GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Animal Models Shared Resource

Director: Christopher Albanese, PhD

Overview

The Animal Models Shared Resource (AMSR) is comprised of three components: rodent, zebrafish, and the Preclinical Imaging Research Lab (PIRL). The Mission of the AMSR is to facilitate the efficient, economical, state-of-the-art use and imaging of animals for the performance of cancer-related studies. The AMSR Specific Aims are as follows: 1) Guide members on designing animal-based studies, 2) Perform *in vivo* studies supporting translational cancer research, 3) Educate and train members on innovative methodologies that best model cancer initiation, progression, and response to therapies in humans.

Key Personnel

Christopher Albanese, PhD, Director

Andrew Nelson, DVM, Manager (Rodent)

Patricia Foley, DVM, Manager (Rodent)

Eric Glasgow, PhD, Manager (Zebrafish)

Key Services

- Consultation on models
- Colony maintenance and diets
- Tumor engraftment
- Injections
- Tissue and blood collection
- Surgery
- Tumor growth monitoring
- MRI
- Ultrasound
- IVIS
- X-ray
- Injecting Zebrafish embryos
- Generating transgenic fish
- Toxicology screening in fish

Major Equipment / Technologies

MRI

Bruker 7T/30
S10 OD025153

Ultrasound

Vevo 3100

Bioluminescence/ Fluorescence

IVIS

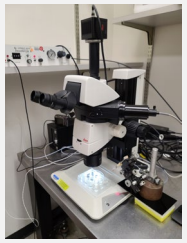
X-ray

Faxitron

Rodent Anesthesia

SomnoFlo

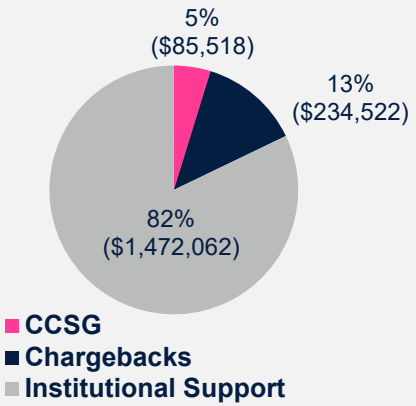
Leica M165FC Microscope



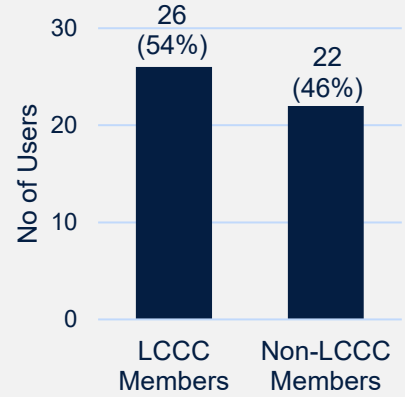
Zebrafish Facility

Usage / Budget (FY22)

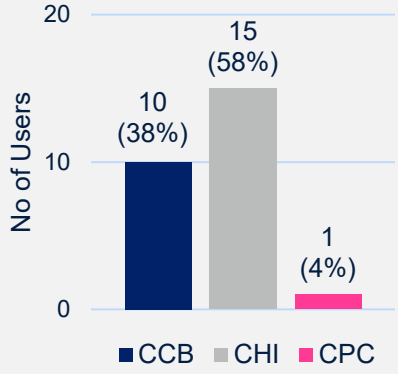
Sources of Support



Usage by Membership



Usage by Program



GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Animal Models Shared Resource

Director: Christopher Albanese, PhD

A Yap-Myc-Sox2-p53 Regulatory Network Dictates Metabolic Homeostasis and Differentiation in Kras-driven Pancreatic Ductal Adenocarcinomas

The AMSR-PIRL performed longitudinal MRI and IVIS bioluminescence imaging to enable **Chunling Yi, PhD**[CCB] to follow PDAC progression, a major scientific priority of the LCCC. This study also used FCSR, MISR, HTSR and GESR.

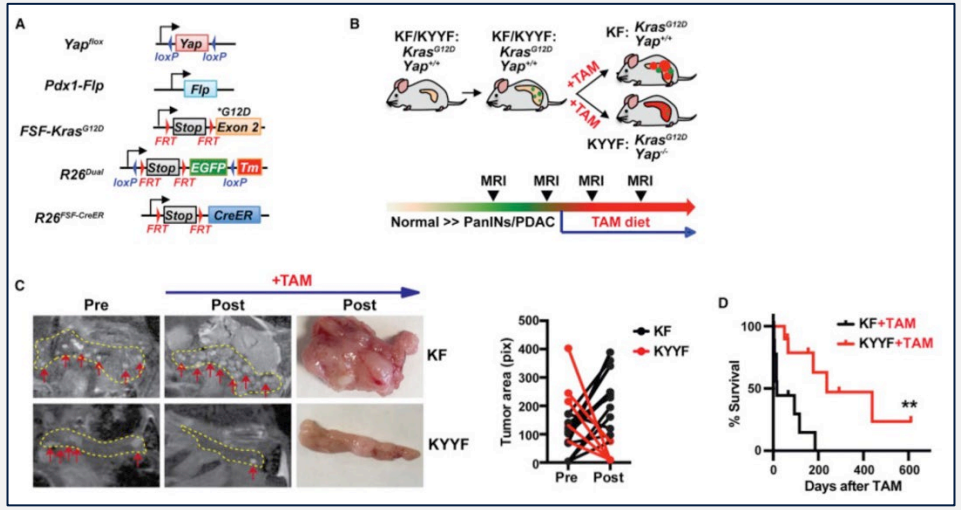


Fig. 1. A) Strategy to activate *Kras*^{G12D} and delete *Yap* in the pancreas. **B)** Experimental design. **C)** MRI and tumor area quantification. Yellow line; pancreas, red arrows; visible nodules. **D)** Kaplan-Meier survival of KF (n = 10) and KYYF (n = 10) mice.

Conclusions: Imaging work done in the AMSR-PIRL helped to establish that PDAC progression is inhibited by YAP deletion. This work also supported TBIO T32 trainee Shannon White.

Grants: R01CA187090, R01CA263630, R21CA258153
Publications: *Dev Cell*, 2019, PMC6783361; *Nat Comm. 2023,* PMC10017707

AIB1 Isoform Alters Enhancer Access and Enables Progression of Early-Stage Triple-Negative Breast Cancer

The AMSR-Zebrafish performed extravasation assays for **Anna Riegel, PhD**[CCB] that demonstrated that a minor subset of AIB1Δ4-expressing breast cancer cells enabled cell invasion when present in a mixed population of cells. These studies also utilized the TCBSR, FCSR, MISR, HTSR, and GESR.

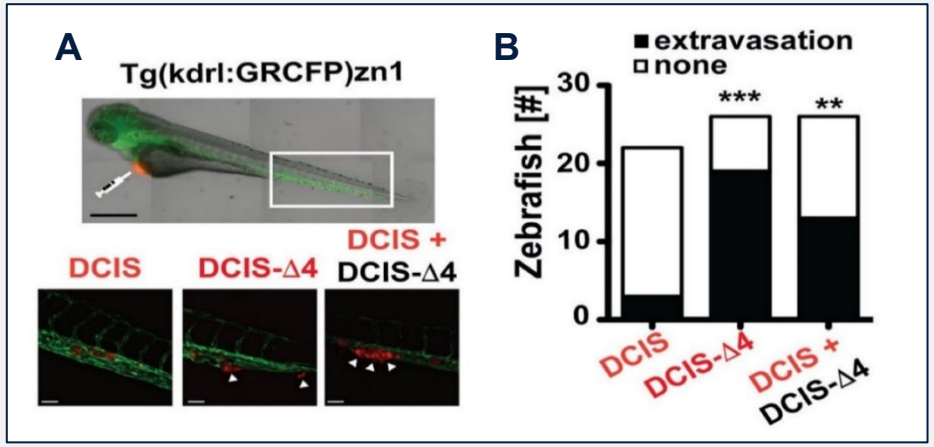


Fig. 2. A) Extravasation of DCIS, DCIS-Δ4 or mixed cells in *Tg(kdrl:GCRFP)* transgenic zebrafish embryos with green florescent blood vessels. White arrow heads; extravasated cells. **B)** The number of zebrafish embryos with extravasated cells.

Conclusions: Work performed by the AMSR-Zebrafish showed that AIB1Δ4 enables bulk tumor cells to become invasive, and selective eradication of this subpopulation might be a way to defeat this cancer. This work also supported TBIO T32 trainees Garrett Graham and Will Keitzman, and T32 and F31 trainee Max Kushner.

Grants: R01CA205632, R21CA226542
Publication: *Cancer Res*, 2021, PMC8373795

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Animal Models Shared Resource

Director: Christopher Albanese, PhD

Environmentally Induced Sperm RNAs Transmit Cancer Susceptibility to Offspring in a Mouse Model

The AMSR-Rodent performed male germline collection (for RNA extraction) and treatments for **Sonia de Assis, PhD**[CHI] to induce carcinogen-induced mammary tumors as well as orthotopic implantation of mammary cancer cells in the resulting DDT RNA-derived IVF offspring.

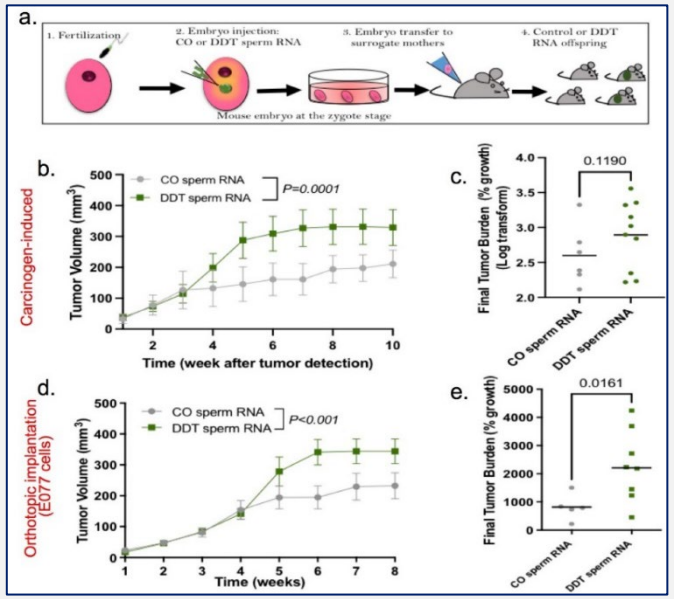


Fig. 3. Treatment with the hepatic enzyme inducer, phenobarbital, leads to reduction of DDT metabolites and normalization of sperm miRNA-10b levels in DDT-exposed males. **A**) Schematic representation of the experimental design, **B**) Expression levels of Cyp2B10, **C**) Levels of DDT metabolites in liver tissues of CO and DDT-exposed male mice treated with PB or vehicle injection, **D**) miRNA expression levels, **E**) Expression levels of miRNA-10b.

Conclusions: These studies performed in the AMSR-Rodent demonstrated that environmentally-induced sperm RNAs can transmit cancer susceptibility to increased tumor growth across generations**.** This work also supported T32 trainee Apsra Nasir.

Grant: R01ES031611
Publication: *Res Sq*. 2023, PMC9934767

Other Key Activities

- Recruited Andrew Nelson, DVM as AMSR-Rodent manager in NJ.
- Facilitated collaboration between **Olivier Loudig**[CCB] and **Sonia de Assis**[CHI] resulting in a publication (*Sci Rep*. 2021, PMC8016877).
- Provided extensive surgical and imaging services for orthotopic oncology models:
  - **David Robbins**[CCB]: implantation and imaging of CRC cells in the ascending colon, caecum and rectum supported grants (R01CA244188, R01CA219189) and a publication (*Nat Commun.* 2021, PMC8421366).
  - Assisted **Nagi Ayad**[CCB] with intracranial implantation and imaging of glioma cells that supported a grant (R01NS118023) and a publication (*Sci Rep*. 2021, PMC8642539).
- New rodent anesthesia equipment and homeothermic support during surgeries.
- Purchased electronic bluetooth-capable calipers for tumor measurements.
- Hosted a full-day in person photoacoustic imaging conference with VisualSonics.

Future Plans

- Expand Zevatar patient-specific chemosensitivity analysis and develop humanized zebrafish for immunotherapy studies
- Build expertise in NJ in ultrasound and vet tech services, and develop zebrafish melanoma metastasis models (genetically modified in NJ and then shipped to DC for testing)
- Upgrade the zebrafish aquatic facility to modernize and double holding capacity in AMSR-Zebrafish (R24OD035428 awarded July 2023).
- Submit LAZR-X VisualSonics Photoacoustic Imaging S10 application.
- Acquire an EchoMRI body composition analyzer.

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Biostatistics & Bioinformatics Shared Resource

Co-Directors: Ming Tan, PhD, Yuriy Gusev, PhD

Overview

The mission of the Biostatistics & Bioinformatics Shared Resource (BBSR) is to support the basic, translational, clinical, and population science research of LCCC Members by providing access to high-quality statistical science and bioinformatics. The BBSR Specific Aims are as follows: 1) Provide biostatistical support and consultation in study design, analysis and manuscript preparation, 2) Support bioinformatics needs and provide Members appropriate access to electronic health record (EHR) data and data-intensive computing, 3) Provide a secure and scalable computational infrastructure for cancer research, and 4) Develop new value-added methodology and education.

Key Personnel

Ming Tan, PhD, Co-Director

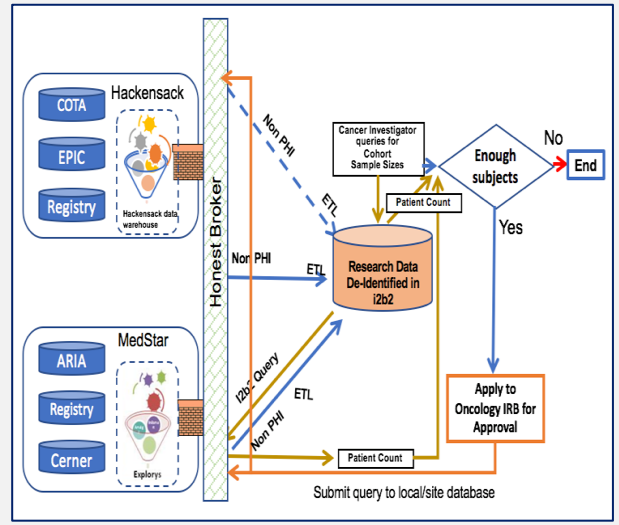
Yuriy Gusev, PhD, Co-Director

Key Services

- Statistical support for experimental design in grant preparation and protocol development for basic, translational, clinical, and population studies.
- Education and training through workshops and courses.
- Support clinical trials through the Protocol Review and Monitoring System, the Clinical Research Leadership Committee, and the Data and Safety Monitoring Committee, as well as access to HIPAA-protected patient records and management of data from multiple CTMS systems (i.e., OnCore, REDCap etc.).

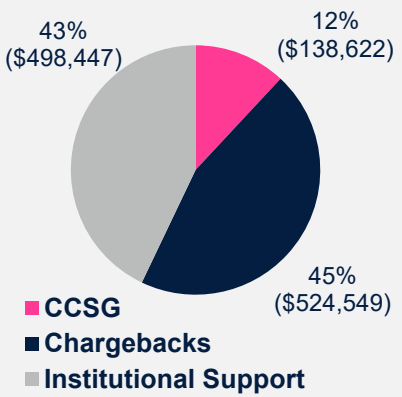
Major Resources / Technologies

- EHR data access for research cohort discovery and trial recruitment
- Standard: SAS, R, Python, Ingenuity
- Special programs developed
  - Adaptive designs SCPRTbin
  - Design and analysis of drug combinations (SynStat)
  - Cancer Mutation Analysis
  - Tumor heterogeneity analysis: DeMix-Bayes, Testing multiple biological mediator: MultiMed E-DRE (enhanced double robust causal estimation)

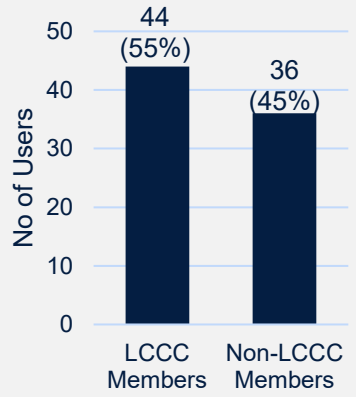


Usage / Budget (FY22)

Sources of Support



Usage by Membership



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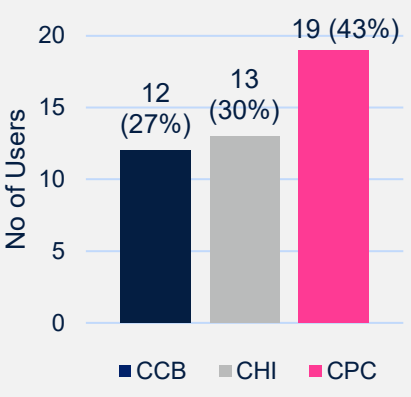


GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Biostatistics & Bioinformatics Shared Resource

Co-Directors: Ming Tan, PhD, Yuriy Gusev, PhD

Harnessing the Thymus for Long-Term Tumor Control with Hematopoietic Stem Cell Derived Naive CAR T Cells

BBSR provided support to **Johannes Zakrzewski, PhD**[CHI] in the design and analysis of a study to harness the thymus for long-term tumor control with hematopoietic stem cell-derived naive CAR T-cells. Animal models have demonstrated superior overall tumor control of several novel NF-kB inhibitors.

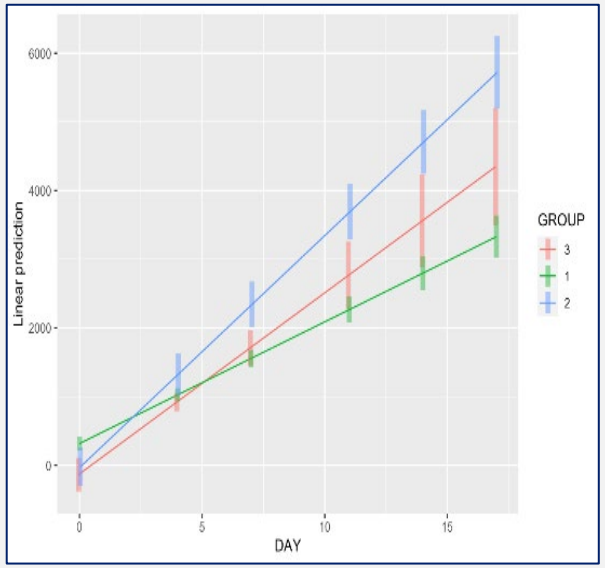


Fig. 1. Analysis of data on longitudinal tumor growth control with hematopoietic stem cell-derived naive CAR T cells.

Conclusions: The BBSR was instrumental in the analysis of data on longitudinal tumor growth control with hematopoietic stem cell-derived naive CAR T-cells that led to a new R37 grant awarded to a consortium LCCC Member.

Grant: R37CA250661

Deficit Accumulation Frailty Trajectories of Older Breast Cancer Survivors and Non-Cancer Controls: The Thinking and Living With Cancer Study

BBSR provided statistical support for **Jeanne Mandelblatt, MD, MPH**[CPC] for the cancer and aging project, Thinking and Living with Cancer, in older breast cancer survivors.

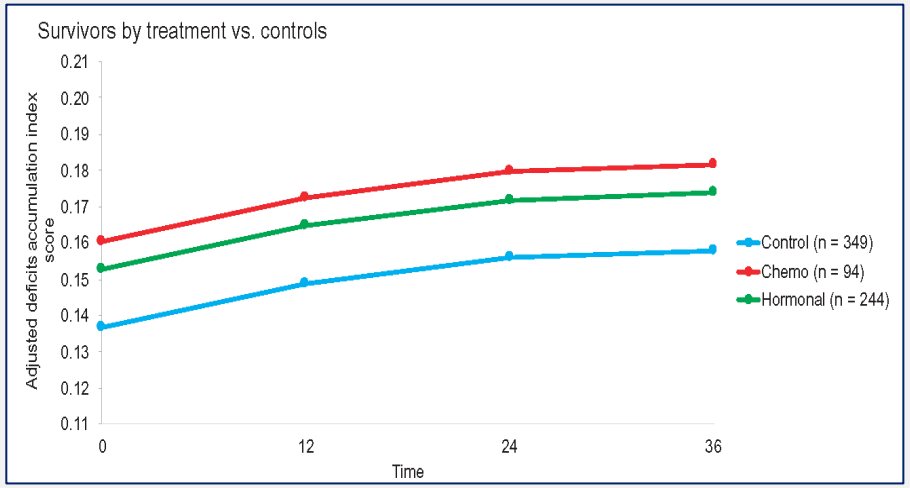


Fig. 3. Adjusted mean deficits accumulation scores for older breast cancer survivors and frequency-matched controls.

Conclusions: Statistical analysis provided by the BBSR supported this multisite prospective study to examine the risk of cognitive decline in older breast cancer survivors. Data revealed significant differences in deficit patterns between survivors and controls.

Grants: R01CA129769, R35CA197289
Publication: *J Natl Cancer Inst.* 2021, PMC8328973

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Biostatistics & Bioinformatics Shared Resource

Co-Directors: Ming Tan, PhD, Yuriy Gusev, PhD

Allele-specific DNA methylation (ASM) is Increased in Cancers

BBSR supported **Benjamin Tycko, MD, PhD**[CCB] to analyze whole genome methyl-seq data on diverse normal cells and tissues from three cancer types to map allele-specific DNA methylation (ASM).

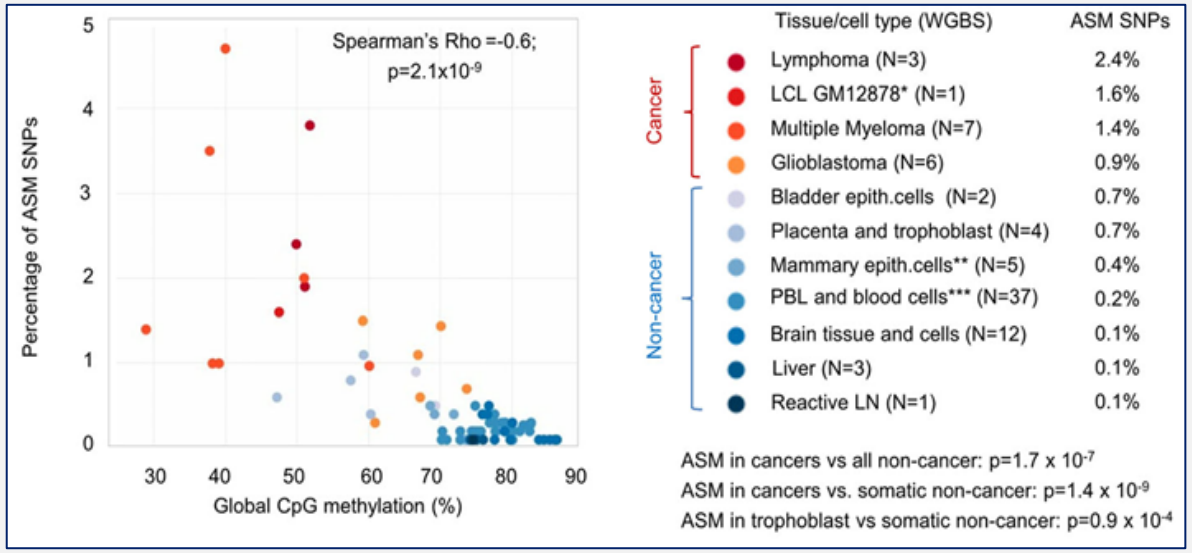


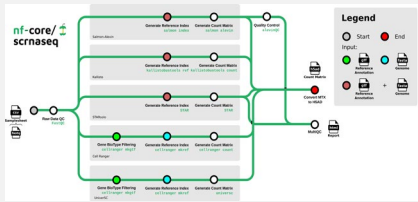
Fig. 2. ASM is increased in cancers and correlates with global DNA hypomethylation.

Conclusions: Statistical analysis performed by the BBSR revealed that ASM is increased in cancers and occurs by a shared mechanism involving SNPs and transcription factor binding sites.

Grants: R01MH092580, R01AG036040, R01AG035020, DP3DK094400
Publication: *Genome Biol.* 2020, PMC7322865

Other Key Activities

- Developed an ImmunoOncology registry that has enabled a study on the impact of immunosuppressive agents on immune checkpoint inhibitor efficacy in advanced melanoma using machine learning methods (*Cancer* 2023, PMID:36951119).
- Developed computational pipelines, tutorials and codes for bulk NGS and SC analysis.
- Supported multiple IITs leading to R21CA270585 to **Ming Tan, PhD**[CCB], and publications (**Sandra Swain, MD**[CCB]: *Breast Cancer Res Treat.* 2022, PMC9633499); (**John Marshall, MD**[CCB] .*Clin Cancer Res*. 2020, PMC10184025).



Future Plans

- Provide study design and reproducible analysis for genomic, translational, and clinical studies to LCCC Members across the Consortium.
- Expand capability in computational biology and reproducible predictive analysis using multi-source data via the computational biology translational working group.
- Support Consortium and Medstar Health collaborations (e.g., data sharing agreement).
- Collaborate with other SRs to support interoperability and enterprise resources.
- Provide management and user support for cloud-based genomics data resources: G-DOC Hub, Cancer Genomic Cloud, AI/ML applications for big biomedical data (e.g., Genomics and Cancer Radiology imaging).

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Flow Cytometry & Cell Sorting Shared Resource

Director: Karen Creswell, PhD

Overview

The Flow Cytometry & Cell Sorting Shared Resource (FCSR) provides flow cytometry (analysis and sorting) and suspension mass cytometry (CyTOF) services to LCCC Members. The FCSR Specific Aims are as follows: 1) Provide state-of-the-art cytometry-based cell sorting and analysis services, and CyTOF services to support high impact cancer research, 2) Provide high-quality education and technical support, 3) Facilitate Member access to mass cytometry (CyTOF XT) services by developing a bank of metal-labelled antibodies commonly used in cancer staining panels.

Key Personnel

Karen Creswell
PhD, Director

Yuanyuan Tian,
PhD, Manager

Zhinuo Jiang,
MS, Technician

Wenshan Tsao,
MS, Technician

Key Services

- Flow Cytometry Phenotyping
- Flow functional assays – apoptosis, ROS analysis, mitochondrial membrane potential, etc.
- Cell cycle analysis
- EV analysis with Nanosight
- CyTOF- high dimensional phenotyping
- Experimental design and data analysis assistance

Major Equipment / Technologies

Flow Cytometry Analyzers

LSRFortessa

Symphony

Cell Sorters

FACSAria

Melody

Mass Cytometer

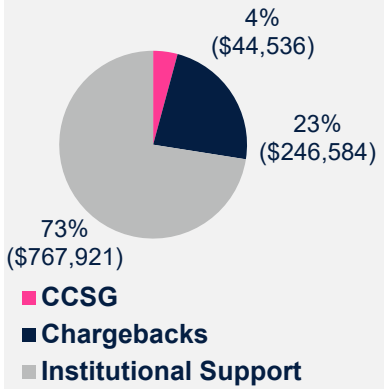
Standard Biotools XT

Nanoparticle Analyzer

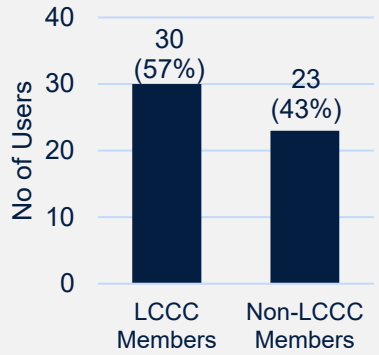
Malvern Nanosight

Usage / Budget (FY22)

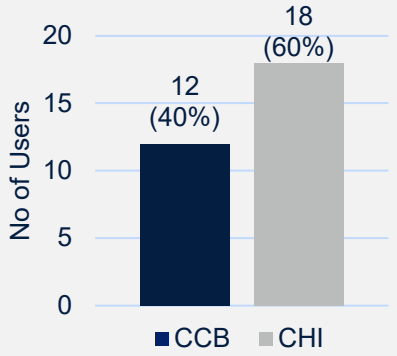
Sources of Support



Usage by Membership



Usage by Program



GEORGETOWN UNIVERSITY

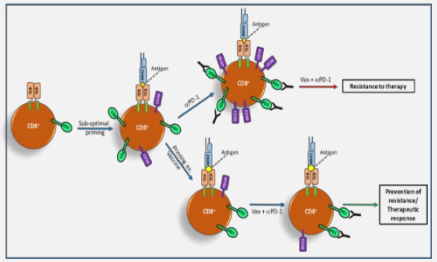
Lombardi Comprehensive Cancer Center

Flow Cytometry & Cell Sorting Shared Resource

Director: Karen Creswell, PhD

A Combination Therapy Strategy to Prevent Anti-PD-1 Therapy Resistance in Metastatic Ovarian Cancer Patients

The FCSR supported the work of **Samir N. Khleif, MD**[CHI] with cell sorting and phenotyping using the new CyTOF XT for a 45 marker phenotyping panel.



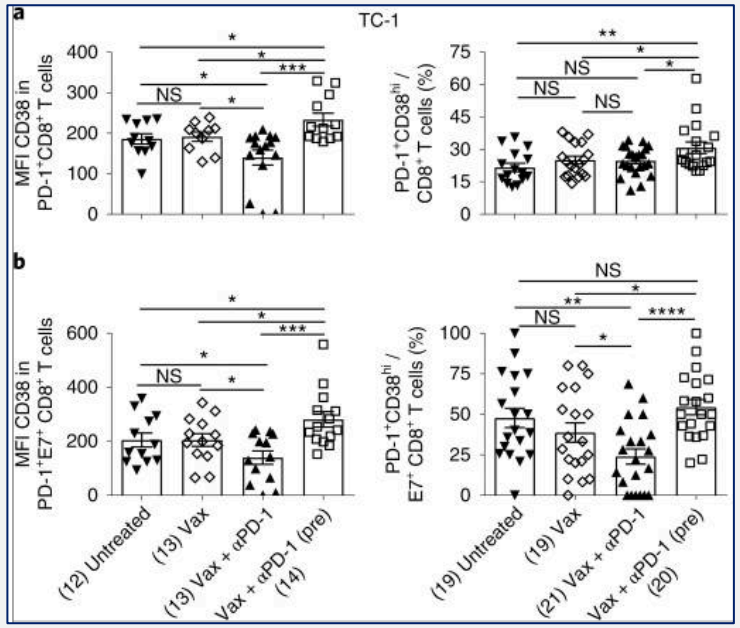


Fig. 1. (above) PD-1 blockade before antigenic stimulation induces PD-1⁺CD38^{hi} CD8⁺ T cells. **Fig. 2.** (right) MFI and frequency of PD-1⁺CD38^{hi} T cells in total (**a**) and antigen-specific (**b**) CD8⁺ T cells in TC-1 tumor-bearing mice at day 13 post-tumor implantation.

Conclusions: FCSR provided critical support for this work, leading to an IIT in ovarian cancer patients, that revealed that treatment with anti-PD1 under sub-optimally primed T cell conditions leads to induction of resistance to anti-PD1 therapy and produces dysfunctional PD-1⁺CD38⁺ CD8⁺ T cells.

Grant: DOD W81XWH2010412
Publication: *Nat Immunol*. 2019, PMC7472661

Tcf1-CTCF Cooperativity Shapes Genomic Architecture to Promote CD8⁺ T Cell Homeostasis

The FCSR provided critical services for phenotyping, cell division assays and cell sorting to **Hai-Hui Xue, MD, PHD**[CHI] in investigating CD8⁺ T cell homeostasis.

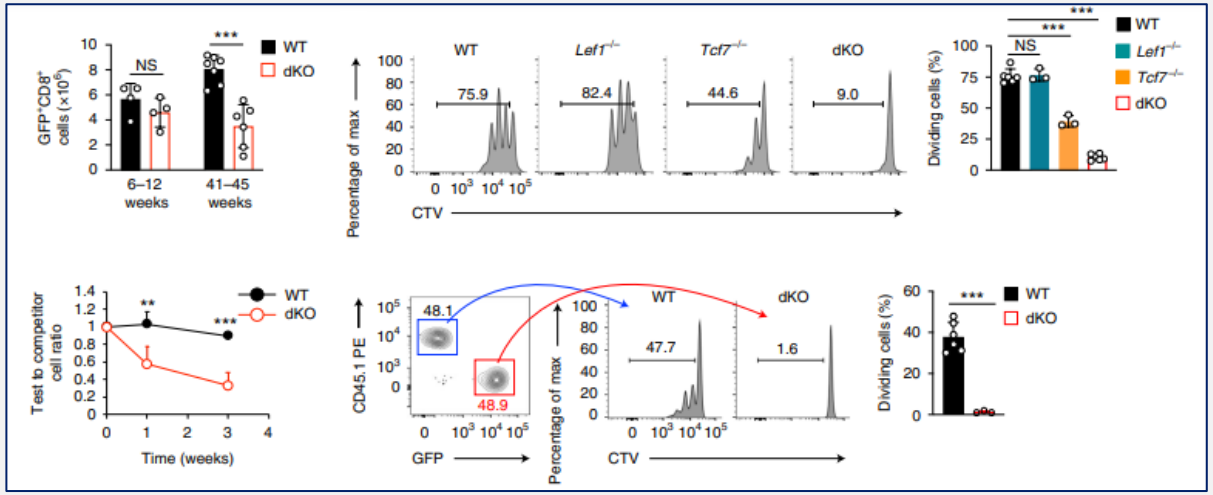


Fig. 3 Tcf1 and Lef1 are required for homeostatic proliferation of CD8⁺ T cells.

Conclusions: FCSR supported the research of a Consortium Member that led to the discovery that CD8⁺ T cell homeostasis is regulated by Tcf1 and CTCF.

Grants: NIH (AI112579, AI121080, AI139874) and the Veteran Affairs (BX005771)
Publication: *Nat Immunol*. 2022, PMC9579964

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Flow Cytometry & Cell Sorting Shared Resource

Director: Karen Creswell, PhD

Temozolomide-induced Guanine Mutations Create Exploitable Vulnerabilities of Guanine-rich DNA and RNA Regions in Drug-resistant Gliomas

The FCSR provided services for cell cycle and apoptosis analysis to **Rebecca Riggins, PhD[CCB]** for her project on Temozolomide (TMZ) resistance in glioblastomas (GBM). This project also used GESR, FCSR, HTSR, MISR, SRBSR and TCBSR.

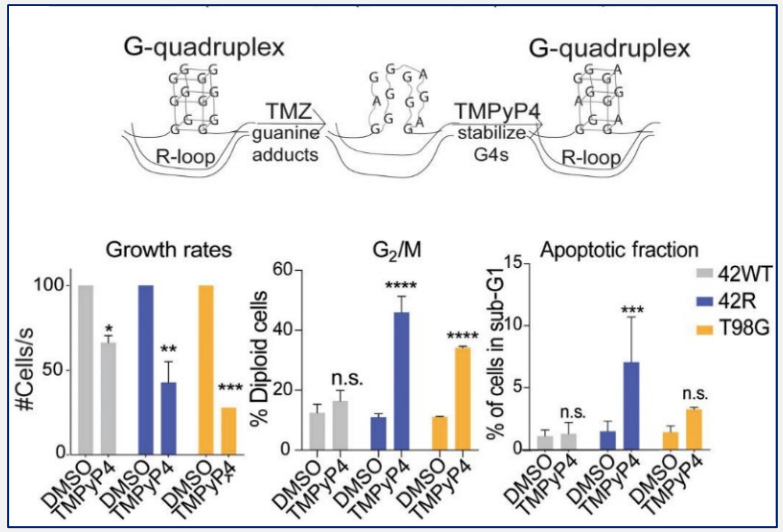


Fig. 4. Guanine mutations in TMZ-resistant GBM cells associate with sensitivity to the G4-stabilizing drug TMPyP4.

Conclusions: FCSR supported research to identify novel vulnerabilities in TMZ-resistant GBM guanine mutations that lead to deregulated alternative splicing in GBM, leading to a new R01. This work also supported T32 trainee Deanna Tiek.

Grants: R01CA256481, NIH T32CA009686
Publication: *Sci Adv.* 2022, PMC9216507

Other Key Activities

- Collaborated with MSAPSR and HTSR to establish CyTOF services at LCCC.
- Installed a second CyTOF instrument in the FCSR, the XT, for multiparameter phenotyping.
- Established new phenotyping panels for LCCC Members (**Cheema[CHI], Fornace[CCB], Khleif[CHI], Slingerland[CHI], Wellstein[CHI]**).
- Validated new phenotyping panels for LCCC clinical investigators for clinical trials (**Reuss[CHI], Weinberg[CHI]**).
- Coordinated with other Shared Resources (GESR, FCSR, HTSR, MISR, SRBSR and TCBSR) on projects for LCCC Members (**Riggins[CCB], Slingerland[CHI], Xue[CHI], Yi[CCB], Zakrzewski[CHI]**).

Future Plans

- Provide educational opportunities to LCCC Members in NJ to demonstrate the CyTOF technology, particularly for suspension-based phenotyping.
- Continue to collaborate with HTSR through the shared management of the CyTOF.
- Develop an antibody bank to maintain cost-effectiveness and encourage investigators to capitalize on CyTOF technology.
- Provide a Standard BioTools CyTOF XT seminar series for user training on data analysis methods and panel design.

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Genomics & Epigenomics Shared Resource

Co-Directors: Habtom Ressom, PhD, Aykut Üren, MD, Benjamin Tycko, MD, PhD

Overview

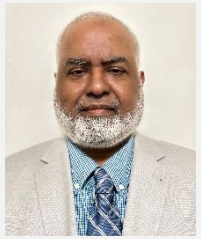
The mission of the GESR is to provide LCCC Members with diverse services for genomic and epigenomic studies, and label-free molecular interaction analysis. The GESR Specific Aims are as follows: 1) Provide access to state-of-the-art services for genomic and epigenomic studies, 2) Offer Surface Plasmon Resonance (SPR) technology platforms for label-free molecular interaction studies, 3) Provide bioinformatics support and training for genomic and epigenomic data analysis to GESR users in collaboration with the BBSR.

Key Personnel

Habtom Ressom, PhD, Co-Director

Aykut Üren, MD, Co-Director

Benjamin Tycko, MD, PhD, Co-Director



Md Islam, MD Manager

Key Services

- DNA/RNA quality assessment
- DNA sequencing
- SNP genotyping
- DNA CNV analysis
- DNA methylation analysis
- mRNA and miRNA expression profiling
- Label-free molecular interaction analysis
- scRNA-seq
- Digital Spatial Profiling (DSP)
- Targeted Methyl-seq
- Bioinformatics
- Training & seminars

Major Equipment / Technologies

GeoMx DSP Protein and RNA assays

NanoString GeoMx Digital Spatial Profiler

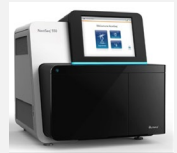
mRNA and miRNA expression analysis

NanoString nCounter Max

Single cell RNA seq and ATAC RNA seq

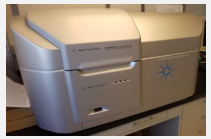
10x Genomics Chromium Controller

Next generation sequencing



Illumina NextSeq 550

mRNA/miRNA expression profiling



Agilent Scanner

Targeted Nextgen Methyl-seq by Fluidigm Juno System

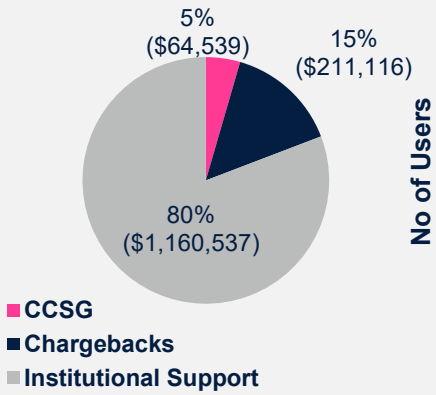
Integrated Fluidic Circuit & Illumina MiSeq

Label-free molecular interaction analysis

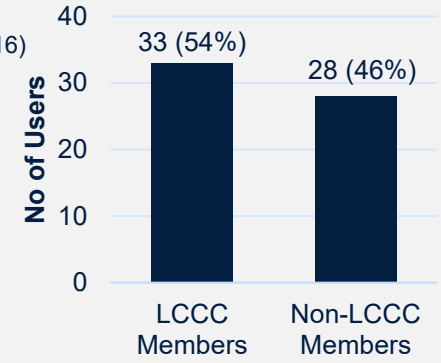
Biacore 4000

Usage / Budget (FY22)

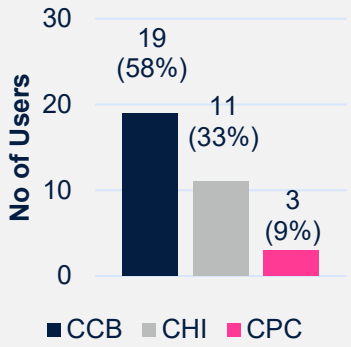
Sources of Support



Usage by Membership



Usage by Program



GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Genomics & Epigenomics Shared Resource

Co-Directors: Habtom Ressom, PhD, Aykut Üren, MD, Benjamin Tycko, MD, PhD

Chlorpromazine Binding to the PAS Domains Uncovers the Effect of Ligand Modulation on EAG Channel Activity

Tinatin Brelidze, PhD[CCB] used the SPR platform in the GESR to investigate the link between up-regulation of ether-a-go-go (EAG) channel activity to cancer and neurological disorders.

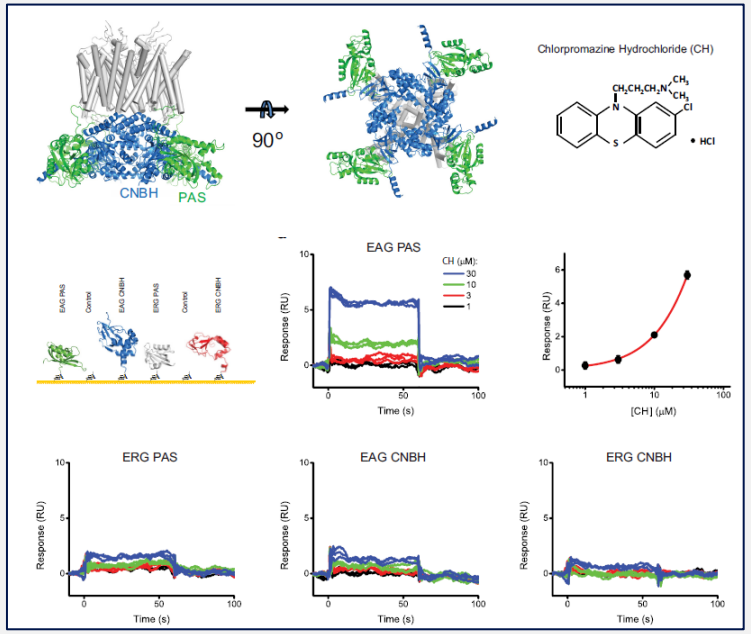


Fig 1. CH binding to the PAS domain of EAG channels detected with SPR.

Conclusions: The GESR provided critical support to a new, internally recruited LCCC Member during the project period. This study raises the possibility of repurposing the antipsychotic drug chlorpromazine for treatment of cancer.

Grant: NIH R01GM12402
Publication: *J BiolChem.* 2020, PMC7105296

NSABP B-41, A Randomized Neoadjuvant Trial: Genes and Signatures Associated with Pathologic Complete Response

Sandra Swain, MD[CCB] used the NanoString platform in the GESR to investigate a large human breast cancer gene expression panel to select candidate prognostic biomarkers for pCR among women treated with trastuzumab in NSABP B-41.

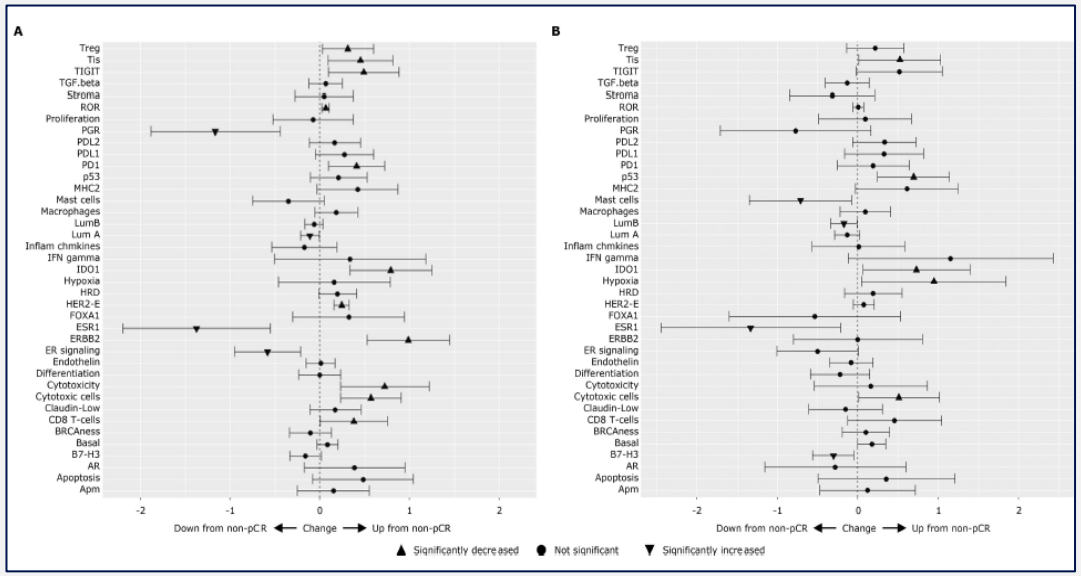


Fig 2. Differences in mean gene expression between patients with and without qCR. (**A**) treated with trastuzumba or trastuzumba + lapatinib. (**B**) treated with lapatinib alone.

Conclusions: In support of correlative science linked to an important NCTN study (NCT00486668), this work identified a panel of genes that predict pathologic complete response (pCR) in women with HER2-positive early-stage breast cancer undergoing neoadjuvant chemotherapy.

Grant: Breast Cancer Research Foundation (BCRF-21-156)
Publication: *Clin Cancer Res.* 2020, PMC7724952

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Genomics & Epigenomics Shared Resource

Co-Directors: Habtom Ressom, PhD, Aykut Üren, MD, Benjamin Tycko, MD, PhD

Experimental Evolution of Pancreatic Cancer

The GESR supported **Alvin Makohon-Moore, PhD[CCB]** with library preparation and targeted sequencing in his work to genetically barcode human cancer cell lines in order to quantify diversity and track clonal dynamics over time under varying nutrient selection pressures during experimental evolution.

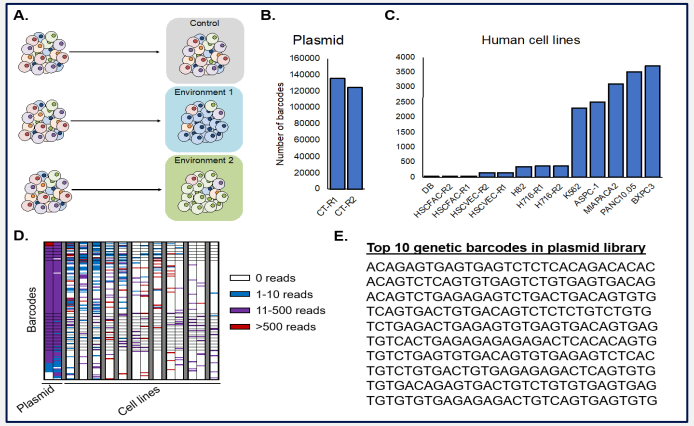


Fig 3. A. Schematic of clonal dynamics in different environments. **B.** Number of genetic barcodes detected in the plasmid library. **C.** Number of genetic barcodes detected across the human cell lines sequenced. **D.** Matrix showing the read counts of the top 10 genetic barcodes in each cell line across the plasmid library and cell lines. **E.** DNA sequencing of top 10 genetic barcodes showing a "Weak-Strong" pattern for 30 bases (weak=A/T, strong=C/G).

Conclusions: A new service in GESR supported the work of a new, early stage, consortium LCCC Member to quantify the number of barcoded cancer cell lineages in each population and to define the specific lineages that survive and adapt to precisely defined nutrient selective pressures.

Grant: NIH R00CA229979

Other Key Activities

- Launched new services such as single-cell RNAseq, digital spatial transcriptomics, and targeted methyl-seq.
- Created a new Surface Plasmon Resonance Database (SPRD), (www.sprdatabase.info) that is publicly available to scientific community to help planning of SPR experiments (*BMC Mol Cell Biol*. 2021, PMC7937274).
- Education and training of SR users in available GESR technologies through symposia and seminars.
- Supported **Amrita Cheema, PhD[CHI]** with RNAseq analysis to investigate the impact of pancreatic cancer derived extracellular vesicles (EVs) on recipient non-tumourigenic pancreatic epithelial cells (*J Extracell Vesicles* 2022, PMC9164146).

Future Plans

- Launch new services to support expanding studies including DNA repair, spatial transcriptomics, epigenomics, and signaling.
- Utilize the cost-effective and complementary resources offered by the Mid-Atlantic Shared Resource Consortium of regional NCI-designated cancer centers that allows reciprocal access to Shared Resources at other centers.
- Collaborate with BBSR and HTSR to assist investigators in multi-disciplinary research, multi-omics approaches, and bioinformatics support that lead to high-impact and innovative science.
- Coordinate initiatives with the new Computational Biology Working Group.

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Histopathology & Tissue Shared Resource

Co-Directors: Brent Harris, MD, PhD, David Kar Fai Chow, MD, Anju Duttargi, MDS

Overview

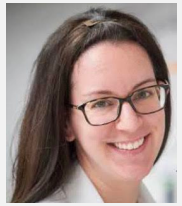
The Histopathology & Tissue Shared Resource (HTSR) provides access to human tissue for translational research and provides comprehensive, high-quality laboratory and interpretive pathology services to LCCC Members. The HTSR Specific Aims are as follows: 1) Provide comprehensive services (e.g., tissue processing, microtomy, cryotomy, staining, immunohistochemistry (IHC), tissue microarray (TMA) construction, immune phenotyping and high throughput analysis), 2) Collect and distribute fresh, frozen, and formalin-fixed paraffin-embedded tissues and select biofluids, 3) Provide technical and pathological support for investigator-driven tissue collection protocols.

Key Personnel

Brent Harris, MD, PhD, Co-Director

David Kar Fai Chow, MD, Co-Director

Anju Duttargi, MDS, MS, Co-Director



Ya'el Kramer, MS, Biorepository Director of Operations

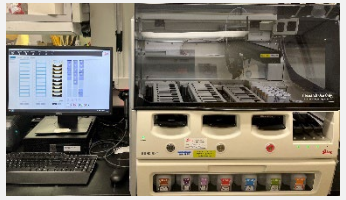
Key Services

- Human tissue and biofluid banking (HIPAA compliant, IRB approved Universal protocols)
- Oversight of Biospecimen Use Committee
- Comprehensive Histology services for human tissues and animal models
- Advanced multiplex IHC
- TMA construction
- Interpretive Pathology consultation
- Whole slide digital scanning
- Staining for downstream spatial biology protocols in other Shared Resources

Major Equipment / Technologies

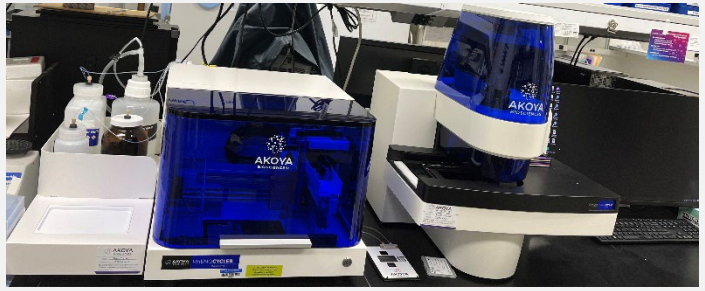
Advanced Tissue Staining

Leica Bond



Multiplex image Analysis

Akoya Phenocycler

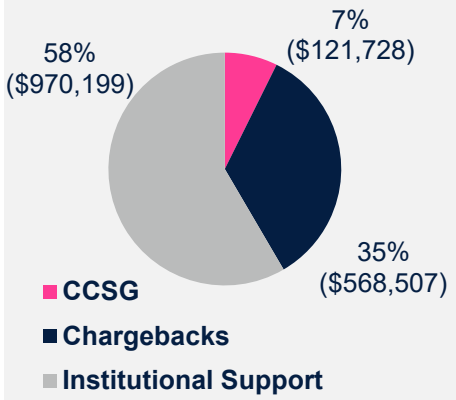


High Throughput Slide Scanner

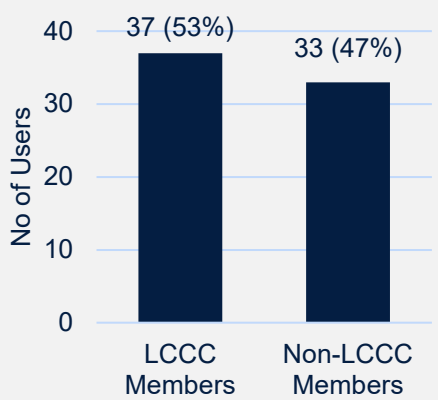
Aperio GT450

Usage / Budget (FY22)

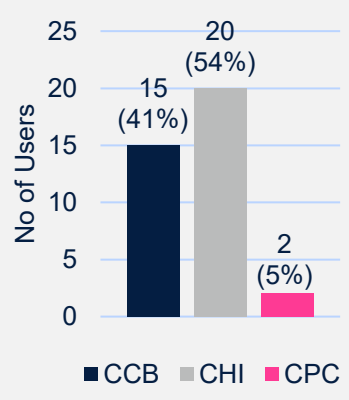
Sources of Support



Usage by Membership



Usage by Program



GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Histopathology & Tissue Shared Resource

Co-Directors: Brent Harris, MD, PhD, David Kar Fai Chow, MD, Anju Duttargi, MDS

Unraveling Mechanisms of PDAC-based Immunosuppression

Louis Weiner, MD[CHI] and graduate student Zoe Malchiodi used 34 metal-conjugated antibodies to assess immune architecture using Imaging Mass Cytometry (IMC) in patient PDAC TMAs; through coordinated SR support (MSAPSR, HTSR, BBSR) and the computational biology translational working group.

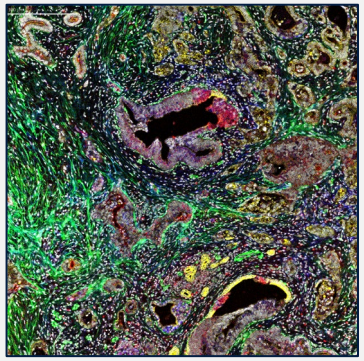


Fig. 1. NK cells associate with PDAC epithelial cells. Representative IMC pseudo-image (Pankeratin (Y), NKG2D (R), FAP (B), aSMA (G).

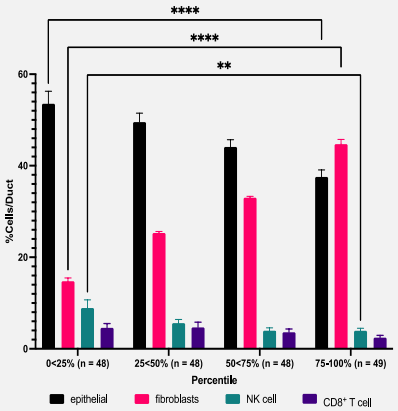


Fig. 2. NK cell content inversely correlates with fibroblast abundance in epithelial-ductal regions of PDAC samples.

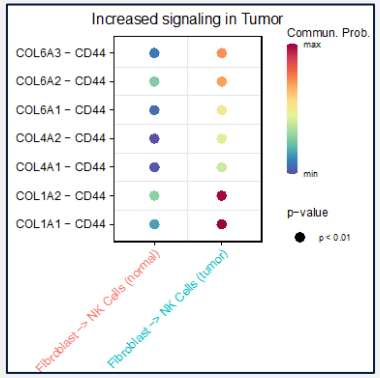


Fig. 3. CellChat analysis of PDAC scRNAseq dataset identifies collagen-CD44 ligand-receptor pairs as potential molecular mediators of CAF inhibition of NK cell migration.

Conclusions: HTSR supported innovative spatial imaging revealing previously unknown influences of cancer associated fibroblasts (CAFs) and their secreted products on NK cell infiltration of malignant epithelial glands in PDAC. Findings prompted evaluation of scRNAseq PDAC dataset to probe critical signals that suppress NK cell migration into malignant epithelial glands.

Grants: PDAC clinical trial (NCT 05558982), F31 CA261125
Publication: *Cancers (Basel).* 2021, PMC7865209

Novel Strategy for Critical Gene Discovery in Clear Cell Renal Cell Carcinoma (ccRCC)

The HTSR supported **Benjamin Tycko, MD, PhD**[CCB] with the collection of frozen tumor and matched normal tissues and blood for further downstream molecular analysis of ccRCCs (DNA copy number analysis, DNA methylation sequencing, and bioinformatic pipeline to identify functional mutations in non-coding regulatory genes, with validation via CRISPR-Cas9 mediated mutagenesis in cell lines).

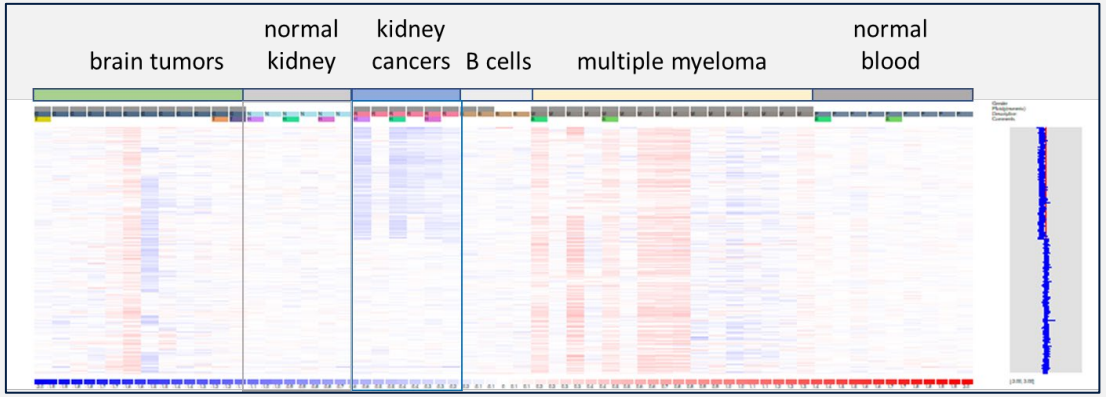


Fig. 4. Illumina Beadchip assays on frozen tissues for DNA CNV for Chr 3. Blue box - clear cell renal cell carcinoma (ccRCC) shows typical loss of 3p region.

Conclusions: The HTSR supported collection of matched frozen tumor and normal tissues in this study to investigate if non-coding mutations are simply neutral passengers or are functional in regulating genes that accelerate tumor progression or confer treatment resistance in ccRCC. These data will have important short-term and long-term impacts in treating patients with ccRCC.

Grant: DoD KCRP Research Grant # KC210215

GEORGETOWN UNIVERSITY

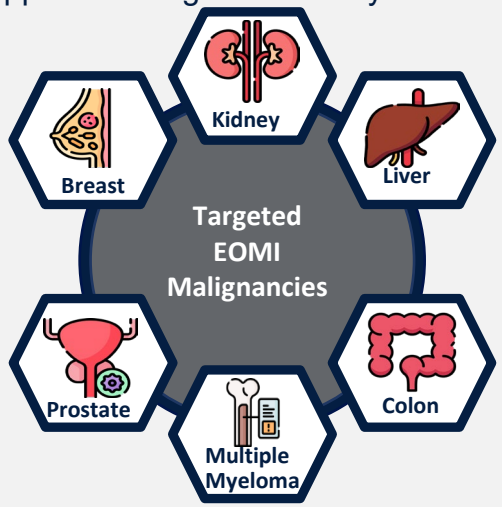
Lombardi Comprehensive Cancer Center

Histopathology & Tissue Shared Resource

Co-Directors: Brent Harris, MD, PhD, David Kar Fai Chow, MD, Anju Duttargi, MDS

Early Onset Malignancies Initiative (EOMI)

Brent Harris[CCB] and **Lucile Adams-Campbell**[CPC] received a supplemental grant for the NCI Community Oncology Research Program (NCORP) Early Onset Malignancies Initiative (EOMI) program to consent individuals having surgery at MedStar Georgetown University Hospital (MGUH) or MedStar Washington Hospital Center (MWHC), abstract pertinent clinical data, and coordinate biospecimen collection to contribute to a nationwide study to investigate the molecular basis of early onset cancer occurring in racially and/or ethnic minority populations through the application of genome analysis technologies.



National Capital Area (NCA) contributions: total accrual numbers (n=21) are as follows:

- Breast (n=4)
- Colon (n=4)
- Kidney (n=10)
- Liver (n=2)
- Prostate (n=1)

Conclusions: HTSR is supporting this study with both retrospective and prospective biospecimen collection. The EOMI will be a step forward in addressing racial/ethnic disparities, in understanding cancer in underserved populations, as well as disparate participation in crucial molecular research to reach that understanding.

Grant: UG1CA239758

Other Key Activities

- Acquisition of Leica Bond and Akoya Phenocycler for multi-plex staining, high throughput miRNA staining, RNAScope and dual ISH and IHC to expand HTSR's existing *in situ* services.
- Created four new TMAs for Colorectal Cancer, Pancreatic Cancer, Invasive Lobular Breast Cancer, and Head and Neck Squamous Cell Carcinoma.
- Efforts to unify Biospecimen Use Committees (BUC) between DC and NJ has expanded access to larger, more diverse biospecimen repositories for translational research. A unified BUC composed of clinical and research scientists, pathologists, surgeons and statisticians in DC and NJ who review research biospecimen use requests has emerged from these efforts since 2021.
- Established Universal Consenting Protocol in NJ.
- HTSR obtained subaward (UM1CA181255 to **Harris**[CCB]) to serve as a site for the NCI AIDS and Cancer Specimen Resource (ACSR).

Future Plans

- Establish a Universal Consent program for biospecimens at the MedStar hospitals similar to the newly established program on the NJ campus.
- Integrate and adapt OpenSpecimen, the new LIMS system, which connects to REDCap and provides outcome and other associated clinical data for all biospecimen resources.
- Continue to support Early Onset Malignancies Initiative (EOMI) of the NCI Community Oncology Research Program (NCORP)

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Microscopy & Imaging Shared Resource

Co-Directors: Michael D. Johnson, PhD, Steven Park, BS

Overview

The mission of the Microscopy & Imaging Shared Resource (MISR) is to support LCCC Members to apply advanced microscopy and imaging-based technologies to their research. The MISR Specific Aims are as follows: 1) Provide access to a comprehensive array of advanced optical imaging platforms and analysis packages, 2) Provide Members with advice and assistance with experimental design, selection of reagents and technology, and analytical approaches, 3) Provide full-service imaging solutions for investigators with imaging and analysis needs, 4) Provide education and training on imaging platforms.

Key Personnel

Michael D. Johnson, PhD, Co-Director

Steven Park, BS, Co-Director

Peter Johnson, Manager

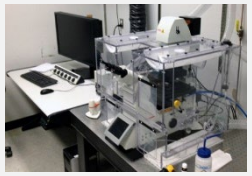
WenShan Tsao, MS, Manager

Key Services

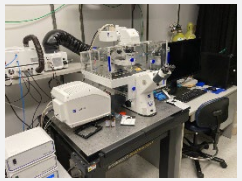
- Consult on experimental design, choice of imaging modalities and systems, and grant submissions.
- Workshops, and initial and ongoing training on imaging platforms, and analysis software.
- Assistance with image acquisition, analysis, and experimental troubleshooting.
- Acquisition and processing of data on some systems (Molecular cytogenetics, DIVER-based studies).
- Supply of optimal supplies, validated imaging reagents, and DNA constructs.

Major Equipment / Technologies

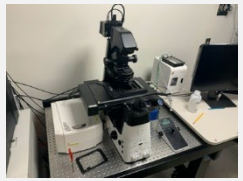
Laser Scanning Confocal Microscopes



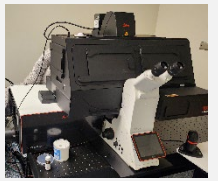
Leica SP8AOBS++



Zeiss LSM 800

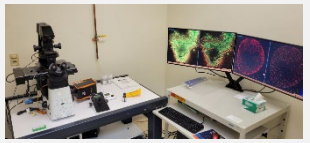


Nikon Ti2 A1R LSM



Leica Stellaris 5 & 8

Super Resolution Platforms

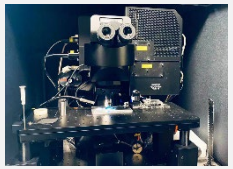


Abberior STEDYCON STED

ONI Nanoimager dSTORM/PALM

Nikon TI2 SD SoRa/Tirf

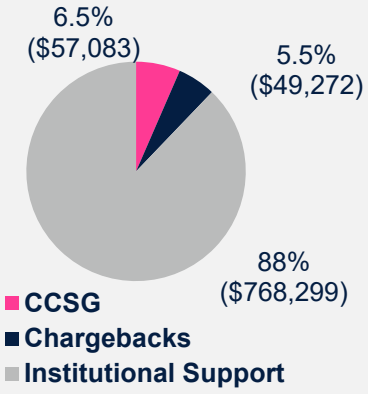
Multi-photon/Phaser FLIM



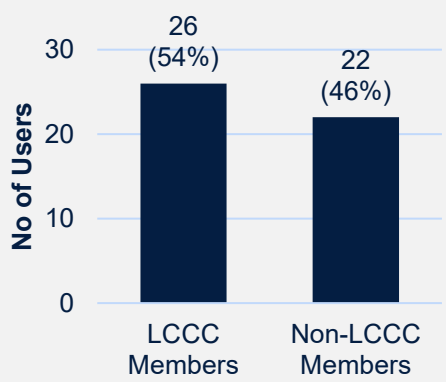
Olympus FVMPE-RS

Usage / Budget (FY22)

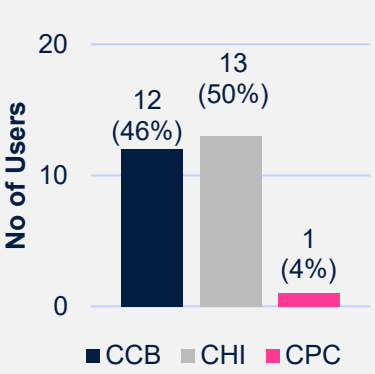
Sources of Support



Usage by Membership



Usage by Program



GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Microscopy & Imaging Shared Resource

Co-Directors: Michael D. Johnson, PhD, Steven Park, BS

Study of Cognitive Function After Cancer Chemotherapy ("Chemo Brain")

William Rebeck[CHI] and **Jeanne Mandelblatt** [CPC] used the MISR in a study working to understand the mechanisms underlying the decline in cognition ("Chemo Brain") experienced by cancer survivors during and after chemotherapy treatment.

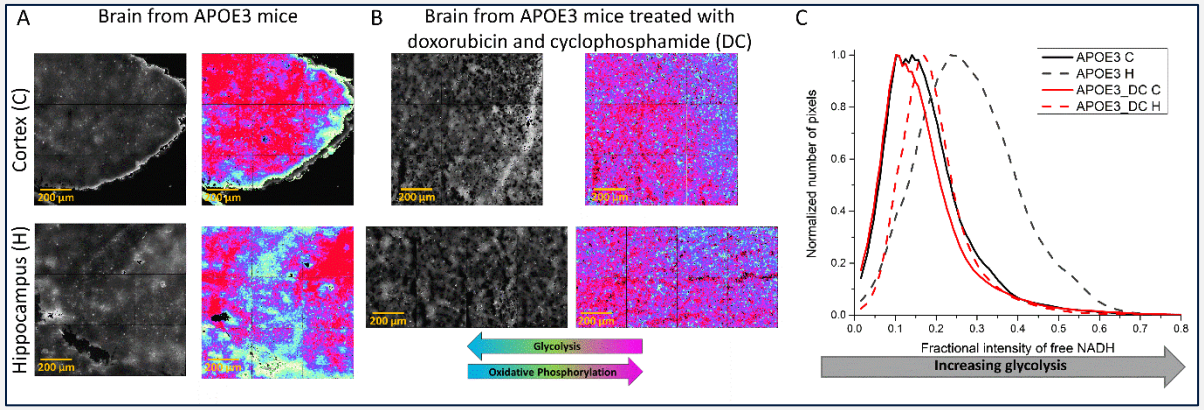


Fig. 1. Brain slices from APOE-3 mice treated with vehicle (**A**) or doxorubicin and cyclophosphamide (**B**) were imaged using the Olympus FVMPERS multi-photon microscope equipped with a SpectraPhysics Insight X3 laser and DIVER imaging system connected to ISS FastFLIM for fluorescence lifetime imaging. (**C**) APOE variants predict glycolytic activity.

Conclusions: MISR supported work that demonstrates chemotherapy causes more metabolic changes, specifically a greater shift to a glycolytic phenotype, in the cortex compared to the hippocampus after systemic treatment with chemotherapy.

Grants: R01 AG067258, R01 CA129769
Publication: *Neurotox Res*. 2019, PMC6333492

Characterization of Exhaled Extracellular Vesicles by Super Resolution Imaging Validates the EV-CATCHER Method on Exhaled Breath Condensates

The MISR supported the work of **Olivier Loudig**[CCB] to evaluate the application of the EV-CATCHER technology for lung cancer screening.

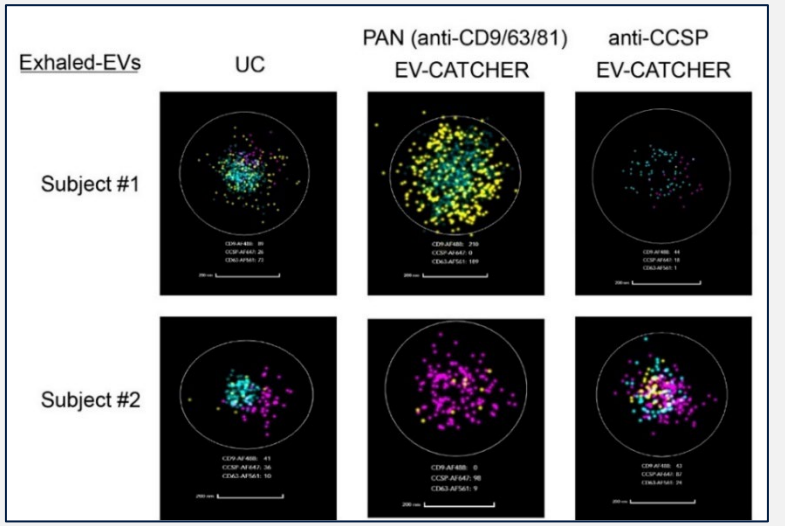


Fig. 2. Extracellular vesicles (EVs) were isolated from exhaled breath condensates by ultracentrifugation (UC) using the EV-CATCHER method and anti-CD9/63/81 or anti-CCSP (Clara Cell Specific Protein). EVs were imaged on the ONI Nanoimager super resolution imaging platform.

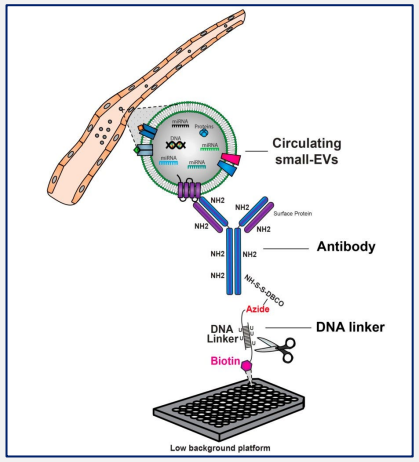


Fig. 3. Schematic of EV-CATCHER technology.

Conclusions: Imaging support provided by MISR demonstrates that deep lung EVs can be isolated using this methodology. In preliminary data, clear differences were seen between smokers/non-smokers and cancer vs control.

Grant: R33 HL156279

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Microscopy & Imaging Shared Resource

Co-Directors: Michael D. Johnson, PhD, Steven Park, BS

Probing Oncogenic Functions of YAP/TAZ by Studying the Dynamics of YAP Mediated Phase Separation and Interaction with Signaling Molecules

The MISR supported **Chunling Yi** [CCB], **Michael Atkins** [CHI] and graduate student Alec McIntosh to study the oncogenic functions of the YAP/TAZ pathway by visualizing the formation of YAP-mediated JUNB condensates in renal carcinoma cells using the Nikon CSR-W1 spinning disk microscope.

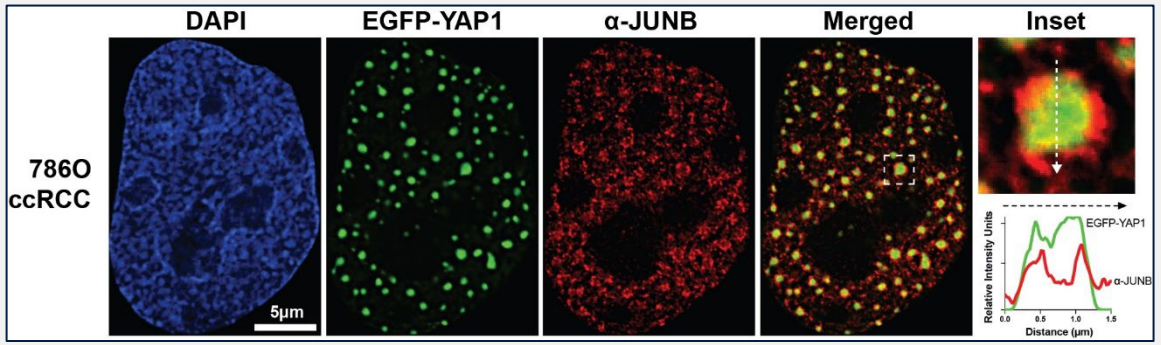


Fig. 4. 786O clear cell renal carcinoma cells were transfected with a YAP1-EGFP fusion construct and stained with an anti-JUNB antibody. JUNB appears to phase separate with YAP1 droplets in these cells. Inset shows an analysis of the co-localization of the signals.

Conclusions: Imaging in the MISR facilitated the discovery that YAP/TAZ silencing is sufficient to induce regression of pre-established NF2-deficient tumors.

Grants: NIH NRSA F30CA264884 to McIntosh, R01 CA187090, Institutional Pilot Funds
Publication: *Dev Cell*, 2019, PMC6524954

Other Key Activities

- Acquisition of 4 new major imaging platforms.
  - Added three new modes of Super-resolution microscopy – SoRa, PALM, dSTORM.
  - Added two new TIRF-capable systems.
  - Two new LSM systems and one spinning disk.
- Significant upgrades to existing instruments and analysis capabilities.
  - Added a converter to the Olympus FVMPE-RS system to function as an inverted microscope to facilitate imaging of tissue culture samples.
  - Enhanced post-acquisition image processing and analysis capabilities with new workstations and new software including Huygens analysis suite and NIS-Elements.

Future Plans

- Construct new custom inverted multi-photon imaging platform to enhance rapid live-cell studies of tissue slices and cultures.
- Add structured illumination microscopy capabilities.
- Acquire additional workhorse confocal imaging platforms to accommodate increasing usage.
- Increase support for full-service image acquisition and analysis.
- Update the TEM to a more modern system.

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Mass Spectrometry & Analytical Pharmacology Shared Resource

Co-Directors: Amrita K. Cheema, PhD, Junfeng Ma, PhD, Claire Carter, PhD

Overview

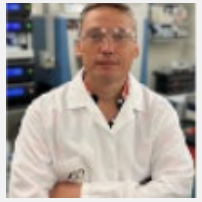
The Mass Spectrometry and Analytical Pharmacology Shared Resource (MSAPSR) functions under a collaborative model organized into four essential components: 1) proteomics, 2) metabolomics, 3) molecular imaging, and 4) PK/PD applications. The MSAPSR Specific Aims are as follows: 1) Provide state-of-the-art multi-omics services for cancer research, 2) Provide imaging mass cytometry (IMC) and mass spectrometry imaging (MSI) Services, 3) Provide computational, data analysis and interpretation support, and training for MSAPSR users in collaboration with the BBSR.

Key Personnel

Amrita Cheema, PhD, Co-Director

Junfeng Ma, PhD, Co-Director

Claire Carter, PhD, Co-Director



Matt Zimmerman, Manager

Meth Jayatilake, Manager

Key Services

- Global metabolomics & lipidomics
- MRM-MS based targeted small molecule and lipid quantitation
- Extracellular flux analysis
- Quantitative proteomics and peptidomics
- Mass cytometry-based tissue imaging
- DMPK studies
- Drug quantitation
- Laser-capture microdissection
- Mass spectrometry imaging of endogenous and exogenous molecules
- Isotopic fine structure analysis

Major Equipment / Technologies

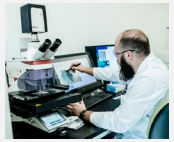
Small Molecule quantitation

7500 QTrap mass spectrometer

Mitochondrial function

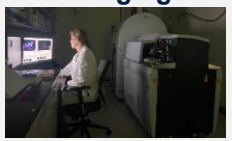
Seahorse Extracellular Flux Analyzer

Laser-capture microdissection



Leica LMD 7

Mass spectrometry imaging



Bruker Solarix 7T FTICR mass spectrometer

Analysis of peptides/proteins

Orbitrap Lumos Tribrid mass spectrometer

Mass cytometry imaging

Hyperion

Pharmacokinetic profiling

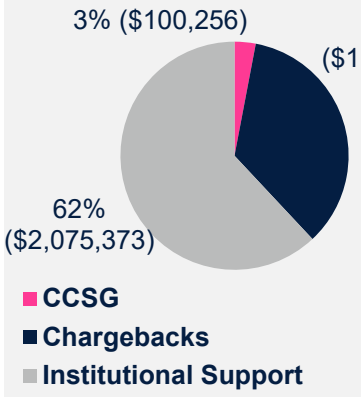
7500 QTrap mass spectrometer

DIA based lipidomics

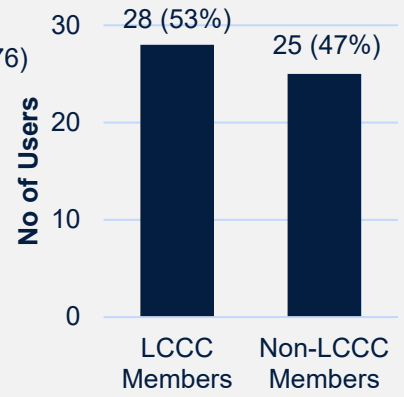
Bruker tims TOF

Usage / Budget (FY22)

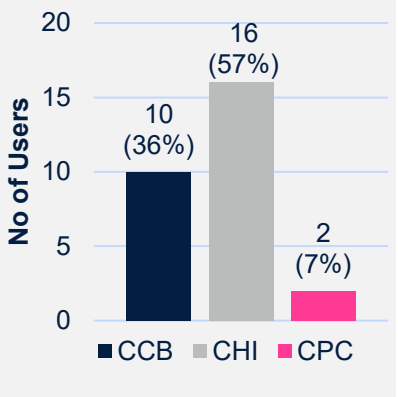
Sources of Support



Usage by Membership



Usage by Program



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Lombardi Comprehensive Cancer Center

Mass Spectrometry & Analytical Pharmacology Shared Resource

Co-Directors: Amrita K. Cheema, PhD, Junfeng Ma, PhD, Claire Carter, PhD

Cardiowatch: Omics-Based Prediction Assay for Radiation Induced Heart Disease

The MSAPSR performed plasma metabolomics-based biomarker discovery to support an IIT led by **Keith Unger, MD**[CCB] that successfully combined metabolomics and prediction modeling to stratify esophageal patients at high risk of cardiac injury following radiation therapy (RT).

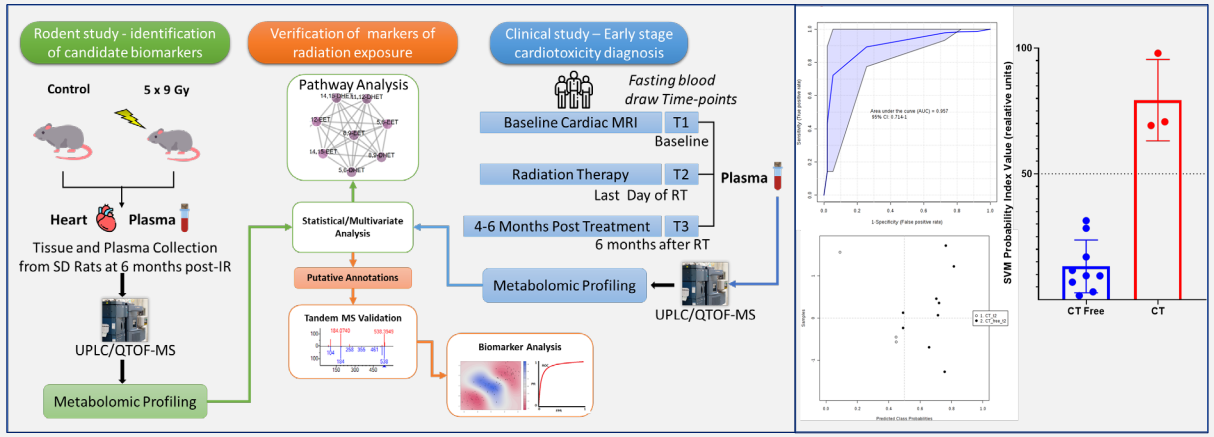

Fig. 1. Overall experimental design integrating a clinical cardiotoxicity study with a rat radiation study to determine biomarkers of radiation exposure.

Fig. 2. Metabolomics panel predictive of radiation induced cardiac toxicity 2 weeks post-RT.

Conclusions: The MSAPSR contributed critical preliminary data that led to the funding of a SBIR Phase II grant and demonstrated the translational utility of a metabolomics approach for developing a biomarker device to predict RT induced normal tissue toxicity.

Grants: NIH R44AI155046, CCSG developmental funds
Publication: *Radiother and Oncol*, 2020, PMC7572465

Cadherin 11 Promotes Immunosuppression & ECM Deposition in Pancreatic Cancer

The MSAPSR provided imaging mass cytometry (IMC) services to support a collaborative study led by **Stephen Byers, PhD**[CCB], **Anton Wellstein, MD, PhD**[CHI], **Michael Atkins, MD**[CHI], and **Louis Weiner, MD**[CHI] that investigated the role of cadherin 11 (CDH11) on ECM modification and immune cell infiltration in the PDAC-TME.

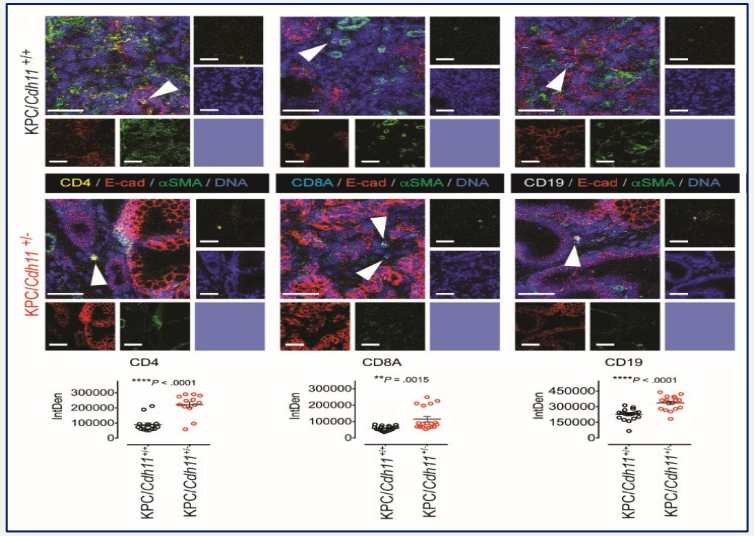

Fig. 3. IMC analysis of PDAC tissues using for CD4, CD8a and CD19 immune cell markers, as well as E-cadherin and αSMA markers helps establish increased anti-tumor immunity, and decreased immunosuppression in KPC/Cdh11+/− mice as compared to KPC/Cdh11 mice.

Conclusions: MSAPSR supported this study that demonstrated the role of CDH11 in mediating interactions among cancer associated fibroblasts (CAFs), the immune system, and pancreatic cancer cells using a transgenic mouse model. This study led to a career development award to Ivana Peran, PhD, an early stage LCCC investigator.

Grant: PanCAN CDA: 23-20-PERA
Publication: *Gastroenterology* 2021, PMC7956114

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Lombardi Comprehensive Cancer Center

Mass Spectrometry & Analytical Pharmacology Shared Resource

Co-Directors: Amrita K. Cheema, PhD, Junfeng Ma, PhD, Claire Carter, PhD

Proteomics and Metabolomics Reveal Functions of O-GlcNAcylation of PRPS1

The MSAPSR performed mass spectrometry-based proteomics and metabolomics in support of a collaborative study, led by **Huadong Pei, PhD**[CCB] and **Gary Kupfer, MD**[CCB] investigating the roles of O-GlcNAcylation of PRPS1 (the rate-limiting enzyme in *de novo* nucleotide synthesis) in lung cancer.

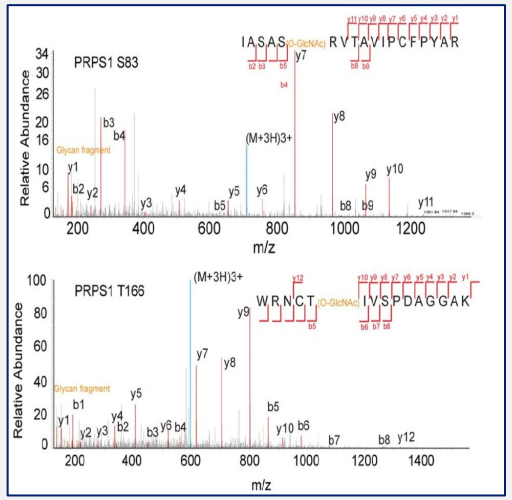


Fig. 4. Mass spectrometry analyses of O-GlcNAcylation sites on PRPS1.

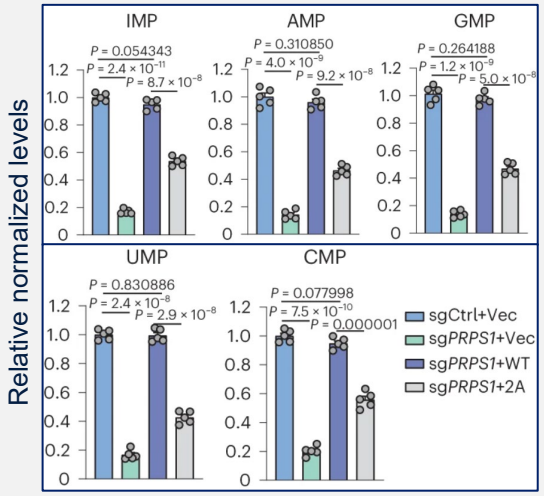


Fig. 5. Analysis of nucleotide levels in xenograft tumor tissues.

Conclusions: MSAPSR supported this collaborative study that revealed that PRPS1 O-GlcNAcylation promotes tumorigenesis and confers resistance to chemoradiotherapy in lung cancer, providing novel molecular mechanisms, and potential therapeutic targets.

Grants: R01 NS121243; R01 CA68135
Publication: *Nature Chem. Biol*. 2023, PMID37308732

Other Key Activities

- Multiple new services developed: IMC, Deep Lipidomics and Mass Spectrometry Imaging.
- Newly installed timsTOF HTMS (Bruker) will enable method development for glycolipid and DIA based metabolomics.
- New services provide cutting edge instrumentation and technical support for cancer-based biomarker discovery and validation studies, and include multiplex imaging, deep lipidomics, and PK/PD studies.

Future Plans

- Add a Desorption Electrospray Ionization (DESI) cyclic ion mobility instrument for pharmacology and cancer biology studies.
- Develop new services including liquid biopsy-based biomarker discovery & single cell proteomics.
- Develop metabolic flux analysis and cancer immune-metabolism capabilities, and extracellular vesicle mediated metabolic modulation of the tumor microenvironment.
- Promote broader use of IMC services for immuno-oncology studies in coordination with HTSR.

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Survey, Recruitment & Biospecimen Collection SR

Co-Directors: Colleen McGuire RN, MSN, Arnold L. Potosky, PhD

Overview

The mission of the Survey, Recruitment & Biospecimen Collection Shared Resource (SRBSR) is to optimize efficiency in the design and execution of studies, facilitate best practices, and enhance collaborative, transdisciplinary studies across the consortium. The SRBSR Specific Aims are as follows: 1) Provide an integrated suite of in-clinic recruitment, survey administration, and biospecimen collection services, 2) Create and maintain a centralized, unified cancer patient registry, and 3) Provide user training and education regarding SRBSR services.

Key Personnel

Arnold L. Potosky, PhD, Co-Director

Colleen McGuire, RN, MSN, Co-Director

Tania Lobo, MS, Data Manager

Key Services

- Study planning and survey design
- Eligibility screening and subject recruitment
- Survey administration
- Development of study tracking databases
- Biospecimen collection and distribution
- Medical record abstraction
- Data entry, merging, and dataset preparation

Major Resources / Technologies

REDCap Research Electronic Data Capture

OPENSPECIMEN a krishagni product

tonic an R1 company

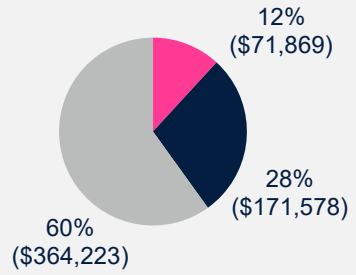
qualtrics XM

Patient Registries

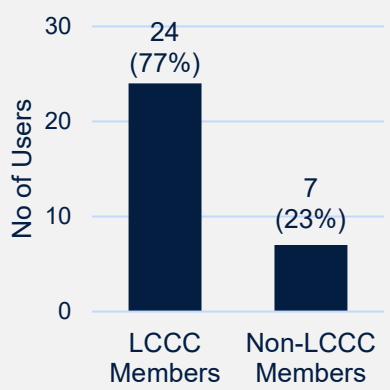
- Georgetown Medstar Research Registry (GMR2)
- Familial Cancer Registry (FCR)
- Tumor Registries

Usage / Budget (FY22)

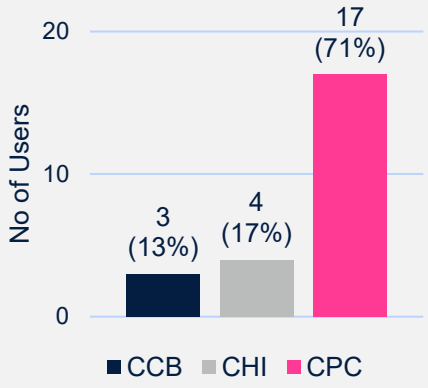
Sources of Support



Usage by Membership



Usage by Program



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Lombardi Comprehensive Cancer Center

Survey, Recruitment, & Biospecimen Collection SR

Co-Directors: Colleen McGuire RN, MSN, Arnold L. Potosky, PhD

Peer Support for Young Adult Women with High Breast Cancer Risk

The SRBSR provided critical support with REDCap database programming, complex two-tiered recruitment strategy and tracking, and training for remote data collection to **Suzanne O'Neill, PhD[CPC]**, **Kenneth Tercyak, PhD[CPC]**, **Claudine Isaacs, MD[CCB]**, and **Beth Peshkin[CPC]** for their cross-consortium multi-site RCT (PeACE) study to assess the effect of a peer support intervention on psychosocial and behavioral outcomes among young women from hereditary cancer families.

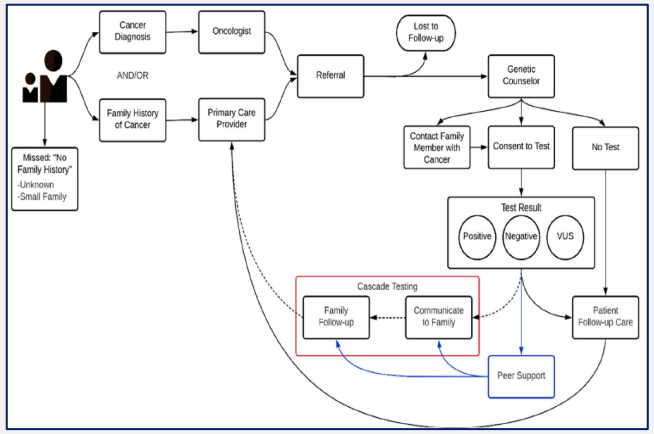


Fig. 1. Process of genetic counseling & testing.

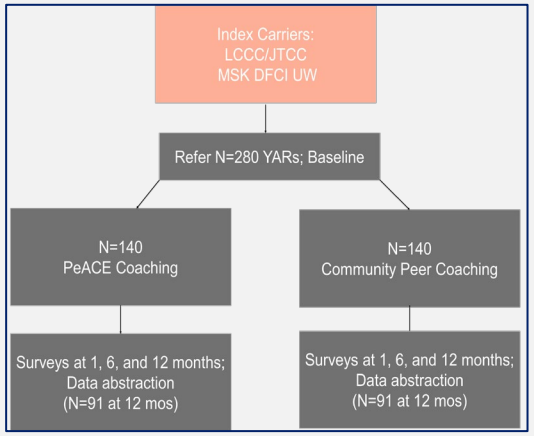


Fig. 2. Trial accrual.

Conclusion: SRBSR supported the pilot study that lead to this cross-consortium trial in the recruitment of BRCA carriers and their family members. This project also led to K-level funding for early-stage investigator **Claire Conley, PhD[CPC]**.

Grants: NIH R01 CA242750, K08 CA270402
Publication: *Hered Cancer Clin Pract.* 2021, PMC8474818

Narrative Video to Improve HBOC Genetic Risk Assessment in Latinas

Alejandra Hurtado de Mendoza, PhD[CPC], Kristi Graves, PhD[CPC], Marc Schwartz, PhD[CPC], Beth Peshkin[CPC], and **Suthee Rapisuwon, MD[CHI]** utilized SRBSR services for REDCap database programming, tracking of >2,000 Latina women screened for HBOC risk in community-based organizations (CBOs), and data cleaning to support an RCT to assess the effect of a culturally targeted narrative video to enhance genetic counseling and testing uptake in Latinas at-risk of HBOC and to integrate cancer screening risk tools at CBOs.

Table 2. Pre-post changes in psychosocial outcomes.

Outcomes	Pre M (SD)	Post M (SD)	p Values
Knowledge			
Knowledge-Main Information Messages 20 items (0–20)	11.00 (2.54)	15.00 (2.24)	$p < 0.001$
Genetic Counseling Knowledge 9 items (0–9)	4.00 (1.72)	4.90 (1.55)	$p = 0.001$
Breast Cancer Genetics Knowledge 13 items (0–13)	5.08 (1.81)	7.13 (1.93)	$p < 0.001$
Intentions			
Intentions-GC (1–5)	4.18 (1.17)	4.69 (0.61)	$p = 0.001$
Intentions-GT (1–5)	4.56 (0.82)	4.59 (0.75)	$p = 0.84$
Attitudes			
Positive Attitudes (1–7)	6.46 (0.76)	6.70 (0.74)	$p = 0.04$
Negative Attitudes (1–7)	1.88 (1.49)	1.44 (0.76)	$p = 0.025$
Norms			
Injunctive Norm (1–7)	6.66 (0.58)	6.82 (0.48)	$p = 0.057$
Descriptive Norm (1–7)	4.03 (2.33)	4.62 (2.46)	$p = 0.09$
Perceived Control			
Confident (1–7)	6.25 (1.21)	6.40 (1.21)	$p = 0.50$
Depend on me (1–7)	6.38 (1.00)	6.50 (1.04)	$p = 0.32$

Table 1. Pre-post changes in psychosocial outcomes.

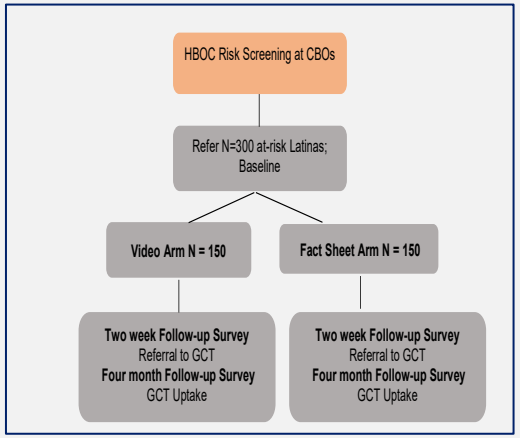


Fig. 3. Trial accrual.

Conclusion: SRBSR supported two pilot studies in the recruitment of an underrepresented minority population in the catchment area, demonstrating that implementing HBOC screening at CBOs is feasible and acceptable. This pilot work led to the first R01 for an early-stage investigator (**de Mendoza**).

Grant: NIH R01 CA248543
Publications: *J Genet Couns.* 2021, PMC10226534; *J Community Genet*. 2020, PMC 6962403; *Int J Environ Res Public Health*. 2019, PMC6926842.

GEORGETOWN UNIVERSITY
Lombardi Comprehensive Cancer Center

Survey, Recruitment, & Biospecimen Collection SR

Co-Directors: Colleen McGuire RN, MSN, Arnold L. Potosky, PhD

Role of γ-OHPdG in Predicting HCC After Curative Resection

SRBSR supported the enrollment, blood collection, and medical record abstraction for a study of 145 hepatocellular carcinoma (HCC) patients led by **Fung-Lung Chung, PhD[CHI]** and **Aiwu Ruth He, MD, PhD[CHI]** supporting development of a non-invasive method to detect HCC derived γ-OHPdG.

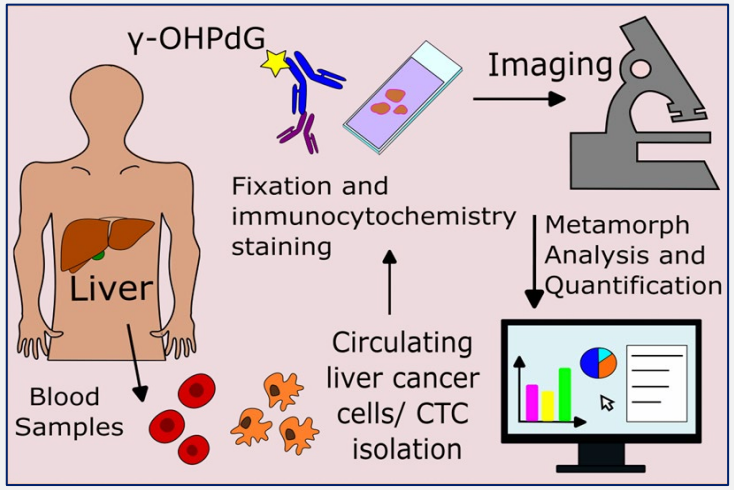


Fig. 4. Isolated HCC CTC using anti-asialoglycoprotein receptor 1 and stain HCC CTC with γ-OHPdG antibody. The γ-OHPdG-positivity, and γ-OHPdG staining intensity are then correlated with clinical factors.

Conclusions: SRBSR supported this successful translational research project investigating a high-priority disease in the LCCC Catchment Area. These data indicate γ-OHPdG- in CTCs is significantly associated with HCC multifocality. This method can be potentially used to validate γ-OHPdG as a prognostic biomarker.

Grants: NIH U01 CA220477

Other Key Activities

- Facilitated cross-consortium collaborative projects.
  - *J Oncology* 2022, PMC8882049 (**Atkins[CHI], Goy[CHI], Potosky[CPC]**)
  - *JHaem* 2021, PMC9176031 (**Biran[CCB], Graves[CPC], Mandelblatt[CPC], Potosky[CPC], Siegel[CHI], Vesole[CHI]**)
  - CCSG Developmental Funds supported pilot project (**Conley[CPC]** and **Derry-Vick[CPC]**)
- Facilitated new R01 CA281752 that will prospectively evaluate PROs among 1600 adults living with metastatic colorectal cancer.
  - SRBSR supported prior grant (R01 NR018841) and publications: *J Ca Surv* 2022 PMC9110561; JNCI 2022, PMC9996211 (**Graves[CPC], Potosky[CPC]**)
- Supported impactful anti-tobacco research.
  - *JNCI* 2022, PMC9552302; led to new R01 CA274716 **(Taylor[CPC])**
  - *Addict. Behav.* 2021, PMC7785681 **(Tercyak[CPC])**
- Maintained and disseminated research registry (GMR2) with >4100 subjects with over 94% consented to re-contact and with biospecimens available.
- SRBSR worked with SRM to enhance their website, streamline access to services, and provide training to users. A new online REDCap service request form was implemented to expedite the process of requesting SRBSR services.

Future Plans

- Expand recruitment services by collaborating with new LCCC members in NJ and at MedStar Washington Hospital Center (MWHC).
- Provide additional training and education on SRBSR services.
- Refine and expand linkage between GMR2 research registry to institutional certified tumor registries at MedStar Georgetown University Hospital (MGUH) and MWHC through a secure interface.

GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Tissue Culture & Biobanking Shared Resource

Director: Bassem Haddad, MD

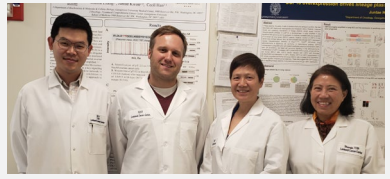
Overview

The TCBSR supports LCCC Members by providing tissue culture, processing and biobanking of blood and other biofluid specimens for their studies. The TCBSR Specific Aims are as follows: 1) Support LCCC investigators with all tissue culture-related aspects of their work, 2) Enable the biobanking of blood and other biofluid specimens obtained from subjects enrolled in multiple LCCC studies, and 3) Provide training in the basic principles of tissue culture and an opportunity to receive hands-on experience.

Key Personnel

Bassem Haddad, MD, Director

Dionyssia Clagett, MS, Manager



Key Personnel: J. Lu; DJ. Murphy; F. Zhu; S. Lu

Key Services

Tissue Culture Services:

- Cell culture and cell banking
- Establishing primary epithelial cell cultures using conditional cell reprogramming
- B-cell transformation with EBV
- Repository of established cell lines
- Quality control assays
- Cryostorage
- Education, training and consultation

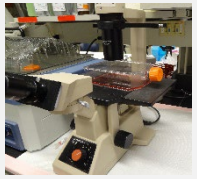
Biobanking Services:

- Processing, banking and disbursing of blood and other biofluid specimens obtained from subjects enrolled in various non-therapeutic, clinical trials, and translational research

Major Equipment / Technologies

- An xCELLigence RTCA System to provide quantitative information on the biological status of cells
- GentleMACS Octo Dissociator for fully automated and heated dissociation/homogenization of tissues
- GelCount System, a mammalian-cell colony, spheroid and organoid counter.
- Max-TL Ultracentrifuge
- Incubators (14), Coulter counters (2), Microscopes (2), Laminar flow hoods (11), liquid nitrogen freezers (19) and -80°C freezers (26)

Shared Laboratories

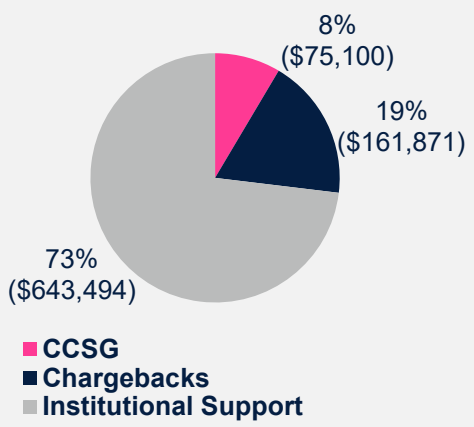


Cryostorage

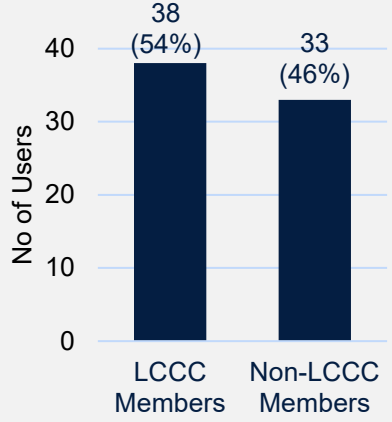
Biobanking

Usage / Budget (FY22)

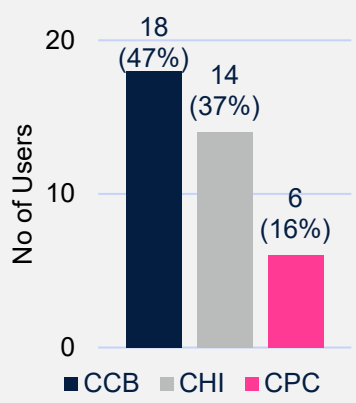
Sources of Support



Usage by Membership



Usage by Program



GEORGETOWN UNIVERSITY

Lombardi Comprehensive Cancer Center

Tissue Culture & Biobanking Shared Resource

Director: Bassem Haddad, MD

Creation of a Living Biobank of Human Primary Non-Malignant Mammary Epithelial Cells from High-Risk Patients

TCBSR provided support to recently retired LCCC Member **Priscilla Furth, MD**[CCB] to use conditional cell reprograming to create a living biobank of human conditionally reprogrammed cell (CRC) cultures of primary non-malignant mammary epithelial cells derived from high-risk patients. In one study, transcriptomes of non-cancerous human mammary epithelial cells at risk for breast cancer development were explored.

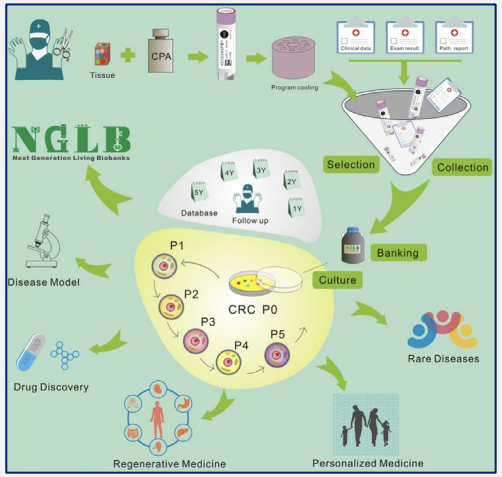


Fig. 1. Process for creating and biobanking conditionally reprogrammed cell (CRC) cultures.

Conclusions: TCBSR provides support to create and maintain a living biobank of CRC cultures from various organs. 96 cell lines from this collection are available to LCCC investigators. In this study, cells from at risk subjects preserved behavioral and transcriptome diversity that could reflect different risk profiles.

Grant: NIH R01 CA112176
Publication: *Sci Rep*. 2022, PMC9033878

DNA Damage and Repair Mechanisms, Obesity, and Breast Cancer Disparities

Chiranjeev Dash, PhD[CPC] and **Rabindra Roy, PhD**[CCB] are evaluating disease and treatment related genotoxicity and health complications in breast cancer survivors in Non-Hispanic Black (NHB) women, who have poorer outcomes in survivorship and a higher mortality compared to Non-Hispanic White (NHW) women. Recruitment is conducted through Community Outreach and Engagement efforts, and all blood samples from study subjects are processed and banked at TCBSR.

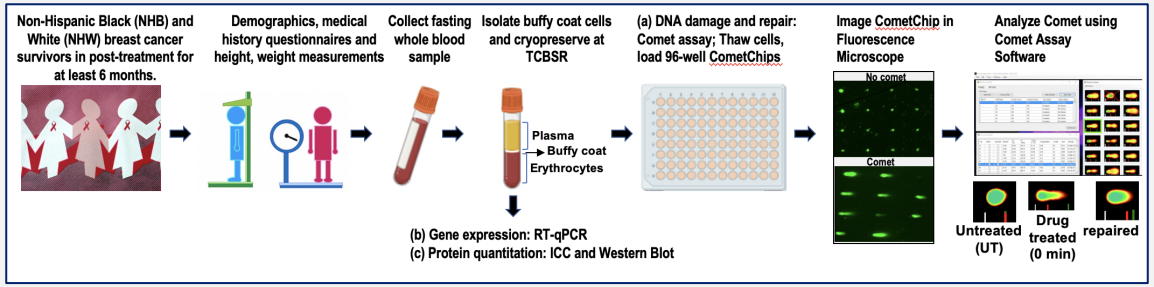


Fig. 2. Recruitment, biobanking, sample processing and DNA data analysis strategy.

Conclusions: TCBSR facilitates health disparities research, a strategic priority of LCCC, by providing specimen processing and biobanking for studies. In this study, systemic differences in basal DNA damage and DNA repair activity and gene expression in circulating leukocytes are being evaluated. Preliminary data detected race-based differences in systemic dsDNA break damage and repair mechanisms.

Grant: NIH R21 CA264489

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Lombardi Comprehensive Cancer Center

Tissue Culture & Biobanking Shared Resource

Director: Bassem Haddad, MD

Role of Cholecystokinin Receptor in Hepatocellular Cancer

Jill Smith, MD[CHI] and **Martha Gay, PhD**[CHI], an early career Assistant Professor, are investigating the hypothesis that hepatocellular carcinoma (HCC) may arise from hepatic stem cells. Dr. Gay uses reagents and cell cultures sourced from the TCBSR, along with the gentleMACS Octo Dissociator to analyze the mouse liver injury model and to assess CD133+ stem cell effects.

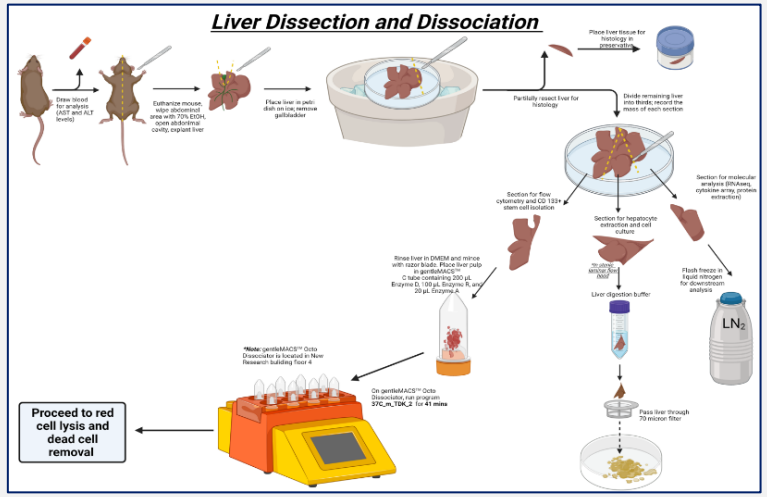

Fig. 3. Strategy for mouse liver dissection and dissociation for downstream molecular analysis.

Conclusions: TCBSR provided cell culture support and the necessary equipment that enabled an early career investigator to complete preliminary studies leading to a K01 award. Preliminary results show that CD133+ liver stem cells express CCK-BRs. Expression was elevated during liver injury and was reduced by proglumide.

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Other Key Activities

- Tissue culture support of all cancer research studies at LCCC.
- Biobanking support:
  - Non-therapeutic research studies (e.g., enable biobanking of blood and biofluid specimens obtained from subjects enrolled by the SRBSR).
  - Cancer related clinical trials managed by the Clinical Protocol and Data Management Office.
  - Serving as the central biorepository for several multi-site studies (e.g., **Mandelblatt, MD**[CPC]'s multi-center Thinking and Living with Cancer study to assess risk for cognitive decline in older breast cancer patients: (NIH R01 CA129769, R35 CA197289, R01 AG068193).
- Deploying a new LIMS system (OpenSpecimen).
- Supporting the work of other shared resources (e.g., SRBSR).

Future Plans

- Expand services to support multi-PI grants and program projects (e.g., provide expertise in biorepository management and tissue culture techniques for P and U grants).
- Work closely with the SRBSR to expand the LCCC biorepository (e.g., hematologic malignancies).
- Continue acquiring new equipment and developing new technologies to support LCCC's research needs and enhance accessibility of the TCBSR resources to NJ-based LCCC Members.