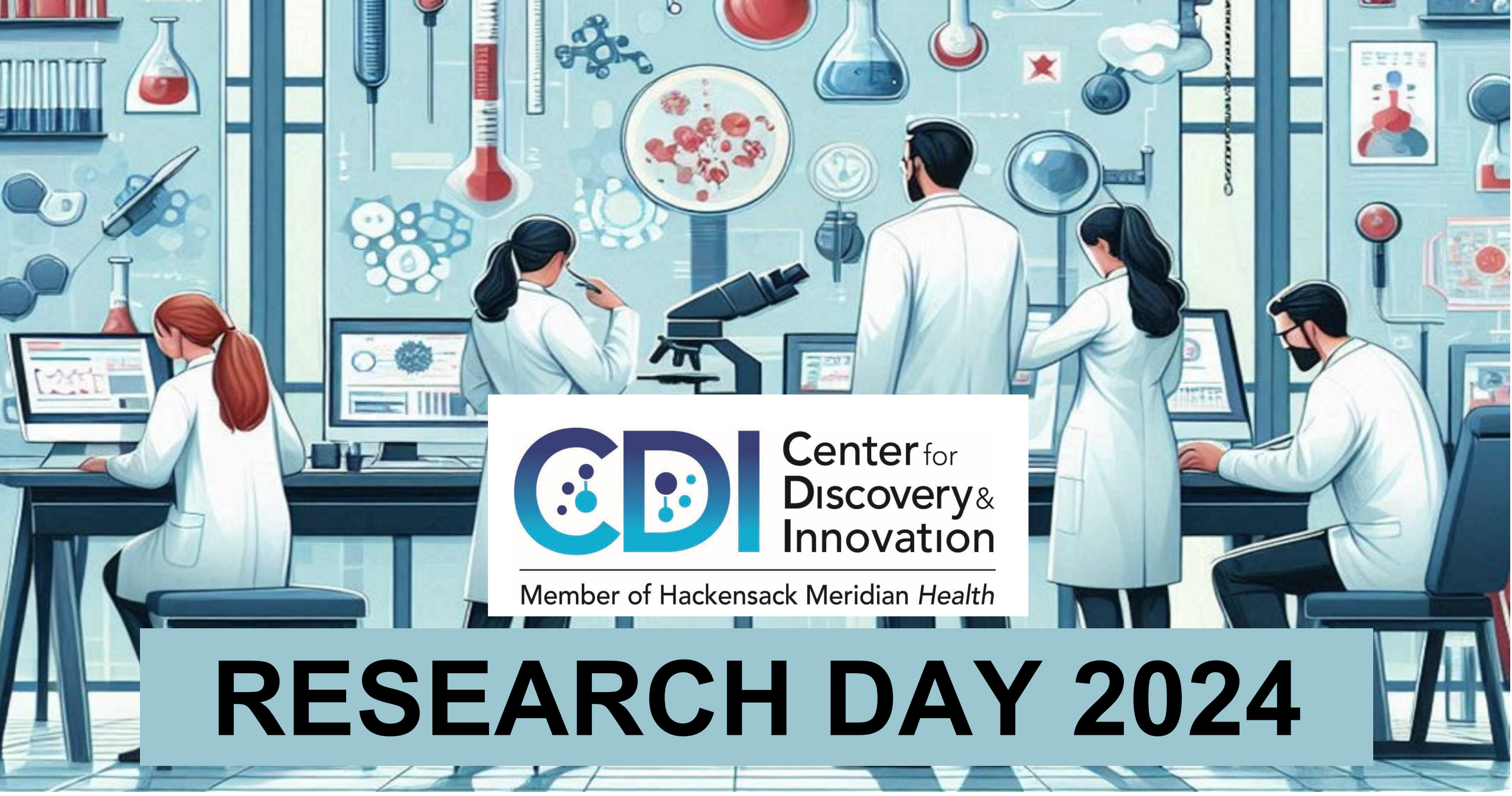

Center for Discovery & Innovation

Member of Hackensack Meridian *Health*

RESEARCH DAY 2024

19 September 2024, Thursday

9:30 am - 6:00 pm

Main Auditorium, Center for Discovery and Innovation

111 Ideation Way, Nutley, NJ 07110 (in-person only)

Keynote Speaker

Dr. Christine Iacobuzio-Donahue, MD, Ph.D

David M. Rubenstein Center Chair for Pancreatic Cancer Research Director, Center for Pancreatic Cancer Research

Evolutionary Biomarkers of Pancreatic Cancer Progression and Outcome

Organizers:

Claire Carter, Ph.D.

Alvin Makohon-Moore, Ph.D.

Erika Shor, Ph.D.

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RESEARCH DAY 2024

19 September 2024, Thursday

Main Auditorium, Center for Discovery and Innovation (in-person only)

9: 00 AM	**9:30 AM**	**BREAKFAST/COFFEE**
9: 30 AM	**9: 45 AM**	**OPENING REMARKS: David S. Perlin., Ph.D., CSO/EVP, CDI**
Oral Presentations | Session 1		
9: 45 AM	**10: 15 AM**	***Immunotherapy of acute myeloid leukemia with a novel trispecific T cell engager targeting cancer-specific non-protein molecular patterns*** 1. Ashley Varkey, D.O.
10: 15 AM	**10: 45 AM**	***Implementing lineage tracing to quantify the clonal evolution of cancer cells*** 2. Yi Zhong, M.D., Ph.D.
10: 45 AM	**11: 15 AM**	***Links between anxiety and symptom burden, quality of life, and patient-clinician alliance among Black patients with advanced lung cancer*** 3. Amanda Khoudary, BA
11: 15 AM	**12: 45 PM**	**POSTER SESSION**
12: 45 PM	**1: 45 PM**	**LUNCH**
Oral Presentations | Session 2		
1: 45 PM	**2: 15 PM**	***Cardiolipin acyl chain remodeling corresponds with altered mitochondrial dynamics and bioenergetics in ETMR*** 4. Evangelos Liapis, Ph.D.
2:15 PM	**2: 45 PM**	***Acute Host Immune Response and Viral Dynamics in SARS-CoV-2 Omicron Subvariant- Infected Transgenic K18-hACE2 Mice*** 5. Vijeta Sharma, Ph.D.
2: 45 PM	**3: 15 PM**	***Human IFN-a2- and PD-L1-modified T cells (ap-T cells) inhibit graft-versus-host disease but retain anti-leukemia activity*** 6. Tian Yuanyuan, Ph.D.
3: 15 PM	**3: 30 PM**	**COFFEE BREAK**
3: 30 PM	**4: 30 PM**	***Evolutionary Biomarkers of Pancreatic Cancer Progression and Outcome*** ***KEYNOTE SPEAKER:*** **Christine Iacobuzio-Donahue, MD., Ph.D.** David M. Rubenstein Center Chair for Pancreatic Cancer Research Director, Center for Pancreatic Cancer Research
4: 30 PM	**4: 45 PM**	**POSTER/ORAL PRIZES & CLOSING REMARKS**
4: 45 PM	**6: 00 PM**	**Wine and Cheese**

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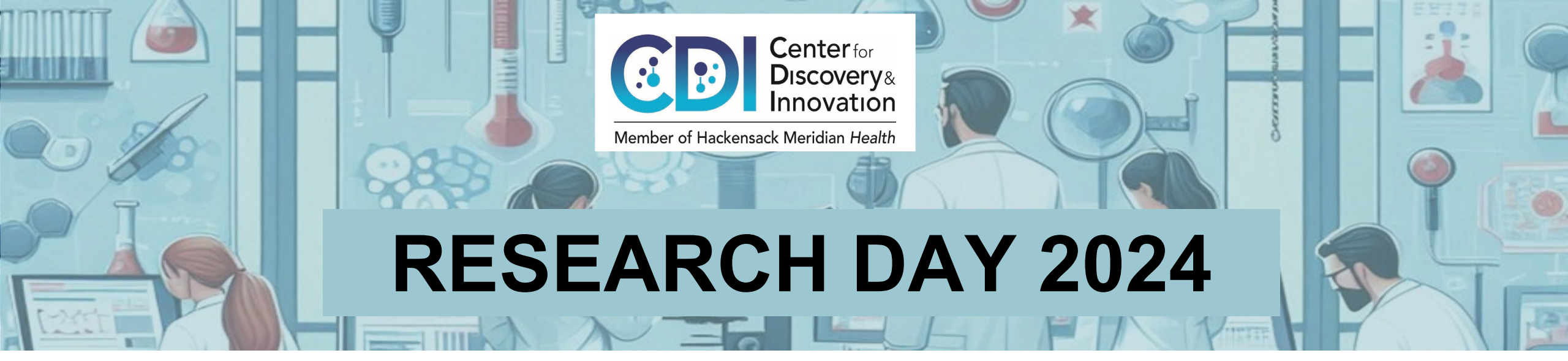

POSTER PRESENTATIONS

Poster #	First Author	Poster Title
1	Narineh Odjourian	Rifabutin central nervous system concentrations in a rabbit model of *Tuberculous meningitis*
2	Alexis Thomas	Unravelling the mechanisms of gyrase inhibitor-mediated killing of nonreplicating persistent *M. tuberculosis*
3	Dhanya Dhanyalayam	Impact of sex differentiated adipomes on regulating foam cell formation in Mtb infection
4	Julianna Cangialosi	A Preclinical Tuberculosis Model for Assessment of Biotin Biosynthesis Targeting Mtb Therapeutics
5	Firat Kaya	Spatial resolution of drug distribution in tissues using Laser Capture Microdissection coupled with LC-MS/MS
6	Irene Gonzalez Jimenez	Study, characterization and development of assays for antiviral drug testing in Respiratory Syncytial Virus
7	Neelam Oswal	A Novel Cellular Tool for Developing Human Pan-Coronavirus Antivirals
8	Nadine Alvarez	Novel Pan-Coronavirus 3CL Protease Inhibitor MK-7845: Biological and Pharmacological Profiling
9	Geselle Cancino-Prado	Multiplex real-time PCR assay for the detection and identification of *Candida auris* and its echinocandin and azole drug resistance markers
10	Sajad Padder	Understanding Drivers of Genome Instability in a Fungal Pathogen: Insights into DNA Double-Strand Break Repair in *Candida glabrata*
11	Raju Shivarathri	Complement C3 promotes sterilizing pulmonary antifungal immunity
12	Wei Hu	Hdac1 promotes effector but limits exhaustion program activation in CD8 + T cell responses to chronic viral infection
13	Ying Wang	Ezh2 and intracellular Ca2+ signals interdependently coordinate GVHD and CAR T cell responses
14	Ying Wang	Dual functions of mitochondrial calcium uniporter in T cell alloimmunity
15	Manpreet Bariana	Multimodal therapy of hematologic malignancies based on targeted drug delivery and photothermal ablation enabled by B cell maturation antigen-directed gold nanoparticles
16	SooJin kim	Uncovering the novel roles of a nuclear envelope protein LAP1 in the development of hepatocellular carcinoma
17	Allison Maas	Ceramide-1-phosphate/CERK are regulators of ETMR growth and offer potential as a new therapeutic target
18	Erin Hirsch	A National Snapshot of Lung Cancer Screening Adherence in Diverse Practice Settings
19	Angelica Castano	Allele-specific methylation mapping and multiplexed CRISPR mutagenesis for identifying functional SNPs in susceptibility loci for cancers and autoimmune/inflammatory diseases
20	Mariam Tariq	RUNX1 shows gain of CpG methylation in gene body sequences and over-expression in human extravillous trophoblast and its gene product affects trophoblast migration and invasion
21	Sanjay Koul	The E2F4 transcriptional repressor is a key mechanistic regulator of colon cancer resistance to irinotecan

Center for Discovery & Innovation

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RESEARCH DAY 2024

Oral Presentation Abstracts

Oral Presentation #1

Immunotherapy of acute myeloid leukemia with a novel trispecific T cell engager targeting cancer-specific non-protein molecular patterns

Ashley Varkey[1,2], Manpreet Bariana[2,3], Elena Cassella[2], Shaina Anuncio[2], Mark Batistick[3], Sonia Sequeira[4], Mahiuddin Ahmed[4] and Johannes Zakrzewski[1,2,3,5]

1. Department of Pediatrics, Hackensack University Medical Center, Hackensack, NJ
2. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ
3. Hackensack Meridian School of Medicine, Nutley, NJ
4. Vitruviae LLC, Nutley, NJ
5. Department of Oncology, Georgetown University, Washington DC

Abstract

Non-protein molecular structures such as carbohydrates or lipids have the advantage of not being subject to some of the common mechanisms of drug resistance development such as genetic mutation or downregulation. Our study introduces the aberrant sugar mannose 9 (Man9) and aberrantly exposed phosphatidylserine (PS) as promising targets for immunotherapy of acute myeloid leukemia (AML). Our biotech partner Vitruviae developed fully synthetic receptor decoy molecules utilizing the lectin receptor DC-SIGN for Man9 binding and T cell immunoglobulin and mucin domain 1 (TIM-1) for PS binding. In flow cytometric assays utilizing these DC SIGN and TIM-1 derived homodimeric as well as heterodimeric molecules, we demonstrated highly specific binding to AML cells (nine AML cell lines and eight samples of newly diagnosed AML patients) while sparing healthy cells such as peripheral blood mononuclear cells. Glycan microarray confirmed that the therapeutic molecules were able to bind to several abnormal glycans but to none of the glycans found in healthy cells. We next created a molecule with high affinity to Man9, PS and human CD3 thus functioning as a trispecific T cell engager directed against Man9 and/or PS positive cancers while lacking affinity to healthy cells. Initial toxicology studies in immunocompetent mice (including human CD3 transgenic mice) showed that high doses of the molecule (up to 1.6 mg/kg) were well tolerated. In vitro efficacy studies (coculture of luciferase transduced target cells with activated CD8+ T cells in the presence and absence of the T cell engager) demonstrated excellent antileukemia activity of the molecule against THP-1 and SET-2 AML cell lines with an IC50 in the 5-10 pM range. We recently conducted a preliminary in vivo efficacy study to evaluate C1498 AML progression in immunocompetent human CD3 transgenic mice treated with three intravenous doses of VP320 and observed an impressive response rate of 66% based on tumor burden assessment by in vivo bioluminescence imaging. In conclusion, we validated two highly promising non-protein targets for AML immunotherapy and developed innovative T cell engager-based Man9/PS dual targeting strategies characterized by the absence of on-target off-tumor toxicity and antigen negative relapse potential. Our anticipated timeline to IND is 18 months with the goal to accelerate clinical development of a first-in-class Man9 x PS x CD3 trispecific T cell engager and ultimately expand access of AML patients to safe and effective immunotherapies.

Oral Presentation #2

Implementing lineage tracing to quantify the clonal evolution of cancer cells

Yi Zhong[1], Alvin Makohon-Moore[1,2]

1. Hackensack Meridian *Health* Center for Discovery and Innovation.
2. Department of Medical Sciences, Hackensack Meridian School of Medicine.

Abstract

Cancer is an evolutionary process characterized by genetic and phenotypic changes that drive disease progression, treatment resistance, and metastasis. Traditional bulk sequencing approaches provide limited insights into the clonal dynamics and heterogeneity within tumors. Our lab aims to implement a comprehensive approach combining deep and precise lineage tracing with multi-omics to monitor tumor evolution at high resolution, enabling the identification of key molecular events and cellular interactions driving tumor progression and therapeutic response. To determine the number of clones we could reliably detect, we exhaustively quantified the barcode diversity of cancer cells across diverse tumor types, finding that both the number and abundance of clones varied across cell lines. To verify barcode sequences and validate clones, we used single cell cloning and barcode sequencing to ultimately detect both relatively common and rare clones. This is important for robustly defining the clonal diversity of each cancer cell population to be evolved with bioreactor technology in a controlled microenvironment, allowing us to gain insights into the impact of the tumor microenvironment and cellular interactions on clonal evolution and treatment response.

Oral Presentation #3

Links between anxiety and symptom burden, quality of life, and patient-clinician alliance among Black patients with advanced lung cancer

Amanda Khoudary[1], Jennifer D. Rodriguez[2], Aishwarya Sridhar[3], Martin Gutierrez[4], Chul Kim[2], Irina Veystman[5], Jennifer Wheeley[3], Claire C. Conley[2], Heather Derry-Vick[1,3]

1. Cancer Prevention Precision Control Institute, Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ
2. Georgetown Lombardi Comprehensive Cancer Center, Washington, DC
3. Hackensack Meridian School of Medicine, Nutley, NJ
4. John Theurer Cancer Center, Hackensack, NJ
5. MedStar Washington Hospital Center, Washington, DC

Abstract

Background

Black patients with lung cancer (LC) experience more unmet supportive care needs for symptoms and distress than non-Hispanic white patients. Prior research suggests distress may exacerbate physical symptoms and complicate clinical interactions. We examined the rate of supportive care service use and the impact of anxiety/depression on symptom burden, health-related quality of life (HRQoL), and patient-clinician alliance among Black advanced LC patients.

Methods

Black adults (n=30) receiving care for advanced LC reported sociodemographic characteristics, anxiety and depression symptoms (Generalized Anxiety Disorder, GAD-2, Patient Health Questionnaire, PHQ-2), and patient-clinician alliance (The Human Connection Scale). After an oncology appointment, participants reported symptom burden (Edmonton Symptom Assessment Scale). One month later, participants reported HRQoL (Functional Assessment of Cancer Therapy (FACT)-General 7-item & FACT-Lung). Research staff collected clinical characteristics from medical charts. We used independent-sample t-tests to determine whether symptom burden, HRQoL, and therapeutic alliance differed between those with any vs. no anxiety/depression symptoms.

Results

Participants were between ages 48 and 81 (mean = 65 years), mostly female (63%), and were diagnosed with Stage IV (73%), non-small cell LC (90%), within the past year (67%). Of the 8 participants with clinically-significant anxiety or depression (GAD-2 or PHQ-2 ≥ 3), 2 (25%) received mental health services and 1 (13%) received palliative care services. Patients with anxiety symptoms reported more intense physical symptoms (p=.02), lower overall HRQoL (p=.004), LC-specific HRQoL (p=.01), and weaker alliance with their oncologist (p=.02) than those without anxiety symptoms. Depression was not significantly associated with symptom burden, HRQoL, or therapeutic alliance (p-values>.23).

Conclusion

These findings expand our understanding of co-occurring psychological and physical symptoms experienced by Black LC patients. Anxiety may contribute to increased symptom burden, reduced HRQoL, and weaker patient-clinician alliances. This study illustrates a need to promote supportive care service use among Black advanced LC patients with psychological symptoms.

Oral Presentation #4

Cardiolipin acyl chain remodeling corresponds with altered mitochondrial dynamics and bioenergetics in ETMR

Evangelos Liapis [1] , Allison Maas [1] , Kelly C. O'Neill [1] , Annapurna Pamreddy [1] , Francesca M. Cozzi [1] , Brent T. Harris [2] , Derek Hanson [1,3,4] , Claire L. Carter [1,5]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, New Jersey, 07110
2. Departments of Neurology and Pathology, Georgetown University Medical Center, Washington D.C. 20007
3. Joseph M. Sanzari Cancer Center, Hackensack University Medical Center, Hackensack, New Jersey, 07061
4. Department of Pediatrics, Hackensack Meridian School of Medicine, Nutley, New Jersey, 07110
5. Department of Pathology, Hackensack Meridian School of Medicine, Nutley, New Jersey, 07110

Abstract

Embryonal tumor with multilayered rosettes (ETMR) is a highly aggressive CNS neoplasm that occurs almost exclusively in infants and is associated with an extremely poor prognosis. Dysregulated mitochondrial bioenergetics and dynamics have been associated with the initiation and progression of diverse cancers and studies have linked metabolic rewiring to chemotherapy resistance. Cardiolipins are mitochondrial-specific lipids that reside in the mitochondrial membrane and their fatty acid composition has been extensively shown to regulate mitochondrial function and dynamics. Despite the known functional significance of cardiolipins, their role in mitochondrial regulation of brain tumors remains ill-defined. Using mass spectrometry imaging, we identified a shift to shorter acyl chain cardiolipins within the rapidly proliferating embryonal tumor cells in patient samples and patient-derived cell lines grown as 3D tumorspheres. Western blot analysis of the enzymes involved in cardiolipin synthesis and remodeling identified a significant increase in the expression of the cardiolipin remodeling enzyme, LCLAT1/ALCAT1, when compared to neural stem cells (NSCs). Orthogonal imaging techniques including immunohistochemistry, transmission electron microscopy and super-resolution microscopy correlated shorter acyl chain remodeling of cardiolipin with fragmented mitochondria (increased fission) and aberrant cristae structure. Further studies identified increased expression of the fission protein Drp1, decreased expression of respiratory chain enzymes and a more glycolytic phenotype in the embryonal tumor cells. Therapeutic targeting of the mitochondrial phenotype in ETMR resulted in selective inhibition of growth and viability.

Oral Presentation #5

Acute Host Immune Response and Viral Dynamics in SARS-CoV-2 Omicron Subvariant-Infected Transgenic K18-hACE2 Mice

Vijeta Sharma [1], Enriko Dolgov [1], Alberto Rojas-Triana [1], Taylor Tillery [1], Camila Mendez-Romero [1], DianaM VillalbaGuz [1], Nadine Alvarez [1], Steven Park [1], Andrew M. Nelson [1], and David S. Perlin [1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA

Abstract

The emergence of Coronavirus disease 2019 (COVID-19) caused by the SARS-CoV-2 virus resulted in a staggering 773 million cases and over 7 million deaths have been reported worldwide (WHO, December 2023). Throughout the pandemic, variants of concern (VoC) such as Alpha, Delta, and Omicron emerged. These variants, particularly Omicron, often have numerous mutations in the spike protein, enabling them to evade immunity and enhance transmissibility. The continual emergence of new omicron variants like BF.5 and BQ.1 displaying Delta-like features, underscores the need for ongoing research. Understanding this rapidly changing virus is crucial to developing effective countermeasures. Characterizing these variants in animal models is essential for preclinical COVID-19 research. Our study used K18- hACE2 transgenic mice, which express the viral entry receptor human Angiotensin-Converting Enzyme 2 (hACE2), to compare the viral infection of VoCs BF.5 and BQ.1 alongside the parent lineage SARS-CoV-2 USA-WA strain up to 4 days post infection (dpi). Omicron subvariant infections exhibited milder clinical signs like body weight loss and altered infection kinetics in mice lung viral burdens compared to the parent strain. Further analysis revealed a temporal shift in peak lung viral burden (7-8 log TCID 50 /g lung tissue, 2 dpi) following infection with BF.5 and BQ.1 subvariants. By 4 dpi, these sub variants exhibited a 2-3 log reduction in viral burden compared to the parent strain, suggesting faster viral clearance. To elucidate the diverse host immune response to different Omicron subvariant infections within the lung, we assessed pro- inflammatory mediators tissue levels. Early antiviral host immune responses revealed elevated levels of pro-inflammatory cytokines and chemokines (IL-6, CXCL10, MCP-1, CCL3, TNF-α) that coincided with peak viral burdens. These mediators have been implicated in lung inflammation, tissue damage, and the development of severe disease, such as ARDS, in COVID-19. Intriguingly, while IL-10 typically counteracts inflammation, its increased levels suggest a complex interplay of inflammatory and anti-inflammatory responses. This immune profile is consistent with those reported in the acute phase of COVID-19 disease patients. The severity of disease was further explored through histopathological changes in lung structure. Lung tissue analysis (H&amp,E stain) showed inflammation and apoptotic regions (TUNEL staining) in perivascular, bronchiolar, and alveolar regions with altered viral staining in subvariant infected lung tissue. These findings significantly contribute to understanding Omicron subvariant-specific host immune responses, paving the way for developing targeted diagnostics, therapeutics, and clinical management strategies.

Oral Presentation #6

Human IFN-a2- and PD-L1-modified T cells (ap-T cells) inhibit graft-versus-host disease but retain anti-leukemia activity

Yuanyuan Tian 1# , Bei Jia 2# , Che Young Lee 1 , Qingrong Huang 1 , Chenchen Zhao 2 , Ciril Abraham 1 , Wenbin Mo 1 , Mimi Chen 1 , Ying Wang 1 , Hong Zheng 2 , Yi Zhang 1

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA
2. Penn State Cancer Institute, Penn State University College of Medicine

Abstract

Current strategies to prevent graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT) primarily utilize pan-immune suppressive agents with significant limitations. Cellular therapies offer targeted options but face challenges in purification, expansion, and efficacy, necessitating new approaches. Our previous studies and others have shown that adoptive transfer of murine plasmacytoid dendritic cells (pDCs) can suppress alloreactive T cell responses and GVHD through IFN-a and PD-L1. We therefore hypothesize that delivering immune suppressive molecules derived from human pDCs using alternative hematopoietic cells could lead to the development of a novel living cell therapy for inhibiting GVHD. We report here that IFN-a2a- and PD-L1-overexpressing human T cells (ap-T cells) acquire potent capacity to inhibit xenogeneic GVHD (x-GVHD) but preserve the ability to eliminate human xenograft leukemia in NSG mice. Our initial studies showed that lower levels of pDCs in donor G-CSF-mobilized allografts correlated with a significantly higher risk of severe GVHD in allo-HSCT patients. Human pDCs expressed high levels of IFN-□ and PD-L1, and dose-dependently suppressed expansion and survival of TCR-activated autologous CD4 + T cells in cultures. Next, we generated lentivirus encoding IFN-α2a and PD-L1 to transduce human T cells, creating αp-T cells to assess their potential immune suppressive function. Compared to vector control, adoptive transfer of these ap-T cells significantly inhibited x-GVHD with a notable reduction in total donor T cells and IFN-g + effector CD4 + T cells in peripheral blood by day 46 after transplantation, as well as donor CD4 + T cells in the bone marrow and spleen at the study&#39,s endpoint. This was accompanied with increased cell death and elevation of PD-1 + TIM3 + terminal exhaustion-like CD4 + T cells in ap-T cell groups. Importantly, treatment by co-transfer of ap-T cells and vector control T cells eliminated human xenograft leukemia in NSG mice challenged by human Raji cells without causing x-GVHD. Notably, ap-T cell treatment increased the frequency of IFN-g + CD8 + T cells. Our findings identified that treatment with human ap-T cells may represent a new and clinically relevant cell therapy strategy to reduce GVHD while preserving potent graft-versus-leukemia effects.

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RESEARCH DAY 2024

Poster Presentation Abstracts

Poster Presentation #1

Rifabutin central nervous system concentrations in a rabbit model of Tuberculous meningitis

Narineh Odjourian[1], Melissa Cristaldo[1], Sean Wasserman[2,3], Rosleine Antilus-Sainte[1], Noha Abdelgawad[4], Maureen Dougher[1], Firat Kaya[1], Matthew Zimmerman[1], Paolo Denti[4], and Martin Gengenbacher[1,5]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, New Jersey, USA
2. Institute for Infection and Immunity, St. George's, University of London, London, United Kingdom
3. Center for Infectious Diseases Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa
4. Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa
5. Hackensack Meridian School of Medicine, Nutley, New Jersey, USA

Abstract

In a small portion of patients with pulmonary tuberculosis, *Mycobacterium tuberculosis* crosses the blood brain barrier to infect central nervous system (CNS) tissues resulting in a severe condition called tuberculous meningitis (TBM), the most severe form of tuberculosis. The mortality rate of TBM is high and often associated with permanent disability. The current chemotherapy regimen for TBM is the standard for pulmonary TB (rifampin, isoniazid, pyrazinamide, and ethambutol), despite the vastly different challenges posed by an infection of the CNS. For most drugs it's unknown whether they penetrate CNS tissues sufficiently to achieve therapeutic concentrations required to kill the pathogen. Drug exposure data at the site of disease are required to develop more effective regimens. New approaches which combine agents with potent antituberculosis activity and enhanced CNS penetration would allow for more effective treatment of TBM. We developed a rabbit model of TBM to study drug exposure in detail. Rifabutin is a rifamycin drug equally potent to rifampin, a core pillar of antimycobacterial chemotherapy. Here, we show that human-equivalent doses of rifabutin achieved potentially therapeutic exposure in relevant CNS tissues in our rabbit model of TBM, supporting further evaluation in clinical trials.

Poster Presentation #2

Unravelling the mechanisms of gyrase inhibitor-mediated killing of nonreplicating persistent M. tuberculosis

Alexis Thomas[1], Priyanka Aswath[1], WenShan Tsao[1], Jansy Sarathy[1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA

Abstract

Tuberculosis is the leading cause of mortality by an infectious agent worldwide. The necrotic debris, known as caseum, that accumulates in the center of pulmonary lesions and cavities contains nonreplicating drug-tolerant Mycobacterium tuberculosis that presents a significant hurdle to achieving fast and durable cure. Fluoroquinolones (FQ) such as moxifloxacin are highly effective at killing this non-replicating persistent bacterial population and boosting M. tuberculosis lesion sterilization. FQ target bacterial DNA gyrase, which catalyzes the negative supercoiling of DNA and relaxes supercoils ahead of replication forks. In preliminary experiments, we compared FQ to several other antibacterial gyrase inhibitors and learned that all non-FQ compounds tested had negligible bactericidal activity against persistent M. tuberculosis in ex vivo caseum. We hypothesize that DNA double strand break (DSB) formation, chromosomal fragmentation and reactive oxygen species (ROS) surge, all of which are induced by FQ treatment, are important drivers of bactericidal potency against this persistent population. Non-FQs often bind to different sites on the enzyme and do not stimulate the formation of DNA-enzyme-drug cleavage complexes. To investigate our hypothesis, we are optimizing methods for the detection of DNA breaks and ROS accumulation in slow growing mycobacteria. We are performing Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) to detect DNA fragmentation in drug treated cells. We are using a variety of fluorogenic dyes such as CellROX far-red to detect the intracellular accumulation of several ROS species. H37Ra, an attenuated M. tuberculosis strain, was used in preliminary experiments. Using flow cytometry, we have demonstrated that treatment with moxifloxacin leads to an increase in TUNEL-positive cells and higher levels of H_2O_2 accumulation in replicating H37Ra cultures.

Poster Presentation #3

Impact of sex differentiated adipomes on regulating foam cell formation in Mtb infection

Dhanya Dhanyalayam[1], Hariprasad Thangavel[1], Kezia Lizardo[1], Jyothi Nagajyothi[1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA

Abstract

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), is the leading global infectious killer. Research in humans and murine models highlights significant sex differences in TB severity, with males often experiencing more severe lung pathology and higher mortality rates than females. Our previous studies identified sex-based differences during the sub-acute stage of infection (30 days post-infection, DPI), where male mice exhibited increased lipid droplet accumulation and apoptosis in the lungs. This was associated with a marked reduction in lipophagy-related gene transcripts, higher colony-forming unit (CFU) counts, and reduced populations of CD45 + cells and macrophages, which are crucial for lung defense against Mtb. Additionally, males showed significant weight and body fat loss, linked to an increased bacterial burden in the lungs. Despite these observations, the mechanisms connecting body fat loss to lung pathology remain unclear. Our recent research suggests that during acute body fat loss, adipocytes release adipomes that modulate various cell types, including macrophages. Given the fat loss observed in Mtb-infected males, we hypothesized that adipomes from male and female mice may differentially regulate macrophage functions, particularly in lipophagy/autophagy and lipogenic gene expression, contributing to foamy macrophage formation and Mtb survival. To test this, we treated RAW macrophages with adipomes from Mtb-infected male and female mice and conducted transcriptomic analysis. RNA sequencing revealed significant downregulation of lipophagy-related genes in macrophages treated with male-derived adipomes compared to female-derived ones. RT-PCR confirmed these findings, showing decreased lipophagy and increased lipogenic transcripts in male-treated macrophages. Bodipy staining further validated the increased lipid droplet accumulation. Lipidomic analysis of adipomes suggests that the lipid cargo biomolecules differ between males and females, potentially driving the observed differences in macrophage regulation. This finding warrants further investigation. Overall, our results highlight sex-dependent differences in how adipomes influence macrophage functions, providing new insights into the mechanisms underlying TB pathology.

Poster Presentation #4

A Preclinical Tuberculosis Model for Assessment of Biotin Biosynthesis Targeting Mtb Therapeutics

Julianna Cangialosi[1], Camilla Folvar[1], Betelhem Tatek[1], Firat Kaya[1], Suyapa Rodriguez, Sindhuja Paruchuri[1], Matthew Zimmerman[1], Courtney Aldrich[2], Qiang Liu[2], Veronique Dartois[1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA
2. Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN, USA.

Abstract

The global TB health crisis necessitates new drugs with novel targets to shorten the treatment period of the current standard of care drug regimens and to find new treatments for drug resistant tuberculosis. 7,8-diaminopelargonic acid synthase (BioA) is an essential enzyme for Mtb biotin synthesis and required for Mtb survival in-vitro in biotin free media. A BioA targeting drug program at the University of Minnesota in collaboration with CDI resulted in the promising antibiotic C111. C111 has a potent MIC50 (0.051μM) against Mtb H37Rv in biotin free GAST media and a favorable oral mouse pharmacokinetic profile. At CDI, subsequent evaluation of C111 for in vivo anti-tubercular activity against H37Rv strain of Mtb in an acute Balb/c mouse efficacy study surprisingly demonstrated no in-vivo efficacy after C-111 treatment. The poor in-vitro to in-vivo correlation was attributed to high levels of endogenous biotin in mouse serum, which have been shown to eliminate the requirement for de-novo synthesis in Mtb. Given the significantly higher levels of biotin in mouse serum (10.6 ng/mL) relative to human serum (0.1-0.8 ng/mL), we aim to evaluate the efficacy of C111 in a model with translatable biotin levels. In the current study biotin depleting chow containing 30% egg white which is enriched with avidin (a glycoprotein that binds with biotin) was given to mice 7 days prior to Mtb infection to lower serum biotin to a humanized level of 0.35 ng/mL. This diet was well tolerated through the entire 42 day efficacy study and resulted in similar in-vivo H37Rv growth kinetics as the mice with biotin supplemented chow. This model will be applied to determine the in-vivo efficacy of C111 and to broadly assess if Mtb biotin synthesis is essential in-vivo and is a promising pathway to leverage for new therapies.

Poster Presentation #5

Spatial resolution of drug distribution in tissues using Laser Capture Microdissection coupled with LC-MS/MS

Firat Kaya [1] , Jacqueline Ernest [2] , Matthew Zimmerman [1] , Rada Savic [2] , Veronique Dartois [1] , Jansy Sarathy [1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA.
2. Department of Bioengineering and Therapeutic Sciences, Schools of Pharmacy, University of California, San Francisco, CA, USA.

Abstract

Introduction:

Laser capture microdissection (LCM) is an advanced and precise technique utilized in molecular biology and pathology for isolating specific cells or tissue regions from a heterogeneous specimen under microscopic visualization. This capability facilitates detailed genetic, proteomic, and transcriptomic analyses of the isolated cell populations. Chronic tuberculosis (TB) disease is defined by diverse pathological manifestations and bacterial phenotypes. Closed necrotic nodules and cavities contain caseum that harbors nonreplicating drug-tolerant Mycobacterium tuberculosis (MTB). Targeting these nonreplicators is critical to achieving a faster and durable cure. Drug distribution at the site of action is appreciated as a major determinant of TB treatment success. By coupling LCM to LC-MS/MS, we can quantify drug exposure in distinct compartments of TB granulomas, thereby informing translational models for the prediction of clinical drug efficacy.

Method:

Single- or multiple doses of anti-TB drugs were administered to TB-infected New Zealand White rabbits, and necropsies were performed after specific time intervals. Lung lesions were collected, frozen and cryo-sectioned (25 µm slices) in a BSL3 suite to perform H&amp,E staining and LCM. A concentric ring dissection method was applied to all the samples to collect separate specimens of uninvolved lung, cellular rim, and sequential rings of caseous tissue. All extracted samples were analyzed by LC-MS/MS outside of BSL3 containment. Modeling analysis of all quantitative PK data was performed at the University of California San Francisco.

Results:

The LCM-LC/MS workflow was applied to the study of bedaquiline&#39,s (BDQ) temporal and spatial distribution in rabbit TB granulomas. BDQ accumulates only at the caseum-cellular rim interface after a single dose, but partitions deeper into the caseous core after steady-state dosing, thereby killing nonreplicating MTB persisters within. Furthermore, BDQ exposure is sustained in caseum beyond 2 years after the end of treatment, a phenomenon that is unique to this class of compounds.

Poster Presentation #6

Study, characterization and development of assays for antiviral drug testing in Respiratory Syncytial Virus

Irene Gonzalez[1], Risha Rasheed[1], Kira Goldgirsh[1], Steven Park[1], Nadine Alvarez[1], David S. Perlin[1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA

Abstract

Respiratory syncytial virus is the most common cause of acute lower respiratory tract infections (LRTI) in the first stages of children&#39,s life. First isolated from chimpanzees in 1956, RSV belongs to the Paramixoviridae family and is composed of an enveloped single RNA strand that translates to 11 proteins. Humans, which are the only known reservoir, can get infected by close contact, inoculation or secretions, especially during late fall or winter, when the peak of infection usually takes place. Clinical manifestations can range from mild to severe, being bronchiolitis and pneumonia the most common clinical outcomes, resulting in a 40-90% and 50% of hospitalizations in infants between 2-6 months, respectively. Although the infection rate is very high, the deaths remain low, but symptomatic reinfections can occur in the elderly and immunocompromised patients, compromising their health. Currently, there are limited prevention and treatment options for RSV. The first vaccine for RSV prevention in neonates and infants, Byfortus (nirsevimab-alip), has been recently approved in Europe, supported by studies that showed a reduction of 70% in LRTI and almost an 80% in hospitalizations in infants that received the dose before the RSV season started. In addition, ribavirin is the only antiviral drug approved by the FDA for treatment of RSV, however, its efficacy remains very low, and its use is only suggested in high-risk patients. Two more monoclonal antibodies are approved but its efficacy is not better than ribavirin. With its elevated infection rate in children, finding new drugs or treatments that target this virus becomes an important matter in global health care. In this study, we explore the potentialities of an RSV strain expressing green fluorescent protein (rgRSV224), as a tool for the in vitro characterization of novel antiviral candidates.

Poster Presentation #7

A Novel Cellular Tool for Developing Human Pan-Coronavirus Antivirals

Neelam Oswal[1], Ching-Wen Chang[1], Madhuvika Murugan[1], Steven Park[1], David S. Perlin[1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA

Abstract

We have developed a newly engineered A549-based cell model to advance the study of human pan-coronaviruses (pan-hCoVs). This cell model, which expresses ACE2 (for SARS-CoV2), DPP4 (for MERS-CoV), and TMPRSS2 (a coreceptor) receptors, is highly susceptible to a wide range of coronaviruses including MERS-CoV, SARS-CoV-2, and seasonal coronaviruses like 229E and OC43. This broad susceptibility makes it an effective tool for studying and measuring the potency, efficacy, breadth of antiviral drugs against various coronaviruses.

Key highlights of this cell model include:

Validation with clinical trial and FDA approved drugs: The model has been validated using known antiviral compounds targeting SARS-CoV-2 main protease (Mpro) such as Nirmatrelvir, Pomotrelvir and Ensitrelvir. These Mpro inhibitors are known to inhibit SARS-CoV-2 replication, but their activity against other coronaviruses is yet to be determined.

Versatility for Drug Testing: The cell line supports the replication of both lethal and seasonal coronaviruses as well as virus-induced cytopathic effect (CPE), allowing researchers to assess drug efficacy across different coronaviruses.

High Throughput Screening: The cell model was used to screen 160 antiviral compounds from the pandemic response box library, leading to the identification of several novel compounds that effectively inhibited SARS-CoV-2 replication.

Potential for Novel Drug Development: The ability to efficiently support high throughput screening and the identification of new antiviral compounds underscores the model's potential for accelerating drug discovery and development for pan-coronavirus therapies.

Overall, this cell line is poised to be a valuable asset for research and development in the field of coronavirus therapeutics, facilitating the discovery of new drugs and improving our ability to combat a wide range of coronavirus infections.

Poster Presentation #8

Novel Pan-Coronavirus 3CL Protease Inhibitor MK-7845: Biological and Pharmacological Profiling

Nadine Alvarez [1] , Gregory C. Adam [2] , Vijeta Sharma [1] , John Howe [2] , Steven Park [1] , Andrew Nelson [1] , Enriko Dolgov [1] , Matthew D. Zimmerman [1] , Risha Rasheed [1] , Valerie W. Shurtleff [2] , Xiaoyan Zhou [2] , Fatima Nizar [1] , Khushboo Sahay [1] , Christine Burlein [2] , John F. Fay [2] , Daniel V. Iwamoto [2] , Carolyn M. Bahnck-Teets [2] , Krista L. Getty [2] , Shih Lin Goh [2] , Imad Salhab [2] ,Keith Smith [2] , Christopher W. Boyce [2] , Tamara D. Cabalu [2] , Nicholas Murgolo [2] , Nicholas G. Fox[2] , Todd W. Mayhood [2] , Mark E. Layton [2] , Craig A. Parish [2] , John A. McCauley [2] , David B. Olsen [2] ,David S. Perlin [1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA
2. Merck & Co., Inc., Rahway, NJ 07065, USA

Abstract

Despite vaccination efforts and the existence of a few treatment options, SARS-CoV-2 remains to be a global health threat due to its ability to evolve and generate new subvariants, leading to new waves of infection. Other coronaviruses like Middle East respiratory syndrome coronavirus (MERS-CoV), represent a persistent additional threat of severe illness to humans. The potential genetic recombination between different strains and the possibility of a new subvariant with higher transmissibility and infectivity, as well as a new class of coronaviruses, pressured the need for the discovery of pan-CoV therapeutic drugs and updated vaccines. Here we present MK-7845, a novel 3CLPro inhibitor discovered through an extensive optimization of an HCV protease inhibitor screening hit. MK-7845 exhibited nanomolar in vitro potency with broad spectrum activity against clinically important human coronaviruses (hCoVs), SARS-CoV-2 and MERS-CoV. Oral dose escalation pharmacokinetic studies in CD-1 mice and therapeutic drug monitoring in infected mice demonstrated highly desirable pharmacologic properties for MK-7845, with a significant oral exposure and plasma concentrations above the PBA EC90. Furthermore, when administered orally, MK-7845 demonstrated a notable reduction in viral burdens by >6 log orders in the lungs of transgenic mice infected with SARS-CoV-2 (K18-hACE2 mice) and MERS-CoV (K18-hDDP4 mice), which equaled or surpassed current approved antiviral agents. Importantly, MK-7845 was highly efficacious without the need for boosting by inhibiting CYP3A4, as is the case with nirmatrelvir. This new class of pan-coronavirus antiviral has important value in addressing the ongoing COVID-19 disease as well as pandemic preparedness for future coronavirus pandemics. The development of drug candidate was part of a multi-year drug discovery partnership between our group and Merck, as part of the NIH national Antiviral Drug Discovery (AViDD) Centers for Pathogens of Pandemic Concern. The goal of this collaboration is to develop a next generation of antivirals with pan-
coronavirus activity.

Poster Presentation #9

Multiplex real-time PCR assay for the detection and identification of Candida auris and its echinocandin and azole drug resistance markers

Geselle Cancino-Prado[1], Milena Kordalewska [1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA

Abstract

OBJECTIVES

Candida auris, a multidrug-resistant fungus, poses a significant global health threat due to its capacity for invasive infections, persistent colonization of human skin and healthcare environments, and hospital outbreaks. *C. auris* clinical isolates often show resistance to azole and echinocandin antifungal drugs, limiting treatment options and resulting in high mortality. Moreover, many laboratories misidentify *C. auris* due to reliance on phenotypic platforms. Here, we developed a molecular-based assay for rapid and accurate detection/identification of *C. auris* and validated mutations conferring resistance to azole and echinocandin drugs.

METHODS

Our *C. auris* diagnostic assay uses triplex asymmetric real-time PCR with allele-specific molecular beacons (MB) labeled with different fluorophores, followed by the melting curve analysis. The assay includes: 1) C. auris species identification using FAM-labeled MB targeting rDNA, 2) Identification of echinocandin resistance-conferring mutations using HEX-labeled MB targeting FKS1 hot-spot 1 (HS1), 3) Identification of azole resistance-conferring mutations using Cy5-labeled MBs targeting ERG11. The specificity of the assay was validated using DNA samples from 37 clinical fungal isolates including 26 *C. auris* of different geographic origins and drug resistance profiles.

RESULTS

We detected and identified *C. auris* isolates with 100% accuracy in the developed *C. auris*-specific assay utilizing FAM-MB. Moreover, in FKS1 HS1 (HEX-MB) and ERG11 (Cy5-MB) real-time PCRs, signature melting profiles and corresponding Tm values were generated for different mutants compared to wild-type strains.

CONCLUSION

We have developed a single-tube triplex real-time PCR assay for *C. auris* diagnostic, enabling simultaneous detection and identification of *C. auris* species and FKS1 and ERG11 mutations associated with echinocandin and azole resistance, respectively. Our assay can be established as a rapid (total turnaround time including setup and results analysis &lt,2 h), highly precise and easily interpretable diagnostic platform that overcomes the limitations of current in vitro tests and guides appropriate antifungal treatment selection.

Poster Presentation #10

Understanding Drivers of Genome Instability in a Fungal Pathogen: Insights into DNA Double-Strand Break Repair in Candida glabrata

Sajad Padder [1], David S. Perlin [1], Erika Shor [1]*

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA

Abstract

Candida glabrata is a yeast species commonly found in the human microbiota, particularly in the gastrointestinal and genitourinary tracts, and an opportunistic pathogen causing life-threatening infections in immunocompromised individuals. *C. glabrata* poses a substantial challenge in healthcare settings due to its increasing resistance to antifungal drugs. Drug resistance in this pathogen is typically caused by genetic variations that confer resistance to the very limited available frontline antifungal agents. *C. glabrata* is also notable for its incredible genetic diversity, both in terms of chromosome structure and DNA sequence (SNPs), which is regarded as a major contributing factor to the rapid evolution of drug-resistant variants. However, the drivers of genetic instability in this pathogen are yet unknown. One of the major sources of genetic instability in biological systems is DNA double strand breaks (DSBs), which facilitate chromosome rearrangements and are associated with high rates of mutations in the nearby regions. Despite its close relatedness with Saccharomyces cerevisiae, we and others have shown that *C. glabrata* responds to DNA damage differently. In particular, the former prefers homologous recombination to repair DNA breaks, while the latter does this predominantly by non-homologous end joining. In this context, we aim to determine the mechanistic basis of genetic diversity in C. glabrata by studying the DNA DSBs (their formation and their repair mechanisms) in this organism. To this end, we are using a Tet-on expression system, where we conditionally induce site-specific DNA DSBs through the expression of Cas9 or NotI endonucleases upon doxycycline treatment. We then track DSB formation and repair using qPCR and the appearance of mutations at the DSB sites using phenotypic assays and DNA sequencing. In my poster, I will be describing in detail the strategy of our conditional gene expression system, its efficiency in inducing DSBs, and our future plans for using this system to elucidate the mechanisms of DNA DSB repair in *C. glabrata.*

Poster Presentation #11

Complement C3 promotes sterilizing pulmonary antifungal immunity

Raju Shivarathri[1] , Adela Karuli[1] , Jigar V Desai[1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA

Abstract

Invasive pulmonary aspergillosis (IPA) is a deadly fungal infection caused by Aspergillus spp. with annual mortality rates exceeding 40% and over half a million cases globally despite concurrent antifungal treatment. IPA is a serious complication in patients with quantitiative and qualitative defects of myeloid phagocytes. . Recent evidence shows that IPA also arises in patients on complement C3/C5-targeting therapies, highlighting the protective roles of C3 and C5. Complement protein C3, a highly abundant, liver-derived, serum-circulating protein. C3 undergoes rapid activation and cleavage upon microbial encounter, producing C3a, C3b and initiating processes such as opsonophagocytosis and further complement activation, culminating in C5 cleavage and C5a production. C3 plays critical roles in systemic antifungal host defense, but its functions and regulation at the pulmonary mucosa for protection during IPA remains elusive. Our data indicate an essential role for C3 in protection during IPA, as C3-deficient mice are highly susceptible to IPA. In addition, locally- produced, pulmonary C3, rather than liver-derived serum C3, is vital for IPA defense, as the mice lacking circulating C3 were competent at pulmonary fungal clearance and did not exhibit enhanced susceptibility. We identified recruited neutrophils, Ly6C hi inflammatory monocytes, monocyte-derived dendritic cells, resident alveolar macrophages, and lung stroma as sources of pulmonary C3. Notably, we observed C3 to be necessary for fungal killing by myeloid phagocytes, not for leukocyte accumulation. Furthermore, we observed the C3 cleavage product C3a to be dispensable for in vivo fungal clearance, highlighting a prominent role for the larger C3 fragments - C3b/iC3b in protection, potentially via signaling through the cognate receptors - CR3 and CD46. Our ongoing work is aimed at uncovering new biological mechanisms of C3-mediated pulmonary antifungal defenses. These insights will improve patient outcomes by informing risk stratification for those on complement-targeted therapies

Poster Presentation #12

Hdac1 promotes effector but limits exhaustion program activation in CD8 + T cell responses to chronic viral infection

Wei Hu[1], Hai-Hui Xue[1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA

Abstract

During chronic viral infection, CD8 + T cells enter a hypofunctional and exhausted state. The exhausted CD8 + T (T EX) cells are heterogeneous, consisting of phenotypically and functionally distinct subsets including Tcf1 + progenitors, Tcf1 – fractalkine receptor (FR) + cytotoxic effectors and Tcf1 – FR – terminally exhausted cells. T EX subset fate decision is made during early response phase, yet the epigenetic determinants remain incompletely understood. Using an in vivo chronic viral infection model, here we show that histone deacetylase 1 (Hdac1) is required for optimal expansion of antigen-specific CD8 + T cells at the effector phase and long-term viral control. Strikingly, genetic ablation of Hdac1 substantially impaired formation of Tcf1 – FR + cytotoxic effectors at the early response phase and resulted in excessive expression of exhaustion-inducing Tox transcription factor, PD1 and Lag3 coinhibitory receptors in all T EX subsets. Genome-wide mapping of chromatin accessibility landscape revealed that loss of Hdac1 caused prevalent increase in chromatin open state, especially at the exhaustion program genes, which became more accessible to exhaustion-inducing Nfat and Nr4a transcription factors. Unexpectedly, Hdac1 deficiency also reduced chromatin accessibility at genes linked to the effector program such as Batf, Tbx21 and Cx3cr1. These data collectively indicate that Hdac1 has dual regulatory functions during early T EX differentiation: to prevent excessive activation of the exhaustion program and to promote induction of the effector program for optimal viral control.

Poster Presentation #13

Ezh2 and intracellular Ca2+ signals interdependently coordinate GVHD and CAR T cell responses

Ying Wang[1],* QingRong Huang[1,2],* Ruqayyah Sanders[1], Yan Zhou[3], Robert Hooper[4], Yuanyuan Tian[1], Tatiana Kent[5], Richard Pomerantz[5], Gennaro Clando[6], Jean-Pierre Issa[6], Jonathan Soboloff[4], Yi Zhang[1,4,#]

1. Center for Discovery and Innovation, Hackensack University Medical Center, Hackensack Meridian Health, Nutley, New Jersey, USA
2. Clinical Immunology Core, Center for Discovery and Innovation, Hackensack University Medical Center, Nutley, New Jersey, USA
3. Fox Chase Cancer Center, Temple University, Philadelphia, Pennsylvania, USA
4. Fels Institute and Department of Cancer Cellular Biology, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA
5. Department Biochemistry and Molecular Biology, Thomas Jefferson University, Sidney Kimmel Cancer Center, Philadelphia, PA, USA
6. Coriell Institute for Medical Research, Camden, New Jersey USA.

Abstract

Despite significant advances in graft-versus-host disease (GVHD) prevention and treatment, calcineurin inhibitor (CNI)-based standard GVHD prophylaxis in allogeneic hematopoietic stem cell transplantation (allo-HSCT) has limited efficacy in controlling acute and chronic GVHD. Thus, inhibition of calcium (Ca2+) signaling is insufficient to suppress the generation and maintenance of alloreactive T cells that mediate host tissue injury. Recent studies suggested that CNI-dependent alloreactive T cells possess great ability to persist and mediate chronic-like GVHD in mice. The molecular events by which these T cells breakthrough CNI inhibition have not been previously defined. Ezh2, a chromatin-modifying epigenetic regulator, silences expression of gene programs critical for multiple cellular processes. Ablating Ezh2 in T cells inhibits GVHD and anti-tumor activity, largely due to massive antigen-activated T cell death. Increased Ca2+ signals in activated T cells are known to induce their cell death and dysfunction. However, the relationship between Ezh2 and intracellular Ca2+ response generation in GVHD has never been previously examined. We report here that Ezh2 and Ca2+-mediated signals operate coordinately to regulate the viability and effector function of GVHD T cells. Blockade of Ca2+ signal by conditional deletion of Stim1, an endoplasmic reticulum (ER) Ca2+ sensor required for Ca2+ entry in T cells, rescued non-viable Ezh2-null alloreactive T cells, as well as restored their capacity to mediate GVHD in mice after allo-HSCT. Moreover, while STIM1-null T cells typically exhibit decreased effector differentiation and function of GVHD T cells, this was restored by deletion of Ezh2 in Stim1-null T cells. These data identify the interdependent roles of Ezh2 and Ca2+ signals in activation, effector differentiation and survival of alloreactive T cells. To understand how Ezh2 acts as ‘brake” for Ca2+ signals in T cells, we performed bulk-RNA-sequencing analysis on Ezh2/STIM1 dual knockout T cells. Ezh2 directly repressed expression Itpr2, which encodes the ER Ca2+ release channel 1,4,5-trisphosphate receptor (IP3R2), thereby interfering with ER Ca2+ release and subsequent cytosolic Ca2+ entry. Combined deletion of Ezh2 and Itpr2 genes restored the inability of allogeneic Ezh2-null T cells to induce lethal GVHD. Furthermore, the co-dependence of Ezh2 and Iptr2 were similarly observed in CD19-directed CAR-T mediated elimination of CD19-expressing C1498 acute myeloid leukemia in mice. Itpr2 loss in Ezh2-null CAR-T cells led to their improved survival, expansion, and production of IFN-□-producing effector CAR-T cells. Collectively, our findings identify that Ezh2 suppresses the expression of Iptr2 to prevent excessive Ca2+ signal generation and antigen-driven T cell death and T cell dysfunction. These observations reveal a potential therapeutic window for the treatment of GVHD focused on increasing intracellular Ca2+ signals to eliminate alloreactive T cells, which is opposing to the current concept of CNI treatment. Furthermore, targeting this Ezh2-Itpr2 axis may have broad implications in the regulation of other types of antigen-driven T cell responses, such as anti-tumor immunity, autoimmunity and graft rejection of solid organ transplantation.

Poster Presentation #14

Dual functions of mitochondrial calcium uniporter in T cell alloimmunity

Ying Wang[1], Alicia Huaze Li[2], Ruqayyah Sanders[1], Yan Zhou[4], Mimi Chen[1], Tien Bui[1], Hongfa Zhu[5], Nasheed Hossain[6], Jonathan Soboloff[2], John Elrod[3], Elizabeth O Hexner[6], Yi Zhang[1,2]

1. Hackensack University Medical Center, Center for Discovery and Innovation, Nutley, New Jersey,
2. Fels Institute for Cancer Research and Molecular Biology
3. Center for Translational Medicine,
4. Fox Chase Cancer Center, Temple University, Philadelphia, Pennsylvania
5. Hackensack Meridian Health, Hackensack, New Jersey, USA
6. Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

Abstract

During the early/intermediate phases of the immune response, calcium (Ca2+) signals are crucial for T cell activation, proliferation and effector differentiation. Yet, inhibiting Ca2+ signaling-activated master transcription factor NFAT has achieved limited success in the long-term restriction of graft-versus-host disease (GVHD), while high remission and infection rates remain major issues. This underscores the need to better understand how different calcium channels coordinate to regulate T cell alloimmunity. Mitochondria is one of the two intracellular calcium stores that buffers and modulates cytosolic Ca2+ response. Mitochondria calcium uniporter (MCU) complex is the sole channel through which Ca2+ enters the mitochondria. The role of MCU in T cell-mediated GVHD remains undetermined. In this study, we report that enforced Mcu expression in donor T cells abolished their capacity to induce lethal GVHD. Using retroviral gene delivery system to induce or constitutively overexpress Mcu in T cells, we discovered that Mcu ectopic expression impaired donor T cell survival. RNA-seq analysis identified the activation of gene programs mediating death of activated T cells. This was induced by persistent alloantigen exposure (restimulation-induced cell death, RICD), leading to massive cell death in the liver, a GVHD target organ. Pharmacological enhancement of MCU function with a natural compound phenocopied this finding while preserving anti-leukemia potency. These data indicate that boosting MCU function in donor T cells could be a novel and effective strategy to mitigate GVHD after allogeneic hematopoietic stem cell transplantation. Interestingly, we discovered T cells naturally downregulated MCU function during differentiation, indicating intrinsic repressive mechanisms that protect T cells from RICD. This led us to interrogate whether MCU is required for T cell alloimmunity. We generated T cell-specific Mcu conditional knockout C57/BL6 mice (Mcu-cKO). Balb/c recipients infused with Mcu-KO B6 mouse T cells developed severe liver GVHD, manifested by the dramatically reduced liver size, bile duct lesion, portal and lobular inflammation associated with massive lymphocyte infiltration. However, these Mcu-KO T cell recipients survived longer with 69% compared to 100% mortality in WT T cell recipients. Mechanistic studies showed Mcu-KO recipients had significantly fewer donor T cells in the spleen and liver, attributed to reduced proliferation capacity, and decreased IFN-□-producing cells. Meanwhile, Mcu-KO donor T cells retained GMCSF- and granzyme B-producing capacity. These data demonstrate MCU promotes T cell alloresponse, distinguishing its dispensable role in autoimmune disease and anti-viral infection models.To delineate the molecular mechanisms through which MCU regulates T cell alloresponse, we performed transcriptome profiling on sort-purified alloreactive CD8 T cells recognizing the alloantigen H60 in balb/b mice. Mcu-KO alloreactive CD8 T cells were characterized with enhanced effector programming, loss of memory potential, positive enrichment of exhaustion feature through gene set enrichment and DEG analysis. In depth mechanistic analysis unveiled STAT5 as the master upstream regulator through which MCU modulated transcriptome. Elevated STAT5 and the expression of its target genes (Bcl2, Tox, Lef1, Gzma, etc) were observed in Mcu-KO CD8 T cells. STAT5 is a predominant IL2 signaling molecule. Mcu deficiency increased the level of CD25, which is required for the formation of high affinity IL2 receptor in CD8 T cells. IL2□ (CD25) is transcriptionally activated by NF□b, the elevated activity of which was identified in Mcu-KO CD8 T cells. These observations suggest that Mcu ablation led to activation of IL2-CD25-STAT5 axis, sustaining the persistence of activated T cells while reinforcing effector programs. Findings from this study elucidate the intricate regulatory network through which MCU regulates T cell alloimmunity, highlighting the fundamental translational potential of enhancing MCU functionality in alloreactive T cells through gene editing or pharmacological approach to reduce GVHD.

Poster Presentation #15

Multimodal therapy of hematologic malignancies based on targeted drug delivery and photothermal ablation enabled by B cell maturation antigen-directed gold nanoparticles

Manpreet Bariana[1,2], Jingyu Sun[3], Ashley Varkey[1,4], Shaina A. Anuncio[1], Elena Cassella[1], Nuo Xu[3], Hongjun Wang[3,5], Johannes L. Zakrzewski[1,4,6]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA
2. Hackensack Meridian School of Medicine, Nutley, NJ, USA
3. Department of Biomedical Engineering, Stevens Institute of Technology, Hoboken, NJ, USA
4. Department of Pediatrics, Hackensack University Medical Center, Hackensack, NJ, USA
5. Semcer Center for Healthcare Innovation, Stevens Institute of Technology, Hoboken, NJ, USA
6. Department of Oncology, Georgetown University, Washington, DC, USA

Abstract

Acute myeloid leukemia (AML) and Multiple Myeloma (MM) are hematologic malignancies with high mortality and morbidity rates and many AML and MM patients have disease that is refractory to currently available therapies. Innovative targeted treatment modalities such as immunotherapies or nanoparticle-based therapeutics have evolved into paradigm-shifting advances in the setting of various solid tumors and blood cancers, but especially nanotechnology-enabled approaches remain an understudied area of research in the blood cancer field. BCMA is a MM associated antigen and one of the most attractive targets for T cell engager or chimeric antigen receptor T cell therapies. The development of successful immunotherapies for AML has been challenging due to a combination of factors including AML associated immune dysfunction and the lack of safe and effective targets on the surface of AML cells. Here we present a novel nano-immuno-molecular platform for gold nanoparticle mediated BCMA-directed drug and photothermal therapy of both MM and AML, and we demonstrate specificity as well as preclinical in vitro and in vivo efficacy of our approach. We fabricated gold nanoframeworks (AuNF) with uniform hydrodynamic diameters (148 ± 17 nm) and showed that the mesopores extended throughout the entire nanoparticle, providing ample surface area for drug loading. Anti-BCMA conjugation of AuNF was performed to maximize uptake of AuNF by BCMA expressing cancer cells. We found that compared with unconjugated control AuNF, anti-BCMA AuNF indeed preferentially accumulated in both MM and AML cells while only minimal uptake was observed in healthy cells (PBMC). IT848 is a small molecule inhibitor of NF-kB which selectively induces oxidative stress in a wide range of blood cancer cells. Using DMSO-based formulations of IT848 (2, 4, 6 μM) we were able to demonstrate dose-dependent antitumor activity against both AML (THP-1, SET-2) and MM (MM.1S, U266) target cells. We next conducted in vitro efficacy studies comparing subtherapeutic doses of free, control AuNF (unconjugated) formulated and anti-BCMA AuNF formulated IT848 and found that the anti-BCMA AuNF formulation was superior to the other two formulations. Longitudinal follow up in the MM.1S xenograft model showed that weekly administration of 1 mg/kg of control AuNF BTZ was subtherapeutic while the anti-BCMA AuNF formulation was highly efficacious. Moreover, we found that PTT in a subcutaneous EL4 lymphoma model not only suppressed tumor growth and altered tumor appearance (causing necrotic and wrinkled appearance), but it also increased tumor infiltration by CD8 T cells with a favorable phenotype (increased frequency of central memory T cells, while decreasing the frequency of exhausted, activated T cells). Collectively, our findings underscore the potential of our strategy to serve as an easily adaptable platform for targeted cancer therapy and to inspire the development of innovative, well-tolerated and effective protocols for the treatment of aggressive hematologic malignancies. Our innovative approach promises to be highly efficacious with infrequent dosing, increasing patient comfort and compliance while simultaneously reducing the risk for adverse effects.

Poster Presentation #16

Uncovering the novel roles of a nuclear envelope protein LAP1 in the development of hepatocellular carcinoma

SooJin Kim [1] , Huh Hyun [1] , Ji-Yeon Shin[1]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ 07110

Abstract

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related deaths worldwide and often arises from a liver condition called non-alcoholic steatohepatitis (NASH). Hepatocarcinogenesis has been linked to alterations in the nuclear envelope, but the specific roles of nuclear envelope proteins in HCC development have not been explored. Our current research is focused on understanding the involvement of a specific nuclear envelope protein called lamina-associated polypeptide 1 (LAP1) in the initiation and progression of HCC. Our preliminary data demonstrate that mice with hepatocyte-specific depletion of LAP1 (L-CKO) showed steatosis, NASH and tumorigenesis in their livers under normal chow diet feeding. Genome-wide transcriptomic analysis with livers from L-CKO mice showed abnormal gene expression changes, particularly in the gene cluster within an imprinted genetic locus known as Dlk1-Dio3, as well as in progenitor cell marker genes. Additionally, we have observed differential expression of LAP1 isoforms in non-tumor and tumor cells. Based on these findings, we hypothesize that LAP1 plays a role in hepatocyte transformation and the loss of LAP1 or isoform switching contributes to hepatic neoplasia. We are investigating the mechanisms by which LAP1 isoforms regulate the initiation and progression of HCC in both in vitro and in vivo models. The results of our current study could potentially lead to new strategies for preventing liver cancer in patients with NASH and other chronic liver diseases.

Poster Presentation #17

Ceramide-1-phosphate/CERK are regulators of ETMR growth and offer potential as a new therapeutic target

Allison Maas [1] , Evangelos Liapis [1] , Kelly C. O'Neill [1] , Annapurna Pamreddy [1] , Francesca M. Cozzi [1] , Brent T. Harris [2] , Derek Hanson [1,3,4] , Claire L. Carter [1,5]

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ 07110
2. Departments of Neurology and Pathology, Georgetown University Medical Center, DC 20007
3. Joseph M. Sanzari Cancer Center, Hackensack University Medical Center, Hackensack, NJ 07110,
4. Department of Pediatrics, Hackensack Meridian School of Medicine, Nutley, NJ 07110
5. Department of Pathology, Hackensack Meridian School of Medicine, Nutley, NJ 07110

Abstract

Ceramide-1-phosphate/CERK are regulators of ETMR growth and offer potential as a new therapeutic target Embryonal tumor with multilayered rosettes (ETMR) is a highly aggressive CNS neoplasm which occurs almost exclusively in infants and is associated with an extremely poor prognosis. To identify functional signaling lipids that contribute to the aggressive nature of ETMR and that can be targeted therapeutically, we carried out mass spectrometry imaging on human ETMR patient samples, the patient-derived cell line BT183, and normal neural stem cells. We identified an accumulation of ceramide-1-photphate (C1P) within the rapidly proliferating embryonal tumor cells in patient samples and the cell model. We also detected high accumulation of C1P within the aberrant vascular proliferations in patient samples. Ceramide-1-phosphate is a pleiotropic bioactive signaling lipid that controls cell fate. C1P and its synthesizing enzyme, ceramide kinase (CERK), are known mitogens that have been shown to regulate cell proliferation in several non-CNS cancers. C1P is known to mediate the upregulation of PI3K/Akt/mTOR, MAPK/MEK/ERK1/2, Rho kinase, JNK, to promote cell survival and migration. After spatially mapping C1P accumulation in patient samples and our in vitro 3D models, we next carried out IHC to correlate lipid accumulation with its synthesizing enzyme, CERK. CERK was found to be abundantly accumulated in both endothelial and tumor cells within the tumor microenvironment of patient samples. Western blot analysis of CERK demonstrated a significant increase in the ETMR 3D tumorspheres compared to the NSC neurospheres. Treatment of the ETMR 3D tumorspheres with the CERK inhibitor, NVP-231, potently (IC 50 , 0.665 μM) reduced cell growth and viability. Western blot analysis of ETMR tumorspheres at 8- and 24-hours post-treatment demonstrated a time-dependent decrease in N-MYC and an increase in p53. Studies are ongoing to elucidate the signaling pathways that C1P/CERK inhibition regulate in ETMR.

Poster Presentation #18

A National Snapshot of Lung Cancer Screening Adherence in Diverse Practice Settings

Erin A. Hirsch[1] , Joelle Fathi[2] , Andrew Ciupek[2] , and Lisa Carter-Bawa [1,3]

1. Center for Discovery &amp, Innovation at Hackensack Meridian Health, Cancer Prevention Precision Control Institute, Nutley, NJ, USA
2. GO2 for Lung Cancer, Washington, DC, USA
3. Georgetown Lombardi Comprehensive Cancer Center Cancer Prevention &amp, Control Program Washington, DC, USA

Abstract

Introduction:
The efficacy of lung cancer screening (LCS) to reduce lung cancer specific mortality is heavily dependent on adherence to recommended screening guidelines. Real-world adherence rates reported in the literature have predominantly represented rates from academic medical centers and have consistently been reported to be <50%, drastically lower than the >90% rates described in clinical trials. The purpose of this research was to determine adherence rates and processes in diverse practice settings, including community and rural LCS programs.

Methods:
Participants for this survey study included a nationally representative sampling of LCS navigators recruited from the GO2 for Lung Cancer Screening Centers of Excellence (COE). Surveys were distributed electronically to all COE contacts using MailChimp. Questions assessed practice location, setting, service area, organization, and tracking processes. Respondents were asked to report no-show, annual, and interval adherence rates for their programs.

Results:
To date, 51 responses have been received, representing programs from 27 states. Of these programs, 86% reported operating in a non-academic medical setting, with 14% primarily serving rural areas, and 47% having a centralized structure. Almost half (43%) of programs use Microsoft or Google products to track patient follow-up, alone or in combination with the electronic health record or commercial software. The no-show rate is tracked by 51% of responding programs, with a median (interquartile range) no-show rate of 10% (5% - 20%) reported. Annual and interval adherence rates were reported by 75% and 82% of programs with median rates of 63% (50% - 75%) and 80% (70 – 90%), respectively. Implications: Adherence rates reported by screening navigators in this study are higher than rates reported in the literature, which is encouraging and indicates more research is needed to understand LCS system and clinic processes that influence adherence rates outside of clinical trial and academic settings.

Poster Presentation #19

Allele-specific methylation mapping and multiplexed CRISPR mutagenesis for identifying functional SNPs in susceptibility loci for cancers and autoimmune/inflammatory diseases

1. **Angelica Castano, Mauricio M. Rigo, Martha Salas, Mariam Tariq, Catherine Do, Benjamin Tycko**
2. **Shima Nejati, Panteha Behboodi**
3. **Samuel Potenciano, Josh Lerman, Patryk Dabek, Sophia Hartley, Jacob Glicksman, Rohan Sarkar**
4. **David Siegel, Noa Biran, Katherine Hamilton, Arunjot Singh, Amanda Muir, Ali Torkamani, Kaushik Ram Ganapathy, Michael Stifelman, Nitin Yerram, Michael Raver, Karim El-Sahwi, Fidi Nagy, Peter L Nagy, Kar Chow, Yael Kramer**

1. Tycko lab current and past members
2. Stevens Institute Graduate Students
3. Summer and Part-time Students
4. Clinical and Other Collaborators

Abstract

BACKGROUND and HYPOTHESIS:
An important challenge in genomic medicine is to go beyond statistical information from genome-wide association studies (GWAS) to pinpoint functional regulatory SNPs (rSNPs) that act in cis to alter the expression of nearby genes and thereby influence disease risk. We and others have shown that mapping of allele-specific DNA methylation (ASM) can be a useful post-GWAS approach for localizing bona fide rSNPs under GWAS peaks, but functional validations are needed. In this project, we hypothesize that (i) CRISPR mutagenesis followed by methyl-seq can validate candidate rSNPs in disease susceptibility loci, and (ii) the results can elucidate whether rSNPs act by creating/deleting specific transcription factor binding motifs, and whether ASM can provide an informative “footprint” of TF binding site occupancies.

METHODS:
Here we combine genome-wide and targeted ASM mapping with multiplexed CRISPR-Cas9 mutagenesis, followed by targeted methyl-seq, to localize and test candidate rSNPs in risk loci for multiple myeloma and ovarian, breast, colon and kidney cancers, and loci associated with susceptibility to celiac disease, Crohn’s disease, and other autoimmune/inflammatory diseases.

RESULTS:
Results were obtained from multiplexed CRISPR-Cas9 transfections, using a large and diverse panel of human cell lines, with changes in local CpG methylation as positive readouts. So far, we have obtained informative CRISPR-mediated small deletions and/or point mutations for candidate rSNPs in a total of 15 disease risk loci, with data from 6 additional loci in progress. Gains of methylation upon mutation/deletion were observed for rSNPs in defined motifs within binding sites for activating transcription factors and the insulator binding protein CTCF, and losses of methylation for rSNPs in binding sites for repressive transcription factors. Interestingly, for several of these loci we find both situations, implying protection of one allele from methylation by an activating TF complex and maintenance of methylation on the other allele by a repressive TF complex, in the same heterozygous cell line. Off-target deletions that were close to but not encompassing the rSNPs did not affect the local CpG methylation patterns. Roles in transcriptional regulation are being tested using RNA-seq of single-cell clones obtained from the CRISPR-Cas9 transfections.

CONCLUSIONS:
Multiplexed CRISPR site-directed mutagenesis is yielding informative data for pinpointing bona fide rSNPs in cancer and autoimmune disease risk loci, and for understanding the molecular mechanisms by which these rSNPs act. The approach that we have developed will be scalable to larger numbers of loci and disease traits.

Poster Presentation #20

A National Snapshot of Lung Cancer Screening Adherence in Diverse Practice Settings

Erin A. Hirsch[1] , Joelle Fathi[2] , Andrew Ciupek[2] , and Lisa Carter-Bawa [1,3]

1. Center for Discovery & Innovation at Hackensack Meridian Health, Cancer Prevention Precision Control Institute, Nutley, NJ, USA
2. GO2 for Lung Cancer, Washington, DC, USA
3. Georgetown Lombardi Comprehensive Cancer Center Cancer Prevention & Control Program Washington, DC, USA

Abstract

Introduction:
The efficacy of lung cancer screening (LCS) to reduce lung cancer specific mortality is heavily dependent on adherence to recommended screening guidelines. Real-world adherence rates reported in the literature have predominantly represented rates from academic medical centers and have consistently been reported to be <50%, drastically lower than the >90% rates described in clinical trials. The purpose of this research was to determine adherence rates and processes in diverse practice settings, including community and rural LCS programs.

Methods:
Participants for this survey study included a nationally representative sampling of LCS navigators recruited from the GO2 for Lung Cancer Screening Centers of Excellence (COE). Surveys were distributed electronically to all COE contacts using MailChimp. Questions assessed practice location, setting, service area, organization, and tracking processes. Respondents were asked to report no-show, annual, and interval adherence rates for their programs.

Results:
To date, 51 responses have been received, representing programs from 27 states. Of these programs, 86% reported operating in a non-academic medical setting, with 14% primarily serving rural areas, and 47% having a centralized structure. Almost half (43%) of programs use Microsoft or Google products to track patient follow-up, alone or in combination with the electronic health record or commercial software. The no-show rate is tracked by 51% of responding programs, with a median (interquartile range) no-show rate of 10% (5% - 20%) reported. Annual and interval adherence rates were reported by 75% and 82% of programs with median rates of 63% (50% - 75%) and 80% (70 – 90%), respectively. Implications: Adherence rates reported by screening navigators in this study are higher than rates reported in the literature, which is encouraging and indicates more research is needed to understand LCS system and clinic processes that influence adherence rates outside of clinical trial and academic settings.

Poster Presentation #21

The E2F4 transcriptional repressor is a key mechanistic regulator of colon cancer resistance to irinotecan (CPT-11)

Sanjay Koul[1,2,5], Junichi Matsubara[3], Yong F. Li[4], Junko Mukohyama[5], Luis E. Valencia Salazar[5], Taichi Isobe[3], Dalong Qian[3], Debashis Sahoo[6], Michael F. Clarke[3], Russ B. Altman[4] and Piero Dalerba[1,5]

1. Center for Discovery & Innovation (CDI), Hackensack Meridian Health (HMH), Nutley, NJ 07110, USA
2. Department of Biological Sc. & Geology, Queensborough Community College (QCC), CUNY, Bayside, NY 11364, USA
3. Institute for Stem Cell Biology and Regenerative Medicine (ISCBRM), Stanford University, Stanford, CA 94305, USA
4. Department of Genetics, Stanford University, Stanford, CA 94305, USA
5. Department of Pathology and Cell Biology, Columbia University, New York, NY 10032, USA
6. Department of Computer Science and Engineering, University of California San Diego (UCSD), La Jolla, CA 92093, USA

Abstract

Colorectal carcinomas (CRCs) are seldom eradicated by cytotoxic chemotherapy. Cancer cells with stem-like functional properties, often referred to as “cancer stem cells” (CSCs), display preferential resistance to several anti-tumor agents used in cancer chemotherapy, but the molecular mechanisms underpinning their selective survival remain only partially understood. Methods. In this study, we used Transcription Factor Target Genes (TFTG) enrichment analysis to identify transcriptional regulators (activators or repressors) that undergo preferential activation by chemotherapy in CRC cells with a “bottom-of-the-crypt” phenotype (EPCAM + /CD44 + /CD166 + , CSC-enriched) as compared to CRC cells with a “top-of-the-crypt” phenotype (EPCAM + /CD44 neg /CD166 neg , CSC-depleted). The two cell populations were purified in parallel by fluorescence-activated cell sorting (FACS) from human patient-derived xenografts (PDX) representative of well-differentiated CRCs, following in vivo chemotherapy with irinotecan (CPT-11). The transcriptional regulators identified as differentially activated were tested for differential expression in normal vs. cancer tissues, and in cell populations enriched in stem/progenitor cell-types as compared to differentiated lineages (goblet cells, enterocytes) in the mouse colon epithelium. Finally, the top candidate was tested for mechanistic contribution to drug-resistance by selective down-regulation using short-hairpin RNAs (shRNAs). Results. Our analysis identified E2F4 and TFDP1, two core components of the DREAM transcriptional repression complex, as transcriptional modulators preferentially activated by chemotherapy in EPCAM + /CD44 + /CD166 + as compared to EPCAM + /CD44 neg /CD166 neg cancer cells. The mRNA expression levels for both genes (E2F4, TFDP1) were found up-regulated in CRCs as compared to human normal colon epithelia, and in normal epithelial cells enriched in stem/progenitor cell-types (Epcam + /Cd44 + /Cd66a low /Kit neg) as compared to goblet cells (Epcam + /Cd44 + /Cd66a low /Kit +) or enterocytes (Epcam + /Cd44 neg /Cd66a high) purified in parallel by FACS form mouse colonic crypts. Most importantly, E2F4 down-regulation using short-hairpin RNA (shRNA) constructs dramatically enhanced the sensitivity of human CRCs to in vivo treatment with irinotecan, across three independent PDX models. Conclusions. Our data identified E2F4 and the DREAM repressor complex as critical regulators of human CRC resistance to irinotecan, and as candidate targets for the development of chemo-sensitizing agents.