary: NACP began another 5-year project period in September, 2014. The Training Core looks forward to continually increasing the number of Native Americans in biomedical careers, so as to reduce the burden of cancer in their communities.
Double-blind, Randomized Trial of Letrozole Dosing Regimens in Postmenopausal Women with Increased Breast Cancer Risk – Effects on Estrogen Suppression and Associated Side Effects

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Background: Aromatase inhibitors (AIs) represent an effective strategy to prevent ER-dependent breast cancers in high-risk postmenopausal women. AIs block the biosynthesis of estrogen from androgens through the inhibition of the aromatase enzyme, resulting in significant reductions in the circulating and tissue estrogen levels. Despite their effectiveness in chemoprevention, AIs are commonly associated with severe adverse events (AEs) – including arthralgias, osteoporosis, depression, hyperlipidemia -- which oftentimes lead to poor quality of life (QoL) and early drug discontinuation.

Methods: We conducted a double-blind, randomized study to determine whether low, intermittent doses of letrozole can effectively suppress estrogen levels and reduce AEs. One hundred and twelve healthy postmenopausal women with a history of lobular carcinoma in situ or Gail risk score greater than 1.67% were randomized to receive letrozole at 2.5 mg once daily (QD, standard dose arm); 2.5 mg every Monday, Wednesday, and Friday (Q-MWF); 1.0 mg Q-MWF or 0.25 mg Q-MWF for a total of 24 weeks. Serum estrogen levels were measured before and after letrozole treatment using liquid chromatography-tandem mass spectrometry assay (LC-MS/MS).

Results: After 24 weeks of treatment, all letrozole dosing regimens (2.5 mg QD, 2.5 mg Q-MWF, 1 mg Q-MWF, 0.25 mg Q-MWF) significantly suppressed serum estrone (E1) and estradiol (E2) levels by an average ranging between 86-92% and 75-77% from baseline, respectively (p<0.0001). The extent of estrogen suppression with the low and intermittent doses of letrozole was not inferior to the standard dose arm. Estrogen levels returned towards baseline six weeks following the discontinuation of letrozole. Serum levels of C-telopeptide, a biochemical marker of bone resorption (a.k.a. circulating bone turnover biomarkers) increased by an average of 37-48% in each treatment arm at the end of the 24-week letrozole intervention; however, there were no differences in the proportional change in C-telopeptide levels among treatment arms (p=0.88). Similarly, there were no differences among the treatment arms with respect to serum lipids profile. QoL assessments were conducted using Menopause-Specific Quality of Life (MENQOL) and Medical Outcomes Study Short Form Healthy Survey (SF-36) and no significant differences in QoL outcomes between study arms were observed.

Conclusion: We demonstrate that low and intermittent doses of letrozole were non-inferior to standard letrozole dosing regimen (2.5 mg QD) in estrogen suppression. The different dosing regimens shared similar AE profiles with respect to QoL outcomes, lipid profile and bone resorption biomarkers. Future studies correlating the degree of systemic estrogen suppression, breast tissue drug activity and AEs are needed to select an optimal AI dosing schedule with effective estrogen suppression and a more favorable side effect profile.
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Activated B-Cell DLBCL with Downstream Activation of Survival Signaling Requires PIM Kinase Activity to Maintain Oncoprotein Translation

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Background: The activated B-cell cell-of-origin subtype of diffuse large B-cell lymphoma (ABC-DLBCL) requires NF-kB pathway activation to maintain the malignant phenotype. NF-kB activation is downstream of B-cell receptor (BCR) stimulation and can become constitutively turned on in ABC-DLBCL through mutations in multiple different BCR signaling intermediates. Drugs targeting upstream signaling proteins such as Bruton’s Tyrosine Kinase (BTK, ibrutinib) or protein kinase C (PKC, AEB071) have shown promising results in other lymphomas driven by BCR activation and are under evaluation in ABC-DLBCL. Many ABC-DLBCL cases, however, have mutations in mediators that are downstream from these targets, particularly coiled-coil domain mutations in CARD11 that stabilize NF-kB activation and A20 loss-of-function alterations that reduce protein turnover of oncogenic NF-kB intermediates.

Results: In this study we explore a potential role for PIM kinase inhibition in ABC-DLBCL and find the clinical pan-PIM inhibitor LGH447 has promising activity in particular against cells carrying these downstream mutations. We find some ABC cells were highly sensitive to LGH447 with IC₅₀ < 0.4 µM (OCI-Ly3 and OCI-Ly10), while some others were completely insensitive with IC₅₀ > 10.0 µM (TMD8 and HBL-1). Strikingly, all ABC lines sensitive to LGH447 carry mutations in either CARD11, TNFAIP3 (encoding A20), or both, while insensitive typically lines lack such lesions.Insensitive lines including TMD8 and HBL-1 instead have upstream mutations in CD79B and are highly sensitive to the upstream inhibitors ibrutinib and AEB071. The PIM1-3 kinases inhibited by LGH447 have multiple targets mediating cell growth and survival, including several that activate cap-dependent protein translation activation. We find LGH447 is toxic to sensitive cells due to lost translational activation. Western blots show reduced phosphorylation of ribosomal protein S6 and 4EBP1, indicating loss of mTORC1 activity. In addition, LGH447, in a manner similar to the potent direct cap-dependent translation inhibitor silvestrol, causes knockdown of key translationally regulated oncoproteins, including c-MYC, MCL1, and Cyclin D3. We also directly monitored protein synthesis through O-Propargyl-puromycin (OP-PURO) incorporation and found a direct effect of LGH447 that was similar to silvestrol, although requiring higher concentrations. PIM’s effects on activation of protein translation therefore are required in LGH447-sensitive ABC-DLBCL cells but dispensable in insensitive cells.

Conclusion: In conclusion, pan-PIM kinase inhibition provides a strong potential therapeutic opportunity in a subset of ABC-DLBCL. Cases that bypass upstream signaling to turn on NF-kB activation more directly also bypass pathways with redundant activation of cap-dependent translation, making them dependent on the therapeutically targetable PIM kinases to carry out this critical process.
ANALYTICAL CHEMISTRY

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Description:
The University of Arizona Cancer Center Analytical Chemistry Shared Resource (ACSR) provides Cancer Center investigators with centralized resources and expertise in performing analytical chemistry assays and pharmacokinetic and pharmacodynamic data analysis and interpretation.

Services:
• Development and implementation of chromatography-based analytical chemistry methods for quantification of cancer therapeutic and preventive agents, nutrients, carcinogens, and endogenous biochemicals
• Quantitative analysis of cancer therapeutic and preventive agents, nutrients, carcinogens, and endogenous biochemicals in clinical and pre-clinical specimens
• Pharmacokinetic study design and pharmacokinetic and pharmacodynamic data analysis

Measurements of the concentrations of cancer therapeutic and preventive agents, nutrients, and carcinogens allow for the assessment of drug/nutrient/carcinogen exposure and disposition. Measurements of the concentrations of endogenous biochemicals could be utilized for risk stratification, diagnosis, disease follow-up, and efficacy evaluation. In addition, these measurements could help define the mechanisms underlying the disease or to develop new strategies for treatment.
BEHAVIORAL MEASUREMENT

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**Description:**
The Behavioral Measurement Shared Resource (BMSR) has more than 20 years of experience as a national leader in providing support to researchers investigating human lifestyle behaviors associated with chronic disease risk such as:
- Behavior change theory and resources
- General behavioral risk factors
- Diet
- Physical activity
- Tobacco exposure
- Quality-of-life
- Sun-safety behaviors
- Sexual practices
- Sleep

In addition, the Shared Resource provides expertise and resources for:
- Lifestyle coaching
- Anthropometric measurement
- Body composition assessment
- Accelerometry
- Multimodal lifestyle behavior change support

The Shared Resource also has experience in developing REDCAP and QUALTRIX surveys/questionnaires for data capture and analysis.

Our services support bilingual (English and Spanish) questionnaires and assessments. Several of our behavioral questionnaires are designed for use in Southwestern populations.

**Services:**
- Consultation for behavioral research design, implementation and assessment
- Validated questionnaires; questionnaire development and validation study design
- Web questionnaire programming and management
- Interviewer training
- Diet data collection – 24-hour dietary recalls
- Accelerometer data collection and analysis
- SMS messaging for behavioral interventions
- Body composition assessment
- Consultation on use of existing health-related quality of life measures
- Statistical summaries, analysis and reports of behavioral assessment data
- Collaboration for peer-reviewed manuscripts, abstracts
- Database creation and management
The BMSR provides technical support for the use of the questionnaires, including optical scanning technology (including image archiving and intelligent character recognition), nutrition assessment software, and light pen entry system for developmental instruments.

The BMSR has ActiGraph Accelerometers/Actigraphy for the collection of physical activity and sleep measures. The accelerometers are small and provide a minimally invasive tool for direct measure of physical activity. BIMSR also has the ActiGraph software tools to analyze activity data collected with the ActiGraph devices.

BMSR has software platforms in place to deliver lifestyle coaching to study participants. Coaches trained in diet, physical activity and tobacco cessation behavior change are available to support research. Software platforms can be modified to meet individual study requirements in collaboration with the Arizona Research Laboratory.

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**BIOINFORMATICS**

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**Description:** Informatics involves management and integration of clinical and/or molecular data sets, while Bioinformatics involves the analysis of high throughput sequence and molecular data for study of cancer genomes. The Bioinformatics Shared Resource provides comprehensive analysis of genomic data to UACC members in support of their research. This contribution can result in short or long-term projects, ranging from one day to many months, depending on the nature and extent of the support required.

The areas of expertise include biological sequence analysis, genome analysis, advanced computational analysis of large data sets such as expression, next-gene sequencing, single nucleotide polymorphism (SNPs), and proteomics data. BISR provides analysis of high dimensional data that use molecular arrays, DNA and RNA sequencing and mined biomedical clinical variables.

The Shared Resource provides all levels of support, from experimental design to analysis and publication of these data. The goal is to provide assistance with data analysis that will lead to testable hypotheses and fundamentally important discoveries in cancer research.

The BISR specializes in the biological interpretation of data that may lead to a new understanding of cancer biology and the discovery of new diagnostic markers, risk genetic markers and drug targets. The staff is well prepared to perform all of these types of analysis.

**Services:**
- Analysis of genome data (e.g. gene expression, non-coding RNAs, CGH, RNAi screens, genome and sequence analysis), genetic data (SNP analysis), proteomics, and other types of molecular data sets of cancer cells and tissues
- Analysis of large cancer molecular datasets and clinical annotated datasets from NIH consortiums, medical institutions or public resources. NIH-TCGA (Cancer Genome Atlas) project, Cosmic (Catalogue of somatic mutations in Cancer), CCLE (Cancer Cell Line Encyclopedia), NIH LINCS project (Library of Network-based Cellular Signatures), and NCBI GEO datasets
• Biological interpretation of the above data, including pathway and ontology analysis, systems analysis, genetic vulnerabilities for drug targeting, predictive patterns for outcome, and data modeling.
• Bioinformatics support for Cancer Center projects and other Shared Resources in the form of molecular databases, genome databases, and data sharing tools

**BIOSTATISTICS**

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**Description:**  
The Biostatistics Shared Resource (BSR) brings together expertise in biostatistics, clinical trials, epidemiology, applied mathematics, statistical computing, and database applications.

BSR personnel have wide-ranging involvement across Cancer Center research activities, including clinical, translational, basic, and population-based research. Biostatistical collaboration and consultation have been provided for investigators in each of the Cancer Center Scientific Programs. The BSR is active in all phases of study design, operation, data management, statistical analysis, and manuscript preparation.

BSR ensures excellent and timely biostatistical support in the design and protocol development of laboratory, translational, clinical, and population-based anti-cancer research studies; provides appropriate state-of-the-art statistical analysis, interpretation, and reporting of anti-cancer research studies. It supports UACC clinical trials by serving on the Scientific Review Committee, and the UACC Data and Safety Monitoring Board.

**Services:**
• Clinical protocol development
• Statistical database applications
• Data analysis and reporting
• Statistical consultation and collaboration
• Statistical support for development of funding applications
CANCER IMAGING

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**Description:**  
The Cancer Imaging Shared Resource (CISR) provides access to turnkey optical, and other pre-clinical and clinical, image acquisition and analysis technologies.

The CISR also offers a developmental component to evaluate and initiate new research projects, and provides access to a range of approaches for small animal experiments including bioluminescence imaging (ISS Ami-X), PET/SPECT and microCT (Siemens) systems. An image analysis facility acts as a central clearinghouse to consolidate and standardize state-of-the-art image processing and analysis routines. The Core also provides experimental design and operator expertise for small animal cancer imaging and spectroscopy on a 7T Bruker magnet owned and maintained by the University of Arizona.

**Services:**
- **Bioluminescence:** Spectral Instruments Imaging Ami-X
- **Confocal microscopy:** Leica SP5-II Resonance Scanning Confocal Microscope
- **MicroCT imaging:** Siemens Inveon
- **Image analysis:** Siemens MicroCT, Definians, Custom Clinical MR on IDL

CENTRALIZED CANCER CLINICAL TRIALS UNIT (C3)

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**Mission, purpose and scope:**  
The Centralized Cancer Clinical Trials Unit (C3) provides centralized infrastructure support for clinical trials conducted at and by the University of Arizona Cancer Center (UACC). C3 is synonymous with the Clinical Protocol and Data Management Program (CPDM) component of the UACC National Cancer Institute (NCI) Cancer Center Support Grant (CCSG).

C3 partners with UACC investigators to provide experienced and consistent human and material resources to support the institution’s clinical research mission, while ensuring that clinical research is conducted in a safe and ethical manner in compliance with applicable rules and regulations.
The UACC C3 unit oversees industry-sponsored, institutionally sponsored, externally peer-reviewed and national group clinical trials. The UACC is a member of several National Clinical Trial Network (NCTN) cooperative groups, including the Southwest Oncology Group (SWOG; full member), NRG Oncology (Breast Alliance, Radiation Therapy, and Gynecologic oncology; affiliate member) and Children’s Oncology Group (COG; full member).

Services:
- Investigator-Initiated Trial (IIT) Development
- Data and Safety Monitoring Board administration
- Quality Assurance and Quality Control (QA/QC)
- Protocol Review and Monitoring System administration
- Budget
- Regulatory
- Operations
- Clinical Trial Informatics
- Cooperative Group Administration

EXPERIMENTAL MOUSE

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http://gemmcore.bio5.org/

Description:
The Experimental Mouse Shared Resource (EMSR) offers a continuum of services from initial consultation and in vivo experimental design, to GEM production through mouse experimentation and data analysis. This enables the investigator to generate and use mouse models of human cancer to their fullest potential, taking advantage of the Shared Resource’s extensive knowledge and expertise at competitive pricing.

The EMSR is a full-service facility which performs preclinical experiments in a wide variety of in vivo and in vitro cancer models. The unit team provides technical and scientific expertise in modeling of cancer disease and offers a variety of services using cancer cell lines.

Services:
- Animal Techniques
- Surgeries
- Injections
- Cell culture
- Administrative support
GENETICALLY ENGINEERED MOUSE MODELS CORE FACILITY

The GEMM Core offers “sequence-to-whiskers” mouse genetic engineering services that include vector design and construction for both transgenic and gene-targeted mice, gene-targeting in ES cells, screening for targeted ES cells, CRISPR/Cas9 targeting for SNP knockins, pronuclear and blastocyst injection, screening for founder and germline chimeric mice, breeding for speed congenics, and consultation on GEM colony management. Also provided are sperm cryopreservation, IVF, and embryo rederivation services.

Facilities:
Vector Construction Facility
Tissue Culture Facility
Mouse Production Facilities

FLOW CYTOMETRY

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Description:
Flow Cytometry is a powerful tool that measures the functional and structural characteristics of heterogeneous mixtures of cells and particles in suspension based on their ability to scatter light. The cell sorting function separates these cells physically into their different classes.

Researchers have the capability to analyze and sort cells by differences in physiology, metabolism, morphology and other characteristics. The ability to distinguish different cell types is limited only by the ability to attach specific fluorescent markers to the cells.

The Flow Cytometry Shared Resource at the University of Arizona Cancer Center supports the research needs of all Cancer Center members by providing state-of-the-art instrumentation for data acquisition, analysis, and cell sorting, and the technical expertise to interpret results and develop methods. We offer information about new techniques and applications of flow cytometry through workshops and seminars, and provide training to interested facility users who wish to run their own samples. Individual consultation services are available to discuss the specifics of each project. The Shared Resource is operated and administered as a partnership between the UA Cancer Center and Arizona Research Laboratories, Division of Biotechnology.
GENOMICS

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Location: Room 3922, University of Arizona Cancer Center

Website: http://azcc.arizona.edu/research/shared-resources/gsr

Description: The Genomics Shared Resource (GSR) is co-sponsored by the UA Cancer Center and the Southwest Environmental Health Science Center. The Shared Resource serves members of our funding centers by providing genomics services based on microarray, next-generation sequencing, and polymerase chain reaction (PCR) technology platforms. Applications include transcriptome expression profiling, exome sequencing, re-sequencing panels, epigenetic analysis, and quantitative real time PCR applications. As part of the commitment to providing high quality genomics service, the Shared Resource has qualified as a member of the Ion Torrent Certified Service Provider Program. The staff of the GSR has extensive experience working with nucleic acids and can assist researchers with nearly any experiment based on analysis of DNA or RNA. In addition to performing analyses, the GSR can assist with sample isolation, quality control, experimental design and analysis.

Services:
• Sample Quality Control
• Experiment Design and analysis
• Sequence analysisDNA analysisRNA expression
• RNA regulation

PROTEOMICS

Director: George Tsaprailis, PhD  
520-626-5461 • tsaprailis@pharmacy.arizona.edu

Location: Room 106, BIO5 Keating Bioresearch Building, 1657 E. Helen St.

Website: http://azcc.arizona.edu/research/shared-resources/psr

Description: The Proteomics Shared Resource (PSR) provides University of Arizona Cancer Center (UACC) investigators with a dedicated facility and expertise in analyzing proteins for their identity, quantity and function via state-of-the art modern mass spectrometry and peripheral analytical instrumentation.
**Services:** A variety of gel-based and solution-based proteomics services are offered at competitive rates to UACC members.

- Gel Electrophoresis – achieves separation of proteins in a gel matrix
- Protein MW determination
- LC-MS/MS
- LC-LC-MS/MS
- Abundant Protein Depletion
- Protein Purification
- Phosphopeptide Enrichment (user provides necessary reagents)
- Biomolecular Interactions by Surface Plasmon Resonance.
- Sample concentration and clean-up
- Aid with experimental design and data/results interpretation

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**TISSUE ACQUISITION AND CELLULAR/MOLECULAR ANALYSIS**

**Director:** Charmi Patel, MD  
520-626-6830 • charmipatel@email.arizona.edu

**Co-Director:** Achyut K. Bhattacharyya, MD  
520-626-6097 • abhattac@email.arizona.edu

**Manager:** Doug Cromey, MS  
520-626-2824 • dcromey@email.arizona.edu

**Location:** Room 0914, University of Arizona Cancer Center

**Website:** [http://azcc.arizona.edu/research/shared-resources/tacmasr](http://azcc.arizona.edu/research/shared-resources/tacmasr)

**Description:**
The Tissue Acquisition and Cellular/Molecular Analysis Shared Resource (TACMASR) provides support and pathology-related services to University of Arizona Cancer Center (UACC) and University of Arizona (UA) researchers on a fee-for-service basis. We strive to maximize research dollars through customized, cost-effective and quality-controlled services.

The Shared Resource’s director and co-director are board certified anatomic pathologists with many years of clinical and experimental research experience.

**Services:**
TACMASR offers two primary types of services to UACC and UA research investigators:

- Cellular and molecular analysis
- Tissue acquisition and biobanking
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