**POSTER #** 1

**Title:** ***Quality of Life and well-being among Breast Cancer patients Lahore Pakistan***

**Author(s):** ***Fiza Ayub****, Tahir Mehmood Khan, Mirza Rafi Baig, Muhammad Usman Amin, Humera Tahir*

**Background:**
Breast cancer has a high incidence rate, emphasizing the necessity of enhanced information of health-related quality of life (HrQOL) in this population of patients. The aim of this study was to exploring the factors influencing the QOL and the patients are suffering in Pakistan.

**Methods:**
A cross-sectional study was conducted on females with breast cancer using the FACIT-B Version 4 questionnaire and a demographic/clinical characteristics section. These instruments were administered to a random sample of 130 Pakistani women. Data analysis included descriptive statistics, independent samples t-tests, and ANOVA.

**Results:**
The patients' mean age was 49.10 (standard deviation (SD) 10.89); 98.5% were married. The mean score was 18.34 for physical wellbeing (SD 5.92; interquartile range (IQR) 11), 16.33 for social/family wellbeing (SD 6.3; IQR 11.25), 13.6 for emotional wellbeing (SD 3.55; IQR 6), 17.13 for functional wellbeing (SD 3.73; IQR 6), and 24.86 for breast cancer subscale (SD 3.64; IQR 4). The study found that age, entitlement, recurrence, marital status, salary, number of doses, duration of cancer treatment, and chemotherapy sessions were significantly related to quality of life (QOL) in terms of physical well-being, as measured by the FACIT-B scale. No association was found between any of the variables and emotional well-being, which is one of the domains of the FACIT-B. Age and marital status were related to the breast cancer subscale and functional well-being, while salary was the only factor associated with social well-being.

**Conclusions:**
Healthcare practitioners must acknowledge and take into account the significance of QOL in addition to therapy for breast cancer patients in order to enhance their health. The findings of this study will aid in filling gaps in current unknown knowledge and identifying sites where patients require additional assistance. Because cancer and chemotherapy clearly have a negative impact on individuals' QOL, oncologists must concentrate on strategies that help cancer patients during their sickness and treatment while also enhancing self-care and QOL. Those with cancer will benefit from emotional wellbeing and adaptation to their disease.

**POSTER #** 2

**Title:** ***p53 Inhibition Differentially Modulates Atovaquone Cytotoxicity in Ovarian and Other Cancer Cell Lines***

**Author(s):** ***Mikaela Bray***, Arvinder Kapur, Manish Patankar, Lisa Barroilhet

**Background:** TP53 is the most frequently mutated tumor suppressor gene in human cancer. Mutations in TP53 result in loss of wild-type function and/or gain-of-function activities that promote genomic instability, chemoresistance, and tumor progression. Understanding how different TP53 mutations influence therapy response is critical for improving patient outcomes. Atovaquone (ATO) is an FDA-approved oxidative phosphorylation (OXPHOS) inhibitor that targets mitochondrial complex III and reduces tumor cell viability across diverse cancer cell lines.

**Methods:**
To evaluate whether p53 modulates ATO-induced cytotoxicity, we examined the effects of pharmacologic p53 inhibition on ATO sensitivity in three ovarian, two pancreatic, and one breast cancer cell line (all harboring TP53 mutations). Cell lines were pretreated with the transcriptional inhibitor pifithrin-α (PFT-α) for 2 hours, followed by 48-hour exposure to ATO at their respective IC50 concentrations. Additional treatments with ATO were performed for 15 minutes, 30 minutes, 1 hour, and 2 hours. Cell lysates were analyzed by Western blot for phosphorylation of p53 at Serine-15.

**Results:**
In ovarian cancer cell lines, PFT-α pretreatment partially rescued viability following ATO exposure, suggesting that even mutant p53 can enhance ATO-induced cytotoxicity through transcriptional activity. This effect was not observed in pancreatic or breast cancer cell lines, indicating possible tissue- or mutation-specific differences in how p53 modulates ATO response. Two of the three ovarian cancer cell lines showed increased phosphorylation of p53 following ATO treatment, an effect absent in the other cancer cell lines.

**Conclusions:**
ATO reduced cell viability across all tested cancer cell lines, confirming its broad anti-cancer activity, potentially through DNA damage and modulation of p53 signaling. p53 inhibition altered the cytotoxic response to ATO in ovarian cancer cell lines, suggesting that while ATO primarily disrupts mitochondrial metabolism, p53 may act as a context-dependent amplifier of cell death in ovarian cancer. Stratifying patients by TP53 mutational status could help predict which tumors are most responsive to ATO.

**POSTER #** 3

**Title:** Amplification of FGFR1 in Liver-Specific ER+ Metastatic Breast Cancer Sites

**Authors(s): Patrick Colegrove**, **Carstyn Joiner**, Gui Ma, Sydney Schjoneman, James Shull, Wei Xu

**Background:** As the most common cancer in women, 1 in 8 women are at-risk of developing breast cancer. The estrogen receptor-positive (ER+) molecular subtype is responsible for 70% of cases with a 50% chance to relapse in the five years following diagnosis. Of those who progress to metastatic disease, the 5-year survival rate is about 27%. Mice models are typically ER- and therefore do not recapitulate human disease, so there is currently a lack of pre-clinical models for ER+ breast cancer. The previously identified August Copenhagen Irish (ACI) rat develops ER+ breast tumors after four months following the implantation of estradiol (E2) pellets. This model is immunocompetent and gene expression resembles that of the luminal B subtype in human ER+ tumors. To generate a cell model suitable for genome testing, we derived the MG-1 cell line from these E2-induced tumors. Although the injection of MG-1 cells into the mammary fat pad of ACI rats did not initially result in metastasis, the injection via the tail vein was able to evade the early steps of immune surveillance and circulation. Consequently, the colonization of the bone, lung, liver, and lymph node was observed, as seen in patients.

**Methods:** To study the molecular events driving site-specific metastasis, the organ tissues were dissociated to generate the MG-1 metastatic subline. As the second most common site of breast cancer metastasis with poor prognosis and a lack of preclinical models, the mechanism of liver-specific metastasis was further explored. In addition to ER, liver metastasis is associated with increased levels of fibroblast growth factor receptor 1 (FGFR1), which is a cell surface protein tyrosine kinase.

**Results:** Knocking down FGFR1 via shRNA, FGFR1 was determined to influence aggressive phenotypes *in vitro.* Further, the R5- and R6-liver clones were derived from the reinjection of the MG-1-R3 cell line into the ACI rat mammary fat pad and dissociation of the resulting metastatic liver cells. The gene profiles of these new derivatives showed increased epithelial-to-mesenchymal transition (EMT) compared to parental cells, indicative of the elevated metastatic potential.

**Conclusions**: Going forward, we hypothesize that these new clones will be proven as a liver-specific preclinical model for ER+ breast cancer. The characterization of the ACI rat and development of MG-1 metastatic subline act as critical models for studying the genetic aberration as drivers for organ-specific metastasis of ER+ breast cancer, as in the case of FGFR, whose amplification may be targeted with FDA-approved pan-FGFR inhibitors.

**POSTER #**: 4

**Title:** ***Inferring blood volume collected on plasma-separating dried blood spots using mass and imaging analysis***

**Author(s):** ***Dietmann EC (1,2,3)***, Stephens MD (1,2,3), Krebs O (4,5), Nkadori EN (3,6), McDonald BR (1,2,3), Tiwari P (3,5,7), McGregor SM (3,6), Murtaza M (1,2,3)

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**Background:**

Analysis of plasma-separating dried blood spots (psDBS) holds promise for cell-free DNA measurement, enabling at-home sample collection, room temperature storage, and easy shipment. However, unlike blood tube collection, it is not feasible to directly measure blood volume collected via psDBS. We evaluated whether analysis of psDBS mass, area, and brightness can infer blood volume and enable downstream measurement of circulating analyte concentrations.

**Methods:**

Whole blood from healthy individuals and cancer patients was collected and used to prepare psDBS with volumes of 50 to 250 µL. psDBS were scanned and weighed. Total area and the mean grey value (MGV) of the erythrocyte region were measured using image segmentation. Using mass, area, and MGV as predictors, we developed a linear regression model to predict whole blood volume. Root mean squared error (RMSE) was used to assess model accuracy. DNA from psDBS was extracted, then quantified using qPCR targeting L1PA2.

**Results:**

We analyzed 478 psDBS samples from 40 healthy individuals and 118 cancer patients. Using 250 samples from 30 healthy individuals, a linear regression model was fit to the data, showing a strong correlation between actual and predicted blood volume (RMSE=10.9, Pearson r=0.95). Using 110 samples from 10 separate healthy individuals and 118 samples from cancer patients, the model predicted blood volume with high accuracy (RMSE=12.2 and 17.1, respectively). There was a strong correlation between plasma DNA concentration in psDBS and matched blood tubes ($R^2$=0.92). Plasma DNA concentration in psDBS was significantly higher in cancer patients than healthy individuals (p-value=0.0036).

**Conclusions:**

Our results suggest estimating blood volume collected on psDBS is feasible using mass and imaging analysis. This approach enables measurement of plasma DNA concentration and other potential biomarkers in psDBS from healthy individuals and cancer patients.

**FLASH TALK** #1/ **POSTER** #5

**Title:** ***RNA-Regulatory Activities of the ATPase DDX41 in Hematopoiesis and Cancer***

**Author(s):** ***Christina M. Jurotich***, Jeong-Ah Kim, Siqi Shen, Kirby D. Johnson, Sunduz Keles, Emery H. Bresnick

**Background:**

The extensive information content of the human genome is considerably amplified by splicing factors, which mediate alternative splicing to generate vast numbers of RNA transcripts. Splicing factors are essential for controlling genome stability, and mutations in genes including *SRSF2*, *SF3B1*, *ZRSR2*, and *DDX41* disrupt pre-mRNA splicing, predisposing to and/or promoting myeloid neoplasms.

Heterozygous *DDX41* germline mutations have been identified in familial myelodysplastic syndrome and acute myeloid leukemia. DDX41 encodes a multi-functional RNA-dependent ATPase regulating RNA splicing, the innate immune cGAS-Sting pathway, and genome stability, but a detailed mechanistic understanding is lacking. Over 700 *DDX41* missense variants are reported in GnomAD, but only 380 have clinical documentation in ClinVar, with 82% being variants of uncertain significance. Despite many variants altering conserved *DDX41* domains, the impacts on pathogenesis and DDX41's cellular functions remain unknown.

**Methods:**

We innovated a genetic rescue system to compare RNA-regulatory activities of wild-type and disease-associated DDX41 variants and applied this strategy to G1E-ER-GATA1 erythroblasts. We conducted an RNA-seq experiment and performed differential gene expression, differential transcript expression, differential transcript usage, and alternative transcription regulation analyses to parse out the activities of DDX41 compared to pathogenic variants. I also analyzed the *DDX41* variants publicly available in the new *All of Us* consortium data, uncovering 120+ previously unreported variants. Further computational analyses of these variants revealed patterns of amino acid alterations unique to different regions of DDX41, and we implemented select variants from this database into our rescue assay to study their activities.

**Results:**

From RNA-seq analysis, DDX41 regulates the alternative splicing of 503 isoforms, regulating more than 1000 alternative splicing events. Many of these transcripts encode genes involved in mRNA processing and RNA splicing, indicating DDX41 may regulate splicing by moderating the ratios of alternatively spliced transcripts. Analysis of the activities of two known disease-associated variants revealed that the mutant proteins lose almost all DDX41 activities, unveiling functional defects that inform pathogenesis. Analysis of three new variants identified from the *All of Us* data revealed partial transcript-regulatory activities of these mutant proteins. We have extended this system into the human erythroid progenitor cell line, HUDEP2, revealing conservation of select transcript regulatory activities.

**Conclusions:**

Through this work, I have discovered DDX41-regulated transcript isoforms that pathogenic DDX41 variants fail to regulate, revealing defects in alternative transcript regulation that may inform how a *DDX41* variant contributes to hematologic malignancy. By incorporating an ensemble of activity metrics, including capacities to control differentiation, gene repression/activation, and splicing, with computational analyses, including differential transcript usage, differential transcript expression, and motif analysis, we are transforming the classification system. Using DDX41-specific criteria will enable high-fidelity clinical curation and reduce the number of variants of uncertain significance to advance genomic medicine.

**POSTER #** 6

**Title:** NIR active dyes for use in dynamic contrast enhanced optoacoustic imaging (DCE-OAI)

**Author(s):** ***David Murphy***, Shaunak Raikar, and Marty Pagel

**Background:**
Optoacoustic imaging (OAI) uses laser-induced thermoelastic expansion of oxy- and deoxyhemoglobin to generate ultrasonic waves, mapping tumor vasculature and oxygen saturation at sub-millimeter resolution. Increased vascular density within tumors can be exploited to highlight regions of interest. Exogenous contrast agents can highlight tumors as these contrast agents are retained in tumors due to their enhanced permeation. Dynamic contrast-enhanced OAI quantifies perfusion or permeability to further delineate tumors. Ideal contrast agents possess a strong OAI signal, do not bind to proteins, and are photostable. In this study, we evaluated and compared a common NIR dye indocyanine green (ICG) with alternatives like ZW800-1 (Curadel, Inc.) and commercially available NIR agents from Revvity.

**Methods:**
To evaluate these potential OAI agents, candidates were tested in an agar phantom within an MSOT inVision 512-echo (iThera Medical) at 37°C. The different dyes were evaluated for their behavior in both phosphate-buffered saline (PBS) and fetal bovine serum (FBS). Assessments of each agent's OAI spectra were done at 20 μM (Figure 1B,C), comparing amplitudes of observable signal (Fig. 1D,E), and photostability.

For in vivo studies, orthotopically placed 4T1 tumors were used to demonstrate the feasibility of detecting surface tumors using DCE-OAI. A tumor model was established in ten 4–8-week-old athymic nude mice by injecting $1x10^6$ cells in the inguinal mammary fat pad. 10 days following tumor cell injection, animals were imaged on the MSOT inVision instrument. The employed DCE-OAI method dynamically measures the MSOT signal in the tumor for 1 minute at 4.8 second temporal resolution. Following the initial 1-minute scan, contrast agent was injected intravenously, and tumor signal was monitored for 10 minutes. Wash-in and wash-out of contrast agent signal in the tumor was analyzed.

**Results:**
ICG's spectral profile is greatly affected by protein interference as the observed signal is 40 percent stronger in FBS than in PBS. OAI signal of ZW800-1 is 3-fold stronger than ICG in PBS, and ~50 percent stronger in FBS. Revvity agents were also compared to ICG. The spectral profile of the Revvity agents were altered by surrounding media with increased signal in most Revvity agents when suspended in FBS compared to PBS. Edema 680 is most affected as its signal increased 2-fold (Figure 1D). In PBS, most Revvity agents exhibited greater signal (3-4.3-fold) than ICG except for Edema 680, which was comparable. Conversely, in FBS, the signal increase ranged from 1-1.55-fold compared to ICG. However, Edema 680 signal was 40 percent lower than ICG. Overall, the difference in signal changes is diminished compared to ICG.

The photostability half-life of ZW800-1 is comparable to ICG in FBS (~33-37 mins) but is lower in FBS (7.4 mins vs 13.9 mins). The effect of FBS on the Revvity agents was minimal compared to ICG and ZW800-1 with only Edema 680 being strongly affected. Notably, the photodegradation of Tomato Lectin was favorable with a half-life of 105 mins in PBS and greater than 120 mins in FBS.

Compared to ICG, ZW800-1 possessed a 40% increase in signal compared to ICG in the ROI. Interestingly, the time to reach maximum signal is ~139 seconds. Due to the increased signal however, the time for ZW800-1 to reach signal levels comparatively to ICG's maximum was 27 seconds vs 37 seconds for ICG.

**Conclusions:**
These results highlight other NIR agents such as ZW800-1 and Revvity dyes are capable for DCE-OAI imaging for tumor detection. These agents possess favorable characteristics even when compared to ICG. Future in vivo studies will evaluate the Revvity agents as well as solidify the ZW800-1 and ICG comparisons.

**POSTER #**: 7

**Title:** ***Tumor DNA analysis in peritoneal fluid from patients with peritoneal carcinomatosis***

**Author(s): Muhammad Talha Nawaz**[1,2,3], Muhammad Talha Waheed[4], Kirsten Dennison[1,2,5], Stephanie McGregor[4,6], Syed Nabeel Zafar[2,5], Bradon McDonald[1,2,5], *Mustafa Raoof[4], *Muhammed Murtaza[1,2,5]

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**Background:**
Up to 30% of patients with gastrointestinal cancers develop peritoneal carcinomatosis (PC). Unlike other body sites, imaging, blood biomarkers such as circulating tumor DNA, and peritoneal fluid (PF)biomarkers such as cytology and CEA, have limited utility and accuracy in patients with peritoneal metastases. To address this gap, we evaluated tumor DNA in PF (peritoneal tumor DNA or ptDNA) as a cancer biomarker in patients with PC.

**Methods:**
We analyzed 63 PF samples collected from 39 patients with PC of appendiceal, biliary, or colorectal origin, undergoing intraperitoneal chemotherapy. cfDNA was extracted from 4 mL PF and quantified using fluorometry. Tumor fraction (TF) was estimated using low-pass whole-genome sequencing and copy number aberration analysis. Clinical variables, including peritoneal cancer index (PCI), cytological status, and overall survival, were compared with molecular findings.

**Results:**
The cohort included 39 patients (53.9% male; median age, 60.5 years) with a median baseline PCI of 19 (range, 0–37). Across 63 PF samples, median cfDNA concentration was 15.8 ng/mL (range, 0.67–135.8 ng/mL). ptDNA was detectable (TF >3%) in 71% of baseline samples; when detectable, median TF was 16.6% (range, 4.3%–88.2%). TF was higher in patients with increased PC burden (PCI 16–39vs <=15; p=0.0062), and in patients with positive PF cytology ($p = 9.6\times10^{-5}$). ptDNA was detectable in45.8% of patients with negative PF cytology. Median overall survival was 17.2 months in the high TF group (>10%) and not reached in the low TF group ($p < 0.001$; HR = 9.7, 95% CI: 1.9–49.0), while one-year OS was 100% vs. 78.7%, respectively.

**Conclusions:**
ptDNA is frequently detectable in patients with PC, and associated with higher PC burden as well as worse prognosis. These findings warrant further evaluation of ptDNA as a biomarker for detection and monitoring of PC in clinical trials of intraperitoneal chemotherapy

**POSTER #** 8

**Title:** ***Development of a new 3D Microfluidic Model of STIC Lesions***

**Author(s):** ***Yalmarie Numan-Vazquez[1]***, Finnbar Reed-McBrain[1], María Virumbrales-Muñoz [1]

**Background:**

High-grade serous carcinoma (HGSC) is the deadliest female reproductive cancer, causing 60% of annual ovarian cancer deaths. Recent studies have established that the fallopian tube is the site of HGSC origin, arising from precancerous STIC lesions. These lesions are small, and there is no standardized procedure for their detection. This limits our access to them, hindering our understanding of their biology. Thus, the molecular mechanisms driving the progression of STIC lesions to HGSC are not well understood. There is a clear and pressing need for *in-vitro* models to study these lesions and prevent their progression. To address this issue, we are developing and characterizing a microfluidic device capable of constructing blind tubular structures (lumens) embedded in hydrogel, mimicking the ovarian fimbria and enabling the study of cell migration.

**Methods:**

Microfluidic Device Fabrication: Standard soft lithography techniques were used to fabricate the microfluidic device. Briefly, a negative mold with the device design was created by spin-coating SU-8 onto a silicon wafer, followed by UV exposure and development to define the device features. The wafer was then used to fabricate the layers of the device by casting polydimethylsiloxane (PDMS). Finally, the device was assembled by aligning the two identical PDMS layers with a rod placed between them, then plasma-bonding the layers to a glass plate. Rods of varying diameters could be utilized to create lumens of different sizes.

Model characterization: The microfluidic device was designed with two distinct chambers and a blind lumen, created by a rod that extends partially into the top chamber. The device incorporates barrier-free confinement using chamber constriction and a phaseguide, eliminating the need for physical barriers like pillars or posts and ensuring cells remain attached to the hydrogel. Fluorescence microscopy was used to visualize the spatial distribution of hydrogel components and confirm both successful lumen formation and the effective barrier-free confinement. To visualize collagen distribution two distinct hydrogel formulations were used, the top chamber was filled with a collagen solution incorporating FITC-labeled collagen, and the bottom chamber with collagen embedded with fluorescent beads. Subsequently, to create the blind lumen and assess its stability, the rod was removed, and the resulting void was filled with a solution of fluorescent beads, enabling visualization of the lumen structure.

Cell migration study: Having established the device's capacity to form lumens, we proceeded to characterize its suitability for cell migration studies. For this purpose, we utilized a standard hydrogel for the top chamber, varying collagen concentration. This variation aims to assess the impact of collagen density on cell migration in the device. Following this, we removed the rod and seeded the half-lumen with fluorescently labeled cells to demonstrate the suitability of this platform for lumen formation and cell migration studies. Subsequently, the lumens were imaged at time 0 and 24 h after seeding.

**Results:**

Fluorescence microscopy confirmed the distinct distribution of FITC-labeled collagen in the top chamber and collagen embedded with fluorescent beads in the bottom chamber, as well as the confinement of the fluorescent beads solution in the lumen, demonstrating successful hydrogel confinement and lumen formation. Varying the hydrogel composition and lumen size enabled the device to replicate different cell migration patterns

**Conclusions:**

We have demonstrated that the hydrogel remains confined within its chamber, confirming the effectiveness of phaseguide and constriction features in creating a barrier-free hydrogel confinement strategy. Moreover, it enabled the formation of a cellular structure resembling the ovarian fimbria, allowing the study of cell migration. In conclusion, this is a versatile platform currently being optimized to analyze cell migration in STIC lesions.

**POSTER #** 9

**Title:** ***Universal CAR T Therapy to Target Multiple Cancer Types***

**Authors:** ***Phuc Q. Pham***[1,2], Amanda G. Shea[1,5], Dan Cappabianca[1], Lei Shi[1,3], Israrul Ansari[1,3], Kwang Nickle[1], Jayden West[1,6], Paul Harari[1], Aaron Lebeau[7], Andrew Baschnagel[1], Paul Sondel[1], Zachary Morris[1], Christian Capitini[3,5], Randall J. Kimple[1], Quaovi H. Sodji[1]

[1]Department of Human Oncology; [2]Cellular and Molecular Pathology Program; [3]Department of Pediatrics; [4]Carbone Cancer Center; [5]Cancer Biology Program; [6]Molecular and Cellular Pharmacology Program, [7]Department of Pathology & Lab Medicine, University of Wisconsin-Madison, Madison, WI

**Background**: Adoptive T cell therapy includes tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy and chimeric antigen receptor (CAR) T cell therapy. Both engineered TCR therapy and CAR T cell therapy involve the transfer of genetic materials to T cells to produce transgenic receptors targeting specific tumor antigen[1,2,3,4,5,6]. However, these CAR T therapies are monospecific and susceptible to antigen-loss escape issues when tumors downregulate the target antigen[7,8]. There have been “universal” CAR NK and T cells designed to bind small molecules and that can be redirected to target specific antigen using small molecule-labelled antibodies[9,10]. *We hypothesize that using such “universal” CAR T cells, designed to target the small molecule Dinitrophenol (DNP), multiple cancer associated antigens can be targeted.*

**Methods**: Primary T cells from different donors were activated for 3 days and transduced using retrovirus to create DNP-CAR T cells. Upon expansion in culture for 14 days, a large bank of DNP-CAR T cells was cryopreserved for future use. Upon thawed, flow cytometry analysis was conducted to confirm DNP-CAR expression one day before killing assays. Killing assays with co-culture of DNP-CAR T cells, DNP-labeled antibodies, and target cancer cells were conducted and analyzed using Incucyte and proliferation assay (CellTiter-Glo - Promega). To target GD2+ CHLA-20 neuroblastoma cell line, the anti-GD2 antibody Dinutuximab antibody was labeled with DNP. To target c-Met+ Detroit 562 (D562) head-and-neck (H&N) cancer cell line, the anti-c-Met camelid antibody 1E7-Fc was conjugated with DNP.

**Results**: Our data showed that only under the presence of DNP-labeled anti-GD2 antibody Dinutuximab, DNP-CAR T cells potently targeted GD2+ CHLA-20 *in vitro*. On the other hand, with the unlabeled antibody, DNP-CAR T cells showed minimal non-specific cancer killing. Similarly, DNP-CAR T cells with DNP-labeled anti-c-Met antibody 1E7 induced high cytotoxicity against the high-c-Met-expressing D562, while this anticancer activity was not significant with the unlabeled antibodies. Moreover, the high antibody concentration was essential to enhance the cancer killing activity of DNP-CAR T cells. All killing assays were validated with different effector-to-target (E:T) ratios (DNP-CAR T cells to cancer cells) and reproducible with T cells from different donors.

**Conclusion**: The “universal” DNP-CAR T cell targeting approach utilized can effectively target multiple cancer types, including neuroblastoma and H&N cancer cell lines, with the presence of the DNP-labeled antibodies as the adapters. For future work, we will characterize the antigen density needed to elicit the cytotoxic activity of the DNP-CAR T cells. We will be poised to evaluate this approach *in vivo* using xenograft mouse model in combination with radiation.

**Reference** *Contact author for citations*

**FLASH TALK** #6/ **POSTER** #10

**Title:** ***Design, characterize and develop IL6/1 fusokine for autologous cell therapy in ovarian cancer***

**Author(s):** ***Sejal Sharma[1]*** *Rahul Das[2] Andrea Pennati[2] Lisa Barroilhet[1], Manish S. Patankar[1], Jacques Galipeau[2]*
*[1]Department of Obstetrics and Gynecology, [2]Department of Medicine, University of Wisconsin Madison, Madison, USA*

**Background:** Our lab has developed novel chimeras of GM-CSF and various γ-c interleukin into a single, bifunctional polypeptide called as fusokines. Fusokines confer immune cells with a gain of function that cannot be explained by the mere sum of their constituent moieties by serving as bispecific ligands to drive unique downstream signaling events. The synergy that arises from these fusions has shown great promise in their ability to modulate the immune response and overcome maladaptive biological processes that underlie diseases such as cancer and autoimmune conditions. Extending these studies, we have now developed IL-6/1, a chimera of IL-6 and IL-1β. Here, we report our initial studies demonstrating the immune activating role of IL-6/1 and develop a cell therapy approach for treatment of high grade serous ovarian cancer (HGSOC).

**Methods:** Human and murine IL-6 and IL-1β gene sequences were cloned into a second-generation lentiviral vector containing a fluorescent reporter and antibiotic resistance gene for selection. IL6/1 transduced HEK293 cells lysates and media were used to run western blot and ELISA to check fusion protein molecular weight and cytokine secretion. T cells isolated from healthy donor PBMCs were activated with CD3/CD28 beads and cultured with IL6/1 and controls were followed by flow cytometry to evaluate T cell proliferation, apoptosis, activation, and memory phenotype. For *in vivo* studies, ID8 murine ovarian cancer cells were transduced with the IL6/IL1β lentiviral construct and validated by ELISA and flow cytometry. Female C57BL/6 mice bearing ID8 tumors were treated with IL6/IL1β-expressing ID8 cells (ID8IL6/1) or Empty Vector (ID8EV) control. Tumor progression was monitored by bioluminescence imaging, and survival analysis was performed.

**Results:** Human and murine IL-6 and IL-1β gene sequences were combined in a single open reading frame either in IL-6/1β or IL-1β/6 (N-C terminal) format downstream of EF1A promoter in a second-generation lentiviral vector. Fluorescent protein markers NeonGreen for human and TurboRFP for mouse IL-6/1 were included in the vector downstream of a T2A cleavage site, respectively, and puromycin antibiotic resistance used for selection. The predicted ~42 KDa MW of the IL-6/1 fusion protein was confirmed by Western blotting. ELISA and western blotting experiments demonstrated that both the anti-IL6 and anti-IL-1β antibodies detected the IL-6/1 fusokine but not the IL-1β/6 chimera. In silico modeling using I-TASSER showed no steric hindrance in the presentation of the IL-6 and IL-1β functional domains in IL-6/1 fusokine. In response to anti-CD3/CD28, increased proliferation and decreased apoptosis of CD4 and CD8 human T cells was observed upon treatment with IL-6/1 ($p < 0.05$). Further analysis showed that the CD45Ra positive memory T cells were proliferating in response to IL-6/1 ($p < 0.05$). Next, we developed ID8 cells that expressed murine IL-6/1 (ID8IL6/1). Luciferase- expressing parent ID8 cells (ID8-Luc) were intraperitoneally implanted in female 6–8-week-old mice and their growth was monitored by bioluminescence imaging. Approximately one week after ID8-Luc administration, when tumors were established, the mice were randomly divided into control and test groups (N=5/cohort). The control groups received a single bolus of ID8 cells transduced with sham control and the test animals received ID8-IL6/1. Weekly bioluminescence imaging demonstrated significantly slower tumor growth in the ID8-IL6/1 group compared to the ID8-EV group, as reflected by tumor radiance slopes (0.02 vs. 0.91 normalized radiance/day, respectively).Additionally, mice bearing ID8-IL6/1 tumors showed a significant improvement in overall survival compared to controls (median survival: 84 vs. 42 days).

**Conclusions:** These findings demonstrate the potent immunostimulatory and antitumor effects of the IL-6/1 fusokine and support its further development as a novel cell-based therapy for ovarian cancer.

**POSTER #**: 11

**Title:** ***Exploring Strategies to Increase Delivery of VLRs to GBM Tumors***

**Author(s):** ***Melanie A Staffenson***, Elizabeth A Appelt, Benjamin J Umlauf, Eric V Shusta

**Background:** Glioblastoma (GBM) is the most common primary brain cancer, afflicting 1 in every 20,000 people and causing 225,000 deaths globally every year[1]. Despite its prevalence, there are no curative treatment options for patients with GBM. Previous work in our lab discovered a novel lamprey-derived variable lymphocyte receptor (VLR), coined P1C10, that targets brain extracellular matrix (ECM) exposed by pathological blood-brain barrier (BBB) disruption[2]. While P1C10 has previously demonstrated successful tumoral delivery of doxorubicin-loaded lipsomes[2], the heterogeneously disrupted BBB prevents diffusion of P1C10 throughout the entire tumor volume. Thus, we investigated increasing tumoral targeting and saturation using different formats (monovalent vs bivalent).

**Methods:** We enabled scalable production of P1C10 using either CHO cells (bivalent) or a modified *E.coli* expression system (SHuffle; monovalent)[4,5]. GL261 cells were intracranially implanted into the right striatum of C57BL/6J mice; tumor engraftment and BBB leakiness were confirmed via T1-weighted MRI scans with gadolinium. At 21 days post implantation, varying ratios of P1C10 were administered via tail vein injection and allowed to circulate for 30min to accumulate in the tumor site. Mice were perfused with sterile saline and brains harvested for immunohistochemical analysis to determine whether brain tumor uptake could be modified.

**Results:** The monovalent form of P1C10 demonstrates a differential affinity for binding brain ECM than a bivalent construct with a fused Fc region detected via both IHC/IF and ECM binding ELISA.

**Conclusions:** As a targeted platform P1C10 has the capacity for a tunable delivery into GBM tumors, with the ability to utilize both lower and higher affinity binding with either monovalent or bivalent formats to drive complete tumoral coverage and subsequent drug delivery. Improving tumoral penetration of P1C10 in GBM tumors would likely enhance the efficacy of conjugated payloads such as immunotherapies, chemotherapies, or other therapeutic approaches.

**POSTER #** 12

**Title:** ***Improved MRI contrast agents for measuring pH***

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**Background:**

The pH of the extracellular microenvironment (pHe) in solid tumors is often acidic, below pHe 7.0, due to upregulated glycolysis caused by dysregulated metabolism. The molecular imaging of tumor acidosis is advantageous as it is associated with tumor growth, invasion, and metastasis. Among various methods that have been employed to measure tumor acidosis, magnetic resonance imaging (MRI) can use pH-dependent contrast agents to change image contrast of the tumor. However, the image contrast also depends on the concentration of the agent in the tumor. Although two MRI contrast agents (two knowns) have been used to evaluate pH and concentration (two unknowns), the design of these agents has been sub-optimal. Also, the precision of measuring $T_1$ and $T_2$ relaxation times to produce a precise estimate of pH should be investigated.

**Methods:**

To overcome this limitation, we have synthesized a pH-dependent Gd(III)-based MRI contrast that contains a sulfonamide group. Protonation of the sulfonamide nitrogen below pHe 7 leads to the dissociation of the arm from the metal core, increasing the $R_1$ relaxation rate of the surrounding water. We measured $T_1$ and $T_2$ relaxation times at 9.4 T magnetic field strength and 37 °C for samples of the Gd-based agent with 0, 0.05, 0.1, 0.2, and 0.5 mM concentrations and pH 4-10 in 0.1 pH increments (total of 305 samples). The difference in $T_1$ and $T_2$ times of each sample relative to $T_1$ and $T_2$ times of control samples with no agent were then converted to $R_1$ and $R_2$ relaxation rates. Plots of $R_1$ and $R_2$ vs. concentration were used to measure $r_1$ and $r_2$ relaxivities. These $r_1$ and $r_2$ relaxivities vs. pH were fit to a modified Henderson–Hasselbach equation using Matlab to produce sigmoidal curves for the Gd-based agent.

We have also synthesized a Dy(III) chelate with a sulfone group that lacks a nitrogen, which has $r_1$ and $r_2$ relaxivities that are independent of pH. We measured $T_1$ and $T_2$ relaxation times of samples of the Dy-based agent at 0, 2, 4, 6, 8 mM concentrations and pH 6.2-7.2 in 0.2 pH increments (total of 30 samples). The $r_1$ and $r_2$ relaxivities were determined from $T_1$ and $T_2$ measurements as described above. The plots of $r_1$ and $r_1$ relaxivities versus pH showed that our Dy-based agent is pH-independent.

**Results:**

We used the Henderson-Hasselbach equation of the Gd-based agent and the pH-independent $r_1$ values of the Dy-based agent to perform simulations that showed how both agents can be used to measure pH, assuming that both agents have a known ratio (i.e., identical pharmacokinetics in future in vivo studies), and the $T_1$ measurements have perfect precision. We then performed the same simulations that had imprecise $T_1$ values to estimate the imprecision in pH estimates if the MRI results lack perfect precision. These simulations allowed us to estimate the precision needed for $T_1$ measurements needed to eventually measure tumor pHe within 0.1 pH units, which is no more than 3% imprecision.

**Conclusions:**

This analysis has set criteria for the next phase of our research project. We have developed MR Fingerprinting (MRF) for measurements of $T_1$ and $T_2$ relaxation times with much greater precision than the precision produced with traditional MRI methods. This improved precision with MRF can overcome a major hurdle when using dual MRI contrast agents for imaging tumor pHe.

**POSTER #** 13

**Title:** Evaluating novel antibody for targeting of capmatinib-resistant MET-altered NSCLC

**Author(s): Will Toscano**, Kwang Nickel, Rachel Minne, Randy Kimple, Andrew Baschnagel, Aaron Lebeau

**Background:**

Lung cancer is one of the most common forms of cancer worldwide, and the most deadly as well. The Mesenchymal Epithelial Transition receptor (MET) is an oncogene that, when altered, contributes to approximately 9% of all Non-Small Cell Lung Cancers (NSCLC). Capmatinib is a common drug used to treat MET-altered cancers and has been proven to be effective in delaying tumor progression. However, as with many cancer drugs, capmatinib does have a weakness: resistance. Resistance comes in two forms: acquired and intrinsic. Acquired resistance occurs when a tumor encounters the treatment but adapts to withstand the treatment, such that it loses its effectiveness. Intrinsic resistance occurs when the tumor is mutated in such a way that the treatment is not effective despite not having been introduced previously.
Our project set out to evaluate one potential avenue of combating resistance: targeted antibodies. vMET1 is a shark-derived antibody that has been shown to target and bind to altered MET receptors in the NSCLC cell line EBC-1. In order to evaluate if vMET1 would be useful in combating resistance to capmatinib, we developed a line of EBC-1 that was resistant to capmatinib, EBC-1-R.

**Methods:**
We evaluated the effectiveness of vMET1 (targeting and binding) on EBC-1-R in four ways: IC50 to verify resistance, Flow Cytometry to assess binding, and Immunofluorescence(IF) assay and Incucyte assay to assess internalization into the cell.

**Results:**

IC50 was able to verify that EBC-1-R was properly resistant, exhibiting significantly less cell death than the sensitive EBC-1 Parental line. Flow Cytometry was able to confirm that the binding ability of vMET1 to the MET of EBC-1 and EBC-1-R is not significantly different, indicating that vMET1 binds just as well to the MET of the capmatinib-resistant cell line as the parental. IF and Incucyte both confirm that not only does vMET1 bind, but it is internalized into the cell as well.

**Conclusions:**

vMET1, as shown by this project, can effectively target, bind, and internalize to MET even in the capmatinib-resistant line of EBC-1. Targeted antibody therapy is effective, with radionuclides such as Lutecium being specifically deployed to cancer cells. Understanding that vMET1 is effective as a targeting antibody on EBC-1 in-vitro, regardless of capmatinib resistance, is an important first step. As we continue to pursue this project through in vivo studies, we hope to learn more about treating capmatinib-resistant NSCLC.

**POSTER #** 14

**Title:** ***Development of a fully humanized vascularized "tumor on a chip" model for prostate cancer liver metastasis***

**Author(s):** ***Katherine Vietor***, Erika Heninger, Gabriel Eades, Adeline B. Ding, Cristina Sanchez De Diego, David A. Quigley, Sheena C. Kerr, Joshua M. Lang

**Background:**
Prostate cancer (PCa) is the most frequently diagnosed cancer in men and metastasizes to bone, lymph node, liver, and lung predominantly. Liver metastatic prostate cancer has the worst outcomes with a median overall survival of 13.5 months and treatment options for CRPC are limited to chemotherapy. Retrospective analysis of liver metastatic biopsies has identified a higher frequency of genetic alterations in *p53*, *Rb1*, and *PTEN* tumor suppressor genes. Current pre-clinical models have limited capacity to model this complex tumor microenvironment (TME) and genomic alterations to understand the lethality of this clinical presentation. There is a critical need to develop new models of liver metastatic PCa for drug development.

**Methods:**
We report the development of a novel fully humanized ‘tumor on a chip” model for the liver metastatic niche that incorporate human liver stroma and PCa organoids. We utilize a microfluidic platform called LumeNEXT which is a closed microfluidic culture device with an embedded endothelial lined lumen.

**Results:**
We have optimized 3D multi-culture conditions to co-culture HepG2 hepatocytes, LNCaP prostate organoids and HUVEC endothelial cells in the LumeNEXT microfluidic device. Confocal microscopy is performed for live cell, multi-parameter analysis of spatio-temporal investigation of the prostate liver TME. On-chip analysis of cell viability is performed with Hoechst nuclear staining, Calcein AM for live cells and Ethidium homodimer dead cell staining. Culture conditions have been optimized for multi-cellular co-culture of LNCaP spheroids, HepG2 hepatocytes, and HUVEC cells that maintained >85% viability in each cell type after 3 days of co-culture. We demonstrate that HUVEC cells are able to form endothelial lined vessels that maintain 3-dimensional structure within this multi-cellular TME.

**Conclusions:**
This platform now enables testing of different cellular compositions of liver TME, including different immune cell and stromal populations. We are also incorporating CRISPR engineered LNCaP cell lines with *p53* and *Rb1* loss compared to wild type control to evaluate the functional impact of the liver TME and high-risk genomic mutations. This novel model system may expedite discovery of the molecular drivers behind this aggressive form of disease and allow identification of targetable mechanisms to develop more effective treatment strategies to improve outcomes for patients with liver metastasis.

**FLASH TALK #** 2

**Title:** ***Predicting Abdominal Deformation During Histotripsy Using Finite Element Modeling***

**Author(s):** ***Grace M. Minesinger***, Paul F. Laeseke, Corinne R. Henak, Katrina L. Falk, Marlin E. Keller, Adrienne Kisting, Michael A. Speidel, Martin G. Wagner

**Background:** Histotripsy is an ultrasound (US)-based focal therapy that is being rapidly adopted for treating liver tumors due to its increased precision and potential to reduce complications compared to invasive, ionizing, or thermal alternatives. Therapy is delivered by emitting high amplitude ultrasound pulses from a concave, multi-element transducer that focus at a point to induce localized, mechanical destruction. To enable ultrasound coupling from the transducer to the patient, a large water bath (10-16 L) rests on the patient for the transducer to submerge into during therapy. Histotripsy is currently guided by diagnostic US imaging, which works well in many cases, but is often limited by blockage or low contrast scenarios, making tumor visualization sometimes difficult or nearly impossible. To overcome such challenges, C-arm cone beam CT (CBCT) guided histotripsy is being developed, but targeting must be performed with the water bath on the patient, as its weight induces deformation of internal organs to a unique position. Unfortunately, the water bath degrades CBCT image quality, so it would be ideal to target tumors using CBCTs acquired *without* the water bath. To enable this, a technique for predicting deformation induced by the water bath is needed. The purpose of this study was to utilize the finite element (FE) method to predict water bath induced deformation during histotripsy in an in vivo swine model.

**Methods:** An FE model (a discretization of the abdomen into tetrahedral elements) was developed to predict water bath deformation for in vivo swine. FE meshes were generated using subject-specific segmentations from CT images and consisted of porcine spine, ribs, and sternum, with the rest of the body as homogeneous material. Deformable tissue (all but the spine) was represented as a nonlinear material model (neo-Hookean: converges to Hooke's law under infinitesimal strains) to more accurately model large deformation. Model parameters (mesh smoothing and resolution, and Young's modulus of the body) were tuned on a swine not used for testing. Water pressure exerted on the anterior abdominal surface was estimated by creating a digital model of the water shape and computing hydrostatic pressure based on the height of the water. These pressure estimates were used as loading conditions to drive the FE-based deformation model. To evaluate accuracy of the technique, deformation was predicted for n=3 in vivo swine and compared to ground truth deformation measured from CBCTs of the same swine with the water bath on. Ground truth deformation was induced using varying water volumes (6-16 L) to measure accuracy in different clinical scenarios, as the volume of water needed depends on patient-specific anatomy and tumor location. For each swine, deformation was then also predicted using the proposed FE model, matching the water volumes used in ground truth scenarios. Accuracy was evaluated by measuring anterior-posterior (AP) deformation on the anterior skin surface centered about the liver. This AP deformation was compared between simulated and ground truth deformation for each water volume.

**Results:** Using the proposed model, the mean absolute difference between simulated and ground truth AP deformation across all water volumes and swine was 3.0±1.7 mm. The mean signed difference was 2.0±2.9 mm (range: [-2.6,6.3 mm]), indicating an overall slight overestimation of deformation in simulations compared to ground truths. For each swine individually, the mean signed differences were 1.1 ± 3.7 mm, 3.5 ± 2.1 mm, and 1.0 ± 2.9 mm. Clinically, if tumors were targeted using CBCT images acquired without the water bath and *no deformation correction was made*, targeting errors would reach ~20-27 mm (extent of AP deformation for 10-16 L). With this predictive algorithm, however, high quality CBCTs could be used for targeting with much lower targeting errors, ranging from <1 to 6mm. Notably, however, deformation predictions for lower water volumes (<14 L) were the most accurate, with higher errors observed with larger loads (14 and 16 L). This may be due to the assumed homogeneous Young's modulus of all soft tissues. Increased accuracy could likely be achieved if more internal structures, such as muscle and gas-filled luminal structures, were explicitly defined and their specific material properties modeled, which will be a subject of future work.

**Conclusions:** This study presented a biomechanical model to predict abdominal deformation due to the weight of the histotripsy water bath used during therapy. Nonlinear finite element modeling was demonstrated to be feasible to predict such deformation for in vivo swine. In the future, these estimates can potentially be used to predict spatial distributions of organs during histotripsy, enabling CBCT-based targeting using higher quality images acquired without the water bath present.

**FLASH TALK #** 3

**Title:** Correlates and Disparities of Cancer Survivors' Formal Conversations about Employment with Healthcare Providers

**Author(s):** ***Hannah Fry***, Kristin Litzelman

**Background:** Despite best practice guidelines, few healthcare providers talk with patients about the impact of cancer on work. Given the importance of work to survivors' health, financial independence, maintaining health insurance coverage, and identity, we examined the prevalence of survivors' conversations with a healthcare provider about working with cancer, and the correlates of having such communication.

**Methods:** Data were obtained from the 2021 Health Information National Trends – Surveillance, Epidemiology, and End Results. The project identified 830 adult cancer survivors who met inclusion criteria (i.e., reported being employed at time of most recent cancer diagnosis, adult ≥ 18 years of age, with cancer diagnosis other than only non-melanoma skin cancer). Multivariable logistic regression was used to examine the associations of sociodemographic and clinical characteristics (e.g., cancer site, cancer-related conditions, years since diagnosis, patient activation, and patient-centered communication (PCC)) and employment discussion with provider. Descriptive statistics (e.g., means, frequency distributions) were used to describe the sample. T-tests and Chi-Square tests were used to compare characteristics among participants by whether they reported employment communication. A multivariable logistic regression was then constructed; we included all variables that differed significantly ($p$<0.10) across employment communication groups identified in the bivariate analyses. For a secondary analysis, the sample was further limited to those who had a health visit in the past 12 months (n=766). Finally, an exploratory analysis stratified all models by gender to assess potential disparities in employment communication. Statistical significance of disparities was tested by including an interaction term, and predicted probabilities were calculated and graphed to aid in interpretability.

**Results:** The sample (mean age: 68.8 years) was mostly female (53.1%), married (72.7%), White non-Hispanic (73.6%), and college-educated (61.5%). Over half (58.3%) reported employment communication. Survivors who were older (OR[CI]=0.98[0.96-1.00]), female (OR[CI]=0.60[0.40-0.88]), had melanoma (OR[CI]=.28[.12-.67]), and were further from diagnosis (i.e., more years since diagnosis; OR[CI]=.97[.95-.99]) were less likely to report such communication. Those with more cancer-related sequelae were more likely to report it (OR[CI]=1.30[1.15-1.48]). Survivors lacking confidence in obtaining health information were less likely to report employment communication (OR[CI]=.52[.34-.79]). Among cancer survivors with a healthcare visit within 12 months of the survey, those reporting better PCC were more likely to report having had employment communication with their healthcare provider (OR[CI]=1.01[1.00-1.03]). Gender moderated several associations. Specifically, about 59% of married men reported employment communications compared to around 40% of unmarried men, married women, and unmarried women ($p_{interaction}$<.05). Furthermore, only 22% women with low confidence in obtaining healthcare information reported employment communications, compared to 51% of women with high confidence, 56% of men with high confidence, and 60% of men with low confidence in obtaining healthcare information (Figure 1, $p_{interaction}$ <.05). Finally, the association between PCC and employment communication differed significantly by gender (Figure 2, $p_{interaction}$ <.05): at low levels of PCC, women were less likely than men to report employment communications, while this disparity diminished at higher levels of PCC.

**Conclusions:** Nearly half of adult cancer survivors report no employment-related communication with providers. Various predisposing, enabling, and need characteristics drive these employment conversations and some are moderated by gender. We revealed that marital status, confidence in obtaining healthcare information, and PCC are differentially associated with employment communication in men compared to women. Gender roles and sexism are both likely contributors to gender disparities in employment communication, as well as the moderation effects we observed. These findings may also be attributed to differences in assertiveness between men and women, with women being potentially less likely to initiate conversations about employment, particularly when they have low confidence in obtaining healthcare information or experience low PCC. Patient activation and PCC may thus be particularly important protective factors for women. Gender differences suggest tailored approaches are needed. Opportunities to support cancer survivors and providers in having these conversations will be discussed.

**FLASH TALK #** 4

**Title:** Development of an anti-MET Shark Antibody to Target Non-Small Cell Lung Cancer

**Authors:** ***Jayden West***, Rachel Minne, Kwang Nickel, Gihan Gunaratne, Andrew Baschnagel, Randall Kimple, Aaron LeBeau

**Introduction:** Non-small cell lung cancer (NSCLC) comprises 85% of all lung cancer cases and is the leading cause of cancer death in the United States. Many NSCLC patients have genomic alterations that can be selectively targeted by molecular therapies. The MET receptor, with its key roles in cell proliferation and wound healing, is one such alteration under study, being altered in 3-4% of and amplified in 3-6% of patients. While two tyrosine kinase inhibitors are approved for such patients, resistance inevitably develops. Novel treatment options for resistant patients are urgently needed.

**Methods:** Variable new antigen receptors (VNARs), isolated from nurse sharks, are small and uniquely shaped, allowing them to form unconventional binding domains. These properties, along with their high penetration levels into solid tumors, make VNARs an excellent platform for developing effective targeting agents. After immunizing a nurse shark and constructing a phage-display VNAR library from the resulting immune cells, we identified a VNAR clone, vMET1, with high MET affinity. We cloned this monomer onto a human Fc domain, and assessed the bivalent vMET1-Fc for its ability to selectively bind and internalize into MET-overexpressing cell lines by flow cytometry and confocal microscopy. Using *in vivo* studies, radiolabeled vMET1-Fc was assessed for its diagnostic and therapeutic efficacy.

**Results:** Shark plasma collected during immunization showed increasing response to MET as measured by biolayer interferometry (BLI). Lead VNAR candidate vMET1 showed fast association and slow dissociation from MET in its bivalent Fc format. Confocal microscopy shows the internalization of vMET1-Fc and colocalization with endosomes in MET-expressing EBC-1 cells, while imaging of cells treated with pH-sensitive pHrodo-vMET1-Fc suggests trafficking to lower pH compartments like lysosomes in MET-expressing EBC-1 and UW Lung-21 cells, versus MET-negative T-47D cells. *In vivo* imaging with [$^{89}$Zr]Zr-vMET1-Fc showed fast, sustained, and selective uptake in MET-expressing xenografted tumors, as measured by PET/CT scanning and biodistribution data. Meanwhile, a therapeutic study performed on mice with MET-positive xenografts and treated with [$^{177}$Lu]Lu-vMET1-Fc showed dramatic delays in tumor growth and significantly longer survival.

**Conclusions:** This is the first VNAR-based attempt at targeting the MET receptor. Our *in vitro* studies show the strong affinity and selectivity of vMET1 for MET. This is recapitulated in our *in vivo* studies, which further show vMET1-Fc's localization to xenografted tumors and ability to significantly delay tumor growth when conjugated to $^{89}$Zr and $^{177}$Lu. A present study will determine vMET1-Fc's biodistribution and immunogenicity in live non-human primates.

**FLASH TALK #5**

**Title:** ***Creating New Models for Male Breast Cancer: Hormone-Dependent Tumorigenesis using the ACI rat***

**Author(s):** ***Kasey L. Mitchell***[1], Carstyn F. Joiner, Rakesh Sarda,and Wei Xu[1]

**Background:** Male breast cancer (MBC) is a rare and understudied disease, accounting for ~1% of all breast cancers and <1% of male cancers, yet it carries a disproportionately high clinical burden. Most MBC tumors are hormone receptor-positive (HR+), with ~90% expressing estrogen and progesterone receptors, underscoring the importance of sex steroid signaling in tumorigenesis. However, due to the historical exclusion of men from clinical trials, treatment protocols for MBC are largely extrapolated from female breast cancer (FBC), despite key biological differences. This mismatch often results in suboptimal outcomes, hormonal disruption, and severe side effects—particularly with endocrine therapies such as tamoxifen, which frequently lead to treatment discontinuation. Compared to FBC, MBC is associated with poorer five-year survival (81% vs. 94.9%) and lower response to chemotherapy (4.9% vs. 9.7%). Routine screening for men is lacking, and biological samples are scarce, leaving no established MBC-specific cell lines or organoid models for studying disease mechanisms or testing therapies in vitro. Although FBC research has advanced significantly—including by our group—MBC remains poorly understood at both the clinical and mechanistic levels. To address this gap, we are developing the first integrated in vivo and in vitro models of MBC using the hormone-responsive August Copenhagen Irish (ACI) rat. Leveraging this system, we are investigating the effects of estrogen and progesterone on mammary tumor formation in males, aiming to uncover the roles of female hormones in driving MBC initiation and progression.

**Methods:** We conducted a controlled hormone supplementation study using 18 male wild-type (WT) and Pvt1-knockout (ACI-KO) rats (n=9 per group: E2 alone, or E2+MPA) and three female ACI rats as positive controls, aged 6–12 months at initiation. Male rats received subcutaneous slow-release pellets containing 27 mg 17β-estradiol (E2), with or without 20–30 mg medroxyprogesterone acetate (MPA) delivered via beeswax pellet, mimicking physiological hormone levels experienced during pregnancy. Female rats receiving E2 alone served as a positive control, given their established tumor response. Mammary glands were palpated weekly, and hormone pellets were replaced at 4 months. Endpoints were determined by tumor burden or overall health. At necropsy, mammary tissues were examined grossly and histologically, with palpable tumors distinct from hyperplasia recorded as MBC development.

**Results:** Preliminary findings showed that both WT and a genetically engineered strain, Pvt1-knockout (ACI-KO) male rats developed mammary tumors only when treated with both E2 and MPA, indicating that progesterone is a necessary cofactor for tumorigenesis in males. Rats treated with E2 alone developed pronounced mammary hyperplasia with visible lactation but did not form tumors, reinforcing the cooperative role of progesterone. Immunohistochemical and immunofluorescence analyses confirmed tumor expression of estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR), consistent with the receptor profile of human MBC. Additionally, we successfully established organoid cultures and epithelial cell monolayers from ACI-KO tumors and hyperplastic tissue, which exhibit stable growth across passages and withstand repeated freeze-thaw cycles.

**Conclusions:** Organoids and cell monolayers derived from these tumors represent the first in vitro models of male breast cancer, providing powerful tools to study hormone-driven male mammary carcinogenesis. This research bridges a critical gap in breast cancer biology and establishes a platform for future translational applications that may ultimately improve our understanding and treatment of MBC.