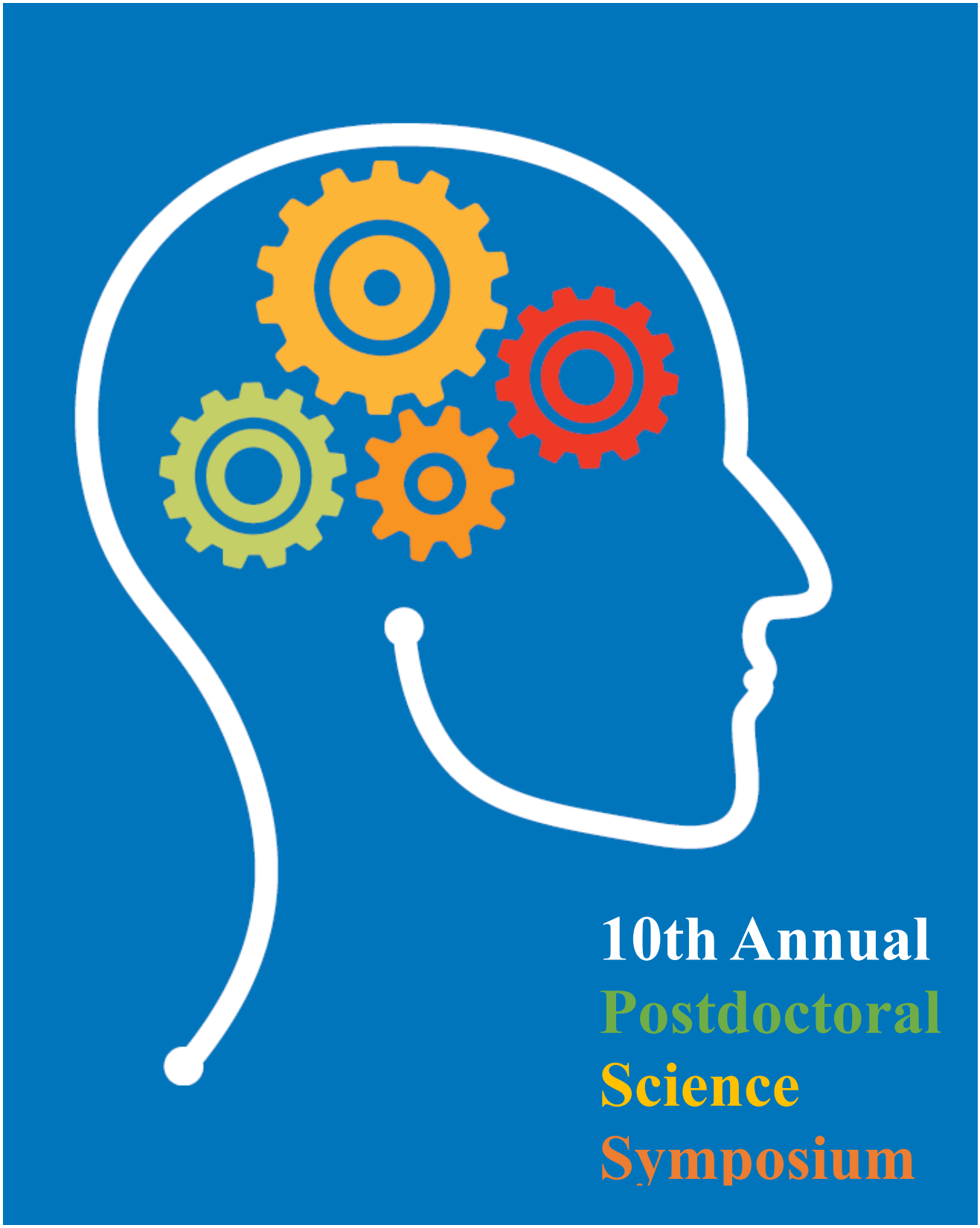
1-28-2020

10th Annual Postdoctoral Science Symposium

University of Texas MD Anderson Cancer Center PostDoctoral Association



| October 27 – 29, 2020 |

Organized by the 2020 APSS Subcommittee of the MD Anderson Postdoctoral Association

Sponsored by the Office for Postdoctoral Fellows, a unit in the Division of Education & Training

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Mission of the Annual Postdoctoral Science Symposium

The Annual Postdoctoral Science Symposium (APSS) was initiated on August 4, 2011, by the MD Anderson Postdoctoral Association to provide a platform for talented postdoctoral fellows throughout the Texas Medical Center to present their work to a wider audience.

APSS is a scientific symposium organized by postdoctoral fellows from The University of Texas MD Anderson Cancer Center that welcomes submissions and presentations from postdoctoral fellows from all Texas Medical Center affiliated institutions and other Houston area institutions. The APSS provides a professional venue for postdoctoral scientists to develop, clarify and refine their research as result of formal reviews and critiques by faculty and other postdoctoral scientists. Additionally, attendees discuss research on a broad range of subjects, engage in professional development sessions, create academic connections and develop new collaborations.

Acknowledgements

The MD Anderson Postdoctoral Association Executive Committee (PDAEC) extends sincere gratitude to Peter WT Pisters, M.D., President of The University of Texas at MD Anderson Cancer Center, for his support and compassionate leadership of the entire MD Anderson community, especially the trainees.

Furthermore, we would like to thank Dr. Diane C. Bodurka, Chief Education and Training Officer, and the Division of Education & Training for their sponsorship and assistance with this event. We appreciate the effort it took to secure the funding necessary to help establish and execute this symposium. The PDAEC would also like to thank the MD Anderson Postdoctoral Advisory Committee for their insights, advocacy and mentorship throughout the year. We also thank Dr. Giulio Draetta, Chief Scientific Officer, for his continued support, encouragement and leadership during these unprecedented times.

We are especially indebted to our distinguished faculty presenters. Dr. Rochelle Buffenstein opened the symposium with a discussion of her intriguing research on the mole rat. Dr. Peter Hotez, recently featured on numerous national news stations for his insights into virology and the novel coronavirus, punctuated this virtual symposium with his presentation on preventing the next pandemic. Also, Nobel laureate and immunotherapy innovator Dr. James P. Allison also shared some of his paradigm-shifting research on immunology and cancer therapy.

We would also like to recognize the contributions of Dr. Victoria McDonnell, Arlincia D. Ned and Paolo M. Mangahas from the Office for Postdoctoral Fellows for their support and guidance as we planned the first fully virtual edition of APSS. Because of COVID-19, organizing the symposium posed new opportunities that required innovation on the part of the planning committee to efficiently and effectively adapt previous processes, align others around the common purpose of convening a virtual symposium and expand our comfort zones to anticipate and seek solutions to perceived and real problems.

We are again thankful for the expertise of scientific editors from the Research Medical Library, namely Dawn Chalaire, Ashli Villarreal and Bryan Tutt. It is their editorial skills that enable us to publish our third APSS abstract publication, which adheres to editorial guidelines.

We are indebted to the faculty and postdoc judges for their constructive evaluation of and feedback to our postdoctoral fellows, their abstracts, and their poster and oral presentations. We would like to extend a big thank you to all the members of the PDAEC, especially our planning committee and the FY20 and FY21

PDAEC co-chairs, Drs. Akosua Badu-Nkansah and Raj Yadav and Drs. Didem Agac Cobanoglu and Jezreel Pantaleon, respectively, whose support helped to make this event possible.

We acknowledge the dedication and support of our faculty mentors, without which this symposium would be impossible. We thank each presenting postdoctoral fellow not only for continuing their research but also for submitting their abstracts as we all navigate this unchartered terrain. And finally, thank you to all who joined us during this three-day virtual symposium. We are very grateful for all the support from the MD Anderson and Texas Medical Center communities.

Sincerely,

Elien Doorduijn, Ph.D.
FY20 Chair
APSS Subcommittee
MD Anderson Postdoctoral Executive Committee

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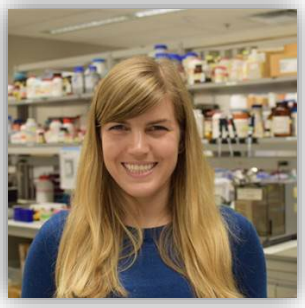
Competition Winners

Nearly 50 abstracts were submitted for the 10th APSS, wherein 12 of the submitting postdoctoral fellows were selected for oral presentations in applied science, basic science, and clinical/translational research.

Oral Competition – First-Place Winners

Applied Science: "*Identifying predictors of opioid misuse in adult trauma patients using a Bayesian data science approach"*

Constanza de Dios, Ph.D. *Mentor: Joy M. Schmitz, Ph.D.*



Basic Science: "*Elucidating the role of purinergic signaling during rotavirus infection"*

Kristen A. Engevik, Ph.D. *Mentor: Joseph M. Hyser, Ph.D.*

Clinical/Translational Research: "*Dual inhibition of glutamine metabolism and oxidative phosphorylation (OxPhos) constitutes a novel approach to target metabolic reprogramming in NOTCH1-driven T-cell acute lymphoblastic leukemia (T-ALL)"*

Natalia Baran, M.D. *Mentor: Marina Konopleva M.D., Ph.D.*

Poster Competition Winners

Shared first position:

"Clinical implication of co-occurring molecular alterations in patients with Philadelphia chromosome-positive B-cell acute lymphoblastic leukemia treated with hyper-CVAD plus dasatinib or ponatinib"

Yuya Sasaki, M.D., Ph.D. *Mentors: Koichi Takahashi, M.D., Ph.D.*
Elias Jabbour, M.D.

"Anti-miR-93-5p therapy prolongs sepsis survival by restoring the peripheral immune response"

Melanie Winkle, Ph.D. *Mentor: George A. Calin, M.D., Ph.D.*

Shared second position:

"The oral poliovirus vaccine restricts rotavirus vaccine replication and infection"

Julia Hankins, Ph.D. *Mentor: Sasirekha Ramani, Ph.D.*

"Brain reactivity and attentional bias to drug cues in cocaine users"

Heather E. Soder, Ph.D. *Mentor: Francesco Versace, Ph.D.*

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Oral Presentations

The following categories were included for oral presentations: Applied Science, Basic Research and Clinical-Translational Science. Each category included up to four presenters, all of whom were required to be first authors of their submitted abstracts.

Only abstracts of presenters who agreed to have their abstracts published are included.

Applied Science

Gold nanorod hydrogel for laser-induced HIPEC in gastric cancer

Karem A. Court[1], Hangjin Yu[1], Diana Chan[1], Elvin Blanco[2], Arturas Ziemys[2], Ashley Holder[3]
[1]Department of Surgery, Houston Methodist Research Institute, Houston, TX; [2]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX; [3]Department of Surgery, University of Alabama at Birmingham, Birmingham, AL.

Background: Cancers that spread to or originate in the peritoneum are termed peritoneal malignancies. These cancers are often deemed unresectable and have limited treatment options, as chemotherapy administered intravascularly has little effect and surgical resection may miss microscopic disease. The standard of care is surgical removal of visible tumor followed by infusion of heated chemotherapy into the abdominal cavity (HIPEC). The existing approach has substantial risk of local recurrence and toxicity to intra-abdominal organs. Gold nanorods (GNR) can be stimulated by near-infrared (NIR) laser radiation to generate heat in a controlled and predictable manner. The goal of this study was to develop an innovative hydrogel film to produce controlled, mild hyperthermia and provide local delivery of chemotherapy to peritoneal malignancies.

Methods: Film was designed with gelatin and glycerol, and its properties were assessed. Cisplatin (CPT) and mitomycin C (MMC) were encapsulated in film; loading and release were measured. GNR concentration was determined to achieve 42 ºC using NIR laser. Human gastric cancer cells (SNU-16) were treated with CPT or MMC film to determine IC_{50}. Cells were treated with combinations of film with GNR, chemotherapy and NIR laser to determine cytotoxicity. Intraperitoneal biodistribution was assessed in a murine model of gastric cancer with peritoneal metastases (SNU-16) using Cy5-GNR, with fluorescence measured by IVIS.

Results: A gelatin film had 90% transparency, had Young's modulus of 685 MPa at 28 ºC and 594 MPa at 37 ºC, and was soluble in water-based solution at 37 ºC. Chemotherapy was successfully loaded with 80% efficiency and released from film, between 2 and 4 hours. The GNR film reached 42 ºC in 30 seconds when exposed to NIR laser. IC_{50} for CPT was 81.4±9.9 μM and for MMC was 0.20±0.04 μM. Treatment of SNU-16 with NIR laser and GNR-chemo film to 42 ºC resulted in similar efficacy to soluble treatment. Preliminary biodistribution results *in vivo* demonstrated that GNR-film at 4 hours after implantation accumulates on the peritoneal surface and metastases.

Conclusions: To date, no study has evaluated nanoparticle platforms for HIPEC. Preclinical evidence of efficacy utilizing a hydrogel GNR-chemotherapy film could lead to clinical trials in peritoneal malignancies. If successful, treatment of other cancers could benefit from this film to decrease the incidence of local recurrence and chemotherapy toxic effects.

Identifying predictors of opioid misuse in adult trauma patients using a Bayesian data science approach

Constanza de Dios[1], Robert Suchting[1], Heather E. Soder1, Jin H. Yoon[1], Danielle Kessler[2], Shweta Kapoor[1], Angela L. Stotts[3], Angela M. Heads[1], John A. Harvin[4], Charles E. Green[1], Scott D. Lane[1], Joy M. Schmitz[1]

[1]Faillace Department of Psychiatry and Behavioral Sciences, UTHealth McGovern Medical School, The University of Texas Health Science Center at Houston, Houston TX; [2]Department of Psychological Sciences, Rice University, Houston TX; [3]Department of Family and Community Medicine, UTHealth McGovern Medical School, The University of Texas Health Science Center at Houston, Houston TX; [4]Center for Translational Injury Research, Department of Surgery, University of Texas Health Science Center at Houston, Houston TX.

Background: Although the use of prescription opioids can alleviate acute pain, it can lead to opioid misuse, a growing public health concern. One population of interest is recent trauma and surgery patients. Identifying variables in existing in-patient assessments can be useful for informing interventions for opioid dependence after discharge. Data science approaches can fulfill the aim of identifying pertinent variables.

Methods: Data were collected from adult patients (N=635) from a Level 1 trauma center in Houston, TX. Thirty-nine items from the Multimodal Analgesic Strategies for Trauma (MAST) database, 13 items from National Trauma Data Standard, and two hospital measurements (morphine milligram equivalents/day and numeric pain rating scale) were used as predictors. The primary outcome was high (19.5% of patients) or low (79.8% of patients) risk level of opioid use, measured at baseline using the Opioid Risk Tool. Generalized linear modeling with Bayesian inference was used to quantify the probability that the effect of each predictor on risk level was greater or less than zero, given the data and weakly informative priors. Risk level was modeled via a binomial distribution with a weakly informative horseshoe prior.

Results: Predictors of risk level in the sample were: younger age; higher numerical pain ratings; prior opioid use; the presence of amphetamines, barbiturates, cocaine, phencyclidine, or THC in the urine drug screen; and being a smoker (posterior probabilities > 75%). The majority of sizable-effect predictors were from the MAST database.

Conclusions: The present research demonstrates the utility of Bayesian data science methods in indicating pertinent measures for predicting risk of opioid misuse in trauma and surgery patients. The findings also support the utility of existing in-hospital assessments for informing interventions appropriate to level of risk.

Examining lung cancer screening: a review of challenges and opportunities in publicly available data

Kristin G. Maki[1], Robert J. Volk[1] and Sanjay S. Shete[2]

[1]Department of Health Services Research, The University of Texas MD Anderson Cancer Center, Houston TX; [2]Department of Biostatistics and Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Screening for lung cancer with low-dose computed tomography (LDCT) is recommended for high-risk individuals meeting specific eligibility criteria. Current guidelines suggest that eligible adults with a heavy smoking history will benefit from periodic LDCT, but due to several associated risks (e.g., false positives, radiation exposure, overdiagnosis), a shared decision-making consultation is required for reimbursement by the Centers for Medicare and Medicaid Services (CMS) and recommended but not required by the United States Preventive Services Task Force (USPSTF) guidelines. Large-scale, national data sets provide a wealth of information about health-related behaviors to inform cancer control efforts at a population level as well as important information regarding uptake of and adherence to recommended preventive behaviors, including cancer screening. However, due to varying methods and survey-level factors, there are challenges related to their use.

Methods: We examined characteristics, intended uses, and variables related to lung cancer screening (LCS) from 4 population-based surveys: 1) National Health Interview Survey (NHIS), 2) Behavioral Risk Factor Surveillance System (BRFSS), 3) National Health and Nutrition Examination Survey (NHANES), and 4) Health Information National Trends Survey (HINTS). The most recent information pertaining to LCS was then compared with the current CMS and USPSTF guidelines. It must be noted that the surveys are not always consistent over time; the BRFSS includes cancer modules that states may opt to use, and other surveys update their questions over time and do not always include the same items from year to year. An overview of 33 publications using these sources is also provided.

Results: None of the data sets included items that fully assess current LCS guidelines for CMS reimbursement. The 2015 NHIS dataset allows analysis of LCS uptake in concordance with USPSTF guidelines.

Conclusions: This examination of NHIS, BRFSS, NHANES, and HINTS identified three key challenges across the surveys. First, the surveys provide varied pieces of information relating to LCS uptake, screening adherence (this is difficult in these cross-sectional surveys with reliance on self-reported data), and patient-provider communication. Second, the majority of the surveys (NHANES excluded) are cross-sectional and rely on self-report (excluding the physical examination portion of NHANES) and may include some level of

bias. Third, the sample size for LCS-eligible individuals may be a small subsample of the population. This may result in difficulties for researchers in adjusting for bias.

Feasibility of an automated radiation treatment planning system for cost-effective cancer therapy in low- and middle-income countries

Kyuhak Oh[1, 2], Bishwambhar Sengupta[2], Carlos Cardenas[1], Laurence Court[1], Eric Ford[2]

[1]Department of Radiation Physics, The University of Texas MD Anderson Cancer Center, Houston TX;

[2]Department of Radiation Oncology, The University of Washington, Seattle, WA.

Background: The global burden of cancer is on the rise, especially the cases in low-and middle-income countries (LMICs) are increasing rapidly. By 2035, the annual number of cancer cases is projected to reach up to 25 million, and 70% of cases are expected to occur in LMICs where there are severe shortages in radiotherapy resources. Our group is developing a cost-effective system for intensity-modulated radiation therapy (IMRT) based on Cobalt-60. However, individual computerized treatment plans are required for each patient. This study explores the tools necessary for automated treatment planning, which may help mitigate these limitations by reducing the planning burden and staff workload, increasing the quality and efficiency of radiation plan creation.

Methods: This novel IMRT technology consists of a Cobalt-60 source and a ring-shaped design of the radiation-compensator system made by a 3D printer and filled with mm sized tungsten beads. For this study, the widely-used Eclipse Treatment Planning System was commissioned using measured data of the Cobalt-60 beam. A linear accelerator machine was used because the IMRT optimization module is not supported for the Cobalt-60 machine. An IMRT treatment plan using compensators were created. A custom python script was used, which creates a unique compensator based on a fluence map extracted from the IMRT optimization module in the Eclipse. A Monte Carlo (MC) program was used for verification. The effects of tungsten beads were studied, the commissioned beams were validated, and the treatment plan was created.

Results: Radiation profiles and transmission of the beads-based compensator agreed with those of solid tin, and it appeared that the reusable tungsten bead was a feasible alternative to the solid compensator. Percent depth dose curves and profiles of Eclipse and MC simulations were investigated and had agreements with differences under 2% and 4%, respectively. Two simple treatment plans for 4 or 5 fields were designed for both the Eclipse and the MC. The resulting doses were consistent for both cases.

Conclusions: Cobalt-60 beam was commissioned in Eclipse planning software, and IMRT plans based on the Cobalt-60 unit combined with reusable compensator materials were created, demonstrating the feasibility of this approach. The results from this cost-effective feasible delivery system meet the current needs for LMICs. This study is still developing, and in upcoming studies the treatment planning system may

be validated in clinical trials and combined with the Radiation Planning Assistant system under development by our group.

Basic Science

Acute myeloid leukemia expands osteoprogenitor-rich niche in the bone marrow and alters bone homeostasis

Anudishi Tyagi[1], Bin Yuan[1], Stanley Ly[1], Fouad El-Dana[1], Vinitha Kuruvilla[1], Qi Zhang[1], Christine Peterson[2], Xin Zhou[3], Benoit deCrombrugghe[3], Michael Andreeff[1], Marina Konopleva[1], Behrang Amini[4], V. Lokesh Battula[1,5]

[1]Section of Molecular Hematology and Therapy, Department of Leukemia; [2]Department of Biostatistics; [3]Department of Genetics; [4]Departmentof Musculoskeletal Imaging; [5]Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Stromal cells in the bone marrow (BM) microenvironment have been shown to contribute to leukemogenesis. We have reported that acute myeloid leukemia (AML) cells induce osteogenic differentiation in mesenchymal stromal cells (MSCs) in the BM to facilitate AML progression in mice. However, the effect of AML cells on various osteo-lineage cells in the BM microenvironment is not known. Here we hypothesized that AML cells alter bone marrow niche by expanding osteoprogenitor cells as well as remodeling the bone.

Methods: We generated triple-transgenic mice by crossing Osx-CreERt2-mice with Ocn-GFP;ROSA-tdTomato-mice. The resulting triple-transgenic mice had the genotype of Osx-CreERt2;Ocn-GFP;ROSA-tdTomato and were used to determine the effects of AML cells on specific subsets of osteoblast lineage. We used AML patient-derived xenograft (PDX) models and bone density data from computer tomography (CT) imaging in AML patients to determine the effect of AML cells on bone density and volume.

Results: In transgenic mice implanted with syngeneic AML cells, we found a 3- to 4-fold increase in Osterix+ cells, but not Osteocalcin+ cells, suggesting that AML cells expand osteoprogenitor cells during short-term exposure. Further, to investigate the effects of AML on bone during long-term exposure, we implanted AML-PDX cells (total 10 models) into NSG mice and analyzed femurs by micro-CT and histological analysis. Interestingly, we observed a dramatic increase in cortical bone thickness and new medullary bone formation in ~50% of the PDX models tested. We also found that increase in the bone volume is associated with less aggressive PDX models (which take 4-5 months to reach 90% peripheral blood [PB] engraftment). Aggressive PDX models (which take only 4-8 weeks to reach 90% PB engraftment) did not develop new bone. To validate this finding we performed Masson-Goldner's trichrome staining and observed web-like trabecular bone formation in mice with less aggressive PDX models, which

was not observed in aggressive AML PDX models. Interestingly, we also observed massive bone resorption associated with osteoclast activation in some PDX models, suggesting high bone turnover in AML bones. Next, we measured bone densities in AML patients and cancer-free individuals by chest CT imaging. We found that bone densities were significantly higher in most AML patients (~70%) compared to healthy individuals and high bone density was associated with good patient outcomes.

Conclusion: Our data suggest that AML cells induce supportive osteoprogenitor-rich niche in the BM. High bone turnover in AML bones suggests altered bone homeostasis in AML patients.

Identifying mechanisms of immune evasion in microsatellite instable endometrial cancers

Brenda Melendez[1], Emily Hinchcliff[1], Nisha Gokul[1], Daniel McGrail[1], Elizabeth Whitley[1], Russell R. Broaddus[2], Rosemarie E. Schmandt[3], Karen H. Lu[1], Melinda S. Yates[1]

[1]Departement of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX; [2]Department of Pathology and Laboratory Medicine, UNC School of Medicine, Chapel Hill, NC; [3]The University of Texas Health Science Center at Houston, Houston, TX**.**

Background: Inherited or sporadic defects in mismatch repair genes (MLH1, MSH2, MSH6, and PSM2) can lead to microsatellite instable (MSI) tumors—most commonly colon, endometrial, or gastric tumors. High mutation rates in MSI tumors have been associated with greater immunogenicity, but these tumors can evade immune response. Recently developed mouse models of MSI endometrial cancer (EC) were used to evaluate immune surveillance mechanisms.

Methods: Uterine-targeted MSH2 knockout ($PR^{Cre+}MSH2^{flox/flox}$) mice were characterized, and it was determined that 22% of mice develop spontaneous EC by 12+ months. MSI was evaluated by PCR. Immune infiltration and function were analyzed by immunohistochemistry and digital spatial profiling. Tumors were divided into TIL^{HIGH} and TIL^{LOW} (<10 TIL/mm^2) groups based on CD8a. Transcriptome analysis of tumor and normal controls was performed. A subset of transcripts was validated by RT-PCR. Cell lines were generated from spontaneous tumors and used for orthotopic syngeneic tumor studies and immune characterization. A gene signature score approach was used to quantify immune-related expression changes by functional categories for tumors from $PR^{Cre+}MSH2^{flox/flox}$ mice and MSI EC patient data from TCGA.

Results: Tumors were MSI and included endometrioid, serous, and mixed histologies. Tumors showed varying degrees of $CD8^+$ T cell infiltration, irrespective of histology. Of 7 tumors, 4 were TIL^{HIGH} and 3 were TIL^{LOW}. TIL^{HIGH} tumors had increased infiltration of dendritic cells suggestive of increased antigen presentation. Type I interferon (IFN) response was upregulated, including genes IFI203 (FC=18.5), IFI204 (FC=11.1), and T cell chemoattractant CXCL9 (FC=5.7). There was no difference in type II IFN response. TIL^{LOW} tumors had low or negligible expression of IFN-associated genes. In conjunction with increases in immunogenicity, TIL^{HIGH} tumors exhibited increased immune exhaustion markers, TIM3 (FC=2.4) and LAG3 (FC=3.2). Tumoral PD-L1 expression was negative (<1% of positive cells). Cell lines from TIL^{HIGH} and TIL^{LOW} tumors used *in vivo* retained the immune profile of the original tumor. Similar to our mouse model, TIL^{HIGH} MSI EC patients from TCGA also showed activation of type I IFN, increased dendritic cell infiltration, and increased exhaustion markers.

Conclusion: The $PR^{Cre+}MSH2^{flox/flox}$ mouse model reflects the spectrum of immunogenicity observed in clinical studies. TIL^{HIGH} tumors express type I IFN-associated factors involved in immunity, while also up-regulating negative immune regulatory molecules. TIL^{LOW} tumors lack key mediators needed for robust immune activation. Generated cell lines will be essential to test the efficacy of immunotherapies, in both immunogenic and non-immunogenic MSI tumors, and to study mechanisms of resistance to treatment.

Generating an immunogenic, protective elephant endotheliotropic herpesvirus (EEHV) vaccine

Jennifer L. Spencer Clinton[1], Taylor Pursell[2], Jie Tan[1], Rongsheng Peng[1], and Paul Ling[1]
[1]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX;
[2]Department of Microbiology and Immunology, Stanford University, Stanford, CA.

Background: Endangered Asian elephants are a keystone species facing many threats, including severe hemorrhagic disease (HD) caused by the elephant endotheliotropic herpesvirus (EEHV). EEHV-HD is the leading cause of death in captive, juvenile Asian elephants in North America and Europe and also affects captive and wild elephants in their natural range countries. While EEHV can also cause lethal disease in captive African elephants, its prevalence remains unknown. Due to the rapid severe onset of EEHV, detection and treatment options are limited. Thus, our goal is to develop a vaccine that elicits strong antibody and cell-mediated immunity (CMI) against EEHV to prevent lethal disease.
Methods: Our vaccine approach will include recombinant modified vaccinia virus Ankara (MVA) vectors expressing key immunoreactive EEHV proteins. Previous studies with EEHV and human herpesviruses indicate that glycoproteins B, H, and L (gB, gH, and gL) are likely to induce protective humoral immunity and CMI. MVA expressing gH and gL will be achieved using a novel approach that incudes expression as self-processing subunits mediated by a picornavirus 2A peptide. Recombinant MVA expressing one or multiple EEHV antigens will be generated by recombination, subsequent sorting, and plaque purification. Mice will be injected intraperitoneally with MVA vaccines with homologous or heterologous prime boosts of each antigen. Vaccine immunogenicity will be assessed by the presence of antigen-specific antibodies and T cell activation via the luciferase immunoprecipitation assay system and flow cytometry, respectively.
Results: We have successfully generated an MVA recombinant that expresses the EEHV gB glycoprotein and have initiated cloning of the gH/gL glycoproteins after verifying that the 2A-linked subunits were processed efficiently. Preclinical studies have shown that MVA-gB vaccinations induce robust anti-gB antibodies and T cell responses after homologous prime boosts in mice. We also demonstrated that a single priming vaccine and one boost are sufficient to induce immune responses and are not significantly different than two subsequent vaccine boosts.
Conclusions: In preliminary studies, we have shown that MVA-gB vaccination can yield significant levels of antibody production and T cell activation in a mouse model. Future studies will incorporate multi antigenic MVA recombinants expressing EEHV gH/gL antigens in addition to gB and will compare heterologous prime boosts with purified antigen subunits and adjuvants. Completion of this study will

provide insights into EEHV vaccine development to protect elephants against lethal HD, as well as highlight MVA as an innovative vaccine platform for other herpesvirus infections.

Elucidating the role of purinergic signaling during rotavirus infection

Kristen A. Engevik[1], Alexandra Chang-Graham[1], Melinda A. Engevik[2], Jacob L. Perry[1], Joseph M. Hyser[1]
[1]Department of Molecular Virology & Microbiology, Baylor College of Medicine, Houston TX;
[2]Department of Immunology and Pathology, Baylor College of Medicine, Houston TX.

Background: Rotavirus is an enteric virus that causes life-threatening diarrhea in children, resulting in ~128,500 deaths each year. Rotavirus infects a limited number of cells at the tips of the villi in the small intestine. Yet, rotavirus influences cell types far away from the site of infection. As a result, the accepted theory in the field is that infected cells signal to uninfected cells through some unknown compound. We recently identified that infected cells release the signaling molecule ADP, which binds to the P2Y1 receptor on nearby uninfected cells. This signaling causes intercellular calcium waves that originate from the infected cell and propagate through uninfected cells. We hypothesized that rotavirus-driven ADP/P2Y1 signaling modulates downstream effectors of infection, such as mucus expulsion, serotonin release, and chloride secretion. We also speculated that ADP/P2Y1 signaling recruits innate immune cells such as macrophages and drives local inflammatory responses.
Methods: To test this hypothesis, we generated cell lines and human intestinal enteroids that stably express cytosolic genetically encoded calcium indicators to characterize calcium signaling throughout rotavirus infection by time-lapse imaging.
Results: We found that intercellular calcium waves required P2Y1 signaling for prorogation, and inhibition of other P2Y receptors were not involved. Using human intestinal enteroids, we observed that P2Y1-mediated signaling was critical for activation of secretory epithelial cells, including serotonin secretion from enterochromaffin cells and mucus expulsion from goblet cells. In monkey MA104 cells, rotavirus infection chemoattracted mouse bone marrow-derived macrophages, which also harbor the P2Y1 receptor. Moreover, application of ADP to macrophages stimulated secretion of the chemokine MCP-1. These effects were blunted by pharmacological inhibitors of the P2Y1 receptor. Consistent with our *in vitro* findings, we observed that murine rotavirus infection increased serotonin secretion and mucin expulsion and drove accumulation of macrophages. These effects were minimized in the presence of P2Y1 inhibitors and in P2Y1 knockout mice. Importantly, P2Y1 knockout and inhibition of P2Y1 by pharmacological inhibitors reduced diarrhea, indicating that P2Y1 is responsible for the global effects of rotavirus infection.
Conclusions: These findings suggest that the signaling molecule ADP and its receptor P2Y1 may be a new target to treat and lessen rotavirus-induced diarrhea.

Clinical-Translational Science

Risk and predictors of late lower cranial neuropathy in long-term oropharyngeal cancer survivors

Puja Aggarwal[1,5], Ryan P. Goepfert[1], Adam S. Garden[2], Naveen Garg[3], Jhankruti S. Zaveri[1,] Xianglin L. Du[5], Michael Swartz[5], Stephen Y. Lai[1], C. David Fuller[2], Renata Ferrarotto[4], Linda B. Piller[5], Katherine A. Hutcheson[1,2]

[1]Department of Head and Neck Surgery, [2]Department of Radiation Oncology, [3]Department of Abdominal Imaging, [4]Department of Thoracic Head and Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX; [5]University of Texas School of Public Health, Houston, TX.

Background: Lower cranial neuropathy (LCNP) is a rare, but permanent, late effect of radiotherapy (RT) and other cancer therapies. LCNP is associated with excess cancer-related symptoms, worse swallowing-related quality of life (QoL) and long-term feeding tube dependence, aspiration pneumonia, and tracheostomy. Previous studies examining LCNP have been case reports or small cohorts of predominantly nasopharyngeal cancer (NPC) survivors. Few studies have investigated risk and predictors of late LCNP among oropharyngeal cancer (OPC) survivors. The overall objective of this paper is to quantify the cumulative incidence of late LCNP and identify clinical predictors of late LCNP among long-term OPC survivors.

Methods: Disease-free, adult oropharyngeal squamous cell carcinoma (OPSCC) survivors who completed curative treatment between January 2000 and December 2013 and consented to participation in research were included in the study. Exclusion criteria: baseline LCNP, recurrent head and neck cancer, treatment at other institutions, deceased status, secondary primary malignancy (SPM)/persistent/recurrent malignancy of the head and neck < 3 months after treatment. This study included 2021 of 3627 OPSCC survivors with median survival of 6.8 years (range: 0.3-18.4; IQR: 4.3-10.2). Late LCNP events were defined by neuropathy of the glossopharyngeal (IX), vagus (X), and/or hypoglossal (XII) nerves ≥ 3 months after cancer therapy. Cumulative incidence of LCNP was estimated using the Kaplan Meier method with adjustment for competing risks using time to event as the underlying metric. Log-rank test was used to assess differences between groups by LCNP status, and multivariable Cox proportional hazard models were fit.

Results: Eighty-eight OPC survivors(4.4%) were diagnosed with late LCNP with median time to LCNP onset after treatment of 5.4 years (range: 0.3-14.1; IQR: 1.6-8.5). Cumulative incidence of LCNP among all

OPC survivors was 0.02 (95% CI: 0.02-0.03), 0.06 (95% CI: 0.05-0.08), and 0.10 (95%CI: 0.07-0.13) at 5 years, 10 years, and 15 years of follow-up, respectively. Multivariable Cox regression identified T4 stage vs T1 stage (HR: 3.82; 95%CI: 1.85-7.86, $p<0.001$) and accelerated RT fractionation vs standard RT fractionation (HR2.15, 95%CI 1.34-3.45, $p=0.002$) as independently associated with late LCNP status, adjusting for age, subsite, T-stage, smoking, and therapeutic modality.

Conclusions: While rare in the population overall, risk of late LCNP progressed over time to 10% cumulative risk over survivors' lifetimes. Our prediction model identified OPC survivors who had T4 tumors and those who received accelerated fractionation RT as having a higher risk of late LCNP. Further efforts are necessary to investigate the risk of and predictors for this disabling late effect of cancer treatment experienced by growing numbers of relatively young OPC survivors who are expected to survive decades after treatment.

Enhancing adoptive T cell therapy by depleting endogenous TGF-β1 in human CD8$^+$ T cells

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Background: Adoptive cell therapy (ACT)—whereby tumor-reactive T lymphocytes are isolated from peripheral blood or tumor tissue and/or genetically engineered, expanded in vitro, and infused into patients—can induce clinical responses in patients with leukemia, lymphoma, metastatic melanoma, and other malignancies. However, a means to generate superior T cells with enhanced anti-tumor efficacy remains highly desirable. Because of the suppressive role of TGF-β1 on the effector functions of CD8$^+$ T cells, T cells have been engineered to express the dominant negative form of TGF-β receptor (TGFBRII-DNR) or to knock out TGF-β receptor in order to block the reception of extrinsic TGF-β1 and maintain the T cells' antitumor function in a hostile microenvironment. However, CD8$^+$ T cells require the TGF-β1 signal to develop into resident memory T cells (T_{RM}), which is potentially crucial to the long-term antitumor effect of ACT. Therefore, these current strategies abrogate CD8$^+$ T cells' ability to form T_{RM}.

Methods: In order to overcome this disadvantage, we developed a novel approach to enhance the effector function of CD8$^+$ T cells without abrogating formation of T_{RM} by CRISPR/Cas9-mediated knockout of endogenous TGF-β1-encoding gene TGFB1 in CD8$^+$ T cells.

Results: A gRNA specifically targeting the 5' of TGFB1 CDS efficiently diminished the levels of both membrane-bound and soluble forms of TGF-β1. Compared with non-target (NT) gRNA control cells, TGFB1-depleted CD8$^+$ T cells induced significantly greater production of cytokines such as IFN-γ, TNF-α and granzyme B after α-CD3/α-CD28 bead stimulation. Importantly, TGFB1 knockout in CD8$^+$ T cells did not dramatically affect the capability to form T_{RM} in response to exogenous TGF-β1 and hypoxia. In the TCR-T cells engineered to express gp100-specific, HLA-A*0201-restricted TCR, we found that TGFB1-depleted TCR-T cells showed dramatically enhanced cytolytic activity and significantly greater IFN-γ, TNF-α and granzyme B when co-cultured with gp100$^+$ tumor cell line Mel526, compared with NT control TCR-T cells. Interestingly, even in the presence of high-dose exogenous TGF-β1, TGFB1-depleted TCR-T cells maintained greater efficiency than NT control in killing Mel526 target cells.

Conclusions: Our findings support the application of endogenous TGFB1 knockout to *in vitro* generation of human antigen-specific CD8$^+$ T cells with enhanced effector properties for adoptive T cell therapy.

Poster Presentations

A total of 36 posters were accepted for presentation during the 2020 Symposium.

Please note only abstracts of presenters who could present during APSS and agreed to have their abstracts published are included.

Physician perspectives on non-alcoholic fatty liver disease

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Background: Non-alcoholic fatty liver disease (NAFLD) is now the leading cause of liver disease in the U.S. Without intervention, some NAFLD patients may progress to more severe forms of the disease, including liver cancer. Primary care physicians (PCPs) are often the first point of contact, and may be the only available resource, for many patients with NAFLD. Early studies suggest inadequate knowledge among PCPs about diagnostic and management approaches for NAFLD. The purpose of this qualitative study was to understand PCP knowledge and practices about NAFLD diagnosis and management.
Methods: We conducted in-depth interviews with PCPs in the greater Houston area. PCPs completed written informed consent and a brief demographics form prior to the interview. Interviews were 30 to 45 minutes in length; they were recorded and later transcribed. Interview questions covered knowledge of NAFLD progression, varying incidence by racial/ethnic status, current clinical practice for diagnosing and managing NAFLD, and perceptions of the burden of NAFLD on patients, among other topics. We used NVivo to analyze the transcripts.
Results: PCPs (n=14) practiced internal or family medicine, with a wide range of experience (1.5 to 30 years). We found variations in NAFLD diagnosis and management across practices and by insurance status. The typical diagnosis process involved assessment of liver enzymes, either as part of annual routine blood panels or workup for other conditions, and, if liver enzymes were elevated, proceeding to liver imaging. For patients with abnormal liver imaging, if they had insurance or were within a safety net health system, PCPs usually referred them to hepatologists/gastroenterologists. For uninsured patients outside the safety net with persistently elevated liver enzymes, PCPs sometimes proceeded with lifestyle modification recommendations, given concerns about ability to access imaging services or see a specialist. There was wide variation in the role PCPs play in the management of NAFLD. Some physicians used motivational interviewing and helped patients set dietary and physical activity goals, while others simply gave verbal recommendations and/or referred patients to a dietician. Several PCPs expressed frustration with the lack of guidance for screening and management of NAFLD.
Conclusions: The process of diagnosing and managing NAFLD appears to vary widely and may be influenced by a patient's insurance status and clinic-specific practices. Given the growing burden of

NAFLD on the U.S. medical system, efforts are needed understand the knowledge and clinical practice patterns of PCPs to support them in their frontline role.

Individual differences associated with lung cancer screening uptake

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Background: Lung cancer is a leading cause of cancer-related death in the United States among males and females. Specifically, less than 1 in 5 (18.6%) individuals with lung cancer will survive 5 years past diagnosis. Lung cancer screening (LCS) with low-dose computed tomography (LDCT) has been shown to reduce lung cancer deaths. This study examines individual factors associated with obtaining LCS among individuals enrolled in a study on the effects of a patient decision aid about LCS.

Methods: This is a secondary analysis of a randomized clinical trial conducted with 13 state tobacco quitlines' clients. Participants who met age and smoking history eligibility for LCS were enrolled (March 2015 to September 2016) and followed up for 6 months (until May 2017). Only participants (n = 204) randomized to the patient decision aid condition were included in the present study. The outcome is dichotomized to reflect obtaining LCS by the 6-month follow-up (1 = obtained screening). Key independent variables include participants' perceived importance of early detection; concern about radiation exposure, false alarms, and overdiagnosis; and anticipated regret. Demographics, practical barriers, and knowledge about lung cancer screening were included as control variables. The data were analyzed using logistic regression in SPSS (version 24).

Results: Participants who knew how to find a screening program were 1.59 times (95% CI: 1.37, 17.50) more likely to be screened than those who were unclear about where to go for screening. Similarly, participants who knew if their insurance covered LCS were 1.55 times (95% CI: 2,14, 10.47) more likely to be screened than those who did not know. Participants who were very concerned (rated as 9-10) and concerned (rated as 5-8) about overdiagnosis were respectively 1.85 (95% CI: 0.04, 0.61) and 1.88 (95% CI: 0.04, 0.53) times less likely to have been screened than those with lower ratings of concern about overdiagnosis. In contrast, participants who expressed a high level of anticipated regret (rated 10) were 1.70 times (95% CI: 1.71, 17.37) more likely to be screened than those who reported lower levels of anticipated regret about not being screened and later being diagnosed with lung cancer.

Conclusions: The study's findings suggest that anticipated regret about not obtaining screening and later being diagnosed with lung cancer is associated with LCS behavior. Conversely, concern about overdiagnosis, and practical barriers, may be associated with not obtaining LCS. Future research will benefit from further examining psycho-social values in this context.

Developing the mesenchymal tissue landscape as a scRNA-seq based metric of liposarcoma differentiation

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Background: Liposarcoma (LS) is a common soft tissue sarcoma originating from white adipose tissue. Multiple LS subtypes exist, but the two most common include well-differentiated LS (WDLS), which is morphologically similar to normal fat, and dedifferentiated liposarcoma (DDLS), an aggressive variant that can appear *de novo* or evolve through dedifferentiation of WDLS later in a patient's disease course. While current treatment strategies using cytotoxic chemotherapy have a modest therapeutic effect, an untested approach would aim to directly intervene in the molecular pathways that promote LS dedifferentiation or relieve the blockade that prevents the orderly differentiation of DDLS. To measure whether candidate therapies modulate differentiation, we need a quantitative metric of cell differentiation that presently does not exist. Herein, we describe a novel scRNA-seq–based mesenchymal tissue landscape (MTL) of cell differentiation, akin to Waddington's landscape, which will be used to decipher the regulatory networks that distinguish between DDLS and WDLS.

Methods: We performed scRNA-seq and scATAC-seq at frequent time points as mesenchymal stem cells (MSCs) were driven toward an adipogenic or osteogenic cell fate *ex vivo* by culturing upon hydrogels of varying stiffness, a property known to regulate MSC lineage commitment. Next, single-cell transcriptomic data were analyzed in the context of a signaling network entropy measure to create the MTL that allows one to quantify and visualize the differentiation states of individual tumor specimens. We hypothesized that: 1) DDLS should cluster closely to early adipocyte differentiation on the MTL, and 2) comparing DDLS and WDLS transcriptome to the gene networks regulated during adipogenesis will identify dysregulated gene networks responsible for differentiation arrest.

R**esults:** As a precursor to the study of LS or other sarcomas, we validated gene signatures of MSCs, adipocytes, and osteoblasts. Next, we identified gene expression programs that are temporally regulated during MSC differentiation. We quantified differentiation by signaling entropy and showed that MSCs had higher differentiation potential than adipocytes and osteoblasts. ScATAC-seq revealed enriched motifs as

potential regulators of adipogenesis and osteogenesis. Having created an MTL enabling our team to quantify differentiation with precision, we are currently sequencing human DDLS and WDLS specimens to identify the dysregulated gene networks and proteins responsible for differentiation blockade.

Conclusions: Understanding the molecular mechanisms that underpin LS plasticity and differentiation may provide a roadmap to develop new biologically targeted therapies able to regulate cell fate. The MTL may also offer new insights for osteosarcoma and rhabdomyosarcoma, pediatric sarcomas that exhibit dysregulated differentiation.

MMP-7 increases migration by decreasing E-cadherin and F-actin localization at cell-cell contacts in prostate cancer micro-tumors formed by perlecan/HSPG2

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Background: Polarized epithelium is maintained by cell-cell interactions via cadherins and cell adhesion molecules (CAMs). Interactions are stabilized further by cell-matrix interactions on the basement membrane, where perlecan/HSPG2 is a major component that controls signaling in resting tissues with unperturbed matrices. Proteolytic cleavage of perlecan increases cell-matrix interactions and dysregulates cell signaling, permitting migration. Previous studies showed that perlecan domain IV-3 (DmIV-3) drives cell cohesion and, when digested with matrix metalloproteinase-7 (MMP-7), drives cell dyscohesion. *In vivo*, prostate cancer (PCa) patients with bone metastases have circulating DmIV fragments, with negative correlation between MMP-7 staining and loss of perlecan in tissue samples. MMP-7 can cleave cadherins and other CAMs disrupting cell-cell adhesions. Also, DmIV-3 fragments generated by MMP-7 cleavage may further induce cell dyscohesion by disrupting interactions between CAMs and/or cadherins.

Methods: To evaluate the impact of MMP-7 and DmIV-3 fragments on cell migration or cluster dyscohesion, uniformly sized PCa cell clusters were pre-formed using a microwell system, enabling control of cluster size and cell number. Pre-formed cell clusters were transferred to DmIV-3–coated wells for 16-24 hours. Clusters then were treated with MMP-7 alone or MMP-7 plus DmIV-3 fragments during live cell imaging. Immunofluorescent staining and Imaris analysis of fixed cell clusters at different stages of cohesion and dyscohesion was performed to determine cytoskeletal architecture. The alignment of E-cadherin and F-actin served as a quantitative measure of cohesion.

Results: PCa cell clusters maintained strong cell-cell contacts with intact DmIV-3. In contrast, pre-formed PCa cell clusters cultured in the presence of DmIV-3 cleaved by MMP-7 showed dyscohesion and increased cell migration initiated at the cluster edges where migratory velocity was highest. Pre-formed PCa cell clusters treated with MMP-7 had increased F-actin reorganization during various stages of PCa cell cluster dyscohesion. E-cadherin and F-actin co-alignment at cell-cell contacts was reduced in PCa cell clusters treated with MMP-7 and DmIV-3 fragments, particularly at the edges where cell-cell adhesion was lowest and cell-matrix interactions were highest.

Conclusion: Following patterns of dyscohesion of pre-formed PCa microtumors provides a good model to study dynamic changes in protein components involved in cell-cell interactions and quantitate cell migration patterns as they can occur in metastasis and circulating tumor cell formation. Actin reorganization promotes a migratory cell phenotype in PCa cell clusters treated with MMP-7 and DmIV-3 fragments. Future studies aim to identify DmIV3 fragment(s) positively associated with tumor dyscohesion that may play key roles in secondary metastasis formation.

Agranular mice expose the mechanistic link between small cerebellar size and neurodevelopmental disorders in preterm infants

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Background: Abnormal cerebellar development instigates motor diseases and neurodevelopmental disorders including ataxia, dystonia, tremor, and autism. These conditions are highly prevalent in premature infants and in newborns with cerebellar hemorrhage, who ultimately attain a smaller cerebellar size compared to children born full-term. The small cerebellar size in surviving preterm infants is likely due to halted granule cell neurogenesis that typically occurs in the third trimester in human and first two postnatal weeks in mice. However, it remains elusive how abnormal granule cell neurogenesis effects cerebellar guided behaviors. Here, we test the hypothesis that granule cell neurogenesis is essential for the structural and functional development of Purkinje cells, which are the primary recipients of granule cell synapses and function as the main computational unit in the cerebellar cortex.

Methods: We used regional genetic manipulations and *in vivo* electrophysiology to test whether granule cells help establish the firing properties of Purkinje cells during postnatal mouse development. We generated mice that lack granule cell neurogenesis and tracked the structural and functional consequences on Purkinje cells. We also investigated how lack of granule cell neurogenesis effects cerebellum-guided motor performance and social behaviors in neonatal pups.

Results: We revealed that without granule cell neurogenesis, Purkinje cells failed to acquire their typical adult connectivity and morphology. In agreement with these anatomical abnormalities, we showed that Purkinje cell firing patterns in 2-week-old agranular pups was different from aged-matched control littermates and instead resembled firing patterns found in 1-week-old control pups. Thus, Purkinje cells need granule cells for their structural and functional maturation. In addition to abnormal Purkinje cell development, agranular pups had abnormal righting reflexes, dystonic postures, tremor, and diminished vocalization when separated from their mothers, a measure for social behavior in neonatal mice.

Conclusions: These data argue that granule cell neurogenesis sets the maturation time window for Purkinje cell function and refines cerebellar-dependent behaviors. Our findings show that abnormal granule cell neurogenesis leads to immature Purkinje cell function and explain how small cerebellums lead to behavioral deficits in surviving preterm infants.

PD-L1-mediated gasdermin C expression switches apoptosis to pyroptosis in cancer cells and facilitates tumor necrosis

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Background: Cleavage of gasdermin D (GSDMD) by caspase-1, -4, -5, or -11 defines the canonical pyroptosis pathway. Among all six human gasdermins, five are associated with important biological functions; gasdermin C (GSDMC) is the only one whose biological function has not yet been identified. Nuclear localization of PD-L1 (nPD-L1) has been reported, but its function and translocation mechanism are unclear. In solid tumors, tumor-associated macrophages, which secrete TNFα, tend to accumulate within necrotic foci. TNFα has long been known to induce tumor necrosis, but the molecular mechanism by which it does so has been poorly understood.

Methods: We used cellular fraction and Duolink assay to determine nPD-L1 expression level under hypoxia. We then established 4T1 and MDA-MB-231 stable cells with enforced expression of PD-L1-WT (wild-type PD-L1), PD-L1-NLS (nuclear localization signal-mutated PD-L1), and PD-L1-NES (nuclear export signal-mutated PD-L1). We treated these cells with TNFα plus CHX under hypoxia to determine cell death patterns and used RT-PCR and immunoblotting to detect the expression of gasdermins. A panel of caspases was used to screen for the one that cleaves GSDMC. GSDMC and caspase-8 were depleted to confirm their involvement in the nPD-L1-mediated pyroptosis pathway. Bioinformatic and mutation analysis was used to determine the cleavage site of GSDMC, followed by functional assay to test the biological function of the N-terminal domain of GSDMC. Biological function of nPD-L1-mediated pyroptosis pathway was investigated *in vivo* using the stable cells above and further confirmed by clinical data.

Results: Here, we showed that PD-L1 switched TNFα-induced apoptosis to pyroptosis in cancer cells, resulting in tumor necrosis. Under hypoxia, p-Stat3 physically interacted with PD-L1 and facilitated its nuclear translocation, enhancing GSDMC gene transcription. GSDMC was specifically cleaved by caspase-8 with TNFα treatment, generating a GSDMC N-terminal domain that formed pores on the cell membrane and induced pyroptosis. We also found that nuclear PD-L1, caspase-8, and GSDMC are required for macrophage-derived TNFα-induced tumor necrosis *in vivo*. nPD-L1-mediated tumor necrosis shortened

mouse survival, which was worsened in an immune-dependent manner. Moreover, high expression of GSDMC correlated with poor survival.

Conclusions: Hypoxia induces nPD-L1 translocation through p-Stat3. PD-L1 harbors transcriptional activity to activate GSDMC expression. Cancer cell pyroptosis via hypoxia/nPD-L1/GSDMC axis causes tumor necrosis. nPD-L1-driven tumor necrosis correlates with poor survival in mice and patients, which is worsened in an immune-dependent manner. The pyroptosis pathway in cancer cells is non-canonical and differs from that in macrophages.

Dinutuximab inhibits triple-negative breast tumor growth by targeting GD2$^+$ breast cancer stem-like cells

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Background: Breast cancer stem-like cells (BCSCs) are reported to be a major contributing factor for metastatic spread of the disease and chemotherapy resistance in triple-negative breast cancer (TNBC). Currently, no available therapeutic tools target BCSCs. We previously reported that the ganglioside GD2 is highly expressed on BCSCs and that inhibition of its expression hampers TNBC growth in xenograft models. Therefore, we hypothesized that the anti-GD2 monoclonal antibody dinutuximab (chimeric 14.18) targets GD2$^+$ BCSCs and inhibits TNBC growth.

Methods: Dinutuximab is FDA approved for the treatment of GD2$^+$ tumors in neuroblastoma. We evaluated the effects of dinutuximab on TNBC cell adhesion, migration, and mammosphere formation. We also investigated the effects of dinutuximab on cell signaling. Finally, we performed *in vivo* studies using TNBC xenograft and patient-derived xenograft (PDX) models to demonstrate the efficacy of dinutuximab in inhibiting tumor growth.

Results: In the present study, we assessed the baseline GD2 expression in more than 25 breast cancer cell lines, including TNBC and estrogen receptor (ER)+, progesterone receptor (PR)+, and Her2+ cell lines as well as TNBC PDX-derived cells and observed a higher median (± standard deviation) percentage of GD2$^+$ cells in TNBC cell lines (6.2 ± 0.7) compared to ER+PR+ and Her2+ cell lines (2.1 ± 0.2). Furthermore, compared with rituximab (as negative control), treatment with dinutuximab significantly decreased adhesion and formation of mammospheres by TNBC cells (MDA-MB-231 and SUM159) as well as the cells' ability to migrate toward a serum-rich medium. Interestingly, treatment of TNBC cells with dinutuximab significantly decreased phosphorylation of mTOR, ERK, and 4E-BP1, indicating that dinutuximab inhibits the mTOR pathway by targeting GD2 in TNBC cells. In addition, treatment with the combination of dinutuximab and NK cells substantially decreased the number of GD2-expressing cells and induced apoptosis in TNBC cells. Moreover, treatment with dinutuximab dramatically reduced tumor growth in an MDA-MB-231 xenograft model using nude mice (15 fold, p<0.0001). Finally, the combination of

dinutuximab and activated human NK cells synergistically reduced TNBC PDX tumor volumes in an NSG mouse model and increased survival ($p<0.0001$).

Conclusion: We demonstrated that GD2 expression is upregulated in TNBC and that targeting GD2 with the anti-GD2 antibody dinutuximab inhibits BCSC function. The combination of dinutuximab and NK cells induces apoptosis in $GD2^+$ TNBC cells through ADCC. The data from TNBC xenograft and PDX models provide proof of concept for the criticality of GD2 positivity in BCSCs and establish dinutuximab as a potentially efficacious therapeutic approach for TNBC.

Sensitivity enhancement of an experimental benchtop X-ray fluorescence imaging system through the firmware upgrade on a commercial single crystal cadmium telluride detector system

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Purpose: To investigate the sensitivity enhancement of an experimental benchtop X-ray fluorescence (XRF) imaging/XRF computed tomography (XFCT) system by implementing the latest firmware on a commercial single crystal cadmium telluride (CdTe) detector system.
Method: A commercial single crystal CdTe detector system with the latest firmware was characterized to operate at a higher bias voltage of 700 V and fast (3.2 μs) peaking time. Using this detector system as well as the existing/old detector system in benchtop XRF/XFCT imaging settings, the Compton/XRF spectra from small (8 mm in diameter) gold nanoparticle (GNP)-containing phantoms were acquired. During these measurements, the GNP-containing phantoms were irradiated with a polychromatic X-ray source operated at 125 kVp/24 mA and filtered with 1.8 mm tin. Single-hole detector collimators with two different aperture sizes (2 and 3 mm) were used. Gold K-shell XRF signals were extracted from the acquired spectra and used to determine the calibration curve for each experimental condition.
Results: The detector system with the latest firmware produced relatively low dead time under high X-ray flux, suggesting its improved photon counting efficiency. Additionally, this detector system was stable and performed well with a spectral resolution of ~0.7 keV FWHM at 69 keV photon energy. With the identical (2-mm aperture) detector collimator and scan time of 10 s, this detector system scored nearly 50% more gold XRF signals than the existing detector system at all GNP concentrations tested. This resulted in the sensitivity enhancement of the XRF/XFCT imaging system, giving rise to the GNP detection limit of 0.02 wt. %, which was better than that achievable with the existing detector system (0.03 wt. %). When combined with the detector collimator with a larger (3-mm) aperture, the detector system with the latest firmware produced drastically more gold XRF signal at a given GNP concentration (e.g., 9 times more for 1 wt. % GNP solution and scan time of 10 s), leading to further reduction in the GNP detection limit (i.e., 0.01 wt. %).
Conclusion: Significant performance enhancement of an experimental benchtop XRF/XFCT imaging system, in terms of the system sensitivity, was achieved by implementing the latest firmware on a commercial single crystal CdTe detector.

Proper platelet generation requires a functional PTIP-MLL3/4 axis-mediated epigenetic modification during megakaryocytic differentiation

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Background: Platelets mediate hemostasis and thrombosis, and various factors promote platelet activation in response to immune signals. Although major signaling pathways and transcription factors have been shown to play crucial roles in platelet biogenesis (thrombopoiesis), the epigenetic regulation of this process and the key epigenetic regulators involved are not as well known. The ubiquitously expressed PAX transactivation domain-interacting protein-1 (PTIP), also known as MLL3/4 complex adaptor protein, is essential for gene regulation and cellular differentiation. However, a role for PTIP in thrombopoiesis has not been previously identified.

Methods: Here we will perform functional and mechanistic assays on *Ptipf/f Pf4-cre*, *Mll3f/f Mll4f/f Pf4cre, Mll3f/f Pf4cre*, and *Mll4f/f Pf4cre* mice to define the overlapping roles of PTIP, MLL3, and MLL4 in platelet generation. These include flow cytometry analysis to characterize different megakaryocyte (MK) populations, electron and fluorescence microscopy to assess MK maturation and proplatelet formation/platelet release, platelet depletion and lifespan assays, and *in vitro* fetal liver (FL)-derived MK cultures. We will validate overexpresses/downregulated genes by modulating their expression in FL-derived MK cultures using CRISPR-Cas9–mediated depletion and lentivirus-mediated overexpression. To define the direct targets of PTIP and MLL3/4 in thrombopoiesis, we will perform RNA sequencing and chromatin immunoprecipitation sequencing (ChIP-seq) with our validated PTIP antibody in wild-type and PTIP-deficient cultured FL-derived MKs to identify PTIP-direct targets.

Results: We found that PTIP deletion led to dramatically reduced blood platelet counts and slightly increased mean platelet volume. MLL4 deletion also affected platelet counts, although the effect was not as strong as that of PTIP deletion. The number of MKs was significantly increased in PTIP-deficient mice. Nonfunctional platelets were constantly cleared from the circulation in the spleen and liver, and PTIP-deficient platelets were rapidly removed. Interestingly, PTIP recruited methyltransferases to chromatin to enable the transcription of target genes.

Conclusions: Taken together, our data identify PTIP as a chromatin regulator of thrombopoiesis that is required for directly modulating thrombopoiesis-specific gene expression together with MLL3/4.

Metabolic stress induces glutamine-dependent GD2+ breast cancer stem cell-like phenotype

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Background: Nutrient deprivation is one of the hallmark conditions of the tumor microenvironment. The rapid growth of the tumor leads to the development of a hypoxic and nutrient-deprived microenvironment within the core of the tumor mass due to an insufficient blood supply. Here we hypothesized that nutrient deprivation-induced metabolic stress results in a breast cancer stem cell-like (BCSC) phenotype in triple negative breast cancer (TNBC) cells.

Methods: TNBC cell lines SUM159 and MDA-MB-231 were cultured under serum- or glucose-deprived conditions for 3-5 days. Expression of a BCSC marker, ganglioside GD2, was measured by flow cytometry. Mass spectrometry analysis was used to study the metabolic pathways in GD2+ cells, followed by carbon and nitrogen tracing experiments with labelled glutamine. Glutathione levels and reactive oxygen species (ROS) in TNBC cells were measured by flow cytometry.

Results: Compared to serum-rich conditions, we found that serum-deprived conditions result in significant upregulation of GD2+ cells: from 9%±2% to 26%±5% in MDA-MB-231 and 7%±2% to 29%±5% in SUM159 cells. Interestingly, this effect was compensated when the cells were supplemented with serum-rich medium. In addition, a positive correlation was observed between GD2+ cell number and tumor volume *in vivo*, suggesting that metabolic stress in the growing tumors results in a GD2+ phenotype in TNBC cells. Next, TNBC cells cultured under glucose deprivation or in the presence of glycolysis inhibitor 2-deoxy glucose (2DG) showed a 2-fold increase in GD2+ cells compared to cells cultured with glucose (6 g/L) or without 2DG, suggesting that glucose deprivation enhances GD2 expression. Global metabolomic profiling by mass spectrometry followed by ingenuity pathway analysis identified signatures such as glutathione-mediated detoxification and glutathione biosynthesis to be more highly upregulated in GD2+ compared to GD2- cells. Furthermore, we found that glutamine, a key component of glutathione biosynthesis, upregulated the percentage of GD2+ cells. Moreover, carbon and nitrogen tracing using 13C- and 15N-labeled glutamine by mass spectrometry showed its direct contribution to GD2 biosynthesis. Treatment with

a glutamine transporter inhibitor, V9302, reduced GD2+ cell number by 70%-80% in a dose-dependent manner. V9302 also inhibited soft-agar colony and mammosphere formation in TNBC cell lines. In addition, V9302 reduced cellular glutathione and increased cellular ROS levels.

Conclusion: Metabolic stress results in a GD2+ BCSC phenotype in TNBC cells. Inhibition of glutamine uptake using V9302 negatively regulates the GD2+ BCSC phenotype and alters redox homeostasis in BCSCs. Targeting glutamine transporter could complement conventional chemotherapy by targeting BCSCs in TNBC.

Differential expression of transcription factors highlights therapeutic vulnerabilities of four SCLC subtypes

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Background: Small cell lung cancer (SCLC) is a highly aggressive neuroendocrine malignancy that accounts for 15% of lung cancer diagnoses. The severity of this disease is exacerbated by the fact that there are few therapeutic options, which mostly offer limited clinical benefit, culminating in a 5-year survival rate of <7% across all stages of disease. SCLC therefore has an urgent, unmet need for both mechanistic understanding of disease progression and the development of novel, targeted therapeutics with high efficacy.

Methods: To identify transcriptional subtypes, we used non-negative matrix factorization of gene expression data from 81 SCLC tumors and identified four subtypes largely based on differential expression of the transcription factors ASCL1, NEUROD1, and POU2F3. We hypothesized that these subtypes may underlie unique therapeutic vulnerabilities. We compared sensitivity of each of these four subtypes to over 500 anticancer therapies. We also examined differential expression of genes that encode surface-expressed proteins that may be targetable by reagents such as therapeutic antibodies or antibody-drug conjugates (ADCs).

Results: We showed that certain subtypes of SCLC had higher sensitivity to targeted inhibitors than others. For example, SCLC-P showed the most sensitivity to standard of care cisplatin and PARP inhibitors, while SCLC-N had the highest sensitivity to AURK inhibitors. Additionally, we identified a total of 60 candidate genes that encode surface proteins that are differentially expressed across the four subtypes of SCLC. Within these 60 candidates, we have identified a few, such as CEACAM5 and SSTR2, for which there exist clinically available, targeted ADCs.

Conclusions: The underlying biology defining our four identified subtypes of SCLC can explain certain vulnerabilities to clinically available treatments such as PARP or AURK inhibitors or cisplatin. It also has revealed a striking number of targetable, differentially expressed surface proteins, some of which already have clinically available reagents that could be repurposed for treatment of SCLC on a subtype-specific basis.

ERK acts as a timer to regulate meiotic progression and oocyte number

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Background: Meiosis-I is essential for genetic diversity and reduction of genomic copy number in all sexually reproducing organisms. This evolutionarily conserved process is governed by a series of highly timed events of chromosome behaviors. An error in any step results in organismal sterility or failures in embryonic development. However, the nature of the timer in space that interprets, coordinates, and monitors the chromosome behavior during meiosis-I remains unknown.

Results: To address this question we used *C. elegans* oogenic germline, in which stages of meiosis-I are spatiotemporally coordinated. We observed that precocious spatial ERK activation in *RAS* gain of function (*gf*) mutants triggered completion of pachytene spatially early and entry into premature gametogenesis and oocyte formation. This led to the formation of multiple small oocytes that displayed high level of sterility and embryonic lethality. Conversely, absence of active ERK in *ERK* loss of function (*lf*) mutant delayed meiotic progression, and germ cells halted at pachytene stage for a prolonged period, resulting in loss of oocyte number as well as sterility and embryonic lethality. Since maintenance of synaptonemal complex (SC) between homologous chromosomes and crossover formation takes place in pachytene-stage germ cells, we hypothesized that the RAS/ERK pathway regulates either or both of these processes. Depletion of *him-3*, an axial element protein of SC, but not *spo-11*, a DNA double strand break protein, by RNA interference rescued the multiple oocyte phenotype in *RAS(gf)* mutants, suggesting that ERK signaling may regulate meiosis progression through SC functioning. *In vitro* ERK2 kinase assays on all of the seven purified recombinant SC proteins identified that ERK directly phosphorylated HTP-1 at Serine 325. Consistent with this, phosphorylated HTP-1 accumulated in a manner overlapping with ERK activation, as assayed with phospho-specific HTP-1(S325) antibody. Lack of HTP-1 phosphorylation (in CRISPR/Cas9-mediated genetically modified phospho-null allele, *HTP-1*[*S325A*]) led to asynapsis and persistence of meiotic double strand breaks, causing delayed meiotic progression. Moreover, *HTP-1(S325A)* rescued the multiple oocyte phenotype in *RAS(gf)*, while phospho-mimetic *HTP-1(S325E)* rescued the loss of oocyte number in *ERK(lf)* mutants.

Conclusions: Taken together, we identified that spatial activation of ERK in the pachytene region acts as a timer of meiosis-I, which coordinates SC maintenance through HTP-1(S325) phosphorylation with the rate of meiotic progression. This work shows a previously unknown link between chromosomal behavior and

oocyte number. Given the conservation of female meiosis-I across species, we speculate that a similar signaling-based mechanism may be operative in mammals to regulate oocyte number.

Dual MEK and AXL inhibition targets tumor cell heterogeneity in NSCLC to prevent resistant outgrowth mediated by EMT

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Background: The epithelial-to-mesenchymal transition (EMT) is a dynamic epigenetic reprogramming event that occurs in a subset of tumor cells and is an initiating step to cancer invasion and distant metastasis. The process is reversible and gives a plasticity to cancer cells to survive under variable conditions, with the acquisition of cancer stem cell-like characteristics and features such as drug resistance. Therefore, understanding survival dependencies of cells along the phenotypic spectrum of EMT will provide better strategies to target the spatial and temporal heterogeneity of tumors and prevent their ability to bypass single inhibitor treatment strategies.

Methods: To address this, we integrated the data from a selective drug screen in epithelial and mesenchymal Kras/p53 (KP) mutant lung tumor cells with separate datasets including reverse phase protein array and an *in vivo* shRNA dropout screen to reveal specific epithelial and mesenchymal survival dependencies. Because EMT is a dynamic process, we also incorporated a dual fluorescence EMT sensor system (the Z-cad sensor) into our murine KP lung cancer models to observe real-time changes in the epigenetic state of tumor cells when treated with drugs identified from the screens. Treatment efficacy was investigated in 2D, 3D and *in vivo* model systems.

Results: The orthogonal approaches combining 3 individual datasets on epithelial and mesenchymal NSCLC tumor cells identified AXL and PARP as potential mesenchymal cell survival dependencies and

MEK and AKT as epithelial dependencies. Further investigation revealed that MEK and AXL inhibitors worked synergistically in decreasing the viability of murine NSCLC cells, likely by differential targeting of EMT subpopulations and prevention of EMT-mediated drug resistance. Similarly, co-targeting MEK and AXL *in vivo* revealed that the combination treatment was more efficacious than either single agent at controlling the tumor growth and metastasis of a highly metastatic murine NSCLC cell line.

Conclusions: Together, our data provide a rational drug combination that co-targets the MEK and AXL signaling pathways in NSCLC tumors. This combination works synergistically to selectively target cells in an epithelial or mesenchymal phenotypic state, leading to significant repression of tumor growth and prevention of resistant outgrowth by targeting EMT-related tumor heterogeneity.

Developing oxidative stress-resistant CAR-T for solid tumors

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Background: High levels of oxidative stress in the solid tumor microenvironment inflict cellular damage and alter functionality of tumor-infiltrating lymphocytes. The hostile environment of solid tumors therefore poses a challenge to immunotherapy, which moderates the action of immune cells to combat tumor growth. As a result, T cells transduced with chimeric antigen receptors (CAR-T) for solid tumors have not shown the success observed for leukemia and lymphoma. To combat the effect of oxidative stress on tumor-specific T cells, we have developed a novel approach to CAR-T protection by incorporating the damage suppressor (*Dsup*) gene from an "extremotolerant" organism, the tardigrade. Tardigrades show extreme oxidative stress resistance to alleviate cellular damage during desiccation. Therefore, we hypothesize that transgenic expression of tardigrade oxidative stress response proteins in CAR-T will enhance their survival and function in solid tumors and will mitigate effects of stress on the antitumor response.

Methods and Results: To this end, we have generated CAR-T expressing tardigrade oxidative stress response gene *Dsup* to explore their functionality in solid tumor environments. To determine if Dsup enhanced T cell survival under metabolic stress, we transduced primary T cells with tardigrade stress response gene *Dsup* or a vector only control. When cultured with H_2O_2 to generate oxidative stress, T cells transduced with *Dsup* have greater viability than T cells receiving only the control vector. To determine if the expression of Dsup affected the antitumor efficacy of CAR T, we cocultured HER2 CAR-T with and without Dsup for 48 hours with HER2-expressing FaDu tumor cells, then quantified tumor cell death by flow cytometry. In preliminary data, we observed HER2-specific CAR-T with Dsup to kill tumor cells in culture. To determine if Dsup improves the ability of CAR-T to target or kill cells *in vivo*, CAR-T transduced with antioxidant gene *Dsup* were used to treat FaDu tumors grown in the chick chorioallantoic membrane model. After treatment with Dsup-HER2.CAR-T, tumors were harvested for analysis, at which time T cell infiltrates were seen throughout the tumor in 3D reconstructions generated by confocal microscopy.

Conclusion: Together, these findings suggest that antioxidant genes have potential to enhance CAR-T efficacy in solid tumors through oxidative stress protection.

Discovery of glucocorticoid receptor degrading PROTACs as therapeutics for advanced prostate cancer

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Background: Advanced castration-resistant prostate cancer (CRPC) is a devastating disease with significant mortality. Inhibition of the androgen receptor with small molecule antagonists such as enzalutamide has become a standard-of-care treatment option for prostate cancer. Unfortunately, most patients invariably develop anti-androgen resistance. Signaling through the glucocorticoid receptor (GR) has emerged as a primary mechanism of resistance to anti-androgen therapies, making it an attractive therapeutic target. However, existing small molecule antagonists of GR suffer from partial agonist activity, which limits their antitumor efficacy. To overcome this problem, we have developed bifunctional small molecules known as proteolysis targeting chimeras (PROTACs) that induce potent degradation of GR.
Methods: Degrading capabilities and detailed assessment of one of our most potent PROTACs, GR-9059, was analyzed in several human prostate cancer models. Bioavailability and tolerability of GR-9059 was also examined in mice, and efficacy in combination with the anti-androgen enzalutamide was determined in a clinically relevant prostate cancer tumor model *in vivo*.
Results: Several novel GR-degrading PROTACs were developed by linking a non-covalent inhibitor of GR (RU-486) to pomalidomide, a ligand to the E3 ligase cereblon. Further study found one of these molecules, GR-9059, to be selective and highly potent at reducing GR levels in CRPC prostate cancer cell lines, regardless of AR expression status. Moreover, GR-9059 was 100-fold superior to the GR antagonist RU-486 in inhibiting cell growth of enzalutamide-resistant CRPC cell lines. Furthermore, pharmacokinetics and pharmacodynamics (PD) studies in mice showed that GR-9059 was readily bioavailable and well tolerated without any overt toxicities. Importantly, GR-9059 had synergistic anti-tumor efficacy in combination with enzalutamide in a mouse xenograft model of CRPC. Finally, transcriptomic analysis of cells and xenograft tumors revealed that the combination of GR-9059 and enzalutamide was associated with downregulation of

key developmental pathways, including Hedgehog and Notch, that are frequently activated during therapy resistance and tumor dormancy.

Conclusions: PROTAC-mediated degradation of GR is a highly powerful approach compared to currently available GR antagonists and supports the notion that targeting GR combined with anti-androgens such as enzalutamide may further improve prostate cancer management and survival.

Autologous breast reconstruction results in better outcomes than implant-based reconstruction in previously irradiated patients who require implant explantation due to infection

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Background: Breast implant explantation is often necessary to treat surgical site infections following implant-based breast reconstruction (IBR). History of radiotherapy further complicates the reconstructive choice. While some plastic surgeons perform autologous reconstruction following failed IBR due to infection, others argue that a second IBR attempt is acceptable. There is no consensus regarding the optimal reconstructive approach for patients with a history of radiotherapy and failed IBR due to infection.

Methods: We conducted a retrospective review of all consecutive patients with a history of radiotherapy who underwent a second reconstructive attempt following IBR and implant explantation due to infection between 2007 and 2019. The primary outcome measure was reconstruction failure (defined as flap loss in the autologous group and a second implant explantation in the IBR group). Secondary outcome measures included overall complications defined as complications at the recipient or donor sites and major complications defined as any recipient or donor site complications requiring reoperation.

Results: We identified a total of 53 patients with a history of radiotherapy who underwent breast reconstruction following IBR explantation due to infection; 27 (51%) received a subsequent autologous free tissue transfer (FF), and 26 (49%) underwent a second IBR +/- a latissimus dorsi (LD) pedicled flap. No significant differences in overall complications (33% vs. 42%, p=0.5), major complications (30% vs. 31%, p=0.9), or breast-related complications (19% vs. 38%, p=0.1) were identified between the FF and IBR groups, respectively. The FF group had a much lower rate of reconstruction failure compared to the IBR group (7% vs. 31%, respectively, p=0.04). In the IBR group, 65% had LD flap in combination with IBR. Reconstructive failure was lower in the LD+IBR group (24%) compared to the IBR-only group (44%); however, this difference was not statistically significant (p=0.38)

Conclusion: Breast reconstruction can be safely performed in women with a history of radiotherapy and implant explantation due to infection. IBR and FF patients had similar rates of overall and major complications. However, reconstructive failure was significantly lower (over 4-fold) in the FF group and potentially higher if IBR was performed without an LD flap. To reduce the risk of reconstructive failure,

plastic surgeons should strongly consider autologous reconstruction in this patient population. When IBR is necessary, the addition of an LD flap may improve reconstructive success.

Autologous versus implant-based breast reconstruction in patients with history of breast-conserving surgery and radiotherapy

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Background: Post-mastectomy radiotherapy is a known predictor of complications following breast reconstruction. However, for women who undergo completion mastectomy after initial breast-conserving therapy (BCT), the literature on the impact of previous radiation on reconstructive outcomes is scarce. The goal of this study was to compare the outcomes of autologous vs. implant-based reconstruction (IBR) in patients with a prior history of BCT undergoing subsequent mastectomy.

Methods: We conducted a retrospective review of all consecutive patients with a history BCT who underwent subsequent mastectomy and ipsilateral breast reconstruction. Inclusion criteria included patients who underwent reconstruction with a free flap (FF) or IBR (tissue expander/implant with or without a latissimus dorsi flap). Overall complications were defined as complications at the recipient or donor sites at any time after surgery while major complications were defined as any recipient or donor site complications requiring reoperation.

Results: A total of 111 breast reconstructions were identified (49% FF, 51% IBR). Mean age was similar between the two groups (52±8 vs. 51±9 years, p=0.5), but mean BMI was slightly higher in the FF group (29±5 vs. 28±6, p=0.007). Hypertension was more common in the FF group (48% vs. 23%, p=0.005), while other comorbidities including diabetes, smoking, and coronary artery disease were similar between the two groups. Follow-up was longer in the FF group (88±50 months vs. 59±51, p=0.002). Reconstruction occurred after a mean of 5±5 years (FF) and 6±5 years (IBR) from the receipt of radiation, p=0.4. No differences in overall complications (59% vs. 51%, p=0.38) and major complications (26% vs. 32%, p=0.51) were identified between FF and IBR. The FF group had a lower rate of major breast-related complications (11% vs. 30%, p=0.015), while overall breast-related complications were similar between the two groups (43% vs. 47%, p=0.6). The FF group had a higher mean number of revision surgeries (1.3±1 vs. 0.4±0.7, p<0.0001).

Conclusion: Overall surgical-site complications were similar following FF or IBR in patients with a history of BCT who subsequently underwent completion mastectomy. However, FF patients had lower risk of

major breast complications and higher likelihood of revision surgery following reconstruction. These findings might be important for surgeons to consider when counselling patients in this population on breast reconstructive options.

Does biologic mesh type impact outcomes in complex abdominal wall reconstruction?

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Background: With the increased use of acellular dermal matrix (ADM) in abdominal wall reconstruction (AWR), numerous types of ADM-based biologic meshes are now available. However, there is a paucity in the literature regarding the differences in outcomes among these ADM types.

Methods: We conducted a retrospective analysis of all patients who underwent AWR using the two most common types of ADM (porcine [Strattice] and bovine [Surgimend]) from 2005 to 2019. The primary outcome measure was hernia recurrence, while surgical site occurrence (SSO) and surgical site infection (SSI) were secondary outcome measures. We excluded patients with human ADM and less than 6 months of follow-up. The cumulative hernia recurrence was estimated using Kaplan-Meier analysis.

Results: We identified 359 patients (49.5%) with porcine ADM and 366 (50.5%) with bovine ADM. There was no difference in age (60±12 vs. 59±11 years in the porcine and bovine groups, respectively, p=0.36) or body mass index (31±7 vs. 32±7 kg/m^2, respectively, p=0.11) between the two groups. Median follow-up was 34 months (range, 6-139 months). Cumulative hernia recurrence at 5 years was similar between the groups (18% vs. 16% in the porcine and bovine groups, respectively, p=0.83). Rates of SSO (27% vs. 26.5%, p=0.88) and SSI (14% vs. 11%, p=0.22) were also similar.

Conclusion: Porcine and bovine ADM have similar hernia recurrence, SSO, and SSI rates for patients undergoing AWR. Surgeons can choose either xenograft mesh with confidence as 5-year recurrence outcomes rival those of synthetic mesh.

Financial toxicity and contralateral prophylactic mastectomy – a propensity-score matched study

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Background: Contralateral prophylactic mastectomy (CPM) is increasingly performed in average-risk patients despite the lack of survival benefit. In an era of heightened awareness of cancer treatment costs, we sought to determine the impact of CPM on financial toxicity in breast cancer.

Methods: We conducted a cross-sectional study of female patients who underwent unilateral mastectomy (UM) with or without CPM for breast cancer over an 18-month period at our institution (2018-2019). We excluded patients with a history of genetic predisposition or bilateral cancer. Propensity-matching was used to create two balanced groups with respect to the likelihood of undergoing CPM. The validated Comprehensive Score for financial Toxicity (COST) evaluated financial toxicity among participants. Multivariable regression analysis evaluated the relationship between CPM and financial toxicity. Relevant domains of the Breast-Q and SF-12 instruments were also examined.

Results: Overall, 104 patients were identified, equally distributed across UM and CPM. CPM was not associated with financial toxicity, as evidenced by comparable COST scores (adjusted difference, 1.53 [-3.24 to 6.29]). Minor complications were significantly lower in UM patients (UM,8%; CPM, 31%). CPM was associated with a significantly higher Breast-Q psychosocial well-being score (adjusted difference: 10.58 [1.34 to 19.83]). BREAST-Q surgeon satisfaction and SF-12 mental and physical component scores were all noted to be similar.

Conclusions: Choice for CPM was associated with a higher percentage of minor complications but led to improved psychosocial well-being without a higher degree of patient-reported financial toxicity in our cohort. Prospective studies are needed to discern the influence of CPM on the incidence and trajectory of financial toxicity.

Self-reported risk factors for financial distress and attitudes regarding costs discussions in cancer care – a single-institution cross-sectional pilot study of breast reconstruction recipients

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Background: High treatment costs associated with breast cancer are a substantial burden to patients and society. Despite mounting awareness, patient perspectives about the value of cost discussions in breast reconstruction (BR) and risk factors for financial distress are unknown.

Methods: We conducted a single-institution, cross-sectional survey of all women who underwent BR following mastectomy or lumpectomy for breast cancer or risk reduction. Questions were derived from previously published survey items, and we leveraged regression analysis to identify patient-level risk factors for major financial distress.

Results: Of 2,293 patients, 647 returned the survey questionnaires (28.2% response rate). Of the 647 respondents, 399 (62%) underwent BR, and of these 35% (n=140) reported that total treatment expenses were higher than expected and 32% (n=129) paid over $5000 in out-of-pocket costs (OOC). Furthermore, 71% (n=284) felt that surgeons should explain the estimated OOC when choosing a type of BR, and 51% (n=205) believed that a financial consultation should be scheduled with every new cancer diagnosis. However, only 13% (n=52) of patients reported having had cost discussions with the treatment team. The incidence of major financial distress was 18% (n=70), and regression analysis showed that higher credit score and annual income were associated with a 66% and 69% risk reduction, respectively.

Conclusion: Recipients of BR demonstrate unanticipated and unplanned financial strain related to out-of-pocket expenses and believe that cost-consciousness should impact treatment decisions. Lower income and credit score are associated with financial distress. Cost discussions may optimize decision making in preference-sensitive conditions.

Cytokine crosstalk within the bone marrow microenvironment as a novel resistance mechanism of Bcl-2/Mcl-1 inhibitor resistance in AML

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Introduction: Bcl-2 inhibitor venetoclax (VEN)-based regimen is a promising therapeutic option in AML, especially for older and unfit patients. Although a high CR rate can be achieved rapidly, the duration of response is limited, and most patients experience relapse within a few months. Considerable effort has been made to investigate the mechanism of resistance to VEN in AML, but the data generated were mainly based on findings in *in vitro* cell lines.

Methods and Results: To investigate the microenvironmental contribution to VEN resistance, we performed experiments *in vitro* co-culturing primary patient samples with mesenchymal stromal cells (MSCs) and *in vivo* using patient-derived xenograft models (PDX). We found that, without MSC co-culture, VEN in general effectively induced apoptosis in both relapsed/refractory and treatment-naive samples. However, such effect was significantly blunted in relapsed/refractory samples when co-cultured with MSCs, suggesting a role of the microenvironment in mediating resistance to VEN. We treated a PDX model, established from a patient who clinically relapsed after VEN/hypomethylating agent treatment, with the Mcl-1 inhibitor AZD5991 alone and in combination with VEN. Although the combination significantly prolonged survival (Carter B. et al, ASH 2018), the mice eventually died of the disease. By combining RNA sequencing and targeted gene sequencing in samples from moribund PDX mice, we found neither upregulation of reported targets that may mediate resistance in cell line screenings nor mutation of BCL2. Instead, we observed that VEN treatment significantly induced a proinflammatory cytokine profile, which includes colony-stimulating factor 1 (CSF1), CSF2, IL1, and IL4. This aberrant expression was even more pronounced in samples from AML that relapsed from the VEN/AZD5991 combination group. Since primary AML cells and macrophages/monocytes in niche possess receptors for these cytokines, our results suggest that AML cells survive apoptogenic stress by a crosstalk involving specific cytokines. In addition, expansion of monocytic subgroups in AML samples was reported to establish VEN resistance. We found that AZD5991 significantly decreased monocytic cell markers, suggesting that Mcl-1 inhibition may effectively eliminate monocytic cells in VEN- resistant/relapsed AML.

Conclusions: We, here, identify a novel mechanism underlying VEN/Mcl-1 inhibitor resistance and are investigating whether targeting aberrantly expressed cytokines such as CSF1 may overcome it.

The oral poliovirus vaccine restricts rotavirus vaccine replication and infection

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Background: Rotavirus is the leading cause of severe childhood gastroenteritis, with 128,500 deaths and 258 million cases worldwide. It is highly prevalent and infects almost every child by 5 years of age. Many live, attenuated oral vaccines have been introduced; however, they are not equally efficacious worldwide. In high-income countries, such as those in the Americas, Europe, and Asia, the vaccine efficacy is approximately 90%. However, in low-income countries, such as those in Africa and South Asia, the efficacy is much lower, approximately 50%-70%. One factor contributing to the reduced efficacy of rotavirus vaccines in these countries is the coadministration of the live, oral poliovirus vaccine. Consistently, coadministration of the two vaccines has been associated with reduced immunogenicity of rotavirus vaccines.

Methods: To understand how poliovirus vaccines interfere with the rotavirus vaccine response, we utilized human intestinal enteroids (HIEs), a non-transformed, physiologically active model of the human intestinal epithelium. We infected HIEs with the oral poliovirus vaccine, Sabin type 1, and the oral rotavirus vaccine, Rotarix, and assessed the effect of coinfection on vaccine infectivity and replication.

Results: Compared to single infections, coinfections resulted in decreased rotavirus replication, while poliovirus replication remained unchanged, suggesting that the reduced response to rotavirus vaccines seen in children may be due to poor replication of this vaccine when poliovirus vaccines are coadministered. Immunofluorescent staining revealed that rotavirus and poliovirus do not infect the same cell. Time-course studies showed that poliovirus replicated more rapidly than rotavirus. Therefore, coadministration of the two vaccines likely favors poliovirus replication and infection over rotavirus; this effect can be remediated by infecting with rotavirus prior to poliovirus.

Conclusions: Our model system recapitulates the inhibitory effect of poliovirus on rotavirus replication, and we are beginning to understand contributing mechanisms. Understanding the mechanisms of interference between rotavirus and poliovirus vaccines will provide evidence to support vaccine dosage or schedule improvements to enhance vaccine efficacy.

Brain reactivity and attentional bias to drug cues in cocaine users

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Background: Previous research has identified two groups of smokers: individuals with increased brain reactivity to drug cues compared to non-drug rewards and individuals with the opposite brain reactivity pattern. Here, to learn whether there are similar groups defined by brain reactivity patterns in other drugs of abuse, we tested a sample of individuals with cocaine use disorder (CUD). We also tested if the two groups of cocaine users display behavioral differences in response to cues.

Methods: Individuals with CUD (n = 43) completed a picture viewing task while we recorded their electroencephalogram. Image categories included pleasant (erotic, romantic, and sweet foods), unpleasant (mutilations, violence, and accidents), cocaine, and neutral images. Brain reactivity was measured as the amplitude of the late positive potential (LPP) over the centro-parietal electrodes from 400-800 ms after picture onset. Participants also completed an eye-tracking task that measured anti-saccade error rates in response to neutral and cocaine cues. Cluster analysis identified groups of participants based on their LPP amplitude. Independent samples t-test identified group differences in error rates on the eye-tracking task.

Results: The cluster analysis revealed two groups of cocaine users similar to previous research on smokers. One group had increased brain reactivity to pleasant compared with cocaine images, while the other had increased brain reactivity to cocaine compared with pleasant images. The second group (cocaine > pleasant) also had higher attentional bias, as measured by increased anti-saccade error rates on drug trials compared with neutral trials, compared with the first group (pleasant > cocaine).

Conclusions: This is the first study to demonstrate two groups of cocaine users defined by brain reactivity patterns. The two groups also differed behaviorally, with the second group (cocaine > pleasant) having a more difficult time disengaging their attention toward drug stimuli. These results suggest that these brain reactivity patterns may cut across addiction populations and provide important information about how the groups may have different behavioral consequences. Future directions include determining if the brain reactivity patterns can be useful in the personalization of treatment.

New translational perspectives for FGFR1 in prostate cancer bone metastases: prognosis and therapy

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Background: Despite effective treatment, no curative therapy is currently available for metastatic prostate cancer. The mechanisms of progression are diverse and include fibroblast growth factor (FGF) axis activation, underpinning the importance of developing FGF receptor (FGFR) blockade as therapy for advanced prostate cancer.
Methods: We performed *in vivo* preclinical approaches and immunohistochemical studies in human samples. To understand the mechanism of FGFR1-induced metastases, we performed RPPA of PC3 cells stably expressing FGFR1 isoforms and analyzed by applying Ingenuity Pathway Analysis.
Results: We found that FGFR1 expression in prostate cancer cells significantly reduced mouse survival and increased the incidence of bone metastases. Accordingly, castration-resistant human prostate cancer bone metastases samples revealed a significant enrichment of FGFR1 expression compared with treatment-naïve, non-metastatic primary tumors. Our studies also showed that FGFR1 induced expression of the relatively uncharacterized anchoring filament protein ladinin 1 (LAD1) in PC3 cells. Furthermore, LAD1 expression was significantly enriched in castration-resistant human prostate cancer bone metastases, supporting the concept that LAD1 mediates, at least in part, the prostate cancer metastatic phenotype.
Conclusions: Our studies indicate that new FGFR1 signatures define pathway activation, and this knowledge will help identify markers of pathway inhibition in human prostate cancer. Our findings also show that FGFR1 underlies the metastatic dissemination of prostate cancer and implicate, for the first time, LAD1 in the metastatic phenotype of a subpopulation of men with prostate cancer and as a target of FGFR1 signaling. Hence, the biological implications of LAD1 expression in prostate cancer pathogenesis warrant further investigation. Since FGFR1 is a major factor involved in disease progression under treatment, the implication of the FGFR1-LAD1 axis in prostate cancer metastatic progression provides a framework of new opportunities for development of FGFR1-LAD1 targeted therapies and/or the identification of markers of progression.

Inhibition of oxidative phosphorylation (OxPhos-i) overcomes NOTCH1-driven chemoresistance in T-cell acute lymphoblastic leukemia (T-ALL)

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Background: The high relapse rate of T-ALL is associated with persistence of chemoresistant cells, harboring activating *NOTCH1* gene mutations. Since Notch1-directed therapies showed limited responses, targeting key oncogenic and metabolic pathways downstream of *NOTCH1* may offer novel approaches. We demonstrated that transformation of thymocytes into preleukemic stem cells (pre-LSC) requires elevated *Notch1* upon the presence of *Scl/*Lmo1; and that survival of *NOTCH1*-mutated T-ALLs depends on OxPhos. OxPhos inhibition (OxPhos-i) using the complex I inhibitor IACS-010759 reduces tumor burden in *NOTCH1*-mutated T-ALL PDX models. Thus, we hypothesized that chemotherapy (Vincristine, Dexamethasone, L-Asparaginase, VXL) aided by OxPhos-i overcomes chemoresistance, depletes LSCs, and combats T-ALL.

Methods: We utilized comprehensive molecular and functional assays: viability, apoptosis, niche-based assay, GSEA of pre-LSCs, NetBid, Seahorse, targeted and untargeted metabolomics, and mass cytometry. The study was conducted on pre-leukemic and leukemic murine models of human Notch1 T-ALL harboring specific genotypes: *LMO1, SCL-LMO1, NOTCH1, LMO1-NOTCH1,* and *SCL-LMO1-NOTCH1*; human T-ALL cell lines (n=9); patient samples (n=5); and PDX models (n=3).

Results: We identified co-occurrence of increased expression of Notch 1 target genes and OxPhos as a highest-ranked gene set at the preleukemic stage (GSEA). In pre-LSC, OxPhos-i reduced viability upon activation of Notch1 by its ligand DL4. Without DL4 activation—among thymocytes harboring *LMO1, SCL-LMO1, NOTCH1, LMO1-NOTCH1* and *SCL-LMO1-NOTCH1* genotypes—only those with the *Notch1* oncogene responded to OxPhos-i. At the leukemic stage, in *SCL-LMO1*-induced murine T-ALL, only oncogenic *Notch1* elevated OxPhos pathway. The same pattern of co-segregation of *NOTCH1* mutations with significant downregulation of apoptosis signaling and upregulation of OxPhos genes occurred in the T-ALL COG patient cohort (n=263, TARGET) prior to chemotherapy. VXL chemotherapy combined with OxPhos-i triggered a metabolic shut-down indicated by reduced OCR and ECAR (Seahorse) with profound reduction of viability (CTG, flow cytometry) *in vitro.* This resulted in metabolic changes and induction of apoptosis in leukemic cells *in vivo*, demonstrated by Metabolomics, RNAseq, and CyTOF analyses. Finally, VXL-OxPhos-i resulted in reduced leukemia burden and extension of overall survival in three aggressive T-ALL PDX models ($p<0.0001$).

Conclusions: In T-ALL, OxPhos upregulation occurs upon activation of *NOTCH1* signaling. Activation of Notch1 sensitizes pre-LSCs and LSCs to OxPhos-i. Targeting OxPhos in combination with chemotherapy facilitates eradication of chemoresistant *NOTCH1*-driven T-ALL through direct targeting of the key metabolic regulators of OxPhos conferred by mutant *NOTCH1* in T-ALL. Clinical trials rewiring metabolism by incorporation of OxPhos-i to standard-of-care therapy in patients with *NOTCH1*-mutated T-ALL are warranted to improve patients' outcomes.

Does enhanced recovery pathway impact opioid use and complications following microsurgical breast reconstruction?

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Background: Enhanced recovery after surgery (ERAS) pathway is a multidiscipline evidence-based approach aimed at improving perioperative patient care and optimizing recovery following surgery. The purpose of this study is to assess the impact of ERAS pathway on opioid use and patient outcomes following microsurgical breast reconstruction (MSBR) at a high-volume center.
Methods: A retrospective cohort study was performed on patients who underwent MSBR from June 2018 to December 2018 for the conventional cohort (CC), and from August 2019 to December 2019 for the ERAS cohort (ERAS). Primary outcome measures included morphine milligram equivalents (MME) and 30-day complication rates. Secondary outcomes included length of hospital stay (LOS) and pain scores.
Results: A total of 186 patients (CC, 108 [58%]; ERAS, 78 [42%]) underwent MSBR during the study period. Similar age (p=0.64) and BMI (p=0.75) were identified in both groups. LOS was slightly shorter in the ERAS group (4.6±1.1 vs. 4.9±0.9, p=0.01) while the complication rate was significantly higher in the CC group (33% vs. 19%, p=0.03). Total MME consumption was significantly lower in the ERAS cohort (18.3±11.2 vs. 32.4±26.8, p=0.001). Despite lower opioid use, average pain scores did not significantly differ between ERAS and CC patients at any time point (2.3±1.4 vs 2.4±1.2, p=0.24). Regarding the intraoperative anesthetic choice, volatile anesthesia was used in 105 patients (56%), total intravenous anesthesia in 21 (11%), and combined anesthesia in 60 (32%) with no significant difference between CC and ERAS. There were no differences in pain scores or MME use between the different anesthesia types. In our multivariate model, ERAS was the only predictor of lower MME consumption.
Conclusions: The implementation of ERAS pathway for MSBR improves patient outcomes by reducing length of stay, total opioid consumption, and incidence of postoperative complications. Type of anesthesia does not seem to impact MME use or pain scores in MSBR patients.

Response to and survival after first-line FOLFIRINOX or gemcitabine/nab-paclitaxel for localized pancreatic ductal adenocarcinoma

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Background: 5-Fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX) and gemcitabine plus nanoparticle albumin-bound (nab)-paclitaxel (GA) are first-line chemotherapy regimens for pancreatic cancer, but their relative efficacy in the setting of localized disease is unknown. The aim of this study was to evaluate radiographic and serologic measures of response to first-line chemotherapy with FOLFIRINOX or GA and to determine the associations between these drug regimens, putative measures of response, and survival.

Methods: Consecutive patients who were diagnosed with previously untreated localized pancreatic ductal adenocarcinoma at MD Anderson Cancer Center between January 1, 2010, and December 31, 2017, and who received at least 3 cycles of first-line chemotherapy with FOLFIRINOX or GA were included. The main outcomes were resection rate, radiographic (RECIST 1.1 and change in tumor volume or anatomic staging) and serologic (CA 19-9 level) metrics of response to first-line chemotherapy, and overall survival.

Results: A total of 485 patients were treated with FOLFIRINOX (n = 285; 59%) or GA (n = 200; 41%) as first-line chemotherapy. Patients treated with FOLFIRINOX were generally younger and had better performance status but more invasive tumors than patients who received GA ($P < .01$ and $P = .01$, respectively). After we conducted propensity score matching to control for these biases, many objective serologic and radiographic metrics of response to FOLFIRINOX and GA—including low rates of local tumor downstaging—did not differ. However, RECIST partial response was more common among patients treated with FOLFIRINOX. Moreover, (chemo)radiation was more commonly administered to, and pancreatectomy was subsequently performed more frequently for, patients initially treated with FOLFIRINOX. The overall survival duration of patients treated with GA was similar to that of patients treated with FOLFIRINOX (HR 1.48 [95% CI 0.97 – 2.26], $P = 0.07$).

Conclusions: In this cohort of patients with localized pancreatic adenocarcinoma who received FOLFIRINOX or GA as their first line of therapy, FOLFIRINOX was associated with higher rates of RECIST PR and subsequent pancreatectomy than GA, but the overall survival associated with these regimens was similar.

Prognostic significance of genetic alterations in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia treated with hyper-CVAD plus dasatinib or hyper-CVAD plus ponatinib

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Background: Clinical outcomes of patients with Philadelphia chromosome (Ph)-positive B cell acute lymphoblastic leukemia (B-ALL) have been significantly improved with the addition of tyrosine kinase inhibitors (TKIs). Treatment with a TKI alone or a TKI with chemotherapy results in morphological complete response in nearly all patients with Ph-positive B-ALL. However, persistence of measurable residual disease (MRD) and disease relapse remain major clinical problems. Identification of predictive and prognostic biomarkers for Ph-positive B-ALL is urgently needed. Recurring genetic abnormalities such as deletions in *IKZF1*, *CDKN2A/2B*, *PAX5*, *BTG1*, and *EBF1* have been identified in Ph-positive B-ALL. Among those, *IKZF1* deletion has been associated with poor prognosis in Ph-positive B-ALL patients treated with imatinib-based or dasatinib-based regimens. However, molecular determinants for clinical outcomes in patients treated with ponatinib (a potent TKI) are not known. Here, we have systematically analyzed genetic alterations in adult Ph-positive B-ALL patients uniformly treated in clinical trials with hyper-CVAD plus dasatinib or hyper-CVAD plus ponatinib regimens and investigated the molecular determinants for treatment outcomes and prognosis.

Methods: We analyzed pretreatment bone marrow or peripheral blood specimens collected from adults with Ph-positive B-ALL who participated in clinical trials with either hyper-CVAD plus dasatinib (NCT00390793, N = 55) or hyper-CVAD plus ponatinib (NCT01424982, N = 50). Targeted capture DNA sequencing of 295 genes (N = 102) or whole exome sequencing (WES, N = 3) was performed. Genome-wide copy number analysis (CNA) was performed using either SNP microarray (N = 102) or WES (N = 3).

Results: The most frequently detected alterations were *IKZF1* deletion. Among the 63 patients with *IKZF1* deletion, we addressed detailed deletion site in 53 cases. Ik6 subtype, which is characterized by deletion of exons 4-7 (N = 28, 23 %) was most frequently detected followed by Ik2 subtype, which involves deletion of exon 2 (N = 20, 32 %). Patients harboring Ik2 subtype deletion or Ik6 subtype

deletion showed significantly worse event-free survival (EFS) (hazard ratio [HR] = 1.91; $p = 0.048$) and overall survival (OS) (HR = 2.36; $p = 0.019$) than patients without *IKZF1* deletion or those with *IKZF1* deletion other than the Ik2 or Ik6 subtypes. We defined the *IKZF1*^{plus} group as the group consisting of the patients with deletion of *IKZF1* and other deletions. Then, we performed univariate analysis for patients with *IKZF1* deletion in order to find the significant deletion partner of *IKZF1* deletion in terms of prognosis, and we defined the *IKZF1*$^{plus(rev)}$ group as patients with *IKZF1* deletion and both *CDKN2A/2B* deletions and *VPREB1* deletion, or at least one of them. The *IKZF1*$^{plus(rev)}$ group showed worse prognosis on EFS and OS than the non-*IKZF1*$^{plus(rev)}$ group. Combining the results of the impact of Ik subtype and *IKZF1*$^{plus(rev)}$ group on EFS and OS, we defined an *IKZF1*-driven high-risk group as patients harboring either alteration of Ik2/Ik6 subtype or *IKZF1*$^{plus(rev)}$. The *IKZF1*-driven high-risk group showed worse prognosis on EFS and OS than the *IKZF1*-driven low-risk group. Next, we performed univariate and multivariate analyses combining baseline features, TKI type, and MRD status to assess their impact on outcome. Multivariate analysis showed that TKI type, MRD status at 3 months, and *IKZF1*-driven high-risk group were independent factors for OS. In patients treated with hyper-CVAD plus ponatinib and categorized into *IKZF1*-driven low-risk group, the 5-year EFS and OS rates were 92.0% and 96.0%, respectively.

Conclusion: In this study, unlike with dasatinib-based therapy, we demonstrated that an *IKZF1*-driven high-risk group was associated with worse EFS and OS than *IKZF1*-driven low-risk in the context of hyper-CVAD plus ponatinib. Evaluation of the prognostic implication of using *IKZF1*-driven risk at the time of the diagnosis may be useful to personalize treatment in order to improve the clinical outcomes of patients with Ph-positive B-ALL in the era of ponatinib.

Anti-miR-93-5p therapy prolongs sepsis survival by restoring the peripheral immune response

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Background: Sepsis remains one of the leading causes of death and currently has no therapy available due to the lack of deep mechanistic understanding and of subject stratification by age, gender and comorbidities in most preclinical and clinical trials. Here, we used diverse human samples and *in vivo* models to define sepsis-associated micro (mi)RNAs that qualify as therapeutic targets.

Methods: To define deregulated miRNAs, we used peripheral blood mononuclear cells (PBMCs) from sepsis patients, whole blood samples from sepsis survivors, and RNA-seq data from healthy individuals. For confirmation of the therapeutic potential of the identified miRNAs, we used multiple animal models: mouse cecum ligation-puncture (CLP)-induced sepsis, mouse viral microRNA challenge, and baboon Gram-positive and Gram-negative sepsis models. We tested the therapeutic potential of sepsis-miRNAs in CLP-induced sepsis in mice of different ages, analyzing survival and, for mechanistic insight, the peripheral immune response. Furthermore, we used an *in vitro* miRNA knockout model to define the exact molecular targets of sepsis-miRNAs using Ago2-immunoprecipitation.

Results: Based on expression is serial human sepsis samples and confirmed data from *in vivo* models, miR-93-5p passed the selection criteria as a potential therapeutic target. MiR-93-5p was significantly overexpressed in plasma and PBMCs from baboons that died early after induction of Gram-positive and Gram-negative sepsis and was downregulated in humans who experienced long-term survival after sepsis diagnosis. Therapeutically, inhibiting miR-93-5p prolonged the overall survival of CLP-induced sepsis in mice, and the effect was stronger in older mice. Targeting miR-93-5p in sepsis reduced the frequency of inflammatory monocytes and increased the frequency of circulating $CD4^+$ and $CD8^+$ effector memory T cells. On the molecular level, miR-93 affected multiple pro- and anti-inflammatory pathways, in accordance with the changes in cell frequencies that we observed.

Conclusions: MiR-93-5p is a potential therapeutic target in sepsis in elderly patients because it affects both innate and adaptive immunity.