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RESEARCH RETREAT

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ABSTRACT BOOK

Carbone Cancer Center
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Abstract #1

CD155 axis modulation promotes natural killer cell-mediated graft-versus-tumor effects against osteosarcoma

Authors:
Monica M. Cho[1], Madison F. Phillips[1], Longzhen Song[1], Amy Erbe-Gurel[1], Christian M. Capitini[1,2]

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Background:
Patients with relapsed/refractory osteosarcoma (OSA) have a poor prognosis with few treatment options. Immunotherapy with allogeneic natural killer (NK) cells after allogeneic bone marrow transplant (BMT) is an attractive approach to employ a graft-versus-tumor (GVT) effect against OSA. However, NK cells have had limited success against solid tumors in vivo. CD155 is overexpressed on OSA and interacts with DNAM-1, an activating ligand, and TIGIT, an inhibitory ligand, on NK cells. The impact of blocking CD155 after allogeneic BMT is unknown.

Methods:
IL-15-expanded C57BL/6 (B6, H-2^b) and BALB/c (H-2^d) murine NK cells were co-cultured with K7M2 murine OSA (H-2^d) after CD155 and CD155 ligand blockade, and analyzed by flow cytometry and cytotoxicity assays. BALB/c mice were also transplanted with T cell depleted allogeneic B6 or syngeneic BALB/c bone marrow, challenged with K7M2 and treated with IL-15-expanded NK cells and CD155 blockade.

Results:
Allogeneic NK cells showed increased degranulation, interferon-gamma production and cytotoxicity against K7M2 OSA in vitro compared to syngeneic NK cells. NK cell activation and cytotoxicity were further enhanced by CD155 and TIGIT blockade, but decreased with DNAM-1 blockade. In vivo, allogeneic NK cell infusion and anti-CD155 treatment decreased tumor growth and increased survival compared to syngeneic NK cell treatment, without induction of graft-versus-host-disease.

Conclusion:
CD155 blockade augments NK cell activation, cytotoxicity and GVT effects against OSA without toxicity, suggesting TIGIT is the dominant CD155 checkpoint during allogeneic BMT. CD155 blockade with allogeneic NK cell therapy after BMT may be an effective combination immunotherapy platform for treating relapsed/refractory OSA.

Abstract #2

Fedratinib and Venetoclax Have Synergistic Activity Against B-Cell Acute Lymphoblastic Leukemia in Vitro

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Treatment of relapsed/refractory B cell acute lymphoblastic leukemia (B-ALL) remains a challenge particularly in patients who do not respond to chemotherapy or immunotherapy. While a subset of these patients can be cured with allogeneic bone marrow transplant, success of the transplant is contingent upon achieving a minimal residual disease negative complete remission. The objective of this study was to assess the efficacy of fedratinib, a semi selective JAK2 inhibitor and venetoclax, a selective BCL-2 inhibitor, on the B-ALL cell lines RS4;11, NALM-6, and SUP-B15. While select studies have examined the efficacy of these drugs on B-ALL as single agents, data is lacking on whether these drugs work optimally as single agents or in combination. Cell lines were treated with either vehicle (DMSO), single agent fedratinib or venetoclax, or the combination of both inhibitors for 48 and 72 hours. Fedratinib doses were tested from 150 nM to 1 uM and venetoclax doses were tested from 2.5 nM to 500 nM. Live/dead discrimination was performed using flow cytometry and a 72-hour MTS assay to assess proliferation. Drug synergy was calculated using CompuSyn software to generate combination index (CI) scores, with a CI score less than 1 reflecting synergy. In RS4;11, the combination of 5 nM venetoclax and 525 nM fedratinib significantly increased cell death compared to single agent venetoclax ($p=0.0451$) and fedratinib ($p=0.0077$). Furthermore, the combination significantly decreased leukemia cell proliferation compared to single agent fedratinib ($p<0.0001$). In NALM-6, the combination of 300 nM venetoclax and 300 nM fedratinib significantly increased cell death compared to single agent fedratinib ($p=0.0103$). Combination therapy also significantly decreased cell proliferation compared to single agent fedratinib ($p=0.0157$). With SUP-B15, the combination of 5 nM venetoclax and 750 nM fedratinib significantly increased leukemic cell death compared to single agent fedratinib ($p=0.0081$). Combination therapy also significantly decreased cell proliferation compared to both single agent venetoclax ($p=0.0012$) and fedratinib ($p<0.0001$). Synergy between venetoclax and fedratinib was seen among respective dose combinations for all three cell lines RS4;11 (CI=0.57258), NALM-6 (CI=0.67991), and SUP-B15 (CI=0.19162). Combination therapy with fedratinib and venetoclax has synergistic activity in multiple B-ALL cell lines. Future studies will explore this combination therapy in vivo and identify signaling pathways impacted by JAK2 and BCL2 inhibition.

Abstract #3

Linking GATA2 Deficiency to Dysregulated Innate Immune Signaling

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Abstract

Cell type-specific transcription factors governing hematopoietic stem and progenitor cell transitions establish networks containing hundreds of genes and proteins. Network complexity renders it challenging to discover essential versus modulatory or redundant components. This scenario is exemplified by GATA2 mechanisms that control hematopoiesis during embryogenesis. Loss of *Gata2* -77 enhancer disrupts the GATA2-dependent genetic network governing hematopoietic progenitor cell differentiation. The aberrant network includes the transcription factor IRF8 and a host of innate immune regulators, including Toll-like receptors (TLRs). Mutant embryonic progenitors lose the capacity to balance production of diverse hematopoietic progeny and generate excessive monocytic progeny. As IRF8 is vitally important for monocytic differentiation, we asked whether IRF8 is essential, contributory, or inconsequential. Using a double-mutant murine system, we demonstrated that reducing *Irf8* in -77 mutant reversed granulocytic deficiencies. In -77$^{-/-}$ E14.5 fetal livers, monocyte progenitors (MPs) increased 2.3-fold and granulocyte progenitors (GPs) decreased 2.2-fold relative to wildtype littermates. Ablating *Irf8* in -77 mutants restored MPs to wildtype levels and reversed the GP deficiency, further increasing GPs 4.2-fold relative to wildtype. We analyzed the mechanistic and biological implications of IRF8 dysregulation concomitant with ectopic upregulation of other innate immune genes in GATA2-deficient embryonic progenitors. Based on TLR upregulation and TLR roles in progenitor mechanisms, we tested whether GATA2 deficiency in embryonic progenitors impacts cellular responsiveness to TLR ligands. Wild type and -77 enhancer-mutant progenitors were treated with increasing concentrations of the TLR1/2 ligand Pam_3CSK_4. The mutant progenitors were hypersensitive to Pam_3CSK_4, which resulted in supra-physiological induction of *Tnf* expression (3.2-fold at 68 nM). Quantitative analyses indicated that hypersensitivity reflected increased Pam_3CSK_4 efficacy, but not potency. GATA2 re-expression in the mutant progenitors attenuated the elevated IRF8 expression and TLR signaling, normalizing *Tnf* and *Ccl3* expression to a near-physiological level (3.9-fold and 2.5-fold decrease, respectively). Thus, GATA2 suppresses TLR signaling in embryonic progenitors. Ongoing studies are elucidating the mechanistic interconnections between IRF8- and TLR-dependent inflammatory networks in GATA2 deficiency during embryonic and adult hematopoiesis in cell populations and single cells, relationships between murine and human mechanisms, and the impact of targeted interventions that modulate these mechanisms.

Abstract #4

Local radiation in combination with CpG and anti-OX40 induces enhanced T cell activation and proliferation.

Author(s): Dan V. Spiegelman[1], Alexander A. Pieper[1], Luke M. Zangl[1], Arika Feils[1], Anna Hoefges[1], Mildred A. Felder[1], Sritha Moram[1], Alexander L. Rakhmilevich[1], Amy K. Erbe[1], Jacquelyn A. Hank[1], Ravi B. Patel[2], Zachary S. Morris[1], Paul M. Sondel[1,3]

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Background: We, and others, have previously shown that the *in-situ* vaccine of hypomethylated CG-enriched oligodeoxynucleotide (CpG) with agonist anti-OX40 antibody (OX40) is effective at curing mice in the A20 lymphoma model [1-4]. In separate preclinical models where CpG+OX40 fails to cause tumor regression, radiation therapy (RT) prior to the *in-situ* vaccine enhances the anti-tumor effect of CpG+OX40 [4]. We investigated the immune response, and specifically the activity of T cells, following treatment with RT+CpG+OX40 in the B78 melanoma model where CpG+OX40 typically fails to cause tumor regression.

Methods: C57BL/6 mice were inoculated with $2x10^6$ B78 melanoma cells on the right flank and allowed to grow until the average tumor size was ~$150mm^3$. In two independent experiments, mice were randomized (n=4-5 per group per experiment) and treated with one of the following: 1) PBS, 2) CpG+OX40, 3) RT, 4) RT+CpG+OX40. 12 Gy external beam RT was dosed to the flank tumor on day 0 and intratumoral CpG (50μg)+OX40 (20 μg) were given on days 5, 7, and 9 after RT. Spleens and tumor draining lymph nodes (TDLNs) were harvested on day 12. T cell activation and proliferation were assessed via flow cytometry.

Results: Compared to all other groups in the study, mice treated with RT+CpG+OX40 demonstrated significantly elevated levels of CD4+ and CD8+ T cell activation in the TDLNs, as measured by interferon gamma expression. Similar trends of CD4+ and CD8+ T cell activation were measured in the spleens. Splenic CD8+ T cells from RT+CpG+OX40 treated mice demonstrated significantly elevated levels of proliferation over PBS and RT, as measured by Ki67.

Conclusions: In B78 melanoma, a weakly immunologic tumor model, combining RT with the *in-situ* vaccine CpG+OX40 enhances the activity of T cells, evidenced by significantly increased CD4+ and CD8+ T cell activation in the TDLN and spleen and elevated CD8+ T cell proliferation in the spleen.

Keywords: radiation therapy, *in-situ* vaccine, combination immunotherapy, T cell co-stimulation

References:

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Dan V. Spiegelman, Alexander A. Pieper, Luke M. Zangl, Arika Feils, Anna Hoefges, Mildred A. Felder, Sritha Moram, Alexander L. Rakhmilevich, Amy K. Erbe, Jacquelyn A. Hank, Ravi B. Patel, Zachary S. Morris, Paul M. Sondel

Abstract #5

Cyclophosphamide augments the efficacy of an *in situ* vaccine in a mouse melanoma model.

Author(s): Noah Tsarovsky[1], Mildred Felder[1], Mackenzie Heck[1], Jacob Slowinski[1], Kayla Rasmussen[1], Sabrina VandenHeuvel[1], Zachary S. Morris[1,2], Amy K. Erbe[1,2], Paul M. Sondel[1,2,3], Alexander L. Rakhmilevich[1,2].

[1]Department of Human Oncology, [2]Paul P. Carbone Comprehensive Cancer Center, and [3]Department of Pediatrics, University of Wisconsin, Madison, WI.

Background:
We have previously shown that a direct intratumoral (IT) injection of the hu14.18-IL2 immunocytokine (IC), an anti-GD2 antibody linked to interleukin 2, can serve as an *in situ* vaccine and synergize with local radiotherapy (RT) to induce T cell-mediated antitumor effects. We hypothesized that cyclophosphamide (CY), a chemotherapeutic agent capable of depleting T regulatory cells (Tregs), would augment *in situ* vaccination.

Material and Methods:
$GD2^+$ B78 mouse melanoma cells were injected intradermally in syngeneic C57BL/6 mice. Treatments with RT (12Gy) and/or CY (100 mg/kg i.p.) started when tumors reached the size of 100-200 mm^3 (day 0) followed by five daily injections of IT-IC (25 mcg) on days 5-9. In some experiments, CY was given prior or after RT. Tumor growth and survival were followed. In addition, tumors were analyzed by flow cytometry.

Results:
A single injection of CY led to an enhanced antitumor effect of IC comparable to that of RT. The strongest antitumor effect was achieved when CY, RT and IC were combined, as compared to combinations of CY+RT, CY+IC or RT+IC. This augmented effect of the triple combination was seen when CY was given on the same day as RT. Flow cytometric analyses showed that CY treatment decreased Tregs and increased the ratio of CD8+ cytotoxic cells to Tregs within the tumors. Moreover, the combination of RT, CY and IT-IC led to a systemic antitumor effect against the untreated tumor in a two-tumor model. Cured mice developed immunological memory as they were able to reject B78 tumor rechallenge.

Conclusion:
CY can augment the antitumor efficacy of IT- IC given alone or in combination with local RT in tumor-bearing mice. These preclinical results suggest the value of initiating clinical testing of the combination of CY, RT and IT-IC as an *in situ* vaccine.

Abstract #6

MET inhibition enhances the effect of radiation in MET mutated non-small cell lung cancer brain metastasis patient derived xenografts.

Author(s): *Shrey Ramesh*,* Kwangok P. Nickel, Saahil Javeri, Nitin Somasundaram, Nan Sethakorn, Randall J. Kimple, Andrew M. Baschnagel

Abstract

In non-small cell lung cancer (NSCLC), the mesenchymal epithelial transition factor (MET) receptor can be altered through both mutation and amplification which occur at frequencies of roughly 3-4% and 1-6% respectively. The most common MET mutation occurs in exon 14, leading to enhanced downstream signaling causing increased cell proliferation, survival, and migration. MET exon 14 skipping (METex14) is linked to poorer outcomes for NSCLC patients. MET is involved in pathways that involve responses to radiation therapy. Recent studies have shown that inhibition of MET increases effectiveness of radiation when treating NSCLC. Capmatinib is a selective inhibitor of the MET receptor which has shown positive results for improving treatment efficacy both in vitro and in vivo when acting as a single agent. However, little is known regarding the synergistic effect with radiation as well as the mechanism of this synergy. We will investigate the mechanism and extent of radiation sensitization due to MET inhibition using capmatinib through experiments in vitro through clonogenic and IHC assays as well as in vivo through NSCLC brain metastasis patient derived xenograft (PDX) models. By gaining a better understanding of the mechanism by which MET inhibition increases radiation sensitization of NSCLC we will move towards increased treatment efficacy and improved patient outcomes.

Abstract #7

MTORC1/2 and HDAC1/2 inhibition as therapy for colorectal cancer with PIK3CA mutation

Author(s): *Autumn Olson*,* Rebecca A. DeStefanis, Alyssa K. DeZeeuw, Susan N. Payne, Cheri Pasch, Linda Clipson, Dustin A. Deming

Introduction
Colorectal cancer (CRC) is a leading cause of cancer-related deaths. PIK3CA mutations are found in 18% of CRCs which lead to the constitutive activation of the PI3K/MTOR pathway and can promote tumorigenesis. We examine the efficacy of the FDA approved inhibitors copanlisib, a PI3K/MTOR inhibitor, and romidepsin, a HDAC1/2 inhibitor, in CRC with a PIK3CA mutation.

Methods
CRC mouse derived cancer organoids (MDCOs) were derived from Apc and Pik3ca mutant mice (Fc1Apcfl/+Pik3caH1047R/+). Brightfield images of the MDCOs were taken prior to treatment with copanlisib, romidepsin, or the combination and 48 hours post-treatment. The change in diameter of each organoid over the 48-hour treatment was determined. Immunoblotting was performed to confirm known targets of copanlisib and romidepsin were altered in response to drug treatment. MTORC1/2 and HDAC1/2 inhibition were also investigated. SW48 and SW48PIK3CA-H1047R (SW48PK) xenograft mice were treated with a vehicle control, copanlisib, romidepsin, or the combination therapy.

Results
In the diameter analysis study, the combination therapy had the largest effect size compared to control (Glass's Δ 2.82). Single agents copanlisib and romidepsin had smaller effect sizes (Glass's Δ 1.75 and 2.04, respectively). Immunoblotting results indicated a decrease in phosphoAKT (Ser473) and pRPS6 (Ser235/236) in the copanlisib treated samples and an increase in H3K27 acetylation was seen in the romidepsin treated samples. There was increased cleaved PARP, an indicator of apoptosis, in the romidepsin and combination therapy treatment groups. In vivo, SW48 xenografts showed a greater response in the combination therapy compared to either single agent therapy (median relative change in tumor volume: control=339%; combination therapy=107%(p-value<0.05), copanlisib=225%(p-value=0.17), romidepsin=200%). A similar trend was seen in the SW48PK xenograft mice (median relative change in tumor volume: control=241%; combination therapy=70%(p-value<0.05), copanlisib=132%(p-value<0.05), romidepsin=177%).

Conclusion
MDCOs with Apc and Pik3ca mutations had an increased response to treatment with the combination therapy as compared to the single agents alone. In vivo studies with human CRC xenografts showed enhanced inhibition of tumor growth with both MTORC1/2 and HDAC1/2 inhibition. These data demonstrate potential for this combination treatment strategy for the treatment of PIK3CA mutant CRC and this combination warrants further investigation in other models and clinically.

Abstract #8

Tumor-Targeting and Efficacy of B7H3/GD2 Bispecific SNIPER Antibodies

Author(s): *Alina Hampton*,* Amy K Erbe, Arika Feils, Zack Rosenkrans, Jessica Wiwczar, Daniel Gerhardt, Bonnie Hammer, Mildred Felder, Mark Bercher, Lizzie Frankel, Dan Spiegelman, Noah Tsarovsky, Alexander Rakhmilevich, Jacquelyn Hank, Bryan Glaser, Reinier Hernandez, @Roland Green, @Paul Sondel (@Co-senior authors).

Introduction: GD2 is expressed on neuroblastoma, melanomas, and other cancers. While it is expressed minimally on normal tissues, it is expressed on nerve cells, causing anti-GD2 mAb (dinutuximab) treatment to cause neuropathic pain. B7H3 is a tumor antigen that is overexpressed on multiple tumor types but is minimally expressed on normal cells and is absent on nerve cells. To increase tumor specificity, we developed a bispecific SNIPER antibody to simultaneously target two tumor antigens (GD2 and B7H3). Our goal for this bispecific SNIPER antibody is to serve as a treatment for neuroblastoma that is at least as effective as dinutuximab, but that causes less pain.

Methods: We tested the in vitro SNIPER specificity by flow cytometry for binding to cells expressing GD2 +/- B7H3. In vivo specificity was also tested in mice bearing variants of GD2/B7H3-expressing tumors using intravenously injected 89Zr-radiolabeled SNIPER after which we monitored the mice via PET imaging. In vitro efficacy testing was performed using an Incucyte S3 to monitor antibody dependent cellular-cytotoxicity (ADCC) capabilities. In vivo efficacy studies of this SNIPER were tested in mice bearing either melanoma or neuroblastoma tumors that express both GD2 and B7H3.

Results: We found that in vitro and in vivo tumor specificity testing confirmed that SNIPER specifically targets B7H3+/GD2+ cells, but it does not bind to GD2+/B7H3- cells (which simulate nerve cells). The SNIPER was as effective at ADCC as the dinutuximab, and an afucosylated version of SNIPER showed significantly enhanced ADCC compared to dinutuximab. Our in vivo efficacy studies found that SNIPER and dinutuximab showed similar anti-tumor efficacy.

Conclusions: The SNIPER binds with strong avidity when both GD2 and B7H3 are present on the same cell. Due to the strong avidity, high tumor specificity occurs between cells co-expressing both GD2 and B7H3, and limits the binding to cells expressing only GD2 (like nerves). With the SNIPER specifically binding to the tumor, it may allow larger doses of SNIPER to be administered compared to that of dinutuximab; increasing efficacy. Ongoing studies include antitumor efficacy testing, nerve binding assays and assessing reduction of pain in vivo with this SNIPER.

Abstract #9

In vivo multiphoton autofluorescence imaging is sensitive to changes in T cell and melanoma tumor cell metabolism during immunotherapy

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Background: Intravital multiphoton microscopy (IMM) of the metabolic co-enzymes NAD(P)H and FAD (optical metabolic imaging, or OMI) provides label-free imaging of metabolic changes *in vivo*. The metabolism of tumor and immune cells is closely associated with cancer progression and immune cell phenotype, so we aim to study metabolic changes during administration of an established, triple-combination immunotherapy within murine melanoma tumors. This therapy includes external beam radiation, intratumoral administration of a hu14.18-IL2 immunocytokine (anti-GD2 mAb fused to IL2), and intraperitoneal administration of anti-CTLA-4 leading to *in situ* vaccination and cure of 90% of GD2+ murine tumors. Previous work has shown that a T cell response is critical to the efficacy of this therapy, so we created an mCherry-labeled T cell mouse model to study T cell response. Here, IMM was used to image concurrent tumor and T cell metabolic and functional changes as well as tumor microenvironment changes.

Methods: We created an mCherry-labeled CD8 T cell reporter mouse model through CRISPR/Cas9 knock-in. We then implanted syngeneic B78 (GD2+) melanoma cells intradermally into the flanks of these mice to induce measurable tumors. Mice were anesthetized, skin flap surgery performed, and tumors imaged at varying time points using IMM. Murine tissues were also harvested and analyzed via flow cytometry and immunofluorescence to confirm mCherry expression in mouse models, study infiltrating immune cell populations, and corroborate IMM findings.

Results: Here we establish that our reporter mouse model enables clear identification and tracking of CD8 T cells. We demonstrate that OMI can distinguish between treated and control tumors based on metabolic changes. We also show changes in protein binding and redox state within treated tumors compared to control. In addition, we show remodeling of the immune infiltrate and collagen structure within the tumor microenvironment during immunotherapy.

Conclusions: Our results suggest that the combination of our imaging techniques and reporter mouse model allows for the tracking and quantification of metabolic changes that occur during immunotherapy in both tumor and CD8 T cells. Overall, this technology enables single cell analysis of metabolic changes *in vivo* to provide insight for immunotherapy development.

Abstract #10

Simultaneous inhibition of Axl and MerTK enhances anti-PDL1 efficacy and creates a proinflammatory tumor immune microenvironment in head and neck cancer

Author(s): *Kourtney Kostecki*,* Mari Iida, Anne Wiley, Seungpyo Hong, Ravi Salgia, Paul Harari, and Deric Wheeler

Background: Head and neck cancer (HNC) is the sixth most common cancer, with approximately 650,000 new cases annually. The receptor tyrosine kinases Axl and MerTK, known for their role on macrophages in regulating clearance of apoptotic cells, are highly overexpressed in HNC. In macrophages, Axl/MerTK signaling leads to M2 polarization, an anti-inflammatory state that promotes tumor growth. Previous studies in our laboratory have shown that Axl is a critical driver of survival, proliferation, metastasis, and therapeutic resistance in HNC, and that MerTK is functionally redundant to Axl. In this study, we investigated the role of Axl and MerTK in creating an immunologically cold tumor immune microenvironment (TIME) by targeting both receptors with INCB081776, a small molecule inhibitor of Axl and MerTK.

Methods: Because Axl and MerTK are expressed on both macrophages and cancer cells, we examined the effect of INCB081776 on each cell type. Polarization state of bone marrow derived macrophages following INCB081776 treatment was determined by qPCR analysis of relevant genes. Additionally, mouse oral cancer (MOC) tumors in syngeneic mice were treated with INCB081776 alone or in combination with an antibody against PDL1 (anti-PDL1), thereby mimicking current standard-of-care treatment. Tumor growth was measured, and tumor immune cell infiltrate was analyzed using flow cytometry and immunohistochemistry.

Results: In macrophages, our experiments suggest that treatment with INCB081776 can reduce M2 polarization and increase M1 polarization, a pro-inflammatory state that promotes tumor cell killing. In both hot (MOC1) and cold (MOC2) HNC tumors, levels of M_1 macrophages increased, levels of M_2 macrophages decreased, and the CD8/FoxP3 ratio increased after INCB081776 treatment, demonstrating that INCB081776 creates a hotter TIME. Interestingly, the levels of both CD8 T cells and TILs increased only in MOC2 tumors, suggesting that INCB081776 has a greater effect on the TIME of cold tumors. Finally, the combination of INCB081776 and anti-PDL1 was superior to either treatment alone in slowing tumor growth.

Conclusion: These studies indicate that INCB081776 cooperates with anti-PDL1 in a syngeneic mouse model of HNC to slow tumor growth and create a pro-inflammatory environment, especially in immunologically cold tumors, suggesting high potential clinical benefit of this therapeutic combination.

Abstract #11

Radionuclide-specific effects of ^{90}Y-, ^{177}Lu-, or ^{225}Ac-NM600 targeted radionuclide therapy on tumor immunomodulation and enhancing immunotherapy response in syngeneic murine tumors

Author(s): *Caroline P. Kerr[1,2],* Amber M. Bates[2], Joseph J. Grudzinski[1], Carolina A. Ferreira[3], Julia Sheehan-Klenk[2], David Adam[3], Maria Powers[2], Wonjong Jin[2], Justin C. Jagodinsky[2], Raghava N. Sriramaneni[2], Paul A. Clark[2], Luke Zangl[2], Anatoly N. Pinchuk[1], Alejandro J. Onate[2], Ria Kumari[2], Cynthia Choi[4], Christopher F. Massey[1,3], Bryan Bednarz[3], Reinier Hernandez[3], Jamey P. Weichert[1,5], Zachary S. Morris[2]

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Background: In preclinical studies, we have demonstrated that delivering low dose radiation to all tumor sites utilizing ^{90}Y-NM600 improves the response to immune checkpoint inhibitors (ICIs). NM600 is an alkylphosphocholine analog that is selectively taken up and retained in murine and human tumors. In this study, the immunomodulatory capacities of three radionuclides (^{90}Y, ^{177}Lu, ^{225}Ac) were compared using immunologically cold murine tumor models: MOC2 head and neck squamous cell carcinoma and B78 melanoma. We hypothesized that physical properties of radionuclides (e.g. emission type, linear
Methods: Dosimetry performed using the Monte Carlo-based RAPID platform determined that 100 µCi ^{90}Y-NM600 or 200 µCi ^{177}Lu-NM600 delivered ~12Gy to MOC2 tumors and ~4Gy to B78 tumors. ^{225}Ac-NM600 was dosed at activities ≤0.5 µCi. Mice bearing MOC2, B78 WT, or *Tmem173* -/- CRISPR deletion B78 (STING KO) tumors were randomized to receive external beam radiation therapy (EBRT), an equivalent tumor dose of ^{90}Y- or ^{177}Lu-NM600, 0.25 µCi ^{225}Ac-NM600, or no radiation +/- dual ICI (anti-CTLA4 and anti-PDL1). Tumors, blood, bone marrow, and spleen were harvested for flow cytometry and RT-qPCR. Additional mice were monitored for tumor growth and survival.

Results: Targeted radionuclide therapy (TRT) and EBRT induced favorable tumor-specific immune cell infiltration (increased CD8/Treg ratio) at day 7 post-treatment. ^{225}Ac-NM600 additionally induced similar changes at day 21, consistent with the longer half-life radioisotope (^{90}Y: 65h; ^{177}Lu: 161h; ^{225}Ac: 240h). Expression of type I interferon (IFN1) response-associated genes (*Ifnβ1*, *Mx1*) was upregulated following 12 Gy EBRT or TRT compared to non-irradiated controls in MOC2 cells and tumors. The timing and magnitude of these effects correlated with radionuclide half-life and LET. ^{225}Ac-NM600+ICI improved overall survival in B78 WT mice over ^{90}Y- or ^{177}Lu-NM600+ICI, ^{225}Ac-NM600 monotherapy, and non-irradiated controls. In the B78 STING KO melanoma cell line, this ^{225}Ac-NM600+ICI survival benefit was decreased.

Conclusion: These studies demonstrate the capacity to deliver immunomodulatory radiation to tumors using TRT. The radionuclide physical properties dictate timing and magnitude of the IFN1 response stimulated by TRT. Understanding these effects may be critical to integrating TRT and immunotherapies in clinical settings.

Abstract #12

Depletion of Tumor Associated Macrophages Enhances Local and Systemic Platelet-Mediated Anti-PD-1 Delivery for Post-Surgery Tumor Recurrence Treatment

Presenting Author: *Zhaoting Li**, Yingyue Ding, Paul M. Sondel, Seungpyo Hong, Quanyin Hu

Background: Clinically, surgery remains the foremost treatment option for patients with solid tumors, while tumor recurrence frequently occurs, leading to a low rate of long-term survival. Cancer immunotherapy, especially immune checkpoint blockade, has been demonstrated as one of the most potent anti-cancer recurrence strategies either as monotherapy or in combinations with other treatment modalities. However, intricate physiological environment changes after surgery, especially wound healing-triggered inflammatory condition and immunosuppression mediated by tumor associated macrophages (TAM), could diminish the efficacy of cancer immunotherapy, and accelerate tumor recurrence and metastasis. Therefore, the combination of TAM depletion and immune checkpoint blockade could be critical for augmenting anti-tumor immunotherapy outcomes.

Methods: A biodegradable dextran nanoparticle was formulated to encapsulate Pexidartinib (PLX). Anti-PD-1 antibodies were conjugated on the surface of platelets, where the inflammatory environment secondary to the surgical exposure could activate platelets to release anti-PD-1 antibodies. Moreover, we developed a biocompatible alginate-based hydrogel encapsulating PLX-loaded nanoparticles and anti-PD-1-conjugated platelets for post-surgery tumor recurrence treatment, to achieve sustained release of PLX and anti-PD-1 antibodies for depleting TAMs and re-activating infiltrated T cells.

Results: We found that the hydrogel-based treatment strategy showed potent anti-tumor efficacy in different tumor recurrence models including the colon cancer, melanoma, sarcoma, and breast cancer. Furthermore, we proved that depletion of TAMs facilitated the migration of $CD8^+$ T cells towards tumor parenchyma by blocking the crosstalk between $CD8^+$ T cells and TAMs. Additionally, the hydrogel-based delivery strategy promoted systemic immune response thus inhibiting tumor recurrence and lung metastasis.

Conclusion: Collectively, we have demonstrated that PLX-NP and P-aPD-1 could be delivered as a combination treatment based on an alginate-based hydrogel delivery system after surgical resection, facilitating the treatment efficacy by leveraging the synergy of TAM depletion and bioresponsive aPD-1 delivery. Moreover, this strategy could be adapted to facilitate other immunotherapeutic modalities whose efficacy has been hindered significantly by the tumor immunosuppressive environment, such as adoptive T cell therapy and cancer vaccination.

Abstract #13

Immune Checkpoint Inhibition and Epigenetic Regulation to Enhance Neuroblastoma Responses

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Background: About 50% of neuroblastoma patients present with high-risk, difficult-to-treat disease. These immunologically "cold" tumors, characterized by minimal expression of Major Histocompatibility Complex I (MHCI), often have poor T cell infiltration. T cells are engaged via interactions with MHC; low MHCI expression may contribute to reduced T cell involvement during immunotherapy. Thus, anti-neuroblastoma memory T cell induction is unlikely, possibly contributing to immunotherapy resistance and/or recurrent disease. We developed variants of the murine 9464D high-risk neuroblastoma model that can be induced to express MHCI with interferon Gamma (IFNγ) stimulation (9464D-MHCI$^+$) or that do not express MHCI (9464D-MHCI$^-$). The "Combined Adaptive-Innate Regimen (CAIR)", that includes radiation together with anti-CD40, CpG, immunocytokine (anti-GD2 linked to IL2) and anti-CTLA4, can cure some mice bearing these 9464D-variants, but response rates (initial response to treatment and memory responses) differ depending on the variant. In the MHCI+ tumors, enhanced T cell infiltration in the tumor microenvironment (TME) may lead to PD-L1 upregulation and checkpoint inhibition.

Methods: Mice bearing 9464D-MHCI$^+$ tumors were treated with CAIR +/- anti-PDL1 and monitored for response; from some mice, tumors were assessed by flow cytometry. In vitro, we treated 9464D-MHCI$^-$ tumors with epigenetic modifier inhibitors (EMis) and monitored expression changes in MHCI and related genes via qPCR and flow cytometry.

Results: PDL1 expression on 9464D-MHCI$^+$ tumors was increased compared to untreated mice, corresponding with decreased PD1+ T cells in the TME. The addition of anti-PDL1 to CAIR significantly enhanced responses in mice bearing 9464D-MHCI$^+$ tumors. Separately, in vitro treatment of 9464D-MHCI$^-$ with EMis+IFNγ expression of MHCI and related genes by qPCR and flow cytometry.

Conclusions: In 9464D-MHCI$^+$ tumors treated with CAIR, PD-L1 expression is increased, suggesting immune checkpoint inhibition pathways are engaged to deter immune responses. By including anti-PDL1 in the treatment, response improved, suggesting immune checkpoint inhibition is overcome. In 9464D-MHC$^-$ tumors, MHCI expression can be restored via treatment with EMis, suggesting the possibility of engaging T cell responses, potentially allowing immunological memory formation. Therapies geared towards restoring MHCI expression, combined with effective immunotherapy regimens may allow for persistent immune responses augmenting the efficacy of anti-neuroblastoma therapies.

Abstract #14

Fulvestrant and radiation modify the tumor immune microenvironment in ER+ metastatic breast cancer and cooperate to enhance response to anti-PDL1 checkpoint blockade

Short title (35 characters including spaces): Radiation and SERD in breast cancer

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Background: Most breast cancer deaths arise from hormone therapy-resistant, metastatic estrogen receptor-positive (ER+) cancers. Many ER+ breast cancers exhibit low tumor mutation burden and lack tumor-infiltrating lymphocytes, rendering them immunologically "cold" and resistant to immune checkpoint-inhibitors (ICIs). Radiation therapy (RT) can augment tumor immune susceptibility but may increase immunosuppressive cell recruitment. Recent studies demonstrate that ER inhibition may antagonize the trafficking and activation of myeloid derived suppressive cells (MDSCs) in tumors. We hypothesized that combining RT and a selective ER degrader (SERD), fulvestrant, would cooperate to relieve immunosuppression, increase tumor immune susceptibility, and facilitate response to ICI in a syngeneic murine model of ER+ breast cancer.

Methods: Using a cell line (TC11) developed in the Schuler laboratory, cells were transplanted to caudal mammary fat pads of female FVB/N mice. When tumors reached ~200mm^3, mice were treated with combinations of vehicle, fulvestrant (250mg/kg sc, weekly), 8Gy RT on 3 consecutive days (8Gy x 3, days -1, 0, 1), and/or anti-PDL1 (200µg, IP, at days 3, 6, and 9). Tumors from these mice were collected at day 28, and cytokines in tumor lysates were evaluated using a multiplex immunoassay. In a similar experiment, tumors were collected at day 10 for qPCR and flow cytometry analysis. An *in vitro* experiment was conducted to investigate how different fulvestrant doses affect the magnitude and duration of type I IFN (IFN1) responses following 8Gy RT in TC11 tumor cells. Cells were collected on days 1, 3, 5, and 10 treated with 0, 1, 5, 10, 100, 500nM of fulvestrant following RT for qPCR analysis.

Results: Therapeutic intervention demonstrated that fulvestrant with RT slowed tumor growth compared to either treatment alone. RT increases tumor cell immune susceptibility by activation of a IFN1 response and combining fulvestrant with RT reduces infiltrating MDSCs in the tumor microenvironment (TME) compared to RT alone.

Conclusion: These results demonstrate a cooperative interaction between RT and fulvestrant in favorably modulating the TME and response to ICIs in an immunologically cold, hormone-therapy resistant, murine ER+ breast cancer model. Further experiments are needed to explore the mechanisms of these immune effects.

Abstract #15

Production and characterization of virus-free, CRISPR-CAR T cells capable of inducing solid tumor regression

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Abstract

Background: Chimeric antigen receptor (CAR) T cells traditionally harbor viral vector-based sequences that encode the CAR transgene in the genome. These T cell products have yet to show consistent anti-tumor activity in patients with solid tumors. Further, viral vector manufacturing is resource intensive, suffers from batch-to-batch variability, and includes several animal components, adding regulatory and supply chain pressures.

Methods: Anti-GD2 CAR T cells were generated using CRISPR/Cas9 within nine days using recombinant Cas9 protein and nucleic acids, without any viral vectors or animal components. The CAR was specifically targeted to the T Cell Receptor Alpha Constant gene (TRAC). T cell products were characterized at the level of the genome, transcriptome, proteome, and secretome using CHANGE-seq, scRNA-seq, spectral cytometry, and ELISA assays. Functionality was evaluated in vivo in an NSG xenograft neuroblastoma model.

Results: In comparison to traditional retroviral CAR T cells, virus-free CRISPR CAR (VFC-CAR) T cells exhibit TRAC-targeted genomic integration of the CAR transgene, elevation of transcriptional and protein characteristics associated with a memory phenotype, and low tonic signaling prior to infusion arising in part from the knockout of the TCR. Upon exposure to the GD2 target antigen, anti-GD2 VFC-CAR T cells exhibited specific cytotoxicity against GD2+ cells in vitro and induced solid tumor regression in vivo, with robust homing, persistence, and low exhaustion against a human neuroblastoma xenograft model.

Conclusions: This proof-of-principle study leveraging virus-free genome editing technology could enable flexible manufacturing of clinically relevant, high-quality CAR T cells to treat cancers, including solid tumors.

*Please see Mueller et al. 2021 (*BioRvix)* for more details.

Abstract #16

Brachytherapy dose heterogeneity primes response to immune checkpoint blockade to generate anti-tumor immunity

Author(s): *Justin Jagodinsky*,* Wonjong Jin, Jess Vera, Raghava Sriramaneni, Paul Clark, Keng-Hsueh Lan, Ishan Chakravarty, Raad Allawi, Sarah Emma, Ian Arthur, Rupak Das, Irene Ong, Jessica Miller, and Zachary Morris

Purpose: The immunologic effects of radiation (RT) are influenced by dose and each may be optimized over a unique dose range. We used brachytherapy (BT) to deliver a heterogeneous RT dose within a single tumor and compared the relative capacities of BT and homogenous dose external beam radiotherapy (EBRT) to enhance the anti-tumor immune response in combination with immune checkpoint inhibition (ICI).

Materials and Methods: We used syngeneic murine models of melanoma (B78) and prostate cancer (Myc-CaP). To evaluate the effect of BT on the microenvironment, mice bearing B78 tumors were randomized to receive BT (^{192}Ir source, 2 Gy to tumor edge), sham insertion, or EBRT (2, 8, or 20 Gy). Tumors were harvested 3 days following RT and in the case of BT, punch excisions were taken at 2, 8, and 20 Gy dose regions for gene expression and immune cell infiltration analysis. To evaluate anti-tumor response, tumor bearing mice were randomized to BT alone, catheter insertion alone, BT+ICI (anti-PD-L1 and anti-CTLA-4, 200 μg IP injection days 3, 6, 9 after RT), EBRT (2 or 8 Gy) + ICI, or ICI alone.

Results: Bulk RNAseq analysis revealed unique gene expression signatures between tissue locations in BT treated tumors. We observed significant differences in number of $CD8^+$ and $FOXP3^+$ cells between tissue locations. In both B78 and Myc-CaP tumor bearing mice, we observed a greater anti-tumor response at the secondary untreated tumor with BT+ICI compared to EBRT+ICI. This effect was dependent on both $CD4^+$ and $CD8^+$ cells and was sensitive to BT dose. Splenocytes harvested from disease-free mice co-cultured with tumor cells showed significant increases in expression of T cell activation markers in both $CD4^+$ and $CD8^+$ cell populations.

Conclusions: We report dose-dependent effects of RT on expression of immune susceptibility markers and immune cell infiltration in a murine tumor model. We observe that a heterogeneous dose of BT results in spatial differences in these effects within a single tumor. This spatial heterogeneity in activation of immune mechanisms may underlie a greater capacity of BT to augment anti-tumor immune response when combined with ICI, as compared to homogenous dose EBRT.

Abstract #17

Impact of intratumoral heterogeneity for DNA mismatch repair on colon tumor development and treatment

Author(s): *Santina Snow*,* Dawn Albrecht, Shane Huebner, Paul Clark, Jamey Weichert, Zach Morris, Rich Halberg

Abstract

Four Consensus Molecular Subtypes (CMS) of colorectal cancer (CRC) were recently developed that distinguish CRC by mutational profile and gene expression signatures. One of the subtypes, CMS1, is enriched for cancers that are deficient for DNA mismatch repair (dMMR), which leads to high tumor mutational burden, creating many neoantigens on the surface of the tumor that can elicit an immune response. Therefore, CMS1 cancers with dMMR are the best candidates for immunotherapy as compared to cancers with proficient DNA mismatch repair (pMMR). However, in early clinical trials, only 30-55% of dMMR metastatic CRC partially responded to immunotherapy. If a tumor has a mixture of dMMR and pMMR tumor cells, then not all tumor cells will respond to immunotherapy, leading to a partial response or resistance. Note this phenomenon could explain why patients with CMS1 CRC have the worst survival after relapse as compared to the other CRC subtypes. Conventional preclinical mouse models that are typically used to test treatment regimens fail to accurately recapitulate intratumoral heterogeneity in human cancers leading to inaccurate predictions of efficacy in the clinic. To better understand how intratumoral heterogeneity with respect to dMMR impacts tumor development and response to treatment, we have developed a new mouse model containing a mosaic colon consisting of both dMMR cells labelled with green fluorescence and pMMR cells labeled with red fluorescence. These mice often develop tumors with three different compositions: homotypic dMMR or immunologically ‘hot’ tumors that are predicted to have the best response to immunotherapy; homotypic pMMR or immunologically ‘cold’ tumors that are predicted to have the worst response to immunotherapy; and heterotypic tumors with a mixture of dMMR and pMMR cells or immunologically ‘lukewarm’ tumors that are predicted to have a partial response to immunotherapy. Surveillance bright field and fluorescent colonoscopy tracks the tumor size and proportion of green (dMMR) and red (pMMR) tumor cells. The novel mouse model allows for the highly detailed characterization of tumor response, especially considering the clonal composition of a tumor, and should greatly facilitate identifying mechanisms of resistance that would not be possible with current, homogenous preclinical models.

Abstract #18

Epigenetic Regulation of MHCI Expression Enhances Response to Immunotherapy

Author(s):

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Background:

Neuroblastoma is the most common extracranial solid tumor in children, with ~50% of patients diagnosed with high-risk neuroblastoma (HR-NBL). An immunologically cold tumor, HR-NBL is characterized by a low tumor-mutation burden, minimal MHC-I expression, MYCN-amplification, and poor T cell infiltration. Loss of MHC-I likely contributes to the poor responses in HR-NBL to immunotherapy as T cells require engagement with MHC-I. The dysregulation of epigenetic modifiers (EMs) in NBL patients may be a potential mechanism for MHC-I downregulation seen on the tumors. Here, we pursued, *in vitro*, the ability of EMs, including inhibitors of DNA methyltransferases (DNMTis), to restore MHC-I expression in a murine 9464D NBL model.

Methods:

Using transduced variants of the 9464D HR-NBL model (an IFNg-inducible 9464D-MHC-I^+ and a non-IFNg-inducible 9464D-MHC-I^-), cells were treated with EMis and assessed by qPCR and flow cytometry for changes in MHC-I and other genes [e.g., antigen presenting machinery (APM)]. Separately, in a cohort of NBL patients, associations between the methylation of genes within the APM and expression levels of such genes were assessed.

Results:

DNMTis combined with IFNg induced MHC-I expression on 9464D-MHC-I^- cells, which previously lacked inducibility. Our qPCR data supports these results, as we observed restored expression of the APM genes, Tap1 and PSMB9, with DNMTi and IFNg treatment. In a cohort of 130 NBL patients with MYCN-amplification, we found evidence supporting the association between methylation of Tap1 and PSMB9 with the loss of expression of these genes.

Conclusion:

These *in vitro* findings suggest that the loss of MHC-I expression in 9464-MHC-I^- variant is caused, in part, by EMs, and thereby reversible with EMis. From our qPCR data, we hypothesize that the shared bidirectional promoter region of Tap1 and PSMB9 may be methylated, leading to gene silencing. The associations found in the MYCN-amplified NBL patients signifies that murine 9464D is a translationally relevant model for investigating the role of EMs in HR-NBL. Future *in vivo* studies are necessary to provide evidence for the potential of EMis, in combination with immunotherapy, to augment T cell engagement and improve the anti-tumor response.

Abstract #19

Liquid biopsy approach for PD-L1 expressing CTCs using multivalent dendrimer-peptide conjugates architecture for applications in companion diagnostics

Author(s): *DaWon Kim*,* Michael Poellmann, Caroline Hopkins, Jiyoon Bu, Seungpyo Hong

Background: Liquid biopsy has been recently emerged as a new, minimally-invasive diagnostic method. Circulating tumor cells (CTCs), which are released from primary and metastatic tumors into peripheral circulation, may be isolated from a routine blood draw using antibodies or peptides that bind to these cells with high specificity. In this study, we aimed to develop a peptide-based capture surface for CTCs expressing programmed death ligand 1 (PD-L1), which is widely expressed in many cancer types and may be used to determine course of therapy.

Methods: We developed a surface with three major components: 1) poly(ethylene glycol) (PEG) linkers as spacers; 2) generation 7 poly(amidoamine) (G7 PAMAM) dendrimers; and 3) either antibodies or peptides targeting PD-L1. The PD-L1-binding peptide (IYLCGAISLHPKAKIEESPGA) was characterized by NMR and MALDI. Atomic force microscopy (AFM) probes functionalized with recombinant PD-L1 were used to measure the binding force of each peptide. Finally, we quantified the capture efficiency of MOC1, a mouse oral carcinoma cell line expressing PD-L1, in the presence of shear stress.

Results: NMR and MALDI confirmed successful synthesis of the PD-L1-binding peptide. Dissociation rate constants from AFM force spectroscopy were measured to be 2.0x10-5 and 3.1x10-5 for dendrimer-antibody and dendrimer-peptide conjugates, respectively. AFM also demonstrated the benefit of using PAMAM dendrimers compared to PEG. In capture assays with MOC1 cells, we observed 3-fold greater capture efficiency on dendrimer-peptide surfaces compared to antibody surfaces. In addition, the dendrimer-peptide surfaces retained 91.6% of MOC1 cells under sustained shear stress.

Conclusion: We demonstrated that a capture surface containing dendrimer-peptide conjugates outperforms antibody surfaces for the isolation of PD-L1+ MOC1 cells. Further development of this system is expected to result in a companion diagnostic for PD-L1 antagonists, including novel nanoparticle drugs of PD-1/PD-L1 immune checkpoint inhibitors.

Abstract #20

Intratumoral MPL augments in situ vaccination generated by radiation and checkpoint blockade

Author(s): *Raad Allawi*,* Justin C. Jagodinsky, Amber M. Bates, Paul A. Clark, Raghava N. Sriramaneni, Thomas C. Havighurst, Ishan Chakravarthy, Erin J. Nystuen, KyungMann Kim, Paul M. Sondel, Won Jong Jin, and Zachary S. Morris

Background: Radiation therapy (RT) is a common cancer treatment that stimulates in situ vaccination in murine models and in cancer patients. RT, however, has not consistently translated into enhanced response to immune checkpoint inhibition (ICI). To optimize antitumor response to RT, we used the vaccine adjuvant, monophosphoryl lipid A (MPL).

Methods: We used syngeneic murine models of melanoma (B78) and prostate cancer (Myc-CaP). Mice bearing B78 or Myc-CaP tumors were randomized to receive RT (12 Gy), RT + anti-CTLA4 (C4; 100 µg IP injection days 3, 6, 9 after RT), MPL (20 µg IT injection days 5, 7, 9 after RT), RT+C4+MPL, or PBS control. To model metastatic disease, 250,000 B16 melanoma cells (parental to B78) were injected via tail vein immediately following RT. To evaluate the effect of MPL on the irradiated tumor microenvironment, a separate cohort of B78 tumor bearing mice from each treatment group were sacrificed 15 days following RT, and tumor, tumor draining lymph node were harvested for immune cell infiltration analysis and cytokine profiling, and serum was collected for analysis of anti-tumor antibody populations.

Results: Combination RT+C4+MPL significantly reduced tumor growth, increased survival and complete response rate compared to RT+C4 in both B78 and Myc-CaP models. MPL favorably reprogrammed the irradiated tumor-immune microenvironment towards M1 macrophage and Th1 CD4 T cell polarization. Furthermore, MPL significantly increased intratumoral expression of several Th1 and M1 associated proinflammatory cytokines. T cells co-cultured with MPL-stimulated macrophages significantly increased expression of the activation marker CD69 in CD8 T cell populations, and the Th1 polarization marker CXCR3 in CD4 T cell populations. MPL treatment significantly increased production of Th1-associated, IgG2c anti-tumor antibodies which were required for and predictive of anti-tumor response to RT and MPL and enabled macrophage-mediated antibody-dependent direct tumor cell killing by MPL-stimulated macrophages. In metastatic models, RT and MPL generated a systemic anti-tumor immune response that augmented response to ICIs.

Conclusions: In melanoma and prostate cancer models, MPL favorably reprogrammed the radiated tumor-immune microenvironments towards M1 macrophage polarization. These findings support the potential for vaccine adjuvants to enhance the efficacy of in situ tumor vaccine approaches.

Abstract #21

^{86}Y/^{90}Y-Labeled Ultrasmall Porous Silica Nanoparticles with Enhanced Pharmacokinetics for Cancer Theranostics

Author(s):
Carolina Ferreira, Shreya Goel, Eduardo Aluicio-Sarduy, Jonathan W. Engle, Weibo Cai

Background
Theranostic nanoparticles hold the potential to significantly improve cancer management. Our group has previously synthesized and characterized sub-15 nm ultrasmall porous silica nanoparticles (UPSN). In this work, we aimed to employ UPSN as a nanocarrier for isotopic pair $^{86/90}$Y for a true theranostic approach (i.e. with the same chemical entity).

Methods
UPSN were successfully synthesized and comprehensively characterized. ^{86}Y-labeled UPSN enabled positron emission tomography (PET) imaging and ^{90}Y-labeled UPSN was used for internal radiotherapy. 4T1 murine breast cancer model was used for in vivo studies. Comprehensive toxicity studies such as histology and blood analysis were carried out: creatinine, blood urea nitrogen (BUN), aspartate amino transferase (AST), alanine amino transferase (ALT), total bilirubin, and alkaline phosphatase (ALP).

Results
UPSN exhibited outstanding in vivo behavior, including super long circulation behavior with signals in the blood pool still observed after 24 hours, similar as monoclonal antibodies. High target tissue accumulation (~12 %ID/g in the 4T1 tumor) as well as evasion from the reticuloendothelial system (RES) organs was observed. *In vivo* PET imaging demonstrated prolonged blood circulation and excellent tumor contrast of ^{86}Y-DOTA-UPSN. Tumor-to-muscle and tumor-to-liver uptake ratios were quite high (12.4 ± 1.7 and 1.5 ± 0.5, respectively, n = 5), which was rarely observed for radiolabeled inorganic nanomaterials in the literature. Cerenkov Luminescence Imaging (CLI) allows noninvasive monitoring of ^{90}Y-DOTA-UPSN tumor uptake throughout the study. Administration of ^{90}Y-labeled UPSN resulted in significantly reduced tumor volumes when compared to the various control groups from as early as day 1 after injection. No normal tissue toxicity was observed for the ^{90}Y-DOTA-UPSN group.

Conclusions
This study represents a true theranostic approach, where the same chemical entity was used for PET imaging (with ^{86}Y) and therapeutic (with ^{90}Y) applications. The use of a safe nanoparticle (silica is “generally regarded as safe” by the FDA) that can achieve high tumor accumulations (no specific targeting was needed in this work) provided a favorable and versatile nanoplatform for future applications in cancer theranostics.

Abstract #22

Development of [55,58mCo]Co-NOTA-NTS20.3 as a versatile theranostic nuclear medicine

Author(s): *Wilson Lin**, Eduardo Aluicio-Sarduy, Todd E. Barnhart, Jonathan W. Engle

Background:

Neurotensin receptors (NTSR1,2,3) are known for stimulating tumor proliferation through neurotensin (NTS) activation and are expressed by a variety of cancers including breast, pancreatic, prostate, colon and non-small cell lung cancers. The high binding affinity, internalization efficiency and internalization rate of the NTS/NTSR1 complex make radiolabeled NTS derivatives interesting for cancer diagnosis and staging. Nuclear localization of NTS/ NTSR1 also suggests therapeutic application with high LET alpha particles and low energy electrons. We investigated [^{55,58m}Co]Co-NOTA-NT20.3 (an NT analog) on NTSR1,3-positive HT29 human colorectal adenocarcinoma cells.

Methods:

^{55,58m}Co were produced via deuteron irradiation of iron and separated with ion exchange chromatography. NOTA-NT20.3 was labeled with ^{55,58m}Co at pH 4.5 in 1 h at 95° C. Gentisic acid effectively mitigated radiolysis. Radiochemical purity was determined by radio-HPLC. Labelled compounds were purified and reconstituted in PBS. Neurotensin internalization rates were evaluated with [^{55}Co]Co-NOTA-NT20.3 in HT29 cells (N=3) at a concentration of 4 nM, and surface bound activity was removed with stripping buffer (0.1 M citrate pH 2). Cytotoxicity studies incubated [^{58m}Co]Co-NOTA-NT20.3 with HT29 cells and evaluated viability with ATP assays at 24h and 48h (N=3). IC50 was computed using GraphPad PRISM version 7.00. A female nude mouse was xenografted with HT-29 cells. Nine days after tumor implantation approximately 5 MBq of [^{55}Co]Co-NOTA-NT20.3 was administered by tail vein injection. PET imaging was performed at 1 and 9 h post injection (p.i.), then major organs were collected and quantified ex vivo to confirm image-derived uptake values.

Results:

HPLC measured radiochemical purity of [^{55,58m}Co]Co-NOTA-NT20.3 was >99% in all cases. [^{55}Co]Co-NOTA-NT20.3 attained >80% of bound activity internalization in HT29 cells after 60 min. [^{58m}Co]Co-NOTA-NT20.3 exhibited cytotoxicity for HT29 cells with IC_{50}=7.0±4.3 MBq/mL. PET imaging with [^{55}Co]Co-NOTA-NT20.3 showed uptake mainly in the kidneys and tumor (7.4%ID/g and 1.5%ID/g, respectively, at 9h p.i.).

Conclusion:

We produced radiochemically pure [^{55,58m}Co]Co-NOTA-NT20.3 suitable for in-vivo applications. Our results demonstrate that [^{55,58m}Co]Co-NOTA-NT20.3 exhibits specific binding and high internalization rates in NTSR1,3 positive HT29 cells. PET imaging quantified kidney and tumor accumulation of [^{55}Co]Co-NOTA-NT20.3. Given the biodistribution profile and cytotoxicity data, [^{58m}Co]Co-NOTA-NT20.3 shows potential for treatment of small cancers and warrants further research.

Abstract #23

Preclinical Evaluation of ^{43}Sc-FAPI PET for Detection of Pancreatic Ductal Adenocarcinoma

Authors: K.V. Becker, P. B. Schwartz, E. Aluicio-Sarduy, J. Jeffery, C. Massey, R. Hernandez, S. Ronnekleiv-Kelly, J.W. Engle, A. Pirasteh

Purpose: Multiple factors contribute to the poor prognosis of pancreatic ductal adenocarcinoma (PDAC): local tumor spread, lack of effective treatments, and failure of conventional imaging techniques in early detection. Positron emission tomography (PET) using ^{68}Ga-labeled fibroblast activation protein inhibitor (FAPI) has been reported to detect PDAC. However, ^{68}Ga's short half-life (67.7 min) and high positron energy (β^{+}_{mean} = 829.5 keV) make it suboptimal for imaging early/small lesions. ^{43}Sc's longer half-life (3.891 h) and lower positron energy (β^{+}_{mean} = 476 keV) enable later time-point imaging and higher PET resolution. Here, we prospectively compared the imaging of PDAC using ^{43}Sc- and ^{68}Ga-labelled FAPI.

Methods: FAPI-46 precursor (SOFIE) was radiolabeled with ^{68}Ga (GalliaPharm) per literature methods. ^{43}Sc was produced by deuteron bombardment of ^{42}CaO (GE PETtrace), purified, and labelled with FAPI precursor. Radionuclidic (RNP) and radiochemical purity (RCP) of ^{43}Sc-FAPI were assayed by HPGe and high-performance liquid chromatography (HPLC). Heterotopic tumors were formed by injecting 10^5 murine PDAC (KPC) cells in the right axilla of 5-8 week old C57Bl/6J mice. After 19-21 days, mice (N=6) underwent imaging on a μPET/CT scanner at 1 h post injection (p.i.) of 2.51 ± 0.03 MBq ^{68}Ga-FAPI. Another group (N=5) were injected with 7.7 ± 0.7 MBq ^{43}Sc-FAPI; 2 mice underwent PET/CT at 8 hours p.i. and 3 were imaged at 1, 2, 5, and 8 hours p.i.. Following imaging, two ^{43}Sc-injected mice were euthanized, and major organs were collected for ex-vivo quantification to validate image-derived data.

Results: HPLC radio-chromatograms of ^{43}Sc-FAPI show quantitative labelling at 74 MBq/nmol and no observable degradation after 12 hours in phosphate buffer. ^{43}Sc-FAPI uptake was higher than ^{68}Ga-FAPI for all tissue in hand-drawn ROIs at 1 hour p.i. There was tumor uptake of both ^{68}Ga- and ^{43}Sc-FAPI in all animals; however, subjective image quality was higher for ^{43}Sc-FAPI than ^{68}Ga-FAPI relative to background and bladder. ^{68}Ga-FAPI images were degraded by artifact from high activity in the urinary bladder and poorer spatial resolution than ^{43}Sc-FAPI images.

Conclusion: While ^{68}Ga-FAPI successfully detects PDAC in a murine model, ^{43}Sc-FAPI produced higher resolution images with reduced artifact and higher potential for detection of smaller lesions.

Abstract #24

Alpha vs. Beta Targeted Radionuclide Therapy (TRT) for the Treatment of Metastatic Castration Resistant Prostate Cancer (mCRPC)

Author(s): Chris Massey*, Carolina Ferreira, Hemanth Potluri, Zachary Rosenkrans, Cynthia Choi, Eduardo Aluicio-Sarduy, Jonathan W. Engle, Jamey Weichert, Douglas McNeel, Reinier Hernandez

Background: Metastatic castration-resistant prostate cancer (mCRPC) is the most lethal form of prostate cancer. Treatments that can extend the overall survival and quality of life for mCRPC patients are urgently needed. Systemic targeted radionuclide therapy (TRT) offers a viable therapeutic avenue to selectively treat mCRPC. Herein, we evaluated the tumor selective alkylphosphocholine analog NM600, labeled with alpha (^{225}Ac) and beta (^{177}Lu) emitters, for TRT in two syngeneic prostate cancer models.

Methods: Male C57BL/6 and FVB mice were implanted with TRAMP-C1 or Myc-CaP cells subcutaneously. Animals received 3.7 MBq of ^{177}Lu-NM600 or 7 kBq ^{225}Ac-NM600, *ex vivo* biodistribution was performed at 4, 24, 72, and 120 h post-injection. For therapy, tumor-bearing mice (150mm^3, n=10) were given an intravenous bolus of 5.5 or 18.5 MBq of ^{177}Lu-NM600, 7 or 20 kBq of ^{225}Ac-NM600, or excipient (control). Tumor growth was followed via caliper measurements and survival was recorded. Toxicity was assessed through complete blood counts (CBC), comprehensive metabolic panel (CMP), and weight change.

Results: Tumor uptake peaked at 8.3 ± 2.7 and 4.2 ± 0.9 %IA/g in Myc-CaP and 10.0 ± 2.5 and 15.4 ±10.1 %IA/g in TRAMP-C1 for ^{177}Lu-NM600 (24h p.i.) and ^{225}Ac-NM600 (120h p.i.) respectively. High-dose ^{177}Lu-NM600 elicited a modest reduction in tumor growth in TRAMP-C1 tumors but no effect in Myc-CaP. Conversely, both ^{225}Ac-NM600 injected activities significantly reduced tumor growth ($p < 0.05$) compared to controls in both tumor models, resulting in significantly improved ($p < 0.05$) overall survival; median overall survival was > 63 d and 51 d (high dose) vs 27 d and 12 d (control) in TRAMP-C1 and Myc-CaP animals, respectively. No overt acute toxicities were noted by CBC, CMP, or animal health assessments.

Conclusion: ^{225}Ac-NM600 significantly improved survival compared to ^{177}Lu-NM600 which showed relatively low efficacy in both murine models of mCRCP. These results, which evidenced the radiobiological advantages of alpha vs. beta emitter to treat mCRPC, warrants further exploration of the mechanism of ^{225}Ac-NM600 efficacy and long-term safety in more clinically relevant models of mCRPC.

Abstract #25

A GMP Process for the Manufacture and Quality Control Release Testing of Metabolically Fit Autologous IFN-gamma-Stimulated MSCs for Xerostomia

Author(s): *Ross Meyers*,* Olga Ganz, Jacques Galipeau

Background: Radiation-induced Xerostomia (RIX) is a long-term side effect of head and neck cancer
(HNC). The first human clinical trial (MESRIX) of adipose-derived MCSs to treat patients with RIX supports the feasibility and likely benefit of MSC auto-transplantation for treating RIX. This study, however, did not examine the use of IFNγ-stimulated MSCs. Prior studies have shown that licensing of MSCs with inflammatory cytokines such as IFNɣ, enhances their immunosuppressive phenotype and leads to their functional maturation.

Methods and Results: We have demonstrated feasibility of MFG clinical doses of MSCs from HNC patients. Bone marrow aspirate is fractionated via Ficoll density gradient centrifugation. MSCs are expanded in human platelet lysate supplemented medium until the target cell dose is achieved. Confluent MSC culture is then stimulated with 1200 IU/mL of IFNɣ for 24 hours. Cells are cryopreserved at ≤ passage 3. In-process quality control (QC) testing is performed to demonstrate Drug Substance sterility, purity, identity and potency prior to cryopreservation. A flow cytometry (FC) method was developed to assess upregulation of immunomodulatory biomarkers (ICAM-1, IDO, PD-L1, MHC I, MHC II). A comparison of IFNɣ-stimulated to untreated MSCs biomarker expression serves as a potency test and confirms MSC augmentation by IFNɣ. Product identity is assessed by FC method demonstrating ≥ 95% expression of MSC phenotypic biomarkers (CD73+, CD90+, CD105+). Purity, as determined by FC, is evaluated based on CD45+ content. Prior to final product (FP) preparation, MSCs are thawed and placed back in culture for 16 hours. We have shown that cryopreserved IFNγ-stimulated MSCs retain potency upon culture rescue, have an immunosuppressive phenotype and robust recovery. Culture-rescued MSCs are formulated for an injection at 10 x 106 cells/ mL, then FP QC release testing is performed. The entire MFG process has been qualified according to GMP protocols. Clinical stability data verified that the Investigational Medicinal Product remained stable for 24 hours at ambient conditions.

Conclusion: We developed a novel GMP and FDA-compliant MFG process that will allow first-in-human, phase 1 clinical study of “fresh” IFNγ-stimulated autologous MSCs for a regenerative medicine indication; treatment of HNC RIX (NCT04489732).

Abstract #26

Dendritic architecture improves thermodynamic stability and drug loading of micelle-based nanocarriers

Author(s): *Caroline Hopkins*,* Jin Woo Bae, Hao-jui Hsu, Adam Drelich, Hao Wu, Lauren Repp, Weiping Tang, Allan R Brasier, Glen Kwon, Seungpyo Hong

Background
Dendron micelles (DMs) are hyperbranched polymeric nanoparticles that have many terminal functional groups and the ability to encapsulate hydrophobic drugs, taking advantage of both active and passive targeting. The building blocks are PEGylated dendron coils (PDCs), amphiphilic polymers that self-assemble into these DMs. They are composed of hydrophobic poly(ε-caprolactone) (PCL) linked to hydrophilic polyethylene glycol (PEG) via a hyperbranched polyester dendron with eight terminal groups. The dendron structures have demonstrated thermodynamically stable self-assembly into micelles due to a significantly lower critical micelle concentration (CMC), the lowest concentration necessary for self-assembly into micelles, than linear micelles. In this study we aimed to optimize thermodynamic stability and drug loading of micelles, allowing for combination nanomedicine that can be tailored for various disease states.

Methods
PDCs with varying weights of the hydrophobic PCL were prepared and characterized using NMR and FT-IR spectroscopy. DMs were formed using various methods, including dialysis and evaporation, to optimize drug loading and encapsulation efficiency of various drugs. Size and morphology measurements were taken of empty and drug-loaded DMs using DLS and TEM. CMC measurements were completed using fluorescence spectroscopy.

Results
Empty PCL3.5k-DMs measured 24.36 nm in diameter and empty PCL14k-DMs measured 37.84 nm. o(LA)8-PTX-loaded DMs were smaller, at 23.54 and 32.67 nm, respectively. Thin film evaporation resulted in significantly higher drug loading of all drugs tested. o(LA)8-PTX was loaded at >10% with 95% encapsulation efficiency, which is also higher in DMs with longer PCL hydrophobic regions.

Conclusions
Hydrophobic-hydrophobic interactions between encapsulated drug and the micelle interior are believed to play a role in the smaller diameter and enhanced drug loading. One of the essential properties that a nanocarrier should have is thermodynamically stable formation, which we see with the dendron micelles. The stable formation, along with effective drug loading allows dendron micelles to serve as a promising functionalizable and tunable nanocarrier for combination therapeutics. Dendron micelles can serve as a platform for active and passive targeting to sites of disease with delivery of small molecule therapeutics.

Abstract #27

Functional Gene Delivery Using Dendron-lipid Micelles for Cancer Immunotherapy

Author(s): *Kaila Javius-Jones*,* Ashita Nair, Jiyoon Bu, Seungpyo Hong

Background: Immunotherapy has become a standard of care in cancer. Immunotherapy approaches modulate the immune response by blocking co-inhibitory checkpoints (e.g. program cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1), increasing cytotoxic T cells via immunostimulatory cytokines (e.g. interleukin-2 (IL-2)), or generating an adaptive antitumor immunity by increasing tumor antigen presentation to antigen presenting cells, amongst other methods. One of the approaches to modulate the immunosuppressive tumor microenvironment (TME) is by altering gene expression within protumorigenic pathways. With our recently developed novel nanoparticle, dendron-lipid micelles (DLNPs), we can create a peptide targeted platform to deliver PD-L1 silencing RNA (siRNA) to cancer cells. We hypothesized the use of PD-L1 peptide functionalized dendron-lipid micelles can help increase intracellular delivery of siRNA while acting as an immune checkpoint inhibitor that can be combined with the delivery of chemotherapeutics, as micelles have drug encapsulation capabilities.

Methods: In this study, we prepared and assessed cytotoxicity of a novel generation 3 (G3) poly(amidoamine) (PAMAM) dendron-lipid micelles, compared to existing non-viral gene carriers, polyethyleneimine (PEI) and G7 PAMAM dendrimers. The PD-L1 peptide conjugated DLNPs were also studied as a targeted gene vector. We were able to formulate 5, 10 and 25 wt % PDL1 peptide conjugated DLNPs and tested the effect of cellular uptake in the PD-L1 overexpressing cancer cell line, MOC1.

Results: The DLNPs showed minimal cytotoxicity compared to PEI and dendrimers as cell viability was reduced more than 80% after a 6-hour incubation in vitro. After conjugating PD-L1 peptides to DLNPs, 5 wt % PD-L1 conjugated DLNPs increased cellular uptake more than 8-fold compared to non-targeted DLNPs.

Conclusions: Our results indicate that the newly engineered dendron-lipid micelles have potential to achieve targeted gene delivery that may be a more effective nanocarrier platform for immunotherapy with lower toxicity concerns, compared to existing ones.

Abstract #28

Single-cell transcriptional landscapes of myeloid cells in HPV+ and HPV- head and neck cancers

Author(s): *Athena E. Golfinos*,* Wei Wang, Paul F. Lambert, Huy Q. Dinh

Myeloid cells are highly plastic immune cells with an emerging understanding of their ontologies, lending to multi-faceted contributions regarding their roles in pathologies such as cancer, including head and neck cancer (HNC). There are two major subtypes of human head and neck cancer based on human papillomavirus (HPV) status. HPV status may contribute to clinical outcomes in HNC, with HPV+ patients typically responding better to treatment than HPV- patients. Reasons for this are largely unknown; however, the tumor immune microenvironment (TIME) may play an important role. Previous studies have extensively characterized the T cell, B cell, and NK cell landscape of HNC, while the highly plastic and heterogeneous myeloid cell compartment is markedly under-investigated. As a result, little is known about the breadth of myeloid cell heterogeneity in HNC and how this heterogeneity may contribute to clinical outcomes in patients. We identified high diversity of myeloid cells in HNC compared to previously reported. We discovered a conventional dendritic cell (cDC2) subset expressing CD1A which is higher in HPV+ tumors than in HPV- tumors. The functional crosstalk of CD1A+ cDC2 with T cells might contribute to the TIME phenotype difference between HPV+ vs HPV- HNC. In addition, we show that SPP1 macrophages found in HNC tumors are angiogenic, and increased expression of SPP1 is linked with worse survival in HNC from analysis with the Cancer Genome Atlas data. Lastly, CXCL9, a predictor of immune checkpoint inhibition (ICI) response, is highly expressed in inflammatory macrophages and immunoregulatory dendritic cells in HPV- and HPV+ cancer. Intriguingly, we found the counterpart of these myeloid cell populations in HNC mouse models, with CXCL9+ macrophages enriched in mice that responded better to ICI. These results provide a baseline understanding of myeloid cells that may contribute to ICI responses. Future research will address the functions of these cells *in vivo* to identify their roles in the tumor microenvironment response to ICI treatment. We also provide potential biomarkers of HPV+ HNC that may contribute to improved prognosis and clinical outcomes in these patients.

Abstract #29

Infrared photoactivation boosts sensitivity of quantitative single-cell proteomics

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Introduction:
Isobaric tagging or peptides facilitates multiplexed experiments that can determine sequences and relative amounts of peptides in biological samples using tandem mass spectrometry. Limited reporter ion generation, however, erodes quantitative accuracy and precision. We supposed that using infrared photoactivation to fragment peptides would eliminate the *m/z* dependence of collisional activation when activating multiple precursors. Further, we implemented ion parking of newly generated reporter ions during photoactivation to limit over-fragmentation and maximize reporter generation from tagged peptides. To highlight improvements in quantitative sensitivity for limited sample amounts, we applied this method to single-cell proteome mixtures.

Methods:
Experiments were performed on a quadrupole-Orbitrap-linear ion trap MS system modified to include a 40 W CO_2 continuous wave laser, allowing for photoactivation of ions within the ion trap. Adjustments to the instrument control code allowed for broadband ion parking at reporter ion m/z (126–131) during photoactivation experiments. Data-dependent MS^2 scans were followed by back-to-back HCD and photoactivation MS^3 scans, allowing direct comparisons.

Results:
This work builds upon the foundation of quantitative proteomics and establishes photoactivation as an ideal method for reporter ion generation. By increasing reporter ion yield, photoactivation boosts the quantitative sensitivity such that a wider dynamic range of analyte concentrations can be quantified. This technology is especially useful for proteomic applications where limited sample starting amounts are available such as single cell applications and enrichments of protein post-translational modification carrying peptides.
We photoactivated individual precursor ions and prevented successive dissociation of generated reporter ions with ion parking, which altogether boosted reporter ion yield by 55%. Further, when synchronously activating multiple precursors, photoactivation with ion parking gives compounded benefits. As an initial proof-of-concept, we show that using photoactivation to generate reporter ions from ten synchronously isolated product ions results in a 2.4-fold increase in reporter intensities across the human proteome, significantly enhancing the sensitivity and dynamic range of quantitation via isobaric tagging. We extend these methods to single-cell proteome mixtures to highlight their benefits for very limited sample starting amounts.

Conclusion:
We demonstrate that photoactivation boosts reporter generation by an average of 2.4-fold relative to state-of-the-art, without perturbing expected quantitative ratios.

Abstract #30

Single-cell myeloid heterogeneity in human fallopian tube and its implications for early high grade serous ovarian cancer

Author(s): *Joshua Brand*,* Marcela Haro, Kate Lawrenson, Huy Dinh

Background: Early detection of high-grade serous ovarian cancer (HGSOC) increases the 5-year survival rate significantly – from under 50% to near 90% with a stage I diagnosis. Accumulating histological and molecular sequencing data indicates most cases of HGSOC originate within the fallopian tube (FT). Much of the work focuses on characterizing epithelial cells within the FT and therefore limits our understanding on how the cellular microenvironment contributes to early tumorigenesis.

Methods: To characterize the cellular landscape of human FTs, we used single-cell RNA sequencing (scRNA-Seq) on tissue samples from 8 non-malignant donors and analyzed them alongside cancer data. First, we developed a computational pipeline to characterize diverse stromal and immune cell types with distinct expression signatures in human FTs. Then to identify their implications in cancer, we examined how the signatures of FT cell types are expressed in publicly available bulk and single cell HGSOC tumors.

Results: Here we show a single-cell transcriptome analysis of non-malignant FT samples, revealing high monocyte diversity that is lost in HGSOC. Some monocyte subsets display inflammatory markers such as IL1A, CCL3/4 and others. Diffusion map method identified distinct trajectories of monocytic maturation in human FTs. Early stages were defined by cytokine/chemokine expression then a gain of MHCII while differentiating into macrophages or dendritic cells. Using FT gene expression profiles, we estimated the abundance of those myeloid subsets in publicly available HGSOC scRNA-Seq data. We found the macrophage-to-monocyte ratio is remarkably elevated in tumors compared to adjacent normal ovary and non-cancerous FT tissues. Intriguingly, analysis of our newly generated FT samples from patients with BRCA1/2 germline mutations revealed a subset of individuals with a ratio more like those found in HGSOC tumors when compared to normal FT. These donors also show altered T cell phenotypes, having fewer resting naïve T cells and consistent with immune activation.

Conclusion(s): Our data represents the first whole transcriptome analysis of broad celltypes residing in the FT. We identified myeloid diversity that is not reflected in macrophage dominated tumor tissue and possibly correlates with early disease. We anticipate this work provides evidence of how myeloid cell plasticity contributes to tumorigenesis and shapes unique tumor microenvironments.

Abstract #31

Quantification and Single Cell RNA Sequencing of Circulating Tumor Cells Using a Highly Sensitive Detection Device

Author(s): *Michael Poellmann*,* Jiyoon Bu, DaWon Kim, Jinwoong Lee, Joseph Caster, Chandrikha Chandrasekharan, Andrew Wang, and Seungpyo Hong

Background: Tumors regularly shed material into circulation. The quantification and analysis of this material, a “liquid biopsy,” has the potential to dramatically improve cancer diagnosis and influence the course of treatment. We have developed a highly sensitive system for purifying circulating tumor cells (CTCs) that employs biomimetic cell rolling and dendrimer-mediated multivalent binding.

Methods: Capture surface mechanics were measured using force spectroscopy techniques. A prototype of our flow chamber has been used to quantify CTCs in several ongoing preclinical studies of patients with oligometastatic cancer, head and neck squamous cell carcinoma, pancreatic and rectal adenocarcinoma, and neuroendocrine tumors. In certain samples, CTCs were efficiently recovered from the surface and sequenced using the 10x Chromium system.

Results: Force spectroscopy measurements shed light on the two-step purification process and provide a mechanistic understanding of how high sensitivity and specificity is achieved in our assay. CTCs were detected at exceptionally high numbers in all cohorts. CTC counts following radiation treatment were predictive of clinical outcomes in oligometastatic and gastrointestinal cancer patients. CTCs recovered from the capture surface had exceptional RNA quality and were successfully sequenced. Preliminary gene set enrichment analysis successfully detected 60 endocrine cells recovered from a single capture surface.

Conclusion: In multiple clinical cohorts, we have demonstrated the ability to isolate high numbers of high-quality CTCs. The ability to sequence high numbers of CTCs from a single sample is likely to illuminate new approaches to treat cancer and provide a deeper understanding of the metastatic process. Force spectroscopy measurements provide a framework for future improvements to surface chemistry and flow conditions.

Abstract #32

MTORC1/2 and HDAC1/2 inhibition promote tumor response through inhibition of MYC

Author(s):
Rebecca DeStefanis,* Autumn M. Olson, Alyssa K. DeZeeuw, Susan N. Payne, Cheri A. Pasch, Linda Clipson, Dustin A. Deming

Background:
Identifying precision medicine strategies targeting *PIK3CA* mutant colorectal cancer (CRC) is of great clinical interest. Previous work from our lab identified MTORC1/2 (copanlisib) and HDAC1/2 (romidepsin) as a potential strategy. We hypothesized that changes in c-MYC protein levels and c-MYC target gene (CTG) might be a potential mechanism.

Methods:
Known CTGs were identified and expression levels examined in *PIK3CA* mutant vs WT CRCs using the cBioPortal Colorectal Adenocarcinoma (TCGA, PanCancer Atlas) dataset. Murine-derived cancer organoids (MDCO) were generated from adenocarcinomas of *Apc* and *Pik3ca* mutant transgenic mice. MDCO results were corroborated using the human 2D cell lines SW48 and SW48$^{PIK3CA\text{-}H1047R}$ and RAS/RAF WT patient-derived cancer organoids (PDCO) generated from patient tumor samples under approved IRB protocols. Immunoblots were used to assess c-MYC levels after treatment across all models. RNA-sequencing of PDCO and SW48$^{PIK3CA\text{-}H1047R}$ cells were treated and changes in 16 CTG were examined in the treated groups vs control. An aggregate score was created for each treatment group where a statistically significantly altered CTGs with log fold change ≥1.5 added one point and ≤-1.5 subtracted one point. All others were scored 0.

Results:
CTGs were examined for differential expression. Using cBioPortal, only 1/16 genes were significantly decreased in *PIK3CA*^{mut} CRC (GADD45A: log ratio -0.21, q=0.01). MDCOs showed a decrease in total c-MYC levels in the combination. These results were corroborated in the SW48 and SW48$^{PIK3CA\text{-}H1047R}$, and multiple PDCOs. c-MYC levels decreased in romidepsin alone and the combination in the isogenic cell lines. However, c-MYC levels didn't decrease as significantly in the PDCOs. RNA-sequencing demonstrated that copanlisib, romidepsin, and combo had a CTG score of 0, -4, and -7 respectively in the *PIK3CA*^{mut} PDCO. Similar results were seen in another RAS/RAF^{WT} PDCO (0, 1, and -4, respectively) and SW48$^{PIK3CA\text{-}H1047R}$ cells (0,-3, and -7, respectively).

Conclusion:
These data indicate known CTGs are not differentially expressed in *PIK3CA*^{mut} vs *PIK3CA*^{WT} CRCs regardless of treatment. A potential mechanism by which this combination promotes tumor response is through a decrease in CTGs and c-MYC protein levels across multiple models of CRC. This decrease in CTGs might be mediated through HDAC1/2 inhibition with a synergistic response in the combination.

Abstract #33

Novel degrader of coactivator-associated arginine methyltransferase 1

Authors: Megan Bacabac, Haibo Xie, Weiping Tang, Wei Xu

Coactivator-associated arginine methyltransferase 1 (CARM1) is a Type 1 protein arginine methyltransferase that produces asymmetrically dimethylated arginine on histone H3 and nonhistone substrates. Amplification and overexpression of CARM1 have been observed in a variety of cancers, including breast cancer, and its overexpression correlates with poor prognosis. Specifically, methylation of BAF155 by CARM1 in triple-negative breast cancer drives cancer metastasis. Potent small molecule inhibitors for CARM1 have been developed, but the effects of inhibiting CARM1 differ from the effects of knocking out CARM1 in cancer cells. For example, CARM1 knockout, but not CARM1 inhibition, decreases cancer cell proliferation. This implies that CARM1 also has non-enzymatic roles in driving cancer progression which necessitates the development of small molecule degraders of CARM1. We have collaborated with Dr. Weiping Tang to develop CARM1-specific proteolysis targeting chimeras (PROTACs). PROTACs contain three components: a protein of interest (POI) ligand, an E3 ligase ligand, and a linker. PROTACs function by bringing an E3 ligase near the POI, leading to its ubiquitination and proteasomal degradation of the protein. Our CARM1-targeting PROTACs contain a CARM1 ligand, TP-064, and either a von Hippel-Lindau (VHL) or Cereblon (CRBN) E3 ligase ligand. Candidate PROTACs with different linker lengths and E3 ligase ligands were screened in breast cancer cell line MCF7. Cells were treated for 24 hours, then CARM1 levels were assessed by immunoblotting. PROTACs with CRBN did not degrade CARM1. Of the VHL-containing PROTACs, the best compound (HX4256) had a linker length of six and degraded CARM1 levels to about 29% after 24 hours. HX4256 treatment also led to a decrease in BAF155 methylation, demonstrating the specificity of the compound to target CARM1-mediated protein methylation. These results suggest that HX4256 is a potent CARM1 degrader. We will investigate the impact of HX4256 on cell proliferation and migration. Based on previous inhibition and knockout studies, I expect that CARM1 degradation leads to decreased cell proliferation and migration. As CARM1 is a well-established therapeutic target, this novel degrader may be used to target CARM1-driven cancers more effectively than currently available inhibitors.

Abstract #34

Acrylamide Treatment in Obese Mice does not Impact Weight Gain but Increases DNA Damage in Mammary Epithelial Cells and Adipocytes

Author(s): *Brenna Walton*,* Katherine Lumsden

Background: Obesity is a rising epidemic worldwide and is associated with increased breast cancer risk, potentially through increasing mammary gland DNA damage. However, the underlying mechanisms are not well understood. There is limited research on how environmental toxin, acrylamide, may act in obesity to further increase breast cancer risk. Acrylamide, a probable carcinogen found in foods common in the obesity-inducing Western diet, has been associated with increased breast cancer risk and suggested to induce weight gain, however the evidence is contradictory.

Methods: To investigate how acrylamide impacts weight gain and the mammary gland under conditions of obesity, three-week-old FVB female mice were randomized to receive a low fat (LFD; 10% kcal from fat) or high fat diet (HFD; 60% kcal from fat). The mice were further randomized to receive 0.7 mM acrylamide water or control water. Body weights were measured weekly for 16 weeks, and collected mammary tissue was probed for oxidative DNA damage (8-OHdG) and double strand DNA breaks (γH2AX and BrdU).

Results: While we saw a significant increase in weight gain of HFD-fed mice compared to controls, the addition of acrylamide did not significantly alter weight gain or mammary gland weight in LFD and HFD-fed mice compared to the respective controls. Additionally, acrylamide did not significantly increase liver fat deposition in the LFD or HFD-fed mice. We did observe a significant increase in DNA damage within the glands of acrylamide-treated mice in both the LFD and HFD-fed mice. This may be due to increases in reactive oxygen species and oxidative stress.

Conclusion: These studies help uncover how acrylamide may be acting as a carcinogen to further increase breast cancer risk.

Abstract #35

FXR suppresses colitis-induced colon cancer progression

Author(s): Xingchen Dong*, Lance Cai and Ting Fu

BACKGROUND: Colorectal cancer (CRC) is the third most prevalent cancer worldwide and the second most common cause of cancer death in the United States. Other than cancer driver gene mutations, inflammatory microenvironments in the intestine is also critical risk factor for CRC initiation and progression. Poor dietary habits and lifestyle, gut dysbiosis which in control of bile acids (BAs) homeostasis, are also considered as major triggers for chronic intestinal inflammation. Farnesoid X Receptor (FXR) is a master regulator of BAs homeostasis whose function has severely compromised in both IBD and CRC patients. Here, we aim to investigate the inflammatory cytokine changes during tumor formation and examine if the BAs-FXR signaling could be manipulated for CRC treatment.

METHODS: We employed a classic inflammation-induced colorectal cancer model, the AOM/DSS model, and profiled BAs and inflammatory cytokines. Moreover, we examined the FXR signaling changes and investigated the potential benefits of Fexaramine D (FexD), an intestinally restricted FXR agonist, in AOM/DSS mice models.

RESULTS: In the AOM/DSS mice, we observed robust tumor formation and significantly increased production of multiple pro-inflammatory cytokines. Moreover, these cytokines stimulate the aberrant proliferation of intestinal organoids, implicating its tumorigenic potential. Meanwhile, we identified marked changes in the production and composition of BAs, as well as a compromised FXR signaling in the AOM/DSS mice. When treated with FexD, the AOM/DSS mice presented restored FXR signaling and BAs homeostasis, alleviated intestinal inflammation, and inhibited tumorigenesis.

CONCLUSION: We uncovered the essential roles of BAs-FXR signaling in restricting intestinal inflammation and tumorigenesis, highlighting the potential of BAs as diagnostic markers and FXR as a therapeutic target for the treatment of colon cancer.

Abstract #36

The Farnesoid X Receptor supresses colitis-induced colorectal cancer

Author(s): *Xingchen Dong*,* Dr. Lance Cai, and Dr. Ting Fu

Background: Colorectal cancer (CRC) is the third most prevalent cancer worldwide and the second most common cause of cancer death in the United States. Other than cancer driver gene mutations, the inflammatory microenvironment in the intestine is also critical for CRC initiation and progression. While the underlying cause(s) are poorly understood, poor dietary habits and gut dysbiosis, which control bile acids (BAs) homeostasis, are considered major triggers for chronic intestinal inflammation. Farnesoid X Receptor (FXR) is a master regulator of BAs homeostasis whose function has been severely compromised in CRC patients. Here, we aim to investigate the inflammatory cytokine changes during tumor formation and examine if the BAs-FXR signaling could be manipulated for CRC treatment.

Methods: We employed a classic inflammation-induced colorectal cancer mouse model, the AOM/DSS model, and examined the FXR signaling changes in the control and AOM/DSS mice. We next profiled BAs and pro-inflammatory cytokines and investigated if pro-inflammatory cytokines could enhance tissue stemness. Moreover, we examined the potential tumor-suppressive benefits of Fexaramine D (FexD), an intestinally restricted FXR agonist, in AOM/DSS mice models.

Results: In the AOM/DSS mice, we observed robust tumor formation and significantly increased production of multiple pro-inflammatory cytokines. Moreover, these cytokines stimulate the proliferation of intestinal organoids, implicating its tumorigenic potential. Meanwhile, we identified marked increases in BAs production and changes in BAs composition, as well as a compromised FXR signaling in the AOM/DSS mice. FexD treatment restored FXR signaling and BAs homeostasis, alleviated intestinal inflammation, and inhibited tumorigenesis in the AOM/DSS mice.

Conclusion: We uncovered the essential roles of BAs-FXR signaling in restricting intestinal inflammation and tumorigenesis, highlighting the potential of BAs as diagnostic markers and FXR as a therapeutic target for the treatment of colon cancer.

Abstract #37

Simultaneous longitudinal assessment of PIK3CA genomic mutations and PI3K pathway activity in circulating tumor cells in metastatic breast cancer.

Author(s): *Marina N Sharifi*[1,2], Kyle T. Helzer[3], Jamie M. Sperger[1], Matthew L. Bootsma[3], Hannah Krause[1], Cole S. Gilsdorf[1], Serena K. Wolfe[1], Zachary Kauffman[1], Mark E. Burkard[1], Kari B. Wisinski[1], Shuang G. Zhao[3], Joshua M. Lang[1]

[1]Department of Medicine, [2]Physician Scientist Training Program and [3]Department of Human Oncology, University of Wisconsin, Madison.

Background: Phosphatidylinositol-3-kinase (PI3K) activating mutations are found in 30-40% of breast cancers, and the PI3K inhibitor alpelisib is FDA-approved for PIK3CA-mutated hormone-receptor positive metastatic breast cancer (MBC). However, rates of intrinsic alpelisib resistance are as high as 40%, and there is an ongoing need for novel biomarkers to help guide patient selection. Circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) may better reflect the heterogeneity of metastatic disease than tissue biopsy, and are amenable to longitudinal analysis. By combining targeted ctDNA sequencing with quantitation of PI3K pathway phospho-proteins, we have developed the first multiplex assay to simultaneously assess genomic alterations and CTC PI3K pathway activity via liquid biopsy.

Methods: Peripheral blood was collected from MBC patients with known PIK3CA mutation status prior to starting new standard therapies and on treatment. Cell-free DNA was extracted from plasma and sequenced using a custom capture panel (IDT). Using VERSA microscale technology, CTCs were captured immunomagnetically followed by staining on chip for phospho-AKT pS473/total AKT or phospho-rpS6 pS235/S236/total rpS6 and quantitative microscopy for single cell phospho/total protein mean fluorescence ratios.

Results: In a pilot MBC cohort (n=14), mean CTC phospho-AKT/total AKT protein expression ratio was higher in patients with PIK3CA mutations compared to patients without mutations (0.37 vs 0.26, $p<0.01$). Among patients with PIK3CA mutations, there was inter-patient heterogeneity in CTC phospho-AKT and phospho-rpS6, consistent with variability in pathway activation. One patient with a tissue PIK3CA mutation 5 years prior did not have the mutation detected in ctDNA at time of liquid biopsy, and had low phospho-AKT in CTCs. In serial sampling of a patient receiving a PI3K inhibitor, CTC phospho-AKT was decreased on treatment, and increased at time of progression.

Conclusion: We have demonstrated the feasibility of simultaneous monitoring of PI3K pathway mutations in ctDNA and PI3K pathway signaling in CTCs in patients with MBC. Future work will prospectively evaluate this assay in patients receiving alpelisib for PIK3CA-mutated MBC. This has the potential to complement PIK3CA mutation status as a biomarker of sensitivity to PI3K therapies, and may also provide a pharmacodynamic assessment of PI3K inhibitor activity in CTCs on treatment.

Abstract #38

Epigenetic modifiers allow restoration of MHC-I and MHC-II in murine B78-D14 melanoma tumors

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[1]University of Wisconsin-Madison

Background
Murine B78-D14 melanoma (B78) is a “cold” tumor model with a low tumor-mutation burden, poor immune cell infiltration, and minimal MHC-I expression. Using an *in situ* vaccine (ISV) regimen (radiation and a tumor-targeting mAb conjugated to IL-2), we can cure some (~50%) B78-tumor bearing mice. We have found that CD4 T cells are essential for anti-tumor responses (initial and memory). While CD8 T cells are present and activated, they are not required for an anti-tumor effect. As a potential route of immune escape, B78s may have induced changes in epigenetic modifiers (EMs) to alter expression of MHC-I/-II. Here, we pursued *in vitro* assays to investigate the ability of EMs, including inhibitors of DNA methyltransferases (DNMTis), histone deacetylases (HDACis), and histone methyltransferases (HMTis), to restore MHC-I/-II expression in B78s.

Methods
To determine the doses of EMis, B78s were treated with various concentrations of EMis and monitored for proliferation and apoptosis. Optimal doses were then used to treat B78s with single-agent EMis, or combinations, +/- IFNg (100U/mL at D5). B78s were assessed by qPCR and flow cytometry for changes in MHC-I/-II and other genes [e.g., antigen presenting machinery (APM)].

Results
With increased doses of EMis, we observed reduced proliferation and increased apoptosis of B78s. Using doses of EMis that we found did not alter proliferation or apoptosis (in an effort to focus on the potential immune modulation), we found that EMis combined with IFNg induced MHC expression. Our qPCR data indicate that much of the APM that control MHC expression is responsive to IFNg and enhanced with EMi treatment. Moreover, the combination of guadecitabine (DNMTi) and entinostat (HDACi) resulted in co-expression of MHC-I and MHC-II on ~40% of B78s.

Conclusions
These *in vitro* findings suggest that certain combinations of EMis may be beneficial to incorporate into our current ISV regimen to augment CD8 T cell engagement and further enhance CD4 T cell activity to improve anti-tumor efficacy. This B78-D14 melanoma is a translationally relevant model for interrogating the roles of MHC-I/-II expression on tumor cells in immune escape and the potential avenues EMs provide to overcome such escape.

Abstract #39

A Novel Nuclear Phosphoinositide Signaling Pathway Regulating a Noncanonical Poly(A) Polymerase

Author(s): *Tianmu Wen**, Mo Chen, Vincent Cryns, and Richard A. Anderson

Background:
Speckle targeted PIPKIα regulated-poly(A) polymerase (Star-PAP) is a noncanonical poly(A) polymerase localized to nuclear speckles. It controls the expression of about 40% of human genes. Previous studies demonstrate Star-PAP being regulated by PIPKIα-mediated phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) binding upon oxidative and genotoxic stress signals, which is required for the cleavage and polyadenylation of the mRNAs for vital proteins such as HO-1, NQO1, and BIK. Other nuclear proteins, including p53 and SF-1, were also found to bind with and be regulated by phosphoinositides (PIs) and their kinases, while HSP27 was found to play a critical role in the p53-PIs regulation pathway, its stability, and nuclear functions.

Methods:
We utilize immunoprecipitation (IP) and proximity ligation assay (PLA) to study the *in vivo* interactions between Star-PAP, PIs, kinases, and small heat shock proteins under stress signals. *In vitro* binding assay and microscale thermophoresis (MST) is used to show direct bindings and binding affinities.

Results:
Here, we show that Star-PAP directly binds with PI4P, PI4,5P2, and PI(3,4,5)P3. When Star-PAP is isolated from cells by IP the PIPs remain very tightly linked to Star-PAP possibly by a covalent linkage. Further, multiple PIP kinases and phosphatase bind to Star-PAP including PI4K2A, PIPKIα, IPMK, PTEN, and they mediate the metabolism between different Star-PAP-PIP complexes. Star-PAP also binds the small heat shock proteins HSP27 and αB-crystallin and their interactions are stimulated *in vivo* upon oxidative and genotoxic stress treatment. We demonstrate here that the binding affinity between Star-PAP and HSP27 is enhanced by PI(4,5)P2 and PI(3,4,5)P3. Knocking down HSP27 and upstream kinase PI4K2A would downregulate the expression of known Star-PAP-PIP2 targets. Furthermore, loss of Star-PAP or HSP27 would downregulate the expression of each other, indicating a function loop.

Conclusion:
It can be implied there is a sequential modification that starts from PI4P, which is phosphorylated to PI(4,5)P2, and further phosphorylated to PI(3,4,5)P3. Our data indicate Star-PAP is modified by a PI stress signaling pathway, where HSP27 plays a key role. This opens up functional studies for the various PIs-binding status of Star-PAP as well as potentially universal PI signaling pathways for nuclear proteins.

Abstract #40

Identifying conserved chromatin modifying proteins that influence H3K27M and EZHIP cancer pathologies in *Drosophila* wings

Author(s): *Tyler Masuda*,* Sam Krabbenhoft, Truman Do, Siddhant Jain, Peter Lewis, Melissa Harrison

Abstract

Central nervous system tumors are the most common cause of solid tumor death in children. Two different pediatric gliomas, diffuse intrinsic pontine glioma (DIPG) and posterior fossa ependymoma type A (PFA) are associated with particularly poor clinical outcomes. Two key molecular events drive nearly all cases of these cancers: a lysine-to-methionine mutation at residue 27 of histone H3 (H3 K27M), and elevated expression of the protein EZHIP in PFA. Both proteins have been found to inhibit Polycomb Repressive Complex 2 (PRC2), causing a near-complete loss of trimethylation of lysine 27 on histone 3 (H3K27me3). By depositing this mark, PRC2 stably silences large regions of the genome, helping to maintain proper gene expression patterns during development. Though the mechanisms of PRC2 inhibition by H3 K27M and EZHIP are well described, it remains largely unknown why this loss of H3K27me3 is pathogenic, as well as the contribution of other chromatin regulatory proteins to these cancers. To better understand these proteins in the context of early metazoan development, we have leveraged the tools available in *Drosophila*, where Polycomb proteins were initially discovered. We have demonstrated in *Drosophila*-derived cell lines and whole tissues that H3 K27M and EZHIP inhibit fly and mammalian PRC2 by nearly identical mechanisms. Since ubiquitous expression of these oncoproteins is lethal, we used tissue-specific expression in the wing, where expression of either protein causes a mutant phenotype. These phenotypes allowed us to perform a screen against 438 conserved chromatin-modifying proteins, looking for enhancement or suppression. Our RNAi screen identified over 50 genes whose knockdown modified the H3 K27M phenotype. The suppressors are robust across multiple tissues, and many suppress both H3 K27M and EZHIP phenotypes. Shared features of these suppressors suggest that restoring normal development requires a precise balance between the repressive H3K27me3 and marks of active chromatin at gene regulatory elements. Ongoing experiments will continue to explore the mechanistic basis for phenotypic enhancement and suppression, as well as potential relevance in mammalian cell culture systems.

Abstract #41

Mad1 Upregulation in Breast Cancer: Causes & Consequences

Author(s): Sarah Copeland*, Jun Wan, Avtar Roopra, Beth Weaver

Abstract

Mitotic arrest deficient-1 (Mad1), an essential component of the mitotic checkpoint, is upregulated in 20% of breast cancer patients. Upregulation of Mad1 causes chromosome missegregation during mitosis, a hallmark of cancer, as well as destabilization of the p53 tumor suppressor. Upregulation of Mad1 is sufficient to promote orthotopic tumor growth in immunocompromised animals. Patients with high levels of Mad1 mRNA expression have a poorer prognosis than patients with intermediate or low levels of Mad1. Unlike many core kinetochore proteins, Mad1 is not transcriptionally regulated by the transcription factor FoxM1. Thus, the mechanism for upregulating Mad1 in cancer, as well as the functional consequences, remain unknown. Bioinformatics analysis identified Histone Deacetylase 1 (HDAC1) as a likely cofactor involved in Mad1 transcriptional regulation. Consistent with this, HDAC inhibition with Trichostatin A (TSA) or Valproic Acid (VPA) increases Mad1 mRNA and protein levels 6- to 10-fold in multiple breast cancer cell lines. HDAC inhibition also induces Mad1 nuclear puncta, a localization pattern seen in primary breast cancer and breast cancer cell lines following Mad1 upregulation. Additionally, CRISPR knockout of HDAC1 in breast cancer cells increases Mad1 protein expression. To identify which transcription factor targets HDAC1 to the Mad1 promoter, we developed a Mad1 promoter reporter assay. By creating truncations, we identified a repressive region of the Mad1 promoter. Ongoing work will identify transcription factor binding sites in this region necessary for negative regulation of Mad1. To determine the consequences of Mad1 upregulation, we have generated a tetracycline (tet)-inducible Mad1 mouse model by inserting a tet responsive promoter and HA tag before the first coding exon of the Mad1 gene. Mice containing one allele of tet-inducible Mad1 and two alleles of a ubiquitously expressed tet-transactivator (rtTA-M2) show inducible expression of HA-Mad1 following one week or one month of exposure to the tet analog doxycycline. Ongoing experiments will define the impact of Mad1 upregulation on mitotic fidelity and p53 levels and determine whether upregulation of Mad1 is sufficient to induce tumorigenesis in an immunocompetent setting.

Abstract #42

The effects of autophagy inhibition on HNSCC sensitivity to CTX

Author(s): *Samantha Bradley*,* Yong-Syu Lee, Justin H Skiba, Jaimee C Eckers, Adam D Swick, Rong Hu, Kwang P Nickel, Zafer Gurel, Pippa F Cosper, Randall J Kimple

Abstract

Autophagy is a naturally occurring mechanism known to play a protective role in cells by degrading damaged organelles and recycling them to create cellular energy, while also maintaining cellular homeostasis and avoiding apoptosis. Cetuximab, a cancer therapy drug, attempts to target the epidermal growth factor receptor (EGFR) overexpression that is highly common in head and neck cancers (HNC), but can inadvertently induce autophagy in the process. When autophagy is induced, it can provide some resistance to further attempts at treating the cancer; understanding this mechanism of resistance is essential for better directing and improving treatment. We hypothesized that autophagy is a major factor in providing HNC cells resistance to chemotherapies such as cetuximab, and that control over this resistance may come by utilizing autophagy blocking drugs. In this study, we examined the efficacy of combining cetuximab with the autophagy inhibitor, SAR405, as well as investigated the specific mechanism of cetuximab-induced autophagy. We utilized assays such as western blotting for LC3, immunofluorescence for LC3, clonogenic colony formation, and CCK8 for cell viability on HNC cell lines A253, SCC1, and SCC1-C5, a cetuximab-resistant derivative. Through this work, we have been able to demonstrate that cetuximab is able to activate autophagy, and that this initiation of autophagy plays a cytoprotective role in HNSCC cells. Additionally, the addition of the autophagy inhibitor SAR405 improved response to cetuximab treatment. These results will help guide future studies investigating effects of additional autophagy inhibitors in combination with cetuximab to treat HNC.

Abstract #43

Quantifying chromosomal instability from karyotype diversity using agent-based modeling and Bayesian inference

Author(s): *Andrew Lynch*,* Nick Arp, Amber Zhou, Beth Weaver, Mark Burkard

Background: Chromosomal instability (CIN) — persistent chromosome gain or loss through abnormal karyokinesis — is a hallmark of cancer that drives aneuploidy. Intrinsic chromosome mis-segregation rate, a measure of CIN, can inform prognosis and is a promising biomarker for response to anti-microtubule agents. However, existing methodologies to measure this rate are labor intensive, indirect, and confounded by karyotype selection reducing observable diversity.

Methods: We developed a framework to simulate and measure CIN, accounting for karyotype selection, and recapitulated karyotype-level clonality in simulated populations. We leveraged approximate Bayesian computation using phylogenetic topology and diversity to infer mis-segregation rates and karyotype selection from single-cell DNA sequencing data.

Results: Experimental validation of this approach revealed extensive chromosome mis-segregation rates caused by the chemotherapy paclitaxel (18.5±0.5/division). Extending this approach to clinical samples revealed the inferred rates fell within direct observations of cancer cell lines.

Conclusion: This work provides the necessary framework to quantify CIN in human tumors and develop it as a predictive biomarker.

Abstract #44

Regulation of the MDM2 Oncoprotein by a Nuclear Phosphoinositide Complex

Author(s): *Jeong Hyo Lee*,* Mo Chen, Tianmu Wen, Richard Anderson, Vicent Cryns

Background
The Mouse double minute 2 homolog (MDM2), E3 ubiquitin-protein ligase MDM2, is an important negative regulator of the p53 tumor suppressor. Our previous data showed that the type Ⅰ phosphatidylinositol phosphate kinase (PIPK Ⅰα) and its product phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2) regulates the stability of p53.

Methods
The Microscale Thermophoresis assays (MST), liposome sedimentation, and Western blot were conducted to indicate interaction between MDM2 and PtdIns(4,5)P2. Western blot and proximity ligation assay were performed to identify association between MDM2 and phosphoinositide kinases including sHSPs. In silico analysis was conducted to figure out whether MDM2 has potential consensus phosphoinositide motifs on the domain.

Results
MDM2 binds to phosphoinositide and binds most strongly to PtdIns(4,5)P2 in vitro. MDM2 tightly associates with PtdIns(4,5)P2 in various cell lines and MDM2 interacts PtdIns(4,5)P2 in vivo. MDM2 also interacts with phosphoinositide kinase and most robustly interacts with PIPK Ⅰα which is support the binding of MDMD2 and PtdIns(4,5)P2. Interestingly, MDM2 interacts with small Heat Shock Proteins (sHSPs) and PtdIns(4,5)P2 regulates the MDM2-sHSPs complex formation and the interaction with p53. In addition, MDM2 has two potential consensus phosphoinositide motifs on the domain and intriguingly both motifs on the ubiquitin ligase region.

Conclusions
MDM2 directly binds with p53, so we tried to figure out whether PtdIns(4,5)P2 also interacts with the MDM2 in vitro and in vivo. Our data demonstrate that PIPK Ⅰα and PtdIns(4,5)P2 indeed interact with MDM2 in vitro and in vivo, and suggest PtdIns(4,5)P2 may modify its interaction with client proteins or function.

Abstract #45

Defining the Mechanism of Enhanced Receptor Tyrosine Kinase Stimulated PI3K/Akt Signaling in Endomembrane in the Absence of p85alpha Adaptor Subunit

Author(s): *Narendra Thapa*,* Mo Chen, Tianmu Wen, Changliang Chen

Abstract

PI3Kalpha enzyme often mutated in cancer and activated by receptor tyrosine kinases is a heterodimer of p110alpha catalytic subunit coupled with p85 adaptor subunits (p85alpha, p85beta, or p55gamma) expressed in 1:1 stoichiometry. Though p85alpha interaction with activated receptor tyrosine kinases is known to bring p110 catalytic subunit to membrane proximity, genetic studies in mice indicate that p85alpha loss promotes insulin-stimulated PI3K/Akt signaling, and p85alpha is downregulated in genomic and protein levels in cancers. Herein, we show unperturbed reorganization (increased coupling of p110alpha with p85beta upon p85alpha loss) of residual p110alpha along microtubules via MAP4 interaction and its integration into internal membrane compartments upon p85alpha loss. The p110alpha shows increased integration into internal membrane compartments via its C2 domain interaction with endosomal PI3P eliciting the highly upregulated PI3K/Akt signaling downstream of activated receptor tyrosine kinases upon p85alpha loss. Deletion of C2 domain or impaired C2 domain-PI3P interaction severely abrogates receptor tyrosine kinase stimulated endosomal PI3K/Akt signaling. The strategies to disrupt p110alpha C2 domain-PI3P interaction could pave a way for spatial targeting of receptor tyrosine kinase stimulated PI3K/Akt signaling in cancers, including p85alpha, downregulated cancers.

Abstract #46

DNMT3A contributes to ibrutinib resistance in mantle cell lymphoma

Author(s): *Nguyet-Minh Hoang*,* Yunxia Liu, Fen Zhu, Li Lu, Apoorv Kondapelli, Lixin Rui

Abstract

Mantle cell lymphoma (MCL), a B-cell tumor comprising approximately 6% of non-Hodgkin lymphoma, remains incurable with the current standard immunochemotherapy. Ibrutinib, a selective inhibitor for Bruton tyrosine kinase (BTK) in the BCR signaling pathway, has elicited responses in 68% of refractory or relapsed MCL patients and promises to be a valuable therapeutic option. Primary or acquired resistance to Ibrutinib, however, impacts the long-term survival of these patients; understanding ibrutinib-resistance and identification of mechanisms to overcome resistance are unmet clinical needs. Our study focuses on *de novo* DNA methyltransferase 3A (DNMT3A) whose expression and activity are higher in ibrutinib-resistant MCL patients. It is also known that ibrutinib resistant cells show elevated expression of MYC target genes and increased oxidative phosphorylation compared to ibrutinib sensitive cells. We hypothesize that DNMT3A is involved in ibrutinib resistance in MCL. We found that DNMT3A physically interacts with MYC in MCL cells. CRISP/Cas9-mediated DNMT3A knockout downregulates both MYC targets and genes that are involved in mitochondrial oxidative phosphorylation. Mechanistically, our DNMT3A ChIP-sequencing demonstrated a significant motif enrichment for MEF2B, a transcription factor that physically interacts with MYC to enhance transcriptional activities of MYC. Functionally, overexpression of DNMT3A increases mitochondrial activities, including increased membrane potential and mitochondrial reactive oxygen species as well as oxygen consumption rate. DNMT3A knockout sensitizes ibrutinib resistant cells to ibrutinib treatment. Conversely, overexpression of DNMT3A induces ibrutinib resistance in ibrutinib sensitive cells. In conclusion, our data provide evidence that DNMT3A overexpression contributes to ibrutinib resistance through the MEF2B-MYC axis, which regulates mitochondria function and oxidative phosphorylation in MCL. The study suggests that targeting DNMT3A is a potential therapeutic strategy to overcome ibrutinib resistance in MCL.

Abstract #47

A potential tumor suppressive role of polo-like kinase 5 in specific neoplasms

Author(s): *Glorimar Guzmán Pérez*,* Shengqin Su, Mary A. Ndiaye, Manish Patankar, and Nihal Ahmad

Abstract

Polo-like kinase 5 (PLK5) is a member of the serine/threonine family of polo-like kinases (PLKs) which play important roles in cell cycle regulation. Although Plk5 was identified in humans over a decade ago, limited information is available regarding its functional significance. Much research to date focuses on brain tissue/tumors, in which PLK5 was found to be downregulated in brain tumors, suggesting potential tumor suppressive functions. The objective of this study was to determine the expression profile of PLK5 in a variety of normal and malignant tissues types to gain an insight into its function in cancer. A search of the ProteinAtlas database showed PLK5 was expressed in several normal human tissue types including brain, eye, lung, testis, fallopian tubes, endometrium, and cervix. We analyzed quantitative immunostaining of PLK5 employing tissue microarrays (TMAs) containing tissue cores from six different organs viz. cervix, endometrium, fallopian tubes, ovary, lung, and testis. Stained TMA slides were scanned using Vectra Imaging System and analyzed with InForm software, yielding quantitative information of PLK5 protein levels in each tissue core for analysis. Our data demonstrated that PLK5 protein levels were significantly downregulated in most malignant tissues vs normal tissues. To corroborate this, we determined expression profiles of PLK5 in cancer and normal tissues using publicly available TCGA (The Cancer Genome Atlas) data via the Genomic Data Commons (GDC) Data Portal. From here, we found that PLK5 had significantly lower expression in cancer than normal tissues in cervix, endometrium, and ovary. Additionally, we used data from the Genotype-Tissue Expression (GTEx) portal, which contains data from non-diseased tissues, to compare PLK5 expression in normal samples from GTEx to tumor samples from TCGA. We found PLK5 levels were higher in normal versus malignant tissues from cervix, endometrium, ovary, and testis; however, there was no difference in terms of PLK5 levels between normal and malignant tissues from lung. Taken together, our results demonstrate that PLK5 levels are downregulated in multiple cancers, suggesting a potential tumor suppressive function of PLK5 in cancer. However, additional detailed studies are required to fully understand the role and functional significance of PLK5 in cancer.

Abstract #48

A Novel Phosphoinositide-NRF2 Complex Regulates Oxidative Stress

Author(s): *Changliang Chen*,* Mo Chen, Narendra Thapa, Suyong Choi, Vincent L. Cryns and Richard A. Anderson

Abstract

Oxidative stress is a condition in which the balance between the production and disposal of reactive oxygen or nitrogen species is altered. Cancer cells have elevated levels of reactive oxygen species due to oncogenic alterations that fuel their rapid growth. The transcriptional activator nuclear factor erythroid 2-related factor 2 (NRF2) is a master regulator of the antioxidant response that is aberrantly activated in many tumors, enabling their survival. Although mutations in KEAP1 stabilize NRF2 in a subset of cancers, additional regulators of NRF2 are likely to contribute to its aberrant activation in cancer. Here, we show that the stability of stress-induced NRF2 is regulated by the type I phosphatidylinositol phosphate kinase PIPKIγ and its product phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2). Nuclear PIPKIγ binds to NRF2 in response to oxidative stress, resulting in the production and association of PtdIns(4,5)P2 with NRF2. PtdIns(4,5)P2 binding promotes the interaction between NRF2 and the small heat shock proteins HSP27 and αB-crystallin. Silencing PIPKI-γ or sHSPs destabilizes NRF2, pointing to a critical role of this complex in regulating NRF2 stability and function. Taken together, we have discovered an unexpected role for phosphinositide kinases and their lipid second messengers in regulating NRF2 and the antioxidant response in cancer, thereby implicating these molecules as novel drug targets.

Abstract #49

A p53-Phosphoinositide Signalosome Regulates Nuclear Akt Activation

Author(s): *Mo Chen*,* Suyong Choi, Tianmu Wen, Changliang Chen, Narendra Thapa, Jeong Hyo Lee, Shane M. Bick, Vincent L. Cryns, and Richard A. Anderson

Abstract

The tumor suppressor p53 and the phosphoinositide 3-kinase (PI3K)-Akt pathway have fundamental roles in regulating cell growth, apoptosis and are frequently mutated in cancer. Here, we show that genotoxic stress induces nuclear Akt activation by a p53-dependent mechanism that is independent from the canonical membrane-localized PI3K-Akt pathway. Upon genotoxic stress a nuclear p53-PI3,4,5P3 complex is generated in regions devoid of membranes by a nuclear PI3K, and this complex recruits all the kinases required to activate Akt and phosphorylate FOXOs, inhibiting DNA damage-induced apoptosis. Wild-type p53 activates nuclear Akt in an on/off fashion upon stress, whereas mutant p53 stimulates high basal Akt activity, indicating a fundamental difference. The nuclear p53-phosphoinositide signalosome is distinct from the canonical membrane-localized pathway and insensitive to PI3K inhibitors currently in the clinic, underscoring its therapeutic relevance.

Abstract #50

Examining the effects of obesity and weight loss on mammary tumor inflammation and fibrosis

Author(s): *Genevra Kuziel*,* Lisa Arendt

Background
Obesity significantly increases risk of breast cancer development, and following diagnosis, obese breast cancer patients have an overall worsened prognosis. The complex relationship of obesity to breast cancer growth and aggressiveness must continue to be examined. Within breast adipose tissue, obesity causes a state of chronic, macrophage-driven inflammation. Chronic inflammation is associated with increased extracellular matrix (ECM) deposition. Increased collagen within the mammary microenvironment is a risk factor for tumor formation, as well as more aggressive tumors, in mouse models. In breast tumors from obese patients, tumor desmoplasia is also found to be increased.

Method
To better understand how obesity affects mammary tumor growth, inflammation, and fibrosis, we used a diet-induced obesity mouse model paired with the estrogen receptor alpha (ERα)+ TC2 mammary tumor cell line in female FVB mice. We observed that TC2 mammary tumors grew significantly faster in obese mice compared to lean mice and had significantly more macrophage infiltration. TC2 tumors from obese mice also had significantly more collagen deposition than TC2 tumors from lean mice, indicating that obesity leads to more fibrotic tumors. To identify how weight loss alters the mammary gland and mammary tumor microenvironments, obese mice were switched from a high-fat diet to a low-fat diet for six weeks, to induce weight loss.

Results
In non-tumor-bearing mammary glands, weight loss led to a significantly decreased number of crown-like structures, a marker of adipose tissue inflammation, similar to observations in human adipose tissue. TC2 mammary tumors grew at an intermediate rate between tumors from lean and obese mice, indicating that weight loss may be a viable option to reduce tumor aggressiveness, particularly for obese patients with other breast cancer risk factors.

Conclusion
Understanding these weight-related changes in the mammary gland and mammary tumor microenvironments will provide greater insight into possible interventions for obese breast cancer patients.

Abstract #51

Validation and analysis of cancer associated fibroblast subtype markers in metastatic colorectal cancer

Author(s):
*Anna Lippert** Katherine A. Johnson, Sean G. Kraus, Philip B. Emmerich, Cheri A. Pasch, Linda Clipson, Kristina A. Matkowskyj, Wei Zhang, Dustin A. Deming

Background:
Cancer Associated Fibroblasts (CAFs) have an impact on immune infiltration in the tumor microenvironment. Two major subtypes of CAFs have been previously identified by literature: myofibroblastic (myCAF) and inflammatory (iCAF). We validated subtype markers and investigate CAF phenotypes in metastatic colorectal cancer patients.

Methods:
Dual-immunofluorescence on formalin fixed paraffin embedded tissue sections was performed to analyze co-staining between combinations of myCAF markers, αSMA and TAGLN, and iCAF markers, PDPN and ICAM1. Tissue microarrays sampling 212 CRC patients spanning all stages of disease, 90 with matched metastatic cores, were stained via immunohistochemistry (IHC) for the CAF subtype markers then quantified on an intensity scale from 0-3+. iCAF and myCAF marker scores were averaged to get a composite score, then split into low (average<2) and high (average≥2) groups. CD8 IHC stains were quantified as number of tumor infiltrating lymphocytes (TILs) per high power field in the epithelial compartment.

Results:
Significant co-staining was observed between iCAF markers PDPN and ICAM1, as well as myCAF markers αSMA and TAGLN. No significant co-staining occurred between combinations of myCAF and iCAF markers. There is no significant difference in abundance of iCAFs or myCAFs in primary site cores of patients with metastatic versus non-metastatic disease (p=0.67 iCAF, p=0.57 myCAF). Of matched primary and metastatic samples, 43.3% decreased in iCAF score from primary to metastatic site while only 18.8% increased. Overall, 34.4% of samples decreased in score by more than 1 and only 2.2% of samples increased by more than 1. The percentage of samples which decreased in myCAF score was 32.2% while 22.2% increased. In all primary cores of patients with metastatic disease, there was higher average $CD8^+$ TILs in those with high iCAF scores compared to those with low iCAF scores (12.0 vs 5.5, p=0.03). There was no significant difference in average $CD8^+$ TILs in those with high myCAF scores compared to those with low myCAF scores (9.3 vs 7.1, p=0.7).

Conclusions:
We validate the myCAF and iCAF markers by demonstrating co-staining between CAFs of the same subtype and exclusion between different subtypes. Additionally, iCAFs correlate with immune infiltration and myCAFs with immune exclusion.

Abstract #52

Effects of tyrosine kinase inhibitors imatinib, dasatinib, and nilotinib on cancer-associated fibroblast phenotypes in colorectal cancer.

Author(s): *Katherine Johnson*,* Anna L. Lippert, Sean G. Kraus, Gracie McGrath, Philip B. Emmerich, Cheri A. Pasch, Linda Clipson, Kristina A. Matkowskyj, Wei Zhang, Dustin A. Deming

Background: Neutralizing the role of cancer-associated fibroblasts (CAFs) in modulating the immune microenvironment of colorectal cancer (CRC) could be the key to enhancing immunotherapy success. TGFβ is a potential functional marker for CAF phenotypes. Imatinib, dasatinib, and nilotinib are tyrosine kinase inhibitors (TKIs) with several kinase targets, including those involved in TGFβ signaling.

Methods: Tissue microarrays spanning 153 CRC patients were stained for αSMA, TAGLN, PDPN, ICAM1, and CD8. CD8 stains were quantified as number of tumor infiltrating lymphocytes per high powered field (TILs/HPF) in the epithelial compartment. Other stains were quantified 0-3+ by intensity. αSMA and TAGLN scores were combined into a myCAF score, and PDPN and ICAM1 into an iCAF score. The LINCS database was used to discover potential drugs to reverse the myCAF gene signature. Primary CAFs derived from CRC patient tumor samples were treated with clinically relevant concentrations of TKIs for 96 hours. RT-qPCR quantified *TGFB1,* the myCAF genes *ACTA2*, *COL11A1,* and *TAGLN*, and the iCAF genes *ICAM1, PDPN, IL1R1, CXCL1* and *CXCL2.*

Results: Cancers with high myCAF but low iCAF scores had the most CD8+ TILs (average 10.2 TILs/HPF; median 1.5; range 0-73), while cancers with low myCAF and high iCAF scores had the least (average 1.5; median 0; range 0-19; $p<0.01$). LINCS analysis identified nilotinib as a top hit to reverse the myCAF gene signature. Treatment with imatinib did not significantly alter expression of myCAF genes, while dasatinib significantly increased expression (control vs. max dose: *ACTA2* 1.4x higher, $p<0.001$; *COL11A1* 2.6x higher, $p<0.01$; *TAGLN* 1.5x higher, $p<0.001$), and nilotinib significantly decreased expression (*ACTA2* 2.2x lower, $p<0.001$; *COL11A1* 1.3x lower, $p=0.05$; *TAGLN* 1.9x lower, $p<0.01$). All drugs decreased *CXCL1*, and all but dasatinib decreased *CXCL2*. Imatinib and nilotinib decreased *TGFB1* (imatinib 1.6x lower, $p<0.001$; nilotinib 1.5x lower, $p<0.05$).

Conclusions: Presence of myCAFs correlates with immune exclusion, indicating these cells as a target for improving immunotherapy. Nilotinib, but not imatinib or dasatinib, is effective at decreasing expression of myCAF genes. Further research is warranted into the mechanisms of this drug on altering CAF phenotypes *in vitro* and *in vivo.*

Abstract #53

PEGylated Functional Upstream Domain (PEG-FUD): as an anti-cancer therapy for breast Cancer

Author(s): *Metti K. Gari*[1], Hye Jin Lee[2], David R. Inman[1], Glen S. Kwon[2], Suzanne M. Ponik[1]

Abstract

As of 2021, breast cancer became the most common cancer diagnosed globally. In breast cancers, the extracellular matrix (ECM) is known to play a key role in disease progression. Work by our group identified a set of changes in the architecture of collagen, an abundant ECM protein, that occurs during tumor progression that are termed Tumor Associated Collagen Signature (TACS). Among the identified TACS, TACS-3 which is distinguishable by straightened collagen fibers that organize perpendicular to the tumor boundary was shown to be a prognostic marker of poor disease free survival rates. Using matrix targeted proteomics we identified a signature of ECM proteins, including fibronectin (FN), that organize with aligned collagen fibers. FN deposition directly contributes to fibrosis and is also known to precede the deposition of collagen, making FN an attractive therapeutic target. Despite multiple efforts in developing therapies that target the ECM, currently there are no effective treatments available due to toxicity and lack of specificity. Thus, we aimed to target fibronectin to enable site specific drug delivery using a bacterial derived peptide known as the functional upstream domain (FUD) which is potent inhibitor of fibronectin assembly. However, due to its small size (6-kDa), FUD has rapid renal clearance limiting its ability to accumulate in vivo. To overcome the pharmacokinetic limitations of FUD, PEGylation was considered. In this study, we used 20-kDa PEG-FUD to assess its potential to target mammary tumors and evaluate its therapeutic efficacy as an anti-cancer therapy. In vivo imaging system was used to assess the biodistribution of PEG-FUD following s.c. injection of Cy5 labeled peptides in 4T1 mammary tumor bearing mice. Our findings indicated that PEG-FUD specifically targets mammary tumors with an increased bioavailability compared to FUD alone. To further understand PEG-FUDs delivery and localization within the tumor microenvironment, we employed intravital microscopy which provides high-resolution images at subcellular scale. As anticipated, we observed greater penetration into the tumor region with PEG-FUD as opposed to FUD which mostly accumulated within the tumor vasculature. Additionally, PEG-FUD treatment significantly reduced tumor growth. These promising results pave the way for PEG-FUDs potential use as a treatment for breast cancer in the future.

Abstract #54

Assessing the risk: How breast density and obesity alter the mammary gland

Author(s): Abbey E. Williams[1], Erica Hoffmann[2], Julia Warren[3], Suzanne Ponik[2,3], and Lisa M. Arendt[1,2,4]

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Background:
Recent epidemiological studies suggest that the risk factors of breast density and obesity together increase breast cancer risk in premenopausal women, and these women have a worsened prognosis following breast cancer diagnosis. Although the underlying causes of increased risk associated with dense breasts and obesity have individually been explored, little is known about how these underlying risk factors interact together to enhance breast cancer risk.
Methods:
To model breast density, we used heterozygous Col1a1^{tmjae} mice, that have a mutation that limits collagen degradation, leading to increased mammary collagen deposition. Heterozygous (het) mice or wild type (wt) litter mates were fed either a low-fat diet (LFD) or a high-fat diet (HFD) to induce obesity for 9, 12, or 15 weeks. Using picrosirius red stain to identify collagen, we observed significantly enhanced collagen deposition surrounding mammary ducts from LFD/het, HFD/wt, and HFD/het mice compared to those from LFD/wt mice. Notably, HFD/het mice had significantly more collagen surrounding ducts than in mammary glands from LFD/het mice, suggesting that obesity further enhances collagen deposition. To identify changes in inflammatory cells, we used immunohistochemistry to identify F4/80+ macrophages and CD8+ T cells in the mammary glands of mice of all groups.
Results:
We observed significantly increased numbers of macrophages in mammary glands of LFD/het, HFD/wt and HFD/het mice compared to LFD/wt mice. Furthermore, CD8+ T cells were significantly reduced in mammary glands of HFD/wt and HFD/het mice compared to either LFD/wt or LFD/het mice. No differences were observed between wt and het mice in either diet group.
Conclusion:
Together, these results suggest that obesity contributes to collagen deposition within dense breast tissue and macrophage-driven inflammation in wild type mammary glands of non-tumor-bearing mice. However, obesity may be the driving factor in decreased immunosurveillance of CD8+ T cells within the mammary gland which may lead to increased breast cancer risk in women with these two risk factors.

Abstract #55

Collagen density primes the mammary tumor microenvironment for early dissemination by promoting macrophage infiltration and an EMT-associated transcriptional program in tumor cells.

Author(s): Erica Hoffmann[1], Ana Rita Nobre[2], Abigail Williams[3], Lisa Arendt[3], Julio Aguirre-Ghiso[4], and Suzanne Ponik[1,5]

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High mammographic density due to increased collagen I deposition has long been recognized as a risk factor for breast cancer progression in women, however the biology underlying this risk factor and the exact mechanisms by which collagen I affects disease progression remain largely unknown. In advanced tumors, stiff, aligned collagen fibers are known to promote epithelial to mesenchymal transition (EMT), invasion of cancer cells, and increase metastatic burden. Tumor associated macrophages are also enriched in collagen dense breast tissue, which further enhance metastatic dissemination. Importantly, we observe key differences between the normal and collagen-dense mammary glands before a tumor is present that may predispose the microenvironment to early tumor cell dissemination. One such factor is the presence of ZFP281, a transcription factor associated with stem cell differentiation and recently recognized as a key factor promoting EMT and cancer progression in multiple cancer types. Using a mouse model with a mutant collagen gene to mimic mammographic density, we identified an increase in periductal/intraepithelial macrophages and expression of the transcription factor ZFP281 in epithelial cells. However, this increase in ZFP281+ cells is lost by the time a primary tumor is formed. Using a 3D collagen gel cell culture model to investigate the effects of collagen I on ZFP281, we observed that increasing collagen density alone is sufficient to decrease protein levels of ZFP281 in breast cancer cells. This affect appears to be primarily mediated by the increased stiffness of the matrix. This previously unrecognized mechanical regulation of ZFP281 expression by collagen I, paired with the timing of fiber accumulation and reorganization, may be an important microenvironmental cue that determines the switch from early dissemination to high proliferation at the primary tumor, a common hallmark of collagen-dense mammary tumors.

Abstract #56

The Impact of GPER Modulation on Extracellular Matrix Formation in Breast Cancer

Author(s): *Shelby A. Fertal*,* Brian M. Burkel, Fern E. Murdoch, Kathy O'Leary, Linda A. Schuler, and Suzanne M. Ponik

The G-protein coupled estrogen receptor (GPER) is a noncanonical estrogen receptor (ER) that is estimated to be present in roughly 50-60% of all breast cancer (BC) subtypes. Previous studies have indicated that the presence of GPER during primary disease aids in disease progression, metastasis, as well as chemotherapy resistance due to the agonistic activity of traditional chemotherapies for ER+ BC, tamoxifen and fulvestrant. It is also well established that the extracellular matrix (ECM), specifically collagen, contributes to the overall risk and progression of BC in patients. Importantly, fibroblasts are one of the primary cell types responsible for ECM deposition in the tumor microenvironment. We hypothesize that the modulation of cancer-associated fibroblasts (CAFs) via estrogen signaling through GPER regulates the deposition and structure of key ECM proteins, such as collagen, to aid in disease progression. CAFs, isolated from human BC surgical resections, were plated at 100% confluency, and incubated with +/- 10nM β-estradiol (E2) and 50ug/mL ascorbic acid daily for 14 days in phenol-free, high glucose DMEM with 10% charcoal stripped FBS to induce matrix deposition. Following the 14-day incubation, cell proliferation was quantified prior to extracting the cells to isolate the CAF-derived matrix (CDM). The composition and organization of ECM was analyzed in CDMs through immunofluorescence (IF) or western blot. Culturing CAFs with E2 resulted in a 10% increase in cell proliferation ($n=3$, $p< 0.05$) and a 1.6-fold ($n=3$, $p< 0.05$) increase in total ECM deposition by western blot. Key ECM proteins such as collagen, fibronectin, and periostin had a 1.8, 1.3, and 1.3-fold increase, respectively ($n=3$, $p< 0.05$), in protein deposition with E2 treatment when normalized to cell number. Collagen fiber analysis of IF stained CDM for collagen resulted in a coefficient of alignment with E2 of 0.76 compared to 0.41 without ($n=3$, $p< 0.001$) and a 0.6- fold decrease ($n=3$, $p<0.0001$) in angle variance when E2 was present. These results demonstrate that estrogen signaling through GPER regulates both deposition and organization of ECM and highlights the need to further investigate how the interplay between the ECM and GPER activation impacts tumor cells to drive BC progression.

Abstract #57

Mesenchymal stromal cells for treatment of radiation induced xerostomia

Author(s): Cristina Paz*, Grace Blitzer MD, Annemarie Glassey, Jayeeta Giri PhD, Andrea Pennati PhD, Olga Ganz, Steven Schreiber, Kwangok P Nickel PhD, Cynthia A. Kelm-Nelson PhD, Vanessa L. Cannaday MS, Robert Pohlman PhD, Tiffany Glazer MD, Ted Lunga MUDr, Daniel Robbins MD, Ryan Mattison MD, Tomy Varghese PhD, Susan Thibeault PhD CCC-SLP, Nicole Rogus-Pulia PhD CCC-SLP, Jacques Galipeau MD, and Randall J. Kimple MD PhD

Purpose/Objectives: There is a **critical need** for a treatment that will safely and effectively alleviate radiation-induced xerostomia (RIX). Head and neck radiation results in hyposalivation and altered sialochemistry which patients experience as xerostomia. We preformed preclinical and a pilot clinical study to determine if IFN-ɣ stimulated marrow-derived mesenchymal stromal cells (MSC(M)) from HNC patients could function as a source of autologous cells for the treatment of RIX.

Materials/Methods: Bone marrow aspirates from HNC patients previously treated with chemoradiation were obtained (IRB #2019-0497, NCT 04007081). MSC(M)s were isolated from bone marrow, expanded in culture, surface marker expression confirmed via flow cytometry, stimulated with IFN-ɣ, and cryopreserved. Thawed MSC(M)s were profiled to evaluate their functionality after cryorecovery. Cells were tested for their ability to stimulate salivary production in a murine model of RIX by injecting $1x10^6$ cells into the submandibular gland (SMG) one week after delivery of a 15 Gy dose of radiation. Efficacy was evaluated by histology and saliva production.

Results: MSC(M)s from six patients were expanded to $>20x10^6$ cells (median 15.5, range of 8-20 days). Post-thaw cultures demonstrated robust growth, with a median doubling time of 3.1 days. Cultured cells showed an MSC(M) phenotype, positive for CD73, CD90, and CD105 and negative for CD14, CD20, CD34, or CD45. IFN-ɣ stimulated MSC(M)s had increased immunomodulatory potential based on expression of IDO, ICAM-1, PD-L1, MHC I and MHC II expression compared with non-stimulated MSC(M)s (391%, 263%, 114%, 70% and 196% percent increase respectively). In a murine model of RIX, delivery of a single 15 Gy dose to the SMG resulted in structural changes evidenced by decreased acinar size, increased fibrosis, and immune cell infiltration and decreased saliva production. Injection of MSC(M)s to the SMG following radiation resulted in improved saliva production, a reduction in fibrosis, and an increase in the size and density of acini within the tissue compared to control.

Conclusions: These data strongly support the feasibility of a first-in-human clinical trial of autologous IFN-ɣ stimulated MSC(M)s to treat RIX and the potential of using human-derived MSC(M)s in future murine studies of RIX.

Abstract #58

Optimizing Mesenchymal Stromal Cells (MSCs) origin for treatment of radiation-induced xerostomia

Author(s): *Anne Marie Glassey*,* Cristina Paz, Abigail Frick, Kwang Nickel, Ilya Gurevic, Sara McCoy, Jacques Galipeau, Randall Kimple

Background

Mesenchymal stromal cells (MSCs) are candidates for cell therapies due to their innate ability to modulate immune responses, enhance epithelial cell proliferation, and reduce inflammatory damage. These properties are attributed to the MSC secretome, which contains tissue-promoting morphogens and anti-inflammatory factors. Through these secretions, MSCs can downregulate inflammation and reduce fibrosis in tissues. Our overall goal is to use MSCs to develop a novel treatment of radiation induced xerostomia. It is unclear how the MSC source impacts their therapeutic potential, so seek to compare adipose tissue and bone marrow derived MSCs to native submandibular gland (SMG) MSCs for the purpose of identifying an ideal tissue of origin. We hypothesize that bone marrow derived MSCs will be a more effective than adipose derived MSCs, while having a similar secretome to SMG MSCs.

Methods

Bone marrow, adipose, and SMG tissues from male and female B6N mice were harvested and MSCs from each isolated. MSC identity is confirmed by flow cytometry (positive for CD73, CD90, STRO1, and CD105; negative for CD14, CD20, CD34, and CD45) and by differentiating MSCs into adipocytes, osteoblasts, and chondrocytes by von Kossa and Oil red O staining. MSC in vitro growth rate and colony formation ability is measured. Secretome analysis for growth factors that promote tissue healing and regeneration like Wnt, GDNF, and R-spondin will be measured through ELISAs of cell lysate and MSC conditioned media.

Results

MSCs identity was confirmed by flow cytometry and by confirming the ability to differentiate into adipocytes, osteoblasts, and chondrocytes. In addition to histologic confirmation, differentiation has been confirmed by demonstrating, by qPCR, expression of markers of differentiation (RUNX2, BSP-II, ALP, SOX9, CEBPα, and PPARɣ). Data on MSC growth rate, colony formation ability, and secreted factors will be presented.

Conclusion

MSCs can be isolated from bone marrow, adipose tissue, and SMGs. This work supports the use of MSCs in the treatment of radiation-induced xerostomia. Data on secreted factors may identify an optimal tissue source for future studies.

Abstract # 59

Characterization of Weakly Hormone Receptor (HR)-Positive, HER2-Negative Breast Cancer and Current Treatment Strategies

Author(s): *Johanna Poterala*,* Kari Wisinski, MD

Background: Hormone receptor (HR) and human epidermal growth factor receptor-2 (HER2) status is critical for determining management of breast cancer. Previous reports of small cohorts with weak HR-positive (HR+)/HER2-negative (HER2-) disease showed similar rates of pathologic complete response (pCR) following neoadjuvant chemotherapy (NAC) as triple negative breast cancer (TNBC). This study aims to further characterize this group of patients, focusing on breast and axillary pCR rates following NAC.

Patients and Methods: Patients with stage I-III, HR+/HER2- breast cancer were identified using the University of Wisconsin Hospital Cancer Registry. Medical records were reviewed for demographics, tumor characteristics with quantification level of estrogen and progesterone receptor (≤33%), treatment and follow-up data.

Results: Data was reviewed from 2,900 patients and a total of 64 patients met inclusion criteria. Eighty percent received chemo, about half with NAC (n=30, 48%). Of 28 patients who had NAC followed by breast and axillary surgery, 12 (43%; 95% CI 25-63%) had pCR (ypT0/Tis/ypN0). Of the 11 patients who had biopsy-proven nodal disease at diagnosis and NAC followed by axillary surgery, 7 (64%, 95% CI 31-89%) patients had pCR at the axilla. For those with recurrent disease, median time to recurrence was 13.6 (5.6–48.7) months.

Conclusions: Breast cancers that are HER2- and weakly HR+ treated with NAC demonstrated an axillary and overall pCR rate more similar to TNBC than breast cancers that are strong HR+. Neoadjuvant approaches may improve pCR rates, which provides important prognostic information. Clinical trials should be developed to focus on this unique patient cohort.

Abstract # **60**

Versican Proteolysis is a Predictive Biomarker of Tumor Infiltrating Lymphocytes within Primary and Metastatic Colorectal Cancer.

Author(s): *Sean Kraus**, Kristina A. Matkowskyj, Philip B. Emmerich, Wei Zhang, Linda Clipson, Cheri A. Pasch, Dustin A. Deming.

Background: A roadblock to immune-therapy response in many solid malignancies is the lack of tumor-infiltrating lymphocytes (TILs) found within their immune micro-environments. Immunologically cold tumors have lower response rates to immune-based therapies and among all colorectal cancers (CRC), only about 6% of patients have an objective response to immune checkpoint blockade (ICB). Versican (VCAN) accumulation correlates with fewer TILs, whereas its proteolysis into versikine (Vkine) correlates with a greater number. This study analyzes the association between VCAN accumulation/proteolysis and TILs in primary and metastatic CRC.

Methods: VCAN, Vkine, and TIL abundance were assessed via immunohistochemistry. VCAN and Vkine were measured using an intensity binning system ranging from 0-3+ and TILs were counted at a 40X magnification. Tumors were designated as high (2 or 3+) or low (0 or 1) for both VCAN and Vkine. Cores that were VCAN low and Vkine high are considered VCAN proteolytic predominant (VPP) and all other combinations are VCAN proteolytic weak (VPW).

Results: 53% of both primary and metastatic tumors were VCAN-low and 47% VCAN-high. 59% of metastases from patients with VCAN-high primary tumors were also VCAN-high, whereas 43% of metastases from patients with VCAN-low primary tumors were designated VCAN-high. It was also found that 37% of primary tumors were VPP but only 26% of metastases were VPP. When comparing proteolysis in a pairwise fashion, it was found that only 37% of VPP primaries had a VPP metastasis. An inverse correlation was found between VCAN accumulation and TILs within primary CRCs. VCAN-high tumors had an average of three TILs/high powered field (HPF), whereas VCAN-low tumors had an average of 16 TILs/HPF ($p=0.007$). VPW primary tumors had an average of 3 TILs/HPF and VPP cores had an average of 21 TILs/HPF ($p=0.009$). VPW metastatic tumors had an average of 8 TILs/HPF, and VPP had an average of 15 TILs/HPF ($p=0.01$).

Conclusion: These data confirm the inverse correlations between VCAN accumulation and TILs in primary CRCs. Additionally, VCAN proteolysis correlates with CD8 infiltration in primary tumors and metastases. Accordingly, VCAN accumulation/proteolysis may prove to be a novel biomarker for ICB response.

Abstract #61

Detecting pulmonary fibrosis activity using fibroblast activation protein targeted positron emission tomography

Author(s): *Zachary T. Rosenkrans**, Anna S. Thickens, Christopher Massey, Ksenija Bernau, Carolina A. Ferreira, Ali Pirasteh, Nathan Sandbo, Reinier Hernandez

Background: The absence of non-invasive biomarkers to sensitively detect pulmonary fibrosis (PF), occurring in up to 40% of lung cancer patients receiving radiotherapy, is a significant burden limiting timely clinical diagnosis. Activated fibroblasts, which selectively express fibroblast activation protein (FAP), drive lung extracellular matrix remodeling during fibrogenesis. To this end, *we assessed the ability of radiolabeled FAP inhibitor (FAPI) to visualize in vivo FAP expression in a mouse model of pulmonary fibrosis by PET/CT.*

Methods: A mouse model of pulmonary fibrosis was established by intratracheal administration of bleomycin (1 U/kg) or saline as control. Pulmonary fibrosis and FAP expression were confirmed histologically. FAP microPET/CT was performed using FAPI-46 radiolabeled with Ga-68 ($t_{1/2}$ = 68 min; [^{68}Ga]Ga-FAPI-46). Mice were intravenously injected with [^{68}Ga]Ga-FAPI-46 at 7 d and 14 d post bleomycin instillation for dynamic PET/CT. Volume of interest analysis of the whole lung quantified lung density in Hounsfield units (HU) and radiotracer uptake in percent injected activity per cubic centimeter (%IA/cc).

Results: CT imaging did not detect differences in whole-lung CT density between mice administered bleomycin or saline at either imaging time point. In contrast, significantly higher [^{68}Ga]Ga-FAPI-46 uptake in the lungs of diseased mice compared to healthy mice was found as early as 15 min post-injection (p.i.) at 7 d and 14 d following lung injury. VOI analysis of the whole at lung 7 d post bleomycin administration revealed a 1.8-fold higher uptake in bleomycin (0.76±0.13 %IA/cc) vs. control (0.43±0.04 %IA/cc mice; p=0.014) at 30 min p.i., and 2.6-fold higher uptake in bleomycin mice (0.33±0.09 %IA/cc) vs. control mice (0.13±0.06; p=0.007) at 60 min p.i. These differences increased to 2.2-fold higher at 30 min p.i. (1.47 ±0.15 %IA/cc vs. 0.66±0.15 %IA/cc; p=0.0025) and 4-fold higher at 60 min p.i. (1.01±0.12 % IA/cc vs. 0.25±0.15 %IA/cc; p=0.0003) in bleomycin vs. controls at 14 d.

Conclusion: FAP-targeted PET with [^{68}Ga]Ga-FAPI-46 is an attractive diagnostic tool for non-invasively detecting early pulmonary fibrosis activity.

Abstract #62

Predicting Survival in Pancreatic Adenocarcinoma: A Concordance Analysis of Two Models

Author(s): *Marcinak C**; Collier A; Aiken T; Zafar SN; and the Central Pancreas Consortium

Background
Pancreatic adenocarcinoma (PDAC) remains highly morbid and difficult to prognosticate. Multiple predictive models of survival following surgical resection of PDAC have been externally validated, but their clinical utility remains unclear. The present analysis aims to evaluate the concordance between predicted and actual survival outcomes of established prognostic models.

Methods
A retrospective analysis was performed using a de-identified dataset of patients who underwent surgical resection of PDAC at seven academic medical institutions across the United States between 2010 and 2020. Patients were included only if their clinical status was known at the time of analysis. We evaluated two prognostic models: the American Joint Committee on Cancer (AJCC) staging system and the Memorial Sloan Kettering Cancer Center Pancreatic Adenocarcinoma Nomogram (MSKCCPAN). The concordance between model-predicted disease specific survival and actual survival was evaluated using the Harrell concordance index. To account for censoring in the data, predicted disease specific survival at 12, 24, and 36 months using the MSKCCPAN was evaluated using the Uno C-statistic.

Results
A total of 323 patients were included in the study. The cohort consisted of 161 (49.8%) males and 162 (50.2%) females with a mean age 65.2 years (SD, 9.2). At the time of surgery, 49 (15.2%) patients had AJCC Stage IA disease, 48 (14.9%) had Stage IB, 55 (17.0%) had Stage IIA, 110 (34.0%) had Stage IIB, and 61 (18.9%) had Stage III. One hundred fifteen (35.6%) patients were alive at last follow-up, while 166 (51.4%) patients had died of disease. Forty-two (13%) patients died of another cause. The Harrell concordance index between predicted and actual survival using the MSKCCPAN and AJCC staging system was 0.61 and 0.62, respectively. The calculated Uno C-statistic was 0.62 for all three evaluated time intervals.

Conclusion
The present analysis suggests that current predictive models correlate poorly with real world survival. Improving risk stratification of patients with PDAC will afford greater opportunities for tailoring precision medicine initiatives and increasing survival. There is an urgent need to develop novel models that better prognosticate outcomes following surgical resection of PDAC.

Abstract #63

Tumor mutational profiles of extreme long-term survivors with metastatic breast cancer

Author(s): *Yang Hu**, Junha Shin*, Stephanie M. McGregor, Kayla K. Lemmon, Sushmita Roy and Mark E. Burkard
* = equal contribution

Background: Extreme population sampling can discover genomic characteristics with a high likelihood of functional significance. Here, we focus on people who live for many years or decades with metastatic breast cancer (MBC). Identification of genetic markers characteristic of this population may allow long-term survival to be predicted and enable de-escalation of treatment and improved care for this subgroup of breast cancer patients.

Methods: We identified women who have MBC and have lived greater than 5 years from initial diagnosis. A total of 14 had archived FFPE metastatic tumor specimens and matched blood available for analysis. We performed whole-exome sequencing (WES) on FFPE tumor specimens (somatic) and blood samples (germline) pairs of 14 long term survivor patients. We used Illumina DRAGEN pipeline for the read alignment and base quality calibration and GATK MuTect2 pipeline for the somatic short variant identification. Common variants were filtered using 1000 Genomes Project, Exome Sequencing Project and gnomAD and annotated using Funcotator of the GATK pipeline.

Results: A cohort of 53 patients, who met criteria, with biopsy proven MBC and long survival were identified in our institution. Among them, 14 patients had sufficient archived tumor for analysis, consisting of 9 HR+/HER2-, 3 HR+/HER2+, 1 HR-/HER2+ and 1 TNBC specimens. Histological type was ductal in 9 patients and lobular in 4 patients. The median age of MBC diagnosis was 53 years with 11 patients diagnosed between ages 35-64 and 3 patients diagnosed after age 65. At the time of study, 13 of these 14 patients are still living. Median time to metastasis after diagnosis was 10.2 years with metastasis occurring in less than 1 year in 2 patients, less than 5 years in 10 patients and greater than 5 years in 2 patients. Metastases to bone were present in 9 patients, to visceral organs in 8 patients and to local and regional lymph nodes in 8 patients. The most common somatic variant identified was ARID1A. When compared with prior analyses of MBC (INSERM, MBC Project), we found that ARID1A mutations were more commonly found, whereas PTEN and ESR1 mutations were never identified, suggesting a somatic mutational profile characteristic of extreme survivors. ARID1A mutations (2 nonsense - p.R1505* and p.Q944*, 1 missense - p.L1496V, 1 frameshift - p.P729fs) occurred in 4 patients, all with ER/PR+ and all HER2- breast cancer. Median survival to date since diagnosis was 30.1 years (range 21.4-39.0). Median survival to date after metastasis was 14.9 years (range 2.7-35.0).

Conclusions: ARID1A mutations are overrepresented in metastatic tumors in extreme long-term survivors with MBC. Further analyses will determine if ARID1A mutations are present at tumor inception or are acquired in metastases in this cohort with indolent disease. Additionally, it is possible that germline genomic profiles may be relevant to long-term survival. Given the variability in this cohort, a larger sample of extreme survivors will be necessary to identify characteristic genetic profiles.

Abstract #64

Highly Sensitive Circulating Cell-free DNA (cfDNA) Detection in Combination with Machine Learning Algorithm for the Accurate Diagnosis and Prognosis of Hepatocellular Carcinoma (HCC)

Author(s): *Piper Rawding*,* Jiyoon Bu, Tae Hee Lee, Seungpyo Hong

ABSTRACT

Background Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide. Although various serum enzymes have been utilized for the diagnosis and prognosis of HCC, the currently available biomarkers lack the sensitivity to detect HCC at early stages and accurately predict treatment responses.

Methods In this study, we utilized a highly sensitive cell-free (cfDNA) detection system to validate the diagnostic capability of cfDNA in patients with HCC. Specifically, we quantified the amount of plasma cfDNA and *alpha-fetoprotein (AFP)* expression in the captured cfDNA from several cohorts, including patients with HCC, alcoholic liver hepatitis, or liver cirrhosis. In addition, we examined the clinical utility of cfDNA in determining the pathological status of a tumor and estimating the survival outcomes by comparing cfDNA with the expression of various serum liver enzymes. To provide more reliable clinical information using our system, we stratified HCC patients into sub-groups based on plasma cfDNA levels and *AFP* expressions using *k*-means clustering, establishing an integrated cfDNA score for HCC patients (cfD_{HCC}) using a principal component analysis. We then validated the diagnostic and prognostic capability of the integrated cfD_{HCC} score for HCC.

Results cfDNA, specifically the *AFP* expression in captured cfDNA, demonstrated the highest accuracy for diagnosing malignancies among all the serum/plasma biomarkers used in this study. The clinical utility of cfDNA was further improved by establishing cfD_{HCC}, demonstrating significantly improved accuracy in determining the pathological features of HCC and predicting patients' survival outcomes in comparison to the other biomarkers.

Conclusions With the application of our developed cfDNA detection platform and machine learning algorithm for the diagnosis and monitoring of HCC, we found our system to be superior at discriminating HCC, determining tumor characteristics, and predicting recurrence and survival outcomes, in comparison to current HCC biomarkers. Our system demonstrated high diagnostic and prognostic capabilities and can be potentially utilized in the clinic as a reliable system for identifying HCC in early stages, guiding therapeutic decisions, and improving overall survival outcomes.

Abstract #65

Analysis of T cell specific predictive biomarkers of graft-vs-host disease and relapse following post transplant cyclophosphamide prophylaxis

Author(s): *Nicholas J. Hess**, Kalyan Nadiminti, Peiman Hematti, Jenny E. Gumperz, Christian M. Capitini

Background: Despite a deeper understanding of the biology of acute graft-vs-host disease (aGVHD) and relapse following allogeneic hematopoietic stem cell transplantation (allo-HSCT), it is currently impossible to predict which patients will relapse or develop aGVHD. It is well known that donor T cells are responsible for both aGVHD and graft-vs-leukemia (GVL) activity. To that end, we investigated the longitudinal functional properties of T cells after allo-HSCT to identify and validate a suite of T cell-specific biomarkers of aGVHD and relapse following post transplant cyclophosphamide (PTCy) based GVHD prophylaxis.

Patients and Methods: After IRB approval, patients who received an allo-HSCT for any hematologic malignancies at UW-Madison were prospectively enrolled from 2020-2021. Blood samples were collected from 35 patients weekly for 98 days, starting day 7 following allo-HSCT. All patients received PTCy based GVHD prophylaxis. Blood samples were RBC lysed prior to flow cytometric staining.

Results: Lower numbers of $CD3^+CD45RO^+$ donor T cells in the first 22 days after allo-HSCT was predictive of relapse ($p=0.025$) with higher numbers correlating with increased aGVHD scoring but failed to reach significance. A different preclinical study from our group also identified a $CD4^+CD8^+$ double positive T cell (DPT) population linked with aGVHD. This study revealed that the percentage of DPT in the first 22 days post-transplant is highly predictive of aGVHD development (all grades, $p = 0.002$) and ≥ grade 2 aGVHD ($p = 0.017$). Additionally, an increase in the DPT frequency two weeks prior to aGVHD diagnosis was also predictive of aGVHD (all grades, $p = 0.003$) and ≥ grade 2 aGVHD ($p = 0.004$).

Conclusion: This is the first study to prospectively monitor T cells, including DPT, that persist after PTCy for allo-HSCT and correlate them with patient outcomes. The number of $CD3^+CD45RO^+$ T cells in the first 22 days may be associated with aGVHD and GVL effects. While the function of DPT is not known, their strong correlation with aGVHD suggests an active role in disease pathology. Validation of these T cell metrics will be needed in multicenter, prospective trials but may one day give clinicians insight into predicting aGVHD and relapse.

Abstract #66

Patient derived cancer organoids predict clinical response for patients with locally advanced rectal cancer.

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Background: Locally advanced rectal cancer (LARC) is a common disease in the US, with a growing incidence in younger patients. Patients undergo multimodality treatments including chemotherapy, radiation, & surgery. To date there is no clinical tool to know the potential benefit of these individual therapies. Patient-derived cancer organoids (PDCOs) are beginning to be used to predict individual patient response to clinical therapies. Here we use PDCOs from LARC patients to compare PDCO response to individual clinical response.

Methods: Fresh LARC tissues from patients undergoing endoscopy & tattooing procedure were obtained following consent on an IRB-approved protocol. PDCOs were cultured using Matrigel & our previously published CRC organoids media. Following culture maturation, PDCOs were treated with control media, 5uM 5FU, 10uM Oxaliplatin, Combination (FOLFOX), XRT (2Gy) or 5FU & XRT. Brightfield imaging was performed at baseline & following 96 hours of treatment. Glass's Δ (GΔ) is used to measure the organoid treatment effect. A GΔ of 1.25 was used as a threshold of treatment response based on prior studies. Clinical imaging was evaluated using standard RECISTv1.1 criteria of the objective response.

Results: LARC biopsy samples were obtained from 22 patients. 11/22 samples were able to be grown as PDCOs. To date 9 cultures have been treated with FOLFOX & 9 have been treated with 5FU & XRT. For those treated with FOLFOX, 3/9 had a GΔ greater than 1.25 & all of these patients has a clinical Partial Response (PR). None of those patients' whose PDCOs did not achieve GΔ of at least 1.25 had PR to FOLFOX clinically. No PDCO's treated with 5FU & XRT achieved a GΔ of >1.25. 3/9 PDCOs had a GΔ >1.0. All of these patients had PR. Of the 7 patients whose PDCOs did not achieve a GΔ of 1 in response to 5FU & XRT, none had a clinical PR (GΔ range 0.6-0.93).

Conclusion: PDCOs hold great promise as a tool to predict clinical outcomes for patients with LARC. Further evaluations need to establish improved methods of PDCO generation from biopsy samples & confirm the optimum response thresholds for prediction of treatment response.

Abstract #67

CH60 complexes with TLR4 at the cell surface to mediate bortezomib resistance in multiple myeloma.

Author(s): *Debayan De Bakshi*,* Shelly M. Wuerzberger-Davis and Shigeki Miyamoto

Abstract

Multiple Myeloma (MM) is a plasma B-cell malignancy considered incurable according to current clinical standards. Newly diagnosed patients receive the drug bortezomib, a clinical proteasome inhibitor, as part of the initial treatment regimen. Bortezomib partially exerts its cytotoxic effect by inhibiting nuclear factor (NF)-κB, a critical mediator of MM pathogenesis and inflammation. MM patients eventually relapse and present bortezomib-refractory disease, with ~70% of MM patients displaying chronic, bortezomib-resistant NF-κB activity that can drive MM progression. The mechanisms of bortezomib-resistant NF-κB activation remain ill-defined. There is a growing understanding that bone marrow mesenchymal stromal cells (BMSCs) are uniquely pro-inflammatory in MM patients, and an important driver of MM pathobiology. Recently, our lab identified hyaluronan and proteoglycan link protein 1 (HAPLN1) as a novel factor secreted by MM-associated BMSCs sufficient to cause bortezomib-resistant NF-κB activation and survival in MM cells *in vitro*. In the current study, we attempted to identify components of the putative receptor complex through which HAPLN1 activates bortezomib-resistant NF-κB signaling in MM cells. Recombinant HAPLN1 protein was purified in *E. coli* and shown to induce bortezomib-resistant tumor growth in an MM tumor xenograft model in mice, recapitulating our published *in vitro* data. We used this biologically active HAPLN1 protein as bait in a cell-surface biotin-labelling assay to tag potential binding partners on MM cells. LC-MS/MS analysis of tagged moieties identified chaperonin 60 (CH60) as the target binding partner of HAPLN1 on the MM cell surface. CH60 shRNA knockdown in MM cells blocked optimal HAPLN1-induced NF-κB activity. We performed co-precipitation analysis with cell-surface CH60 to determine other members of the putative receptor complex activated by HAPLN1. These experiments revealed that toll-like receptor 4 (TLR4), an innate immune receptor overexpressed in MM cells, specifically interacted with cell-surface CH60. Chemical antagonism and genetic knockout of TLR4 abrogated HAPLN1-induced NF-κB activity and bortezomib-resistant survival in MM cells, highlighting the critical role of TLR4 in HAPLN1-mediated drug resistance. Collectively, our findings report a previously undescribed CH60-TLR4 receptor complex on MM cells which protects against bortezomib-induced cytotoxicity, thereby presenting potential targets to overcome drug resistance in MM.

Abstract #68

Pharmacological DDX3 inhibition in a cellular model of castration resistant prostate cancer induces a senescent-like phenotype

Author(s): *Hannah N. Miles*,* Teresa T. Liu, William A. Ricke, Lingjun Li

Background:

The growing emergence of castration resistant prostate cancer (CRPC) – in which cancer no longer responds to androgen deprivation – underscores the critical need to discover novel biomarkers distinguishing cancer stages. DEAD-box helicase 3 X-linked (DDX3) is involved in RNA metabolism and its overexpression has been implicated in tumorigenesis and cancer progression.[1, 2] Previously, we established that DDX3 mediates the formation of castration resistance through posttranscriptional regulation of androgen receptor (AR) in a cellular prostate cancer progression model.[3] Here, we found via transcriptomic and proteomic analysis that DDX3 inhibition in our CRPC cellular model led to the induction of a senescent-like phenotype relative to treatment in benign prostatic epithelium and thus may open avenues towards further targeting CRPC clinically.

Methods:

The CRPC model $BCaP^{MT10}$ and benign BPH-1 lines were grown in biological quadruplicates and treated with 2 µM RK-33 for 48 hours, at which time cells and conditioned media were harvested.[4] Quantitative PCR (qPCR) was carried out on TRIzol-extracted RNA using the BioRad CFX Manager in technical triplicates. Cells were homogenized via sonication in urea buffer, then denatured with dithiothreitol and iodoacetamide, followed by trypsin digestion. Conditioned media was concentrated and precipitated using 80% acetone, then processed and digested as above. Both sample types were analyzed using LC-MS/MS on a nanoAcquity UPLC coupled to an Orbitrap Elite mass spectrometer. Peptides were fragmented using higher-energy collision dissociation (HCD), identified, and quantified using Proteome Discoverer.

Results:

Previous analyses have demonstrated increased CDKN1A expression levels as a marker of senescence in cells, as it inhibits cell cycle progression. Our qPCR results demonstrated a significant increase in CDKN1A transcript expression when the $BCaP^{MT10}$ cells were treated with RK-33 relative to DMSO serum-free and FBS-containing controls. We expect this upregulation of senescence markers to carry over into our proteomic results, both intracellularly and secreted, as these markers should be significantly increased relative to DMSO controls as well as FBS-treated control cells.

Conclusions:

Altogether, we expect to see multiple markers of senescence upregulated at the transcriptional and proteomic levels, indicating a senescent phenotype upon DDX3 inhibition that may open additional therapeutic avenues in the treatment of CRPC.

Abstract #69

DEVELOPMENT OF CRISPR-CAS9 MEDIATED PROTEIN KNOCKOUT CELLS AS A TOOL AND RESOURCE FOR PROSTATE CANCER RESEARCH

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INTRODUCTION AND OBJECTIVE: Since its discovery in 2012, CRISPR-cas9 has become a staple technique amongst molecular biologists. Using the cas9 endonuclease, precise DNA strand breaks can be introduced to allow for a wide variety of downstream applications including insertion of new DNA sequences, removal of sequences, and knockout (KO) of protein expression. Here, we introduce a method combining stable CRISPR-cas9 expression and transient puromycin resistance to allow for development of cell lines with multiple KO proteins.

METHODS: Utilizing Lentiviral hCMV-Blast cas9 Nuclease, PC3, LAPC4 and other prostate cells were transduced overnight. The following morning, complete growth medium was added to quench the viral infection. Cells were allowed to recover for 24 hours, and then media was removed and replaced with media containing blasticidin for selection.

We selected one subclone from each parental cell line for transfection. Pre-designed synthetic guide RNAs (sgRNAs) were purchased for genes of interest, including FGF-5, DDX3X, and ER-α. Cells were co-transfected with varying concentrations of sgRNA and a plasmid containing both GFP and puromycin resistance. Cells were monitored for GFP expression and treated with puromycin for selection once GFP was observed. After 48 hours of puromycin treatment, media was refreshed, and cells were allowed to recover.

Following puromycin selection, we seeded cells into 96-well plates to isolate clonal populations. Gene KO was verified using immunofluorescence (FGF-5) or Western blot, and qRT-PCR.

RESULTS: Using our method described above, we were able to successfully generate multiple blasticidin-resistant cas9-expressing prostate cell lines, which can be used in combination with sgRNAs to yield KO cells for a protein or combination of proteins of interest. To date, we have confirmed successful knock out of FGF-5 in both PC3 and BCaP MT10 cells.

CONCLUSIONS: We present here a method for generating prostate cell lines that stably express cas9 endonuclease, while only transiently expressing resistance to puromycin during transfection with sgRNAs. This allows for multiple successive rounds of sgRNA transfection and clonal selection; allowing researchers to generate double, triple, and potentially even quadruple (or more) KO cell lines with identical genetic modifications and complete ablation of target proteins.

Abstract #70

FGF-5 stimulates metastasis and anchorage-independence in prostate cancer

Author(s):
Mary Stangis,* Dalton T. McLean, Teresa T. Liu, William A. Ricke

Background:
While localized prostate cancer has a five-year survival rate of over 99%, five-year survival rates drop to approximately 30% once metastases develop. Fibroblast growth factor-5 (FGF-5) has been shown to be overexpressed in many forms of cancer and has been implicated as a factor promoting metastasis in hepatocellular carcinoma. In this study, we propose that FGF-5 expression stimulates metastasis and increases anchorage-independence, suggesting that FGF-5 may be a suitable therapeutic target for the treatment of metastatic prostate cancer.

Methods:
The BCaP prostate cancer progression model was utilized to study changes in FGF-5 expression as cells move towards an increasingly metastatic phenotype. We also utilized an overexpression vector and CRISPR-cas9 sgRNAs to generate multiple prostate cancer cell lines either overexpressing FGF-5 or with FGF-5 loss. Protein expression was verified by western blot. Recombinant human FGF-5 (rhFGF-5) protein was used to treat cells to confirm that increased FGF-5, not just transfection, caused observed changes. To demonstrate the tendency of each cell line towards metastasis, both scratch assays and agar invasion assays were utilized.

Results:
Using qPCR, we found that FGF-5 expression is significantly increased in aggressive and metastatic prostate cancer BCaP sublines compared to non-tumorigenic at the RNA (p=0.001, p<0.000 respectively) level. We also found that FGF-5 expression is generally increased in the tumorigenic BCaP sublines compared to benign prostate tissue. Scratch assays demonstrated increased motility in cells treated with rhFGF-5; untreated cells were not able to close the scratch completely by the observation point, where treated cells (1 nm and 2 nm rhFGF-5) had completely closed it. To bolster this finding, we also performed agar invasion assays and found that cells overexpressing FGF-5 had a modest increase in number of colonies compared to cells transfected with empty vector.

Conclusions:
Our study shows that FGF-5 expression increases with prostate cancer progression and promotes invasion and anchorage-independence. While further study in vitro and in vivo is needed, our results suggest that targeting FGF-5 is a promising strategy for the treatment of metastatic prostate cancer.

Abstract #71

The mechanisms of Ibrutinib resistance and treatment strategies in DLBCL

Author(s):*Yunxia Liu**, Shuichi Kimpara, Yangguang Li, Nguyet M Hoang, Fen Zhu, Lixin Rui

Abstract

Lymphoma is the most common hematological cancer, and more than 20,000 deaths caused by lymphoma annually in the United States. The chronic activation BCR/BTK signaling pathway is a hallmark of many B-cell lymphoid malignancies, including diffuse large B-cell lymphoma (DLBCL), mantle cells lymphoma (MCL) and chronic lymphocytic leukemia (CLL). Targeting BTK has been used as an effective therapeutic strategy in lymphoma. Ibrutinib, a selective BTK inhibitor, has been used clinically and achieved an initial response rate of between 30% and 70% in lymphoma. The primary and acquired resistance to ibrutinib frequently occur. However, the mechanism of ibrutinib resistance remains uncertain. In this study, we established two ibrutinib-resistant (IB-R) cell lines by gradually exposing ABC-DLBCL cells (TMD8 and OCI-Ly10) to ibrutinib. RNA-seq analysis were performed between parental and IB-R cells, we observed upregulated oxidative phosphorylation (OXPHOS) pathway and PI3K/AKT signaling pathway in the IB-R cells. Oxygen Consumption Rate (OCR) assays were used to confirm increased OXPHOS in IB-R cells under glucose and pyruvate condition, not glutamine condition. Interestingly, vibrant PDH activities were found in the IB-R cells via WB assays. Knock down of EGR1, one of BCR gene signature enriched in these resistant cell lines, could suppress the OXPHOS pathway. Further assays indicated that EGR1 could promote the PDP1 expression in the transcriptional level to active the PDH activity. Metformin, the complex 1 inhibitor, serves as OXPHOS inhibitors. The IB-R cells were more sensitive to response metformin treatment compared with parental cells. Combination metformin with ibrutinib significantly suppressed the cell viability. Together, our findings reveal a novel mechanism of ibrutinib resistance and to characterize EGR1 as a novel biomarker for drug resistance in aggressive lymphoma. Targeting EGR1 transcriptional activity could be exploited by rational therapeutic strategy for effectively treating ibrutinib resistant lymphoid malignancies.

Abstract #72

Screening of histone post-translational modifications in castration-resistant prostate cancer reveals CHD1 gene deficiency engenders a distinct epigenetic profile.

Author: Tanaya Purohit

Abstract: Alterations in epigenetic signaling play a significant role in the development and progression of prostate cancer (PC). Moreover, genes encoding epigenetic modifiers are frequently altered in PC. Linking a tumor's epigenetic phenotype to its genotype can be useful to develop targeted therapies against specific histone post-translational modifications (PTMs) and their enzymes. To interrogate this hypothesis, we screened ~100 histone PTMs in patient-derived xenografts (PDXs) during the transition from hormone-sensitive PC (HSPC) to castration-resistant PC (CRPC) using quantitative liquid chromatography coupled to tandem mass spectrometry. We found a unique signature of histone PTMs and changes in their histone enzymes linked to chromo domain helicase DNA-binding protein 1 (CHD1) deficiency. CHD1 is a chromatin remodeler that is commonly deleted in 15-25% of PC. This novel study provides an unbiased identification of unique epigenetic states and aberrant histone enzyme activity associated with the genetic alteration of CHD1 in PC.

Abstract #73

The RNA-binding Protein DDX3 Regulates Androgen Receptor Expression in the Castration-Resistant Prostate Cancer

AUTHOR(s): *Han Zhang[1], Teresa Liu[3,4,5]Peng Liu[4,6,] Irene Ong[4,6,] William A. Ricke2[,3,4,5]*
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INTRODUCTION AND OBJECTIVE: *Recently, AR-low/negative (ARL/-) CRPC subtypes that lack expression of AR protein have been identified. These ARL/- CRPCs cannot be targeted by hormone therapy, leaving few medical treatments available for patients. Our previous studies indicated that the RNA helicase, DDX3X, binds to the AR mRNA at stress granules and inhibits AR translation, leading to hormone resistance. However, the detailed binding mechanism of DDX3X and AR mRNA remains unclear. In addition, it is unknown whether there are other mRNAs that contribute to the development of ARL/- CRPC under the regulation of DDX3X. Here, we evaluated DDX3X binding in non-tumorigenic and tumorigenic human-derived cells. Our long-term goals are to determine factors that contribute to the development of ARL/-CRPC, as well as potential DDX3X binding sites on AR mRNA.*

METHODS: *We collected whole cell lysate from non-tumorigenic prostate epithelial cells (BCaP-NT1) and tumorigenic ARL/- CRPC cells (BCaP-MT10). DDX3X-bound RNA was precipitated, and reverse transcribed to cDNA, followed by quantitative-PCR (qPCR) and sequencing. Then, we performed RNA-immunoprecipitation (RIP) analysis based on the Sigma Imprint Analysis Calculations. In the future, through RIP analysis and gene ontology (GO) analysis, the genes/pathways altered in ARL/- CRPC and putative DDX3X binding sites on AR mRNA will be identified.*

RESULTS: *We identified different mRNAs that bound to DDX3X in non-tumorigenic BCaP-NT1 cells and tumorigenic BCaP-MT10 cells. The GO analysis is expected to identify pathways involved in the development of ARL/- CRPC under DDX3X-mediated regulations. DDX3X is expected to bind to AR mRNA consensus sequences identified from RIP analysis.*

CONCLUSIONS: *Beyond AR mRNA binding, there are likely other mRNAs that contribute to the development of ARL/- CRPC through DDX3X-mediated mRNA binding. Whether DDX3X recognizes mRNA consensus sequences, and whether the binding mechanism is direct or indirect remain to be determined.*

Abstract #74

The Influence of Chronic Graft-Versus-Host Disease on Psychological and Physical Function in Allogeneic Hematopoietic Cell Transplant Patients

Author(s): *Jenna Hansen*,* Mark B. Juckett, Mikayla A. Foster, Meredith E. Rumble, Keayra E. Morris, Peiman Hematti, and Erin S. Costanzo

Background: Chronic graft-versus-host disease (cGVHD) is a common, late adverse effect of allogeneic hematopoietic cell transplantation (HCT). Few studies have examined quality of life concerns among individuals with cGVHD, and there has been no comprehensive evaluations of psychological functioning. The current study sought to address this gap in the knowledge by comparing psychological and physical function between adult HCT survivors with and without cGVHD and by evaluating the influences of disease site and severity.

Method: Participants with (n=57) and without (n=19) cGVHD completed self-report measures of depression and anxiety (IDAS), fatigue (FSI), insomnia (ISI), pain (BPI), cognition (PROMIS), and sexual function (PROMIS). In addition, participants with cGVHD underwent a comprehensive cGVHD clinical evaluation using NIH consensus scoring criteria.

Results: Results indicated that participants with grade 3 (severe) cGVHD reported significantly higher levels of depression (t=-2.13, p=.04, d=.67) and pain intensity (t=-2.12, p=.04, d=.72) compared to HCT survivors with no cGVHD. Participants with grades 1 (mild) and 2 (moderate) cGVHD had comparable scores to those with no cGVHD on most of the measures. Those with cGVHD in the skin and in the GI tract had the poorest psychological and physical function when compared to those with no cGVHD. When compared to those with no cGVHD, participants with skin cGVHD reported higher levels of depression, fatigue intensity and interference, pain intensity and interference (all p<.05). Participants with GI tract cGVHD reported greater depression, somatic anxiety, fatigue intensity and interference, pain intensity, and sexual dysfunction (all p<.05). Participants with cGVHD in the eyes, mouth, lungs, and joints had comparable psychological and physical function to those with no cGVHD on most measures.

Conclusion: The results suggest that patients with severe cGVHD and those with cGVHD manifesting in the skin and GI tract are the most at risk for poorer psychological and physical function as compared to other allogeneic HCT survivors and may benefit from proactive biobehavioral interventions to improve quality of life and function.

Abstract #75

Biobehavioral Mechanisms Underlying Psychological and Physical Function in Cancer Patients with Chronic Graft-Versus-Host Disease

Author(s): *Jenna Hansen**, Meredith E. Rumble, Christopher L. Coe, Mikayla A. Foster, Mark B. Juckett, Daniel Dickson, Keayra E. Morris, Peiman Hematti, and Erin S. Costanzo

Background: Chronic graft-versus-host-disease (cGVHD) is a common complication of allogeneic hematopoietic cell transplantation (HCT) that involves allogeneic reactions and autoimmune dysregulation and can lead to poor psychological and physical function. Little is known about the biobehavioral mechanisms that may underly poor psychological and physical function in cGVHD patients. This study examined the extent to which circadian rest-activity rhythms and inflammation are associated with psychological and physical function in patients with cGVHD.

Method: Adults with cGVHD (*N*=53) wore a wrist actigraph for 7 days, provided a blood sample, and completed self-report measures of depression and anxiety (IDAS), fatigue (FSI), insomnia (ISI), pain (BPI), cognition (PROMIS), and sexual function (PROMIS). 24-hour circadian rest-activity indices (mesor, amplitude, acrophase, R-squared) were derived from actigraphy. Cytokine and chemokine levels relevant to cGVHD (IL-6, IL-8, TNFα, MIF, MCP-1, MIP-1 alpha) were measured in peripheral blood plasma using Mesoscale Discovery multiarray panels. Multiple regression was used to evaluate the extent to which rest-activity indices and inflammatory biomarkers predicted psychological and physical function.

Results: Results showed few associations between actigraphy indices and outcomes. The exception was that participants with a later daily activity peak (acrophase) reported poorer sexual function (β =-.30, *p*=.044). Higher levels of circulating IL-8 and MIP-1 alpha were associated with greater depression (β=.34, .33) and poorer sexual function (β =-.35, -.37), and MIP-1 alpha was also associated with greater insomnia severity (β=.37), all *p* values<.05. MIF was associated with greater somatic anxiety (β=.30) and fatigue intensity (β=.35), all *p* values<.05. Il-6, TNFα, and MCP-1 showed few associations with psychological and physical function. All models were re-run covarying for patient age and cGVHD severity, and a similar pattern of results was seen.

Conclusion: Results suggest that pro-inflammatory cytokine and chemokines, IL-8, MIP-1 alpha, and MIF, associated with cGVHD may contribute to poorer psychological and physical function, identifying a biobehavioral mechanism that may be an important target for future interventions

Abstract #76

Association of albumin and colorectal cancer incidence in the Southern Community Cohort Study

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Keywords: Adiposity, biomarkers, colorectal cancer

Abstract:

Purpose: Increased inflammation is a proposed mechanism defining the association between obesity and increased colorectal cancer (CRC) risk. We assessed the association between albumin, a biomarker of inflammation, and CRC incidence in a cohort that consists predominantly of African American and low-SES participants, two sub-populations disproportionately affected by CRC and obesity in the U.S.

Methods: Participant data arise from 904 Southern Community Cohort Study participants, where 69% of participants were African American, and 55% had income <$15,000. Eligible participants were diagnosed with incident CRC and had serum albumin measurements; controls were matched 2:1 on age, sex, and race. Conditional logistic regression determined odds ratios and 95% confidence intervals for the associations of serum albumin concentration with CRC incidence, overall and stratified by sex, race, time since blood draw, and obesity status (BMI≥30). All analyses were adjusted for education, smoking, physical activity, alcohol intake, CRC screening, and family history of CRC.

Results: Serum albumin concentration was inversely associated with CRC, where the OR for per-standard-deviation increase was 0.86 (95% CI 0.74-0.99). Associations were strongest in participants with BMI ≥30 (OR 0.79, 95% CI 0.66-0.94), who were female (OR 0.80, 95% CI 0.65-0.98), African American (OR 0.81, 95% CI 0.68-0.97), and diagnosed 5 or more years from blood draw (OR 0.77, 95% CI 0.64-0.93).

Conclusions: Our results support the role of an inflammatory mechanism in the association between obesity and CRC risk as low serum albumin can indicate a systemic inflammatory state, particularly in overweight and obese individuals. Our results show a consistent association between lower albumin concentrations and increasing CRC risk that is more prominent in obese participants. Previous studies have also reported lower average serum albumin in African Americans in comparison to whites, so the stronger association between low albumin and CRC risk found in African Americans in our study may also represent a contributing factor to the disproportionate burden of CRC in this sub-population.

Abstract #77

Prognostic Impact of Common Pathologic Alterations in Pancreatic Ductal Adenocarcinoma from the Veterans Health Administration

Author(s): *Eric G. Mehlhaff*,* Syed N. Zafar, Dyan M. Lesnik, Noelle K. LoConte, Sam J. Lubner, Jeremy D. Kratz

Background: The Veteran Health Administration's (VHA) National Precision Oncology Program was established to provide comprehensive molecular profiling for US military veterans with advanced cancers. There is an urgent need for precision strategies in pancreatic ductal adenocarcinoma (PDAC), as it is a leading cause of cancer-related mortality. We hypothesized that contributions of molecular alterations in PDAC would fail to stratify overall survival (OS), as current strategies are largely dependent on the activity of cytotoxic chemotherapy.

Methods: A retrospective, multicenter cohort of 342 veterans with PDAC were identified from 01/2016-03/2021 who underwent comprehensive next-generation sequencing of tumor using FoundationOne CDx (UWIRB#2020-0696). Subjects were stratified by localized (L) or metastatic (M) disease at the time of diagnosis. Molecular alterations were compared by disease presentation using chi-squared analysis and the clinical outcomes of overall survival (OS) using student's t-test.

Results: Baseline characteristics were representative of the VA population across 79 independent sites. The cohort was male-dominant (97%) with a median age of 69 years at diagnosis. Of this sample, 55% had M disease (n=189) compared to 45% with L disease (n=153). Median OS for M PDAC was 8.9±10.2 months (mo) v. L PDAC with median OS 22.5±18.4 mo ($p<0.00005$). Primary driver alterations were representative of PDAC and comparable between L and M on presentation, respectively; these included *KRAS* (92% v. 91%), *TP53* (73% v. 80%), *CDKN2A* (29% v. 32%), *SMAD4* (18% v. 23%), *ARID1A* (15% v. 16%) and *BRCA2* (9% v. 12%). Primary driver alterations did not confer differences in OS across the population when comparing mutant (mt) to wildtype (wt) for *KRAS* (10.7 v. 11.8 mo, n=312), *TP53* (10.3 v. 11.8 mo, n=263), *CDKN2A* (10.2 v. 10.9 mo, n=105), *ARID1A* (10.8 v. 10.9 mo, n=53), *SMAD4* (11.3 vs 10.7 mo, n=72), and *BRCA2* (13.8 v. 10.7 mo, n=37).

Conclusions: Using the largest report of molecular profiles in veterans with PDAC to date, current therapeutic strategies fail to differentiate clinical outcomes by common molecular alterations with cytotoxic chemotherapy. The molecular profiles of veterans are representative of PDAC and do not vary significantly between localized and metastatic disease. There remains a persistent unmet need for therapeutic strategies including ongoing investigations of novel metabolic and immune-based therapies.

Abstract #78

Anthropometric Obesity Measurements and Colorectal Cancer (CRC) Risk in the Southern Community Cohort Study (SCCS)

Authors: Zoe Walts*, Lisa Parlato, Ronni Brent, Qiuyin Cai, Mark Steinwandel, Wei Zheng, William J Blot, Shaneda Warren Andersen

Background

Colorectal cancer (CRC) is the third most common cancer in the US. Obesity, Non-Hispanic Black (NHB) race, and socioeconomic status (SES) have been identified as CRC risk factors. Consequently, CRC disproportionately burdens low-SES, NHB populations. For NHB Americans, BMI may not accurately measure visceral adipose tissue (VAT), where cancer-related metabolic changes likely occur. However, NHB compared to NH-White Americans tend to have less fat at the same BMI. Waist Circumference (WC) may better measure VAT and better predict obesity-associated risk. This study quantifies associations between obesity anthropometrics (BMI and WC) and CRC risk in a majority low-income and NHB cohort.

Methods

At baseline, 69966 and 9281 participants from the Southern Community Cohort Study (SCCS) had available BMI and WC. BMI quintiles used standard cutoff. WC quintiles/ tertiles were established by participant distribution. Cox proportional hazards models were calculated to determine associations between anthropometrics and CRC risk overall and stratified by sex, race, and BMI.

Results

Race stratification revealed significant associations. NHB participants in the third compared to the first WC quintile had an 87% increased risk (HR:1.87; 95% CI:1.13-3.07). Female participants in the second compared to the first WC tertile had a 73% increased CRC risk (HR:1.73; 95%CI:1.11-2.69). In the highest categories, and for males, trends indicated positive associations between risk and WC, however HRs crossed 1. NHW participants with an underweight (<18.5) compared to normal (18.5-24.9) BMI had a 199% increased risk (HR:2.99; 95%CI:1.27-7.02) which remained significant after adjustments (HR:2.78; 95%CI:1.27-7.02).

Conclusion

Small BMI was associated with CRC risk in NHW participants. CRC-related mechanisms of underweight BMI need further investigation. Large WC tended to increase CRC risk. The strongest associations occurred in NHB and female participants. Null associations in the largest quintiles may be due to small case numbers. These findings represent the complicated relationship between obesity anthropometrics and CRC risk. Previous studies comparing anthropometric utility in Black populations are limited, but researchers tend to find null associations between anthropometrics and CRC risk for NHB women. The present results, however, support WC as a potential obesity risk indicator for female, low-income, or NHB populations where BMI may not apply.

Abstract #79

Disaggregating Data on Asian Americans with Liver Cancer: A nationwide population-based analysis

Authors: *Molinna Bui**, Kajua Lor, Pharm D, John Hampton MS, Noelle LoConte MD

Background: Primary liver cancer includes hepatocellular carcinoma (HCC) and cholangiocarcinoma. Previous research indicates higher prevalence of hepatocellular carcinoma in Asian patients due to higher rates of chronic viral hepatitis. We aimed to analyze the National Cancer Database (NCDB) database for liver cancer survival including disaggregating Asian American ethnicities / subgroups.

Methods: Patients (N= 193,934) with liver cancer diagnosed between 2004 – 2018 within the database were extracted and stratified by ethnic group: Chinese, Japanese, Korean, Filipino, Hmong, Vietnamese, etc. that are diagnosed with liver cancer were analyzed from NCDB. NCDB is a nationwide oncology outcomes database for more than 1,500 Commission of Cancer-accredited cancer programs in the U.S. & Puerto Rico, and it includes data on patient demographics / characteristics, cancer staging and tumor histological characteristics, type of treatment administered, and outcomes. Median survival in months was examined with each Asian American subgroup and compared to their White counterparts using Stata.

Results: Of the 193,934 patients, 159,739 (74.3%) were White and 80 (0.04%) were Hmong. The average age was 64, and nearly 75% of patients were male. Nearly half were on Medicare (46.2%), and a little more than half were in a metropolitan area of about 1 million individuals and more (57.1%). Hmong individuals had significantly poorer median survival (months) in comparison to White individuals (2.79 vs. 11.86 months; P <0.001) and other Asian American subgroups (e.g., Chinese: 33.48, Japanese: 13.17, Filipino: 10.97, Korean: 16.03, Vietnamese: 17.08, Thai: 14.98, Asian Indian: 15.34, Pakistani: 28.94). Relative to White individuals, Hmong individuals had higher rates of death (2.01 vs. 1.07; [CI] 1.57-2.58, 1.05-1.08; p<0.001). Similarly, Laotian individuals had higher rates of death with a median survival of 4.73 months and a hazard ratio of 1.46.

Conclusion: Compared with White individuals, the Hmong and Laotian individuals were more likely to present with advanced liver cancer and had poorer cancer survival. Furthermore, Hmong have worse health outcomes than any other Asian American subgroups; however, because datasets combine all Asians into one race ethnicity category, this distinction is missed. To our knowledge, this is the first NCDB study to disaggregate outcomes for Asian Americans and liver cancer. Further work should disaggregate data for cancer for Asian Americans to improve prevention and treatment of cancer. Wisconsin is home to many citizens from the Hmong diaspora and these findings can drive community outreach and clinical efforts.

Abstract #80

Multilevel determinants of tobacco dependence treatment program implementation in NCI-Designated cancer centers in the Cancer Center Cessation Initiative

Authors: Bird JE, Hohl SD*, Nguyen C**, Pauk D, Minion M, Adsit RT, Burris J, D'Angelo H, Fiore M, Minion M, Nolan MB, Ostroff JS, Rolland B

Purpose: Providing tobacco dependence treatment to cancer patients who smoke improves tobacco and cancer outcomes, but treatment is not consistently offered. Here, we examine determinants of tobacco dependence treatment implementation across NCI-Designated cancer centers in the Cancer Center Cessation Initiative (C3I).

Methods: We conducted a mixed-methods study of survey data and semi-structured interviews conducted among Program Leads from 20 C3I-funded cancer centers. We calculated descriptive statistics of survey data and applied directed content analysis to interview transcripts. We organized coded data into constructs representing the Consolidated Framework for Implementation Research. We then grouped centers based on survey data of intervention, inner, and outer setting characteristics (e.g., treatment components offered, length of time since program initiation, patient smoking rate) to identify determinants of implementation.

Results: The most offered tobacco treatment interventions were in-person (85% of centers) and telephone (70%) counseling and pharmacotherapy (80%). Over half (53%) had been implementing their program for over two years. Smoking prevalence rates across centers ranged from 4% to 47%. Most cited barriers to implementation included inner setting challenges related to leadership and provider buy-in, program compatibility with workflows and IT systems, need for physical space and limited staff dedicated to providing treatment. Facilitators to implementation included outer setting characteristics (e.g., insurance coverage for tobacco treatment services) and process characteristics, including presence of a program champion and support from external change agents such as NCI.

Conclusions: Obtaining leadership and staff buy-in, allocating resources to support IT and workflow integration, and hiring and retaining tobacco treatment specialists were key determinants of successful implementation across C3I centers. These multilevel factors that influenced implementation across a diverse set of NCI-Designated Cancer Centers in C3I can guide NCI, other cancer centers, and community oncology practices in successful implementation of tobacco dependence treatment programs and enhance patient outcomes.

Keywords (2-3):

Tobacco Dependence Treatment Programs; Implementation Science; Cancer Survivors

Topics:

Lifestyles Behavior, Energy Balance & Chemoprevention

Survivorship & Health Outcomes/Comparative Effectiveness Research

Abstract #81

Relationship Between Incarceration & Preventable Cancer: Cervical Cancer in the Female Incarcerated Population

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Background: Increasing evidence points towards incarceration causing poor health, with one study estimating that every year in prison is associated with a two-year reduction in life expectancy. Cervical cancer is the most common cancer among incarcerated females. No study has examined whether this increased rate of cervical cancer diagnosis persists after release. We created a novel data set combining medical and social science records. We then assessed the risk of cervical cancer diagnosis compared to non-screenable cancer diagnoses for females with and without a history of incarceration.

Methods: Data from a large academic cancer center was matched to data from the state's Department of Corrections. Patients diagnosed with breast, colorectal, and lung cancer were excluded from the data; our final analysis included only those diagnosed with cervical or a non-screenable form of cancer. Logistic regression was used to estimate the odds of cervical cancer relative to non-screenable cancers among those with and without a prior history of incarceration. Models were expanded to include race and age.

Results: Females with a history of incarceration had greater odds of being diagnosed with cervical cancer versus a non-screenable cancer relative to those who had not been incarcerated (OR=7.04; 95% CI: 4.4-11.0, p<0.001). There was no significant difference in risk by race group. After adjusting for race and age at diagnosis, the odds ratio remained large and significant (aOR=3.86; 95% CI: 2.3-6.3, p<0.001).

Conclusions: Our findings suggest that the increased risk of cervical cancer previously reported in actively incarcerated females persists after release. Despite limitations, this analysis provides actionable data to support efforts to improve the health of women who have been incarcerated, arguing for a continuum of care in the post-release period. Other cancer control measures might include increased HPV vaccination during incarceration. Despite the documented increased risk of cervical cancer in females who are incarcerated, the Federal Bureau of Prisons recommends vaccination only up until age 26. On a broader scale, policy makers should be encouraged to view decarceration as a cancer control mechanism. Given the inherent inequities in our criminal justice system, dismantling our country's unique structure of mass incarceration could have significant health impacts.