Baylor College of Medicine

ADVANCED TECHNOLOGY CORES

2022-2023 CATALOG

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ADVANCED TECHNOLOGY CORES



I am pleased to present the BCM Advanced Technology Cores catalog for 2022-2023. This publication is designed to help you access the high-end instrumentation and specialized technologies you need for your research. The Advanced Technology Cores (ATC) at BCM expand the research capabilities of all researchers and essentially create unlimited research opportunities.

Each of the cores is staffed by faculty level directors and dedicated research technicians with highly specialized expertise in the technologies provided. A range of research support services are provided such as access to shared instrumentation, analysis of research samples provided by investigators and experiments with Core personnel performing specialized portions of the project. In addition to technical procedures, Cores provide consultation on experimental design, data analysis and training.

This catalog provides an introduction to each of the Cores including services and major instrumentation, technology platforms, core leadership, contact information and examples of scientific research supported by core. For more information about any of the Cores, visit www.bcm.edu/research/atc-core-labs.

On behalf of all the faculty and staff in the Cores, we look forward to working with you to advance science across all areas at BCM.

— Mary E. Dickinson, Ph.D. Senior Vice President and Dean of Research

CORE LEADERSHIP



Dean P. Edwards, Ph.D. *Executive Director*

Dr. Edwards provides scientific oversight and guidance and establishes policies for governance and funding.



Jennifer McCullough, MBA Director of Business Operations

Ms. McCullough administers financial and accounting policies, and provides strategic planning and guidance for business operations.

ACKNOWLEDGEMENTS

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INSTITUTIONAL SUPPORT

Dan L Duncan Comprehensive Cancer Center Baylor College of Medicine Seed and Capital Funds Office of Research: Advanced Technology Cores unit

GRANT SUPPORT

NCI P30 Cancer Center Support Grant (CCSG) NIH P30 Digestive Disease Center (DDC) NIH U54 Intellectual & Development Disabilities Research Center (IDDRC) Cancer Prevention and Research Institute of Texas (CPRIT) Core Facility Support Awards NEI P30 Instrumentation Module Center NIH UM1 Consortium for large-scale production and phenotyping of knockout mice NIH S10 Shared Instrument Grants NIEHS P42 Superfund Project NIEHS P30 Gulf Coast Center for Precision Environmental Health (GC-CPEH)



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ANTIBODY-BASED PROTEOMICS

This Core provides customized services for protein profiling by antibody-based affinity platforms. These platforms provide targeted quantitative assays both for validation and protein biomarker discovery research, particularly for low abundance regulatory proteins, activation states of proteins with antibodies to specific phosphorylation sites and epigenetic core histone post-translation modifications. Services provided include reverse phase protein arrays (RPPA) and Luminex bead technology for multiplex quantitative analyses of intracellular and extracellular signaling proteins.

MAJOR EQUIPMENT

- Bio-Plex 200 Luminex bead reader (Bio-Rad)
- Luminex bead washer (Bio-Tek ELx405)
- Quanterix 2470 Microarrayer (Quanterix)
- Dako Autostainer Link 48 (Agilent)
- Axon Array Scanner 4200AL and GenePix software (Molecular Devices)
- TissueLyzer II (Qiagen)
- Molecular Devices Spectramax 340PC Plate Reader

SERVICES

- Consultation and experimental design.
- Protein sample preparation.
- Reverse Phase Protein Array assays. High density microarrays spotted with researchers' protein lysates and probed with validated specific antibodies (>260) antibodies to proteins and phosphorylation sites of major protein signaling pathways).
- RPPA epigenetic panel. Profiling of a wide range of histone post-translational modifications (PTMs) and histone and chromatin modifier proteins.
- Luminex bead assays (Luminex xMAP technology) for highly sensitive quantitative measurement with very small protein lysate or serum samples.
- Image analyses of protein/antibody microarrays.
- Data management and analysis (Q/C, normalization, statistics and differential analysis).



CORE LEADERSHIP



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Profiling of histone post-translational modifications and chromatin modifying proteins by reverse phase protein array(RPPA)

Epigenetic variation plays a significant role in normal development and human diseases including cancer. In this study, we have adapted and validated an antibody-based reverse phase protein array (RPPA) platform for profiling 20 histone post-translational modifications (PTMs) and the expression levels of 40 proteins that modify histones and other epigenomic regulators (Figure 1A). The specificity of the RPPA assay for histone PTMs was validated with synthetic peptides corresponding to PTMs of histone N-terminal tails and by detection of endogenous histone PTM changes in response to inhibitors of histone modifier proteins in cell culture experiments. RPPA was compared with middle-down Mass Spectrometry (MS) (Figure 1B). Our data demonstrated the complementary nature of RPPA and MS and the overall higher sensitivity by RPPA than MS in histone PTM profiling when validated antibodies are available. Experimental validation of the epigenetic RPPA was demonstrated with different experimental models including induction of pluripotent stem cells (Figure 1C). This RPPA epigenetic platform includes a rapid microscale method for histone isolation and partially automated workflows for analysis of histone PTMs and histone modifiers that can be performed in a high-throughput manner with hundreds of samples. This method has potential for translational research applications through the discovery and validation of epigenetic states as therapeutic targets and biomarkers.



Figure 1. Detection of histone posttranslational modifications and chromatin modifying proteins by RPPA. A) A schematic description of epigenetic RPPA; B) Histone PTM profiling by RPPA and Mass Spectrometry (MS) on SUM159 cells treated with or without 5µM sodium butvrate for 2 hours (n=3). After the treatment, histones were extracted and H3.1 was isolated for RPPA (left panel) and MS (right panel) analyses. RPPA data were normalized and expressed as the mean ± SD. The significant differences between sodium butyrate treated and untreated samples are evaluated by unpaired *t*-tests; (*p< 0.05, **p< 0.01, ***p< 0.001) C) Profiling of histone posttranslational modifications and expression levels of chromatin modifying proteins during somatic cell reprogramming: Heat map of changes in histone PTMs (left panel) and differential expression levels of histone modifier and chromatin remodeling proteins (right panel) between iPSCs and fibroblasts identified by unpaired *t*-tests p<0.05, adjusted p value (FDR) =<0.1, and a cut-off of a 1.5 fold difference.

Wang X, Shi Z, Lu HY, Kim JJ, Bu W, Villalobos JA, Perera DN, Jung SY, Wang T, Grimm SL, Taylor BC, Rajapakshe K, Park H, Wulfkuhle J, Young NL, Li Y, Coarfa C, Edwards DP, Huang S. High-throughput profiling of histone post-translational modifications and chromatin modifying proteins by reverse phase protein array. J Proteomics. 2022 Jun 30;262:104596. PMID: 35489683.

BIOENGINEERING

The goal of the Bioengineering Core is to provide investigators custom scientific instrumentation needed to conduct elegant experiments and ask truly cutting-edge research questions, and also to provide clinicians custom, one-of-a-kind, medical devices to create innovative solutions for health care. The core is staffed with an experienced bioengineer and a machinist who can work with investigators and clinicians to design complex devices, identify suitable off-the-shelf devices, manufacture custom parts and integrate the apparatuses/instruments into the research workflow or clinical practices.

MAJOR EQUIPMENT

- Hermle 5-axis CNC (Computer Numerical Control) Milling machine center capable of cutting solid materials such as metal, plastics, and wood into parts with complex geometries up to a size of $24'' \times 18'' \times 18''$.
- Haas CNC Lathe capable of machining custom cylindrical parts up to 14" diameter and 14" long.
- Hardinge manual precision lathe.
- Bridgeport manual milling machine.
- Vertical band saw and horizontal cutoff saw.
- Epilog Laser cutter capable of cutting plastic, wood, or paper sheets up to $32'' \times 20''$ with 3/4'' thickness and engraving plastic, leather, metal, and glass.
- Stratasys 3D printer capable of printing ABS plastics and supporting material up to a size of $8'' \times 8'' \times 6''$.
- Thorlabs optical workstation equipped with vibration isolation optical table, laser diode mount, laser controller, and power meter allowing design and tests of optical devices.

SERVICES

- Customized instrumentation design and manufacture.
- Customized electronics/optics design and manufacture.
- High precision mechanical manufacture.
- 3D design and printing.
- Laser cutting and engraving.
- Stockroom of fasteners and raw materials such as aluminum, stainless steel, and plastics.
- Consultation for biomedical engineering projects.



CORE LEADERSHIP



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The Core has produced custom parts for several microscopes used to study neural information processing at the network level and the microenvironment of tumorassociated vessels in whole intact mouse brains.

Picture of an electrophysiological recording and imaging setup used for studying interactions between brain regions in mice.

Chen G, Kang B, Lindsey J, Druckmann S, Li N (2021) Modularity and robustness of frontal cortical networks. Cell 184(14):3717-3730





The design and fabrication of a custom apparatus for light-sheet microscopy

Light-sheet microscopy images of progressive morphological and functional changes in tumorassociated vessels. A whole intact mouse brain at P65 (**A**, close ups in **B**, **C**) or P80 (**D**, close ups in **E**, **F**), with tumor-derived cells labeled by GFP (magenta), and vessels labeled by fluorescent lectin (teal).

Carlson JC, Cantu Gutierrez M, Lozzi B, Huang-Hobbs E, Turner WD, Tepe B, Zhang Y, Herman AM, Rao G, Creighton CJ, Wythe JD, Deneen B (2021) Identification of diverse tumor endothelial cell populations in malignant glioma. Neuro Oncol. 23(6):932-944.

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BIOSTATISTICS AND INFORMATICS SHARED RESOURCE

The goal of the Biostatistics and Informatics Shared Resource (BISR) is to provide state of the art biostatistical, bioinformatics, multi-omic analysis and computational support for clinical, translational and basic science research.

MAJOR EQUIPMENT

Highly-available cluster with >900 physical CPUs in a single compute node architecture with a 10 Gb Ethernet connection to 495 TB of extensible Tier 1 directattached, rapid I/O data storage. Queues are managed with the PBS scheduler. The system is maintained by an expert HPC system administrator in a Tier 3 data center under standard governance structures.

SERVICES

- **Biostatistics and Analytics:** Experimental design; assistance with design and conduct of clinical trials; data analysis, including integrative bioinformatic analyses
- **Multi-Omics Bioinformatics:** Data analysis for 'omics' core facilities including downstream integrative bioinformatic analyses
- **High Performance Computing (HPC):** cluster management and storage allocation; user training, central software library maintenance; troubleshooting
- **Other:** Assistance with grant applications; education; statistical review for the Protocol Review and Monitoring and Data Review Committees; deposition of 'omics-scale' datasets

Investigators needing assistance with the following, please use the indicated contacts:

- HPC Cluster (cluster-help@breastcenter.tmc.edu)
- Biomedical Informatics & Research IT (dowst@ bcm.edu)
- OnCore[®], & Clinical Trials Data Management (oncore-support@breastcenter.tmc.edu)
- Acquire and Biobanking Data Management (acquire-support@breastcenter.tmc.edu, biobanksupport@breastcenter.tmc.edu)
- Software licensing for Oncomine[™], Ingenuity[®], SAS[®], and SPSS[®] (licensing@breastcenter.tmc.edu)

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Sex Chromosome vs Hormone in Neonated Lung Injury

A) Mice from the Four Core Genotype model; XXF (chromosomally and gonadally female), XXM (chromosomally female, gonadally male), XYF (chromosomally male, gonadally female), and XYM (chromosomally and gonadal male) were exposed to hyperoxia (95% FiO, from post-natal day (PND)1-4) during the saccular stage of lung development and euthanized on PND5 and PND21. Whole lung mRNA was subjected to RNA-Seg analysis. B) The total number of up-and down-regulated differentially expressed genes (DEGs). C) Transcriptome profiles of 578 healthy human adult lung samples compiled by the GTEx consortium to assess genotype/transcriptome relationships in phenotypically healthy individuals. We computed summed z-scores for each human individual and each FCG signature and assessed inter-signature correlations. There is a clear separation between the PND5 and the PND21 hyperoxia signatures. D) Gene Set Enrichment Analysis (GSEA) to quantify enrichment of GO Biological Process pathways. Hierarchical clustering and heatmaps were generated for the transcriptomic footprints using the significant normalized enrichment scores (NES). Pathway-based clustering of response to hyperoxia across all genotypes show striking and distinct clustering between the PND5 and PND21 responses. E) Blood transcriptome at PND28 from a cohort of human newborns evaluated for development of BPD was obtained. The distribution of summed z-scores for hyperoxia gene signatures from all our murine models, at PND21, was evaluated against four clinical variables: gestational age, birth weight, BPD status and need for oxygen at 28 days of post-natal age. F) Distribution of summed z-scores for hyperoxia signatures in murine models at PND21 are shown in the female human newborn blood samples collected at PND28, stratified by biological sex and by BPD status.

Grimm SL, Dong X, Zhang Y, Carisey AF, Arnold AP, Moorthy B, Coarfa C, Lingappan K. Effect of sex chromosomes versus hormones in neonatal lung injury.JCI Insight. 2021 Jul 8;6(13):e146863. doi: 10.1172/jci.insight.146863.

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CORE FOR ADVANCED MAGNETIC RESONANCE IMAGING (CAMRI)

The Core for Advanced Magnetic Resonance Imaging (CAMRI) is a state-of-the-art resource for the Houston research community that makes possible advanced imaging studies of the function, physiology and anatomy of humans and animals, with special expertise in human blood-oxygen level dependent functional MRI (BOLD fMRI). Conveniently located in the heart of BCM main campus, the center houses two cutting edge MR imaging systems.

MAJOR EQUIPMENT

- Two Siemens 3 Tesla Prisma^{Fit} MRI Scanners with 80/200 gradients.
- A wide variety of equipment for functional brain imaging studies, including sensory stimulation devices, response buttons, eye trackers, and MR-compatible transcranial magnetic stimulation (TMS).
- Multiple MRI coils with the ability to scan all body parts.
- Additional space available for animal preparation, TMS, behavioral testing, and stimulus recording.
- Flywheel scientific data management system to make data easily accessible and shareable.

SEQUENCES

- Functional MRI (fMRI), including multiband acceleration
- Diffusion tensor imaging (DTI)
- Single and multi-voxel magnetic resonance spectroscopy (MRS)
- Arterial spin labeling (ASL), both pulsed and continuous
- High-resolution structural imaging: FLASH, TSE, FLAIR, etc.
- All Siemens standard sequences for whole body

SERVICES

- Imaging technologist available to assist in data collection
- Analysis Support: Includes consultation, data management, and possibility of collaboration on MRI projects
- Operator training available to enable safe use of MRI equipment by new users
- Access to the instruments for fully trained users is available 24/7, facilitating subject recruitment and retention
- Monthly journal club and seminar series, details on our wiki at http://openwetware.org/wiki/CAMRI

CORE LEADERSHIP



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Drs. Sameer Sheth, Kelly Bijanki, and their colleagues collected MRI data at CAMRI in patients with treatmentresistant depression who would then go on to have deep brain stimulation (DBS) systems inplanted to treat their disease. As part of this clinical trial, the patients were monitored in the hospital with electrodes to record electrical activity in their brain that resulted from stimulation to the DBS leads. In their recent paper, the team compared the electrical activity to diffusion tractography, a commonly used MRI technique that is used to find the locations of white matter pathways in the brain. The figure below shows the correspondence of the two techniques for one of their patients.

Figure adapted from Adkinson JA, Tsolaki E, Sheth SA, Metzger BA, Robinson ME, Oswalt, D, McIntyre CC, Mathura RK, Waters AC, Allawala AB, Noecker AM, Malekmohammadi M, Chiu K, Mustakos R, Goodman, W, Borton D, Pouratian N, Bijanki KR. Imaging versus electrographic connectivity in human mood-related fronto-temporal networks. Brain Stimulation. 2022. 15(3): 554-565.

Dr. Ramiro Salas and his colleagues



collected MRI images at CAMRI from more than 500 psychiatric inpatients being treated at the Menninger Clinic. They also collected data about the symptoms of the patients and their genetics. The group is using these data to learn about brain correlates of a variety of psychiatric disorders and their treatment outcomes, as well as findings that more generally address methods for conducting psychiatric research. The group recently demonstrated the importance of careful recruitment and characterization in studies of psychiatric populations (Gosnell SN, Meyer MJ, Jennings C, Ramirez D, Schmidt J, Oldham J, Salas R. Hippocampal Volume in Psychiatric Diagnoses: Should Psychiatry Biomarker Research Account for Comorbidities? Chronic Stress. 2020 4:1-10.) and developed a method for finding associations between genetic markers and brain imaging (Poblete GF, Gosnell SN, Meyer M, Fang M, Nguyen T, Patriquin MA, Nielsen D, Kosten T, Salas R. Process genes list: an approach to link genetics and human brain imaging. Journal of Neuroscience Methods. 2020 Jun 1;339:108695.) The figure below shows the work of Dr. Meghan Robinson and colleagues using these data to compare processing methods for resting state functional MRI, a common technique in MRI that compares activity of brain regions to determine networks. They applied different strategies for cleaning up noise in the data, and found differences in how effective those strategies were at detecting different types of patient diagnoses.



Figure adapted from Robinson, ME, Poblete G, Abdallah CG, Salas R. Choice of nuisance regression in functional connectivity impacts detection of group differences in mental health conditions. Presented at Organization for Human Brain Mapping, 2022. Submitted.

CELL-BASED ASSAY SCREENING SERVICE (C-BASS)

C-BASS strives to provide cutting-edge technologies and the latest genomic tools for cell-based functional genomics studies, and to aid with individual gene function, pathway identification, and large-scale genome-wide screens. The cell-based services offered are built upon interconnected and complementary technology platforms of RNAi-based functional genomics and CRISPR/Cas9-mediated genome editing. Services include generating knockout (KO) and knock-in (KI) cell lines using CRISPR/Cas9, providing cDNA and shRNA vectors individually or as custom libraries, and consultation and expert advice on genome-wide or sub-genome-targeted genetic screens. Through education and on-going improvement and optimization, we enable BCM researchers to carry out drug discovery screens using a variety of platforms.

MAJOR RESOURCES

- An arrayed lentivirus-based shRNA library that targets the human genome
- An arrayed lentivirus-based shRNA library that targets the mouse genome
- A human cDNA library in a Gateway[®] compatible vector
- A mouse cDNA library
- An arrayed lentivirus-based CRISPR sgRNA library that targets the human genome
- A vector collection for CRISPR/Cas9-mediated genome editing and other functional applications

SERVICES

- Individual vectors
- Pre-assembled shRNA sub-libraries (e.g., kinase, transcription factors, etc.)
- Pre-assembled CRISPR sgRNA sub-libraries (e.g., kinase, transcription factors, etc.)
- Custom sub-libraries (gene collection designed by investigator)
- Whole-genome shRNA/cDNA collection (human and mouse)
- Lentiviral production and infection (individual or 96well format)
- Gene editing through CRISPR/Cas9
- Consultation and experimental design for genome editing
- Vector design, construction, and testing
- KO and KI cell line generation and validation

EQUIPMENT

- Biomek FX^p automated liquid handling workstation
- Biomek NX^p automated liquid handling workstation
- Biomek 3K automated liquid handling workstation



CORE LEADERSHIP



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p53 loss activates prometastatic secretory vesicle biogenesis in the Golgi

Cancer cells exhibit hyperactive secretory states that maintain cancer cell viability and remodel the tumor microenvironment. However, the oncogenic signals that heighten secretion remain unclear. The C-BASS core generated p53 knockout in A549 lung carcinoma cells for Dr. Jonathan Kurie's group, mRNA levels of Golgi associated genes were assessed comparing to wild type cells. Loss of p53 activated prometastatic secretory vesicle biogenesis in the Golgi and upregulates the expression of a Golgi scaffolding protein, progestin and adipoQ receptor 11 (PAQR11), which recruits an adenosine diphosphate ribosylation factor 1-containing protein complex that loads cargos into secretory vesicles. PAQR11-dependent secretion of a protease. PLAU, prevents anoikis and initiates autocrine activation of a PLAU receptor/signal transducer and activator of transcription-3-dependent pathway that



up-regulates PAQR11 expression, thereby completing a feedforward loop that amplifies prometastatic effector protein secretion. Pharmacologic inhibition of PLAU receptor impairs the growth and metastasis of p53-deficient cancers. Blockade of PAQR11-dependent secretion inhibits immunosuppressive processes in the tumor microenvironment. Thus, Golgi reprogramming by p53 loss is a key driver of hypersecretion in cancer.

p53 loss activates prometastatic secretory vesicle biogenesis in the Golgi. Tan X, Banerjee P, Shi L, Xiao GY, Rodriguez BL, Grzeskowiak CL, Liu X, Yu J, Gibbons DL, Russell WK, Creighton CJ, Kurie JM. Sci Adv. 2021 Jun 18;7(25):eabf4885. doi: 10.1126/sciadv.abf4885. Print 2021 Jun. PMID: 34144984

Hormonal modulation of ESR1 mutant metastasis.

Estrogen receptor alpha gene (ESR1) mutations occur frequently in ER-positive metastatic breast cancer (MBC), and confer clinical resistance to aromatase inhibitors (Als). Dr. Suzanne Fugua's group discovered that mutations within the hormone binding domain (HBD) of ESR1 including Y537S can be found at high frequencies in MBC patients. The C-BASS core created MCF cells carrying the homozygous ESR1 Y537S mutation using the CRISPR knock-in technology. These ESR1 Y537S mutant cells exhibit enhanced migration and invasion potential in vitro. When small subpopulations of Y537S ESR1 mutant cells were injected along with WT parental cells, tumor growth was enhanced with mutant cells becoming the predominant population in distant metastases. Y537S mutant primary xenograft tumors were resistant to the antiestrogen tamoxifen (Tam) as well as to estrogen withdrawal. Y537S ESR1 mutant primary tumors metastasized efficiently in the absence of estrogen; however, Tam treatment significantly inhibited metastasis to distant sites. Gu G et al. identified a 9-gene expression signature that predicted clinical outcomes of ER-positive breast cancer patients, as well as breast cancer metastasis to the lung. Androgen receptor (AR) protein levels were increased in mutant models, and the AR agonist dihydrotestosterone (DHT) significantly inhibited estrogen-regulated gene expression, EMT, and distant metastasis *in vivo*, suggesting that AR may play a role in distant metastatic progression of ESR1 mutant tumors.

Hormonal modulation of ESR1 mutant metastasis. Gu G, Tian L, Herzog SK, Rechoum Y, Gelsomino L, Gao M, Du L, Kim JA, Dustin D, Lo HC, Beyer AR, Edwards DG, Gonzalez T, Tsimelzon A, Huang HJ, Fernandez NM, Grimm SL, Hilsenbeck SG, Liu D, Xu J, Alaniz A, Li S, Mills GB, Janku F, Kittler R, Zhang XH, Coarfa C, Foulds CE, Symmans WF, Andò S, Fuqua SAW. Oncogene. 2021 Feb;40(5):997-1011. doi: 10.1038/ s41388-020-01563-x. Epub 2020 Dec 15. PMID: 33323970

CRYO ELECTRON MICROSCOPY (CRYOEM)

The Cryo Electron Microscopy (CryoEM) Core is a state-of-the-art resource for near-atomic resolution 3-D analysis of the structure and dynamics of macromolecules and assemblies, either purified or within cells. This includes the established technique of single particle analysis, wherein images of tens of thousands to millions of isolated macromolecules are reconstructed to produce one or more 3-D structures at resolutions as high as 0.2 nm (near atomic resolution), as well as *in-situ* electron cryotomography which permits the 3-D study of cells or regions of cells at nanometer resolutions 100x better than optical microscopy. Single particle analysis is a direct alternative to X-ray crystallography, and can provide additional information about dynamics and compositional variability, which crystallography cannot access. We can also work with users to optimize specimens and provide preliminary data to gain free access to the new 'beamline' style CryoEM facilities sponsored by the NIH.

MAJOR EQUIPMENT

- ThermoFisher Glacios 200 keV instrument with a field-emission gun, Falcon 4 and Apollo detectors and Krios-compatible autoloader. Equipped with MAPS software compatible with Krios at UTHSC.
- ThermoFisher Aquilos 2 A dual-beam cryo-Focused Ion Beam (FIB)/SEM instrument capable of milling thin lamella from vitrified whole cells and tissue for imaging with one of the TEMs.
- JEOL-3200FSC 300 keV instrument with a field-emission gun, energy filter and a K2 summit direct detector. Capable of single particle reconstructions beyond 3 Å resolution, and nanometer resolution cellular tomography of thin specimens. Fully automated for 24-hour operation.
- JEOL-2200FS 200 keV instrument with a field-emission gun, phase plate, energy filter, Gatan CCD camera and a DE-20 direct detector. Workhorse instrument for single particle reconstruction at subnanometer resolution, able to look at particles smaller than the 300 keV instrument.
- JEOL-2100 200 keV instrument with DE-12 direct detector. This is our primary cryo screening instrument.
- JEOL-1230 120 keV instrument with 4k Gatan CCD for negative stain and fixed section imaging. No cryo specimens.
- FEI Mark IV Vitrobot with 2-sided blotting for specimen preparation.
- Leica EMGP automatic plunge freezer with 1-sided blotting for specimen preparation.
- Fischione Model 1070 Nanoclean plasma cleaner for grid preparation.
- PELCO easiGlow™ Glow Discharge Cleaning System.
- ÄKTA Pure HPLC for on-site size exclusion chromatography.

SERVICES

- CryoEM/CryoET project consultation.
- Near-atomic resolution CryoEM single particle analysis. We can support all stages of the pipeline from specimen preparation through computer reconstruction.
- Cellular CryoET to provide 3-D structure of intact cellular material ~5 nm resolution in bulk leading to ~1 nm after averaging.
- Cryo-FIB milling of thick cells/tissue, as specimen preparation for CryoET.
- Screening and optimizing new specimens for CryoEM and/ or CryoET.
- Training students and staff in all aspects of the CryoEM/ CryoET pipeline.
- Room temperature TEM imaging of negative stain or fixed specimens.
- Standard Transmission Electron Microscopy (TEM).



CORE LEADERSHIP



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Structural variations in platelets in cancer at nanometer resolution by CryoET (Nakada, Ludtke and Wang Labs. Genetics & Biochemistry Depts)

Traditional TEM studies generally involve chemical fixation of the cells followed by some type of labeling. While these strategies can be very useful in looking at cytoskeletal features and overall organization, they are clearly highly perturbative at the molecular level. In CryoET, whole cells are rapidly vitrified to LN2 temperatures, and can then be visualized in 3-D at nanometer resolution, revealing perfectly preserved organelles and cytoskeletal features down to the level of individual macromolecular interactions in the cytosol.

Facilitated by new electron cryotomography (cryo-ET) methods in the CryoEM/ET core, we performed ultrastructural analysis of platelets in a leukemic (AML) mouse model at different time points after induction. We characterized morphological changes and quantitively measured subcellular organelles within platelets, characterizing early-stage AML development. Five populations of platelets were visualized in this study, drawn from: normal wild-type mice, irradiated mice with normal bone marrow transplants at two time points, and irradiated mice with MLL-AF9-transformed HSPCs at the same time points. We found that platelets with structurally abnormal mitochondria emerge during the preleukemic phase of AML, preceding detectable changes in blood cell counts or detection of leukemic blasts in blood. A large proportion of platelets exhibited changes in the overall shape and depletion of organelles in AML.



Figure 1. Cryo-ET of platelets from normal and AML mice. **A**) Pre-AML platelets (**top**) had α granules, dense granules, λ granules, mitochondria, the plasma membrane, microtubule, glycogen particles, and OCS. However, some platelets had abnormal mitochondria with less cristae. (scale bar 1 µm). The platelets of leukemia (**second from top**) showed small round or oval shape without pseudopods. There were only glycogen particles and OCS inside. (scale bar 500 nm). Two control platelets (**top**) had typical features including α granules, dense granules, λ granules, mitochondria, membrane, microtubule, glycogen particles, and open canalicular system (OCS) (scale bar 1 µm). **B**) an enlarged view of abnormal mitochondria in pre-AML platelets. Some mitochondria in pre-AML platelets had abnormal that had less cristae and matrix (scale bar 100 nm).

Specifically, 23% of platelets in pre-leukemic cells exhibit abnormal, round mitochondria with unfolded cristae. Surrounding the abnormal mitochondria is a cluster of empty spherical vesicles. These changes in mitochondria arise before abnormal blood cell counts can be detected, and do not appear in control cells exposed to a similar radiation treatment. If this observation can be confirmed in human AML, this is potentially a new diagnostic for an otherwise extremely difficult to diagnose condition. In the model a large enough fraction of platelets appears to be impacted, that it should be quite feasible to leverage this observation to develop a cost-effective test for this often-fatal disease.

CYTOMETRY AND CELL SORTING

Cytometry is an integral part of BCM faculty research across all disciplines. The technology, including flow, mass and image cytometry, continues to develop at a rapid pace driven by advances in instrumentation, labeling reagents, and computational capabilities. The Core provides state-of-the-art instrumentation, technologies, and exceptional specialized expertise and training in cytometry. Services include analysis by mass cytometry, imaging cytometry and flow cytometry as well as fluorescence-activated cell sorting (FACS) supported by an in-house bank of validated antibodies. Additionally, services include large particle sorting, magnetic cell separation, automated cell counting and viability, consultation, data analysis and training. Access to instruments in the facility for fully trained users is 24/7.

MAJOR EQUIPMENT

- Fluidigm Helios Mass Cytometry with Hyperion mass imaging platform
- Cytek Aurora Full Spectrum 4-laser Cytometer
- BD Symphony A5 30+ Parameter Flow Cytometer
- Amnis ImageStreamX MKII, a 4-laser imaging cytometer providing a multispectral image for every cell
- Seven Flow Cytometric Cell Analyzers; two 5-laser BD LSRs, one 4-laser LSRII, and a 3-laser LSRII, two 3-laser BD Canto IIs (one violet and one yellow-green), and 4-laser Invitrogen Attune NxT
- High Through-put Flow Cytometric Analysis; High throughput systems available on flow cytometric analyzers
- Four Flow Cytometric Cell Sorters; two 5-laser BD SORP Aria IIs, a 4-laser BD Aria IIu and a 4-laser Sony MA900
- Union Biometric BioSort Large Particle Cell sorter; 30 300um objects using Blue and YG lasers
- Viability Analyzer; Beckman Coulter Vi-CELL
- Magnetic Cell Separator; Miltenyi AutoMACS Pro
- Cell Tissue Dissociator; Miltenyi gentleMACS Octo Tissue Dissociator
- Three Computer Work Stations including an Mass Imaging Cytometry Work Station

SERVICES

- Cellular Analysis: Assisted and unassisted flow cytometric and viability analysis using up to five separate lasers and 30 parameters for multiple assays including small particles.
- Cell Sorting: Assisted and unassisted flow cytometric and magnetic cell sorting services that include parity with analyzers so any project capable of analysis can be moved to cell sorting.
- Mass and Fluorescent Antibody Bank for highparameter cytometry
- Data Analysis: Assisted and unassisted data analysis including a dedicated server for data storage, workstations for data analysis as well as Astrolabe, VisioPharm and FlowJo software site licenses available to investigators.
- Training: Didactic Flow Analyzer course as well as individual training on cell sorting and other instrumentation, software or equipment updates.



CORE LEADERSHIP



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Spatiotemporal evolution of tumor-supporting PD-1HIGH TAMs occurs through PD-1/PD-L1 interaction. (Dr. Hyun-Sung Lee and Dr. Bryan Burt, Michael E. DeBakey Department of Surgery)



The CCSC's imaging mass cytometry and cell sorting was used to garner the spatiotemporal evolution of tumorsupporting PD-1^{HIGH} TAMs occurs through PD-1/PD-L1 interaction. The solid component contained PD-1^{HIGH} TAMs, and the lepidic component had PD-1^{LOW} TAMs, expressing with PD-L1 and a phagocytic marker, CD44. A) Spatial dynamics of the tumor-immune microenvironment during the tumorigenesis of lung adenocarcinoma. B) PD-1^{HIGH} TAMs interact with cancer cells expressing PD-L1 to form a solid fortress in invasive adenocarcinoma. C) IMC-based SPACE maps to show cellular interactions of cancer cells, PD-1^{HIGH} TAMs, and PD-1^{LOW} TAMs during the tumorigenesis. D) Cytokine release on SPACE map. TGF β and IL10 were released from cancer cells, PD-1^{HIGH} TAMs and PD-1^{LOW} TAMs on late-stage tumors. **E)** Effect of PD-1^{HIGH} TAMs on cancer cells. PD-1^{HIGH} and PD-1^{LOW} TAMs from mice at a 4-week time point were sorted and cultured with sorted tumor cells (CD45 human EGFR.). After 72 hours, the number of apoptotic tumor cells was significantly fewer in the coculture with PD-1^{HIGH} TAMs. All in vitro experiments in triplicate were repeated. F) Scheme of 3D-spheroid formation assay to test cell-to-cell interaction. G) Disruption of spheroid formation by inhibiting PD-1 and PD-L1 interaction in spheroid formation assay. Single cells obtained from invasive adenocarcinoma at a 7-week time point formed a spheroid. The spheroid formation assay showed that blocking PD-1/PD-L1 interaction disrupted a spheroid formation with the wider spheroid area. The spheroid size was evaluated by measuring the spheroid perimeter using Fiji software, in which area was converted into % relative area based on the size of the intact spheroid. Results are expressed as mean pixel measure ± SEM from three independent experiments in four replicates. The IgG2a isotype antibody was the control isotype for both the anti-PD-1 and anti-PD-L1 antibodies.

Hee-Jin Jang; Hyun-Sung Lee; Wendong Yu; Maheshwari Ramineni; Cynthia Y. Truong; Daniela Ramos; Taylor Splawn; Jong Min Choi; Sung Yun Jung; Ju-Seog Lee; Daniel Y. Wang; Joel M. Sederstrom; Massimo Pietropaolo; Farrah Kheradmand; Christopher I. Amos; Thomas M. Wheeler; R. Taylor Ripley; Bryan M. Burt. "Therapeutic Targeting of Macrophage Plasticity Remodels the Tumor-Immune Microenvironment." Cancer Res (2022) 82 (14): 2593–2609. https://doi.org/10.1158/0008-5472.CAN-21-3506

GENE VECTOR (REORGANIZED 2022)

The Gene Vector Core (GVC) assists investigators with the production of gene transfer vectors, which can be used for studying gene function by over-expression, ectopic expression, gene silencing, or gene editing. Recombinant viral vectors retain the native features of viruses that have been tested in nature for millions of years but can also take on unique desired features. The Vector Development Laboratory in the Center for Cell and Gene Therapy has merged with the GVC providing expanded services such as first-generation adenovirus (FGAd) production and several quality assurance assays available to all BCM investigators. The GVC has undertaken a variety of initiatives aimed at increasing productivity, cutting costs, developing quality control assays, improving existing services, and expanding the repertoire of viral vector-based research tools. The core offers several popular viral vector platforms and has extensive experience in the production of viral vectors including adeno-associated virus (AAV), FGAd, helper-dependent adenovirus (HDAd), lentivirus (LV), and retrovirus (RV). Our core is vigilant in keeping pace with recent advances in viral vector technology, provides expert consultation and advice, and works together with investigators to provide services most suited to each individual project.

SERVICES

- Packaging and purification of AAV (serotype 1, 2, 5, 6, 7, 7M8, 8, 9, 10, DJ, DJ8, 2-Retro, and PHP.eB) at various scales.
- Rescue, and/or amplification/purification of FGAd (serotype 5 and 5/35) and HDAd (serotype 2, 5, 5/11, and 5/35).
- Packaging and concentration/purification of VSVG-, MokolaG-, LCMV-Arm53b-Pseudotyped integrating or non-integrating LV with 2nd, or 3rd, or 4th generation packaging systems.
- Packaging of ecotropic, amphotropic, and pantropic VSVG-Pseudotyped RV
- Subcloning into viral transfer vectors and preparation of plasmids for viral vector production.
- Other supporting services: infectious titer, HPLC analysis, tests for RCA, sterility, endotoxin, and mycoplasma.
- The customer provides transfer vectors for transfection. Packaging plasmids or helper viruses are provided by the Core.
- Off-the-shelf packaged vectors are available in the catalog.
- Common viral transfer plasmid vectors developed by the Core have been deposited to Addgene https://www.addgene.org/Kazuhiro_Oka/. These plasmid DNAs are available from the Core.

MAJOR EQUIPMENT

 Ultraspeed Centrifuge (Beckman Optima XPN 90 and Sorvall WX80)





CORE LEADERSHIP

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Corinne Sonnet, Ph.D. *Co-Director Assistant Professor, Department of Medicine* 713.798.1238

Hippo Pathway Effector Tead1 Induces Cardiac Fibroblast to Cardiomyocyte Reprogramming (PI: Todd Rosengart, M.D, Department of Surgery)

Heart failure remains a leading cause of cardiac death. Cardiac regeneration strategies are promising but challenging options. The conversion of fibroblasts into induced cardiomyocytes could regenerate myocardial tissue from cardiac scar tissue through in situ cell differentiation. A wide variety of cardiac reprogramming cocktails are based on the core cardiac transcription factors Gata4, Mef2c, and Tbx5. However, reprogramming cardiac fibroblasts to contractile cells is inefficient. Singh et al. examined Hippo pathway intermediates to enhance induced cardiomyocyte generation. Genes encoding transcription factors were transferred via lentivectors supplied by the Gene Vector Core. The addition of Hippo pathway effector Tead1 (TEA domain transcription factor 1, Td) but not Yap (yes-associated protein) or Taz (transcriptional activator binding domain) induced cardio-differentiation factor cTnT in mouse embryonic and adult rat fibroblasts compared with those treated with Gata4, Mef2C, and Tbx5. Furthermore, the replacement of Tbx5 with Td (GMTd) induced the greatest cTnT expression. The authors found that the expression of GMTd increases the expression of the trimethylated lysine 4 of histone 3 (H3K4me3) mark at the promoter regions of cardio-differentiation genes and mitochondrial biogenesis regulator genes. A similar enhancement was also observed in human cardiac fibroblasts. These results suggest that the Hippo pathway intermediate Tead1 is an important regulator of cardiac reprogramming and that a cocktail of GMtd has potential applications in *in situ* cardiac regeneration.

Figure. Tead1 enhances contractile function of reprogrammed cardiac fibroblast. **A)** Immunofluorescence of rat cardiac fibroblasts infected with lentivector encoding GFP, Gata4, Mef2C, and Tbx5 (GMT), or GMTd. Scale bar: 100 μm. **B)** Cell contraction (**top row**) and Ca2+ transient (**bottom row**) in cells treated with GFP, GMT, or GMTd. **C)** Quantification of the number of spontaneously beating lentivector-treated cells. GMT: Gata4, Mef2C, and Tbx5. GMTd: Gata4, Mef2C, and Tead1. iCMs: induced cardiomyocytes.

Singh VP, Pinnamaneni JP, Pugazenthi A, Sanagasetti D, Mathison M, Martin JF, Yang J, Rosengart TK. Hippo Pathway Effector Tead1 Induces Cardiac Fibroblast to Cardiomyocyte Reprogramming. J Am Heart Assoc 10: e022659, 2021



GENETICALLY ENGINEERED RODENT MODELS (GERM) CORE

The Genetically Engineered Rodent Models (GERM) Core possesses specialized expertise and state-of-theart equipment for providing essential mouse services to investigators at Baylor College of Medicine and collaborative investigators of other institutes. Our core assists investigators with projects involving the production of transgenic, targeted knockout, and targeted knock-in mouse lines. Knockout and knock-in mouse lines can be generated using gene targeting in embryonic stem (ES) cells with chimera production or CRISPR genome editing in mouse zygotes. For projects involving CRISPR genome editing, the GERM Core offers a genome editing design service (guide selection, donor DNA design, and genotyping design) in addition to an on- and off-target mutagenesis genotyping service. The GERM Core also performs cryopreservation of mouse embryos and sperm for long-term storage of mouse lines, mouse line rederivation, in vitro fertilization, and mouse colony expansion.

SERVICES

Transgenics

- Generation of transgenic mice by traditional construct microinjection
- Generation of transgenic mice by bacterial artificial chromosome microinjection

Traditional Gene Targeting

- Gene targeting in mouse ES cells and chimera production
- Rosa26 targeting in mouse ES cells and chimera production
- Chimera production from investigator provided ES cells

CRISPR Genome Editing

- Guide RNA testing in mouse zygotes
- Generation of knockout mice
- Generation of knock-in mice using single-stranded oligodeoxynucleotides (ssODNs)
- Generation of knock-in mice using long singlestranded DNA (IssDNA) or double-stranded DNA (dsDNA)
- CRISPR-assisted ROSA26 targeting using doublestranded DNA (dsDNA)
- Founder and N1 animal PCR genotyping
- Founder and N1 animal Sanger sequencing
- Targeted analysis of off-target mutagenesis

Cryopreservation and Embryology

- Mouse sperm cryopreservation
- Mouse embryo cryopreservation
- Mouse in vitro fertilization
- Mouse colony expansion
- Mouse strain rederivation

CORE LEADERSHIP





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MAJOR EQUIPMENT

- Nikon Eclipse Te300 Microscopes with Hoffman objectives
- Nikon Diaphot inverted microscopes
- SMZ 800 and 1000 dissecting microscopes
- Embryoscope Plus
- CEROS II Animal Sperm Analysis System
- Narishige micromanipulators
- FemtoJet microinjectors

- Gene Pulser Xcell BioRad electroporation systems
- Nuaire laminar flow hoods
- MagMax Express-96 Well Magnetic Particle
- Qiaxcel Advanced System
- Qiagility
- QuantStudio 7 Flex Real-Time PCR System
- QX100 ddPCR system

CORE SUPPORTED RESEARCH

CRISPR-mediated deletion of Gremlin-2 alters estrous cyclicity and disrupts female fertility in mice (PI: Stephanie Pangas, Department Pathology & Immunology)

The differential screening-selected gene aberrative in neuroblastoma (DAN) protein family, which includes GREM1 and GREM2, are developmentally conserved extracellular binding proteins that antagonize bone morphogenetic protein (BMP) signaling. While BMPs play essential roles in ovarian follicle development, the role of DAN family in female reproductive physiology is less understood. Mice null for Grem2 were generated to determine its role in female reproduction. A) The GERM Core designed and implemented a CRISPR genome editing approach that deleted the open reading frame (ORF) of Grem2 in mouse embryos. **B)** *Grem2* expression was not detected in the ovary or lung of homozygous knockout mice. C) Grem2 homozygous knockout mice had an incisor defect. D) Loss of Grem2 reduced female fecundity as measured by reduced litter number per month and reduced litter size later in life (not shown). E) Grem2 knockout females had irregular estrus cycles and a reduced number of days in estrus days per month (not shown). F) Grem2 knockout females had significantly reduced production of ovarian anti-Müllerian hormone (AMH) from small growing follicles, leading to a significant decrease in serum.



Rydze RT, Patton BK, Briley SM, Torralba HS, Gipson G, James R, Rajkovic A, Thompson T, Pangas SA. Deletion of Gremlin-2 alters estrous cyclicity and disrupts female fertility in mice. Biology of Reproduction. 2021, 10(5):1205-1220. PMC8599072

GENOMIC & RNA PROFILING (GARP)

The mission of the Genomic & RNA Profiling (GARP) Core facility is to provide the most cutting-edge genomic technologies to research investigators with an emphasis on personal service and quality. In order to provide whole genome profiling we offer multiple Next-Generation Sequencing platforms (Illumina iSeq 100, NextSeq 500, NovaSeq 6000 and Oxford Nanopore Promethion) and targeted NanoString nCounter assays.

MAJOR EQUIPMENT

- Illumina NovaSeq 6000 Sequencer
- Illumina NextSeq 500 Sequencer
- Illumina iSeq 100 Sequencer
- Oxford Nanopore Promethion 24 Sequencer
- Hamilton NGS STAR (Library Prep Automation System)
- Nanostring nCounter Digital Quantification System
- ABI ViiA7 Real Time PCR/qPCR instrument
- Agilent Bioanalyzer
- Covaris Ultrasonicator

SERVICES

- Next-Generation Sequencing
 - » Sequencing only
 - » Library preparation
 - » RNA-seq (polyA-selected, whole transcriptome, small RNA)
 - » Spatial Transcriptomics (10X Genomics-Visium)
 - » ChIP-seq
 - » Whole Genome Bisulfite Sequencing
 - » Long-Read Sequencing (cDNA-seq & gDNA-seq)
- Targeted NanoString nCounter assays (up to 800 multiplexed assays/sample)
- Nucleic acid quality check



CORE LEADERSHIP



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Spatial transcriptomics to explore the tumor microenvironment of high-grade serous ovarian carcinoma (HGSC)

The GARP Core, in collaboration with Human Tissue Acquisition and Pathology Core (HTAP) performed spatial transcriptomics on tissue collections of 12 chemotherapy-naive high-grade serous ovarian carcinoma samples. Spatial gene

expression was compared between poor responders and excellent responders to chemotherapy.

A) Schematic representation of the study for tissue collection in patients with HGSC.

B) Representative image of a hematoxylin-andeosin-stained section (top) and unsupervised clustering (bottom). Not all clusters were identified between the two groups or in all samples within each group. Overlaying the cluster perimeters onto the hematoxylin-and-eosinstained images revealed that distinct patterns of stromal and epithelial areas identified through gene expression mirrored the stromal and epithelial areas defined by morphology.

C) Heatmap of putative cell composition of each cluster in the excellent responder (ER) and poor responder (PR) groups. 20 cell types were identified, many within the same cluster.

D) Dot-plot representing differential gene expression

of known ovarian cancer genes (COSMIC) in bulk RNA sequencing and in spatial transcriptomics of the main clusters (0-4). Black boxes are shown around the point when the gene set enrichment analysis adjusted p value is <0.1.

Spatially resolved transcriptomics of high-grade serous ovarian carcinoma. Elaine Stur, Sara Corvigno, Mingchu Xu, Ken Chen, Yukun Tan, Sanghoon Lee, Jinsong Liu, Emily Ricco, Daniel Kraushaar, Patricia Castro, Jianhua Zhang, Anil K Sood. iScience. 2022 Feb 14;25(3):103923.



HUMAN TISSUE ACQUISITION AND PATHOLOGY (HTAP)

The Human Tissue Acquisition and Pathology (HTAP) Core provides services for collecting and processing of tissues for research. HTAP serves as the primary centralized tissue bank at BCM and provides human specimens to BCM researchers and others for IRB approved research. Inquiries for tissue requests can be made by sending an email to biobanking@bcm.edu.

Pathology Services are performed on both human and animal tissues by expert technical staff with the assistance of pathologists who provide consultation and review of slides and images. Histology, tissue microarray development, immunohistochemistry (IHC), RNAScope, and imaging are available on a fee-for-service basis.

MAJOR EQUIPMENT

- Vectra3 imaging system with inForm software
- Nikon slide scanning and imaging system
- Shandon Excelsior ES Tissue Processor
- Shandon HistoCentre Embedding System
- Sakura TissueTek SCA Coverslipper
- Shandon Varistain Gemini Slide Stainer
- Microm HM 315 Microtome
- Epredia Cryostar NX50

SERVICES

- Human Tissue Procurement Collection and quality review of human tissues [live, frozen, FFPE] from BCM affiliated hospitals. For large funded projects, investigators must have an IRB approved research protocol and cost sharing is expected. Small projects that require no associated patient data are distributed at cost for sectioning and/or preparation.
- **Histology** Tissue processing, embedding, cutting, and staining of human and animal tissues.
- **Specialized Tissue Sectioning** for 10X Genomics Spatial transcriptomics.
- Immunohistochemistry (IHC) and TUNEL Assays-IHC for proliferation and apoptosis are performed using methods and antibodies provided by the Core. Investigator supplied antibodies are used for other IHC assays which are optimized for performance.
- Multiplex immunofluorescence Standardized 5-color tumor/lymphocyte marker panel for human studies.
- **RNAScope & BaseScope** Advanced Cell Diagnostics Technology for detection of RNA in paraffin embedded tissue.
- **Digital imaging** State-of-the-art imaging of tissue sections or TMAs using the Nikon slide scanner or Vectra imaging system with Nuance FX multispectral camera. Image analysis using inForm software or Nikon Elements for pattern recognition analysis and quantitative scoring.
- **Tissue microarray (TMA)** TMAs are developed using the Core's archival FFPE or tissues provided by individual researchers.
- **Consultation** with pathologists. Experienced pathologists will assist with review of stained slides.



CORE LEADERSHIP



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HIV-1 Viral Protein R Couples with WAT Thermogenesis and Diabetes (PI: Ashok Balasubramanyam, M.D., Department of Medicine/Endocrine Division).



The figure above features histological samples from Vpr transgenic mice that were prepared and stained by the HTAP core for the publication cited below. This study investigates how persons living with HIV (PLWH) manifest chronic disorders of brown and white adipose tissues that lead to diabetes and metabolic syndrome. HIV accessory protein viral protein R (Vpr) circulates in the blood of PLWH even during antiretroviral therapy and causes hyperlipidemia, and glucose intolerance. A feature of metabolic dysregulation described in PLWH is the presence of adipocytes positive for uncoupling protein 1 (UCP1) in white AT (WAT) deposits. This paper describes the UCP1-positive adipocytes in subcutaneous WAT of PLWH to assesses the levels of UCP1, the defining marker of thermogenesis, in subcutaneous AT of PLWH and Vpr mice. Vpr mice show evidence of white-to-beige adipocyte conversion. Histological examination of WAT of Vpr-Tg and sVpr treated mice showed morphological changes similar to classical BAT that were not observed in their respective controls, including Ucp1-positive adipocytes that were smaller in size and contained multilocular lipid droplets. Ucp1 IHC on white adipose tissue (WAT), from transgenic mice confirmed the gene expression results. IHC shows increased Ucp1 protein expression in the iWAT of Vpr-Tg and sVpr-treated mice Specific expression in the iWAT.

Agarwal N, Iyer D, Saha P, Cox AR, Xia Y, Utay NS, Somasundaram A, Schubert U, Lake JE, Hartig SM, Balasubramanyam A. HIV-1 Viral Protein R Couples Metabolic Inflexibility With White Adipose Tissue Thermogenesis. Diabetes. 2021 Sep;70(9):2014-2025. doi: 10.2337/db20-0888. Epub 2021 Jul 7. PMID: 34233931; PMCID: PMC8576429.

HUMAN STEM CELL AND NEURONAL DIFFERENTIATION CORE (REORGANIZED MERGER 2022)

The Human Stem Cell and Neuronal Differentiation Core (HSCNDC) represents a merger of the prior Human Stem Cell Advanced Technology Core and the Human Neuronal Differentiation Core of the Neurological Research Institute (NRI). The merged core provides a wide range of products and services related to human pluripotent stem cell (hPSC) research, as well as hands-on training classes for basic and advanced stem cell culture techniques. We offer cost-effective solutions to generate and characterize new induced pluripotent stem (iPS) cells for in vitro disease modeling, employing non-integrating vector technologies such as Sendai virus-based and episomal vector-based reprogramming. We also offer customized research support for experimental design and validation assays, as well as genome editing of hPSCs using CRISPR/Cas9. The newly merged core specializes in deriving neural progeny from iPSCs, including protocols for directed differentiation and direct conversion of iPSCs to neurons.

MAJOR EQUIPMENT

- EVOS XL and FL inverted microscope systems
- Lonza 4D-Nucleofector transfection system
- NuAire In-VitroCell CO2 Incubators with O₂ control
- Beckman Coulter Allegra X-14R centrifuge
- ABI StepOnePlus Real-Time PCR system
- MVE TEC 3000 LN2 cryostorage system
- NanoCellect WOLF Cell Sorter and N1 Single-Cell Dispenser
- Keyence BZ-X810 epifluorescence microscope
- Proteins Simple WES for automated western blot
- Axion Maestro Pro multielectrode array system
- CellInk Bio X 3D bioprinter
- Calcium imaging and patch clamp systems

SERVICES

- Hands-on training classes and workshops
- Human pluripotent stem cell (hPSC) culture services
- Generation of induced pluripotent stem (iPS) cell lines
- Stem cell line characterization (PluriTest & KaryoStat assays)
- Mycoplasma testing
- Consultation on experimental design
- Customized genome editing of hPSCs using CRISPR/Cas9
- Generation of cancer cell iPSC models
- Generation of iPSC derived neural models





CORE LEADERSHIP



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The IncRNA H19 alleviates muscular dystrophy by stabilizing dystrophin.

This study by the groups of Chunru Lin and Liuqing Yang aimed to elucidate the proteomic regulation of Dystrophin in muscular dystrophies (MDs). They reported that a long noncoding RNA (IncRNA), H19, associates with dystrophin and inhibits E3-ligase-dependent polyubiquitination at Lys 3584 (referred to as Ub-DMD) and its subsequent protein degradation. In-frame deletions in BMD and a DMD non-silent mutation (C3340Y) resulted in defects in the ability of the protein to interact with H19, which caused elevated Ub-DMD levels and dystrophin degradation. DMD C3333Y animals, induced-pluripotent-stem-cell-derived skeletal muscle cells from patients with Becker MD and mdx mice subjected to exon skipping exhibited inhibited dystrophin degradation, preserved skeletal and cardiac muscle histology, and improved strength and heart function following AGR-H19 or nifenazone treatment. These studies pave a way for developing targeted therapeutics for Becker MD and for a subset of patients with Duchenne MD.



H19 mimics and NIF attenuate Ub-DM.D. **F)** Representative immunofluorescence images using indicated antibody (upper) and statistical analysis of DM.D. staining intensities (lower) of hiPS-SkMC derived from a healthy donor or patients with BM.D. after indicated treatments. **G)** Representative immunofluorescence images using indicated antibodies (upper) and statistical analysis of Ub-DM.D. (Lys 3584) staining intensities (lower) of hiPS-SkMC derived from a healthy donor or patients with BM.D. after indicated control and patient-specific iPSC lines used in this study.

Zhang Y, Li Y, Hu Q, Xi Y, Xing Z, Zhang Z, Huang L, Wu J, Liang K, Nguyen TK, Egranov SD, Sun C, Zhao Z, Hawke DH, Li J, Sun D, Kim JJ, Zhang P, Cheng J, Farida A, Hung MC, Han L, Darabi R, Lin C, Yang L. The IncRNA H19 alleviates muscular dystrophy by stabilizing dystrophin. Nat Cell Biol. 2020 Nov; 22(11):1332-1345.

INTEGRATED MICROSCOPY

The Integrated Microscopy Core (IMC) is a state-of-the-art imaging facility that provides extensive training for users on all instruments, and consultation for new and ongoing project development and troubleshooting for a wide range of light microscopy imaging needs for samples from 2D cell culture models, 3D organoids and tissue sections. Key distinguishing activities of the IMC include support for assay development, high content analysis leading to high throughput microscopy, and a new BigPharma scale high throughput spinning

disk confocal with 4 color simultaneous imaging. IMC instrumentation supports multiplexing channels for live or fixed cell confocal, deconvolution, super-resolution (SIM) microscopy, or automated high throughput microscopy. A full suite of image analysis, statistics and reporting software is available for data mining and management.

MAJOR EQUIPMENT

- Yokogawa CV8000 high throughput spinning disk confocal microscope
- Nikon A1-Rs laser scanning spectral confocal microscope
- Cytivia DeltaVision deconvolution microscope with large sCMOS camera
- Olympus IX83 automated epifluorescence microscope with image deconvolution with near IR capabilities
- Cytivia OMX Blaze super-resolution instrument (SIM) with TIRF capabilities
- Biotek Cytation 5 microscope-in-a-box (fluorescence, color, slide scanning, live imaging), plus plate reader (fluorescence, absorbance, luminescence)
- Sartorius IncuCyte S3 long term live imager
- Nikon Ci-L upright brightfield microscope with color camera

SERVICES

- One-on-one training for all instruments and assisted use
- Assay development and project consultations
- Fully automated and assisted high throughput microscopy for 96/384 well plates
- Image Analysis (limited): custom or pre-set (i.e., cell count, subcellular localization, spot counting, translocation, cell cycle, toxicity, live/dead, apoptosis)







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Imaging examples using the Yokogawa CV8000 high throughput spinning disk confocal.

The CV8000 is capable of both live and fixed high throughput imaging of 2D and 3D cell culture models. It can image up to five fluorescence channels, plus brightfield/phase, with objectives ranging from 4x to 60x/water. The Cell Pathfinder high content analysis software is available for basic and advanced image analysis.

Unpublished images courtesy of Matthew Penna, Sara Johnson and Dr. Thomas Cooper, Department of Pathology, Baylor College of Medicine; and Dr. Dan Gorelick, Center for Precision Environmental Health



MACROMOLECULAR X-RAY CRYSTALLOGRAPHY

Single crystal X-ray diffraction is the most powerful technique to determine the atomic structure of biologically important macromolecules and their functional complexes with small molecules or natural ligands. The Macromolecular X-ray Crystallography Core provides a cost-efficient solution for researchers and trainees at Baylor College of Medicine and its neighboring institutions to pursue high-resolution structural studies. Unlike other structural analysis techniques, X-ray crystallography is not limited by the size or chemical composition of the specimen, making it possible to determine the 3D structure of small molecules and nucleic acids to large, multi-subunit macromolecular assemblies. Furthermore, X-ray crystallography allows the 3D structure determination of macromolecules bound to an agonist or antagonist often with little additional effort. The atomic structures of these complexes are highly valuable and can be exploited for rationale structure-based drug design. Access to core equipment for fully trained users is 24/7.

MAJOR EQUIPMENT

- Mosquito Crystallization robot
- Formulatrix Rock Imager 2 with UV/multi-fluorescence option (New NIH S10 Shared Instrument Grant).
- Rigaku Ultimate Home Lab X-ray diffraction system

SERVICES

- **Consultation:** Custom service to develop a structure solution strategy, provide answers to protein expression, purification, and crystallization needs, discuss data collection requirements.
- **Training:** Provide training for unassisted use of the crystallization robot, imager, and X-ray home source.
- **Crystallization setup:** Assisted and unassisted crystal growth screening in 96-well plate format using the hanging- or sitting-drop vapor diffusion technique.
- **Crystal imaging:** Assisted and unassisted service to capture and record crystallization experiments.
- **Crystal optimization:** Assisted service to optimize crystal growth.
- **Cryo optimization:** Assisted service to identify cryoprotectants for X-ray diffraction experiment.
- X-ray data collection (home source): Assisted and unassisted use of the in-house X-ray source for data collection.
- X-ray data collection (National Synchrotron facility): Assisted data collection using the high-intensity synchrotron radiation beamline at the Argonne National Laboratory.
- **Data processing:** Assisted data processing of X-ray diffraction data.
- **Structure determination:** Custom service to determine the crystal structure of a macromolecule of interest.

Researchers are responsible for making their own macromolecule in purified form (1-10 mg scale). Expression and purification of recombinant proteins of interest are available as services through the Protein and Monoclonal Antibody Production Core.

CORE LEADERSHIP



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Atomic Structure of the Leishmania spp. Hsp100 N-domain

Leishmaniasis is a vector-borne, neglected tropical disease caused by the Leishmania parasite that is transmitted to humans through the bite of an infected female sandfly. Approximately 1.3 million new cases are reported annually, including ulcerative skin lesions (cutaneous leishmaniasis) and systemic infections (visceral leishmaniasis) that can be fatal if left untreated.

Leishmania parasites are flagellated protists that cycle between extracellular promastigotes and intracellular amastigotes that are responsible for parasite replication and all clinical manifestations of leishmaniasis. It was previously reported that the *Leishmania spp*. Hsp100 unfoldase is required for full amastigote development, supporting a role as an essential virulence factor and potent drug target. To inform on Hsp100 function, we determined the 1.06-Å resolution crystal structure of the Hsp100 N-domain from *L. mexicana*, which represents the highest resolution 3D structure of a microbial Hsp100 N-domain to date. The structure suggests that disrupting the structural integrity of the N-domain could potentially abolish Hsp100 function and may be exploited for the development of new anti-leishmanial drugs.



Figure. Crystal structure of LmHsp100_N. **A)** Enlarged view of the hydrophobic cleft showing the location of the six methionines (green) as well as of Trp8 and Phe111 (purple), with the tryptophan side chain being solvent exposed. **B)** Phe111 is surrounded by methionine residues that likely protect this critical residue from oxidative damage. Four out of five methionines are shown for clarity. A section of the composite 2Fo-Fc electron density map is shown as purple mesh and contoured at the 1.0 σ level.

Mercado, J.M., Lee, S., Chang, C., Sung, N., Soong, L., Catic, A., and Tsai, F.T.F. (2022). Atomic structure of the Leishmania spp. Hsp100 N-domain. Proteins 90:1242-46.

MASS SPECTROMETRY PROTEOMICS

The Mass Spectrometry Proteomics Core offers services for quantitative proteome-wide profiling of cells and tissues, isolation and characterization of protein complexes and other affinity-based pulldowns, post-translational modification (PTM) analysis, and routine or targeted identification of purified proteins. We specialize in providing comprehensive project-based support that includes experimental design, optimization of biochemical procedures for sample preparation, state-of-the-art mass spectrometry technology, and custom data analysis to address specific challenges of different proteomics approaches. The core offers specialized support for preclinical PDX cancer models with mixed-species proteomes and clinical sample processing for multiomics.

MAJOR EQUIPMENT

- Thermo Scientific Mass Spectrometers:
 - » Orbitrap Fusion Tribrid
 - » Orbitrap Lumos ETD Tribrid
 - » Orbitrap Exploris 480
 - » Orbitrap Eclipse
- EASY-nLC1200 and EASY-nLC1000 UHPLC Systems
- Agilent 1260 Infinity II HPLC System
- Agilent AssayMAP Bravo Protein Sample Robotics System

SERVICES

- Proteome Profiling (label-free or TMT-based) services that combines efficient non-detergent sample preparation procedure with dual reverse phase fractionation procedure and optimized mass spectrometry acquisition methods to allow identification and quantification of up to 10,000 proteins from as little as 100,000 cells or 20 micrograms of tissue lysate.
- Phosphoproteome Profiling (TMT-based) service is offered as matched proteome and phosphoproteome profiling based on a NIH Clinical Proteomic Tumor Analysis Consortium (CPTAC) TMT10/11 harmonized protocol.
- Affinity Purification/Mass Spectrometry service is a suite of assays for characterization of immunoprecipitated protein complexes, enrichment and identification of proteins that assemble on immobilized DNA baits, and characterization of protein targets of small molecules. The core's unique emphasis is in purification of endogenous complexes. Custom data analysis against BCM's own complexome database and filtering of non-specific precipitants is included in this package service.
- Post-translational modification (PTM) analysis includes identification and quantification of phosphorylation, ubiquitination or acetylation sites on purified proteins.
- Routine MS sequencing of purified protein samples for single-protein identification or targeted verification via parallel reaction monitoring.
- Consultation, experimental design and data analysis.



CORE LEADERSHIP





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Mutations in Hcfc1 and Ronin result in an inborn error of cobalamin metabolism and ribosomopathy. (Dr. Ross Poche's Laboratory)

Dr. Ross Poche's laboratory used Proteome Profiling services of the Core to characterize expression of proteins in MEF cells derived from mutant Ronin^{F80L/F80L} mice. Ronin is a transcriptional regulator that affects expression of Methylmalonic Aciduria type C and Homocystinuria (MMACHC) gene, which harbors mutations that cause combined methylmalonic acidemia and homocystinuria (cblC) disease. In Ronin mutants, the laboratory found developmental defects that were previously observed in cbIC and uncovered additional unexpected pathophysiology consistent with ribosomopathies and increased protein translation. To explore the mechanisms of these additional phenotypes, the core performed global mass spectrometry analysis on lysates from Ronin^{F80L/F80L} and wild type MEFs and found almost 200 proteins that were significantly increased in the mutants. GO analysis showed the expected upregulation of enzymes that reflect perturbations of MMACHC-dependent cobalamin metabolism. And unexpectedly, while RNA-seq showed reduction of ribosomal components, proteomics suggested overrepresentation of ribosome biogenesis factors and ribosomal subunit components, some of which are Ronin target genes. This finding was consistent with increased translation inferred from polysome enrichment and puromycin incorporation. Mass spectrometry data also revealed an increase in proteins known to function in unfolded protein binding, indicating that Ronin F80L mutants may suffer from pathological changes in translational quality. The core then performed mass spectrometry analysis of E18.5 wild type and Ronin^{F80L/F80L} brains (not shown), revealing similar increase in proteins involved in ribosome biogenesis and protein synthesis. These findings echo recent report of budding yeast ribosome protein mutants that uncovered similar perplexing phenomenon, providing insight into fundamental mechanisms of ribosomal dysregulation.



Figure (adapted from published Figure 7): The partial figure from the publication shows differential protein expression analysis results from core's proteome profiling data (upper left). Their pathway analysis (bottom left) highlights enrichment of protein biosynthesis, ribosome biogenesis, and ER stress pathways in Ronin F80L/ F80L MEFs. Polysome profiling and Western blot of increased protein ubiquitination, implying protein folding defects, in RoninF80L/F80L versus WT E18.5 brains are also shown on the right.

Chern T, Achilleos A, Tong X, Hill MC, Saltzman AB, Reineke LC, Chaudhury A, Dasgupta SK, Redhead Y, Watkins D, Neilson JR, Thiagarajan P, Green JBA, Malovannaya A, Martin JF, Rosenblatt DS, Poché RA. Mutations in Hcfc1 and Ronin result in an inborn error of cobalamin metabolism and ribosomopathy. Nat Commun. 2022 Jan 10;13(1):134. doi: 10.1038/s41467-021-27759-7. PMID: 35013307; PMCID: PMC8748873.

METABOLOMICS

The Metabolomics Core provides targeted metabolic profiling for discovering and validating biomarkers of various diseases with state-of-the-art high throughput mass spectrometry as the main platform. Metabolites can be measured in tissues, cell lines, fecalsample, and biofluids. The entire process starting from sample preparation to mass spectrometry is monitored using spiked isotopic standards that have been characterized for their chromatographic behavior as well as fragmentation profile. Biostatisticians are available for further analysis of the resulting output data.

MAJOR EQUIPMENT

- Agilent 6495 Triple Quadruple (QQQ) Mass Spectrometry
- Agilent 6495B Triple Quadruple (QQQ) Mass Spectrometry
- AB SCIEX 5600 Triple TOF Mass Spectrometry
- 1290 and 1260 Series HPLC Systems

SERVICES

Targeted metabolite steady-state profiling: The Core has the capability of identification, quantification and, characterization of over 600 metabolites using the targeted multiple reaction monitoring approaches (MRM) developed for different chemical classes of compounds. Data can be reported either in absolute concentrations or as intensity ratios to internal standards.

Metabolic Flux: Isotope flux and metabolite profiling help formulate and test hypotheses about the metabolic consequences of various changes to guide further integrative systems biology analyses of the underlying mechanisms in disease. The Core has the capability of characterizing [13C] Glutamine and [13C] Glucose flux using LC-QQQ Mass Spectrometry.

Lipidomics: Using an ABSCIEX 5600 Triple TOF MS, identification of lipids is accomplished by data-dependent production (MS/MS) information of human plasma, tissues, and urine and in both positive and negative ionization modes. MS/MS acquisition or MS/MS ALL acquisition provides information on the nature of the lipid head group and/or neutral loss of the head group from the molecular ion adducts. Information on the fatty acid composition of the lipids is obtained in the negative mode.

Data Analysis:

- Pathway mapping using OCM, GSA or NETGSA
- Developing classification models
- Integration with other OMICS datasets



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CLASSES OF STEADY-STATE METABOLITES MEASURED BY MRM ANALYSIS

Assay 1	Amino sugars
Assay 2	Amino acids
Assay 3	Prostaglandins
Assay 4	Carnitines
Assay 5	Polyamines
Assay 6	Glycolysis Intermediates

Assay 7	TCA
Assay 8	Nucleotides
Assay 9	Vitamins
Assay 10	Steroids
Assay 11	Bile acids
Assay 12	Short-Chain Fatty Acids

Assay 13	Methylated Metabolites
Assay 14	Fatty acids
Assay 15	Neurotransmitters and related metabolites

CORE SUPPORTED RESEARCH

CHAF1A Blocks Neuronal Differentiation and Promotes Neuroblastoma Oncogenesis via Metabolic Reprogramming (PI: Eveline Barbieri, Baylor College of Medicine)

In this study, the Dr. Barberi Lab showed that CHAF1A gain-of-function supports cell malignancy, blocks neuronal differentiation in three models [zebrafish neural crest (NC), human NC, and human neuroblastoma (NB)], and promotes NB oncogenesis. CHAF1A upregulates polyamine metabolism, which blocks neuronal differentiation and promotes cell cycle progression. Targeting polyamine synthesis promotes NB differentiation and enhances the anti-tumor activity of retinoic acid (RA). This study identified a novel function of CHAF1A in reprogramming polyamine metabolism to block neuronal differentiation and support oncogenesis. Inhibiting polyamine synthesis promotes NB differentiation and enhances the anti-tumor activity of RA.



Figure: CHAF1A blocks neuronal differentiation and promotes neuroblastoma oncogenesis via metabolic reprogramming. Schematic presentation of the polyamine pathway with metabolite changes in SHEP cells with or without CHAF1A overexpression for 24 h (red = upregulated metabolites, $p \le 0.05$; blue = downregulated metabolites, $p \le 0.05$). Right: polyamine levels in SHEP cells with or without CHAF1A overexpression for 24 h.

Tao L, Moreno-Smith M, Ibarra-García-Padilla R, Milazzo G, Drolet NA, Hernandez BE, Oh YS, Patel I, Kim JJ, Zorman B, Patel T, Kamal AHM, Zhao Y, Hicks J, Vasudevan SA, Putluri N, Coarfa C, Sumazin P, Perini G, Parchem RJ, Uribe RA, Barbieri E. CHAF1A Blocks Neuronal Differentiation and Promotes Neuroblastoma Oncogenesis via Metabolic Reprogramming. Adv Sci (Weinh). 2021 Oct;8(19):e2005047. doi: 10.1002/advs.202005047. Epub 2021 Aug 8. PMID: 34365742; PMCID: PMC8498874.

MHC TETRAMER

MHC Tetramer technique has become a "gold standard" for the quantification of T cell immune responses. The T cell antigen-receptor (TCR) recognizes antigens through peptides bound to major histocompatibility complex molecules (MHC), also called HLA (human leukocyte antigens) in humans. Joining multiple copies of the MHC/antigen complex into a single probe resolves the difficulties presented by the low affinity of the MHC molecule for the CD8 receptor. This unique technique can offer exquisite antigen specificity and sensitivity to monitor T cell responses, which makes it suitable for basic and clinical studies including cancer prevention, cancer therapy, cell and gene therapy, immunotherapy, and non-cancer related immunology research. The mission of the core is to provide customized MHC/peptide tetramers for identification of antigen specific T lymphocytes by flow cytometry.

STANDARD SERVICES

- Standard or customized MHC/peptide class I tetramers
- We offer more than sixty human, mouse, macaque, and chimpanzee alleles for customized production of class I MHC reagents with desired epitopic peptides. Researches will also have two fluorescent labels choices: R-phycoerythrin (PE) or allophycocyanin (APC).
- Biotinylated or non-biotinylated MHC/peptide class I monomer. Customers can be provided with labeled tetramers with fluorophores of their own choice or can use monomers in several special applications.
- Customers can be provided with labeled tetramer with fluorophores of their own choice or can use monomers in several special applications

SPECIAL SERVICES

- CD8 binding-deficient MHC Class I tetramers: Containing mutations in the MHC α3 domain that ablate CD8 binding; can be used to quantitate and to sort CD8independent T cells.
- Chimeric Tetramer: Containing MHC Human/Mouse chimeric heavy-chain
- MHC Monomers for generating TCR-like antibodies
- Developing high-affinity soluble antibody molecules endowed with a TCR-like specificity toward tumor epitopes, termed TCR-like antibodies, recently became a hot topic for cancer immunotherapy studies.
- MHC Class I monomers designed for ligand exchange
- When trying to analyze multi epitopes of an antigen, generating individual tetramers can be very time consuming and less practical. The MHC monomers specially designed for ligand exchange can address this issue well. We now have ligand exchange monomers designed for six commonly used HLA alleles.
- SARS-CoV-2/COVID-19 tetramer reagents
- We produceda set of SARS-CoV-2/COVID-19 tetramer reagents based on published works. Epitopes were previously identified in SARS and are 100% conserved in SARS-CoV-2. These tetramer reagents can be used to study human, mouse and macaque T cell responses to SARS-CoV-2.



CORE LEADERSHIP



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Identification of immunogenic epitopes for SARS-CoV-2 by mass spectrometric analysis of MHC-eluted peptides.

Severe acute respiratory syndrome coronavirus 2 (SARSCoV-2), the highly transmissible respiratory virus responsible for the COVID-19 pandemic outbreak, continues to render significant, lasting impact on global public health and has created an urgent need to develop accurate immunodiagnostics, and effective treatment strategies. For the prevention and treatment of COVID-19, the T cell responses are equally if not more important than humoral responses in mediating recovery and immune protection. One major challenge in developing T cell-based therapies has been the identification of immunogenic epitopes that can elicit a meaningful T cell response. This study defined several SARS-CoV-2 epitopes for the membrane glycoprotein (MGP) and the nonstructural protein (NSP) of the virus by directly analyzing peptides eluted from naturally processed MHC-peptide complex using tandem mass spectrometry. The immunogenicity of these MS-defined peptides were validated by in vitro generation of MGP and NSP13 peptide-specific cytotoxic T cells (CTLs) and T cell recognition of MGP or NSP13 was confirmed in endogenously expressing cell lines. SARS-CoV-2/COVID-19 tetramer reagents produced by the Core were used to sort and validate antigen-specific T cells against various SARS-CoV-2 targets.

The figure below shows the generation of TCR engineered T cells (TCR-T) specific for the MGP-65 peptide. The whole length of the MGP-65 TCR alpha chain and beta chain linked with fusion peptide FP2A was inserted into retrovirus vector pMSGV1, and resultant recombinant retrovirus vector was used to infect the OKT3 cells activated HLA-A0201 allo-PBMCs (**left panel**). After 5 d of infection, CD8+ and MGP-65-Tetramer+ T cell populations were analyzed by flow cytometry (**middle panel**). CD8+ and MGP-65 Tetramer+ cells were then sorted and expanded with a rapid expansion protocol (REP). After expansion for 2 weeks, high-purity CTLs (Tetramer+ population over 90%) were generated (**right panel**).



Pan K, Chiu Y, Huang E, Chen M, Wang J, Lai I, Singh S, M. Shaw RM, MacCoss MJ, and Yee C. Mass spectrometric identification of immunogenic SARS-CoV-2 epitopes and cognate TCRs. PNAS, 2021 Vol. 118 No. 46 e2111815118, PMID: 34725257, PMCID: PMC8609653

MOUSE METABOLISM AND PHENOTYPING CORE

The Mouse Metabolism and Phenotyping Core (MMPC) provides equipment, services, and resources for the expert characterization of whole animal and organ systems phenotypes within rodent models. The MMPC has standardized key methodologies and can expedite comprehensive research analyses on diseases related to cancer, cardiovascular dysfunction, metabolic disorders, rodent models of human disease and drug studies. The MMPC also provides advanced analysis of metabolic pathways and related physiological and biochemical parameters in mice and rats, as well as in isolated/cultured cells in vitro. Workstations for image reconstruction and data analysis are available with MMPC personnel providing consultation on selecting the appropriate tests and procedures, and the interpretation of data.

MAJOR EQUIPMENT

- Vevo F2 Ultrasound (VisualSonics) (New NIH S10 Grant)
- 7.0T Pharmascan MRI (Bruker)
- eXplore CT 120 (TriFoil Imaging)
- Ms-FX Pro Optical Imager and X-Ray (Bruker)
- Unrestrained Whole Body Plethysmography (Buxco)
- Oxymax FAST Indirect Calorimetry System (Columbus Instruments)
- Comprehensive Lab Animal Monitoring System [CLAMS-HC] (Columbus Instruments)
- UltraFocus [X-Ray and Body Composition Analyzer] (Faxitron)
- EchoMRI-100[™] [Body Composition Analyzer] (EchoMRI)
- Non-Invasive Blood Pressure (IITC Life Sciences)
- Blood Pressure and ECG Telemetry (DSI)
- 6-lane treadmill (Columbus Instruments)
- Running wheels (Minimitter)
- Metabolic cages (Techniplast)
- ECG-Mouse Monitor (Indus Instruments)

SERVICES

- Hyperinsulinemic-euglycemic clamp in conscious mice: direct quantification of the role of specific organs and tissues in glucose-insulin homeostasis
- Cellular oxygen consumption & glycolysis monitoring of cultured cells by use of the Seahorse instrument
- Metabolic monitoring of food intake, energy expenditure and real-time body temperature for small animals (mouse) after 72 hours of acclimation using the Comprehensive Laboratory Animal Monitoring System (HC-CLAMS)
- Echocardiography
- Home cage activity monitoring
- Hypoxia chamber animal housing
- Plasma/Blood parameter Analysis
- Lipid Metabolism
- Glucose metabolism
- Glucose tolerance test
- Insulin tolerance test
- Pyruvate tolerance test (gluconeogenesis)
- Telemetry Device Implantation
- Osmotic Pump Implantation
- Additional Surgical Services (by request)
- Intact Animal Phenotyping Services: consultation for behavior, physiology, and metabolic assay testing battery design, technical services for data collection and analysis.

- Pulse oximetry (Indus Instruments)
- Grip strength meter (Columbus Instruments)
- XFe96 Seahorse Analyzer (Agilent)
- XF24 Seahorse Analyzer (Agilent)
- Versamax System (Accuscan)
- Vessel Doppler (Indus Instruments)
- Rectal probe for body temp measurement
- Isoflurane anesthesia stations

CORE LEADERSHIP



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REV-ERB in GABAergic neurons controls diurnal hepatic insulin sensitivity

Rev-erb in GABAergic neurons regulates rhythmic hepatic insulin sensitivity independently of locomotor and consummatory behaviors. a) RNAscope analysis of Rev-erb α gene expression in a cross-section of the mouse brain harvested at ZT6-9. VGAT (Slc32a1) served as the GABAergic neuron marker. Scale bar, 500 μm. b) Wheel-running activity in LD, n = 4. c) Wheelrunning activity in DD, n = 4. **d)** Food intake in LD, n = 4 cages. e) Body weight, n = 10. f-h) Glucose tolerance tests (GTT) at the indicated ZT,



n = 8. I) Serum insulin levels, n = 8. j-l) Glucose infusion rate (GIR), hepatic glucose production (HGP), and blood glucose disposition rate (GDR) in hyperglycemic-euglycemic clamp analyses at indicated ZTs, n = 4. Data are mean \pm S.E.M. * p < 0.05 by two-sided t-test.

Ding G, Li X, Hou X, Zhou W, Gong Y, Liu F, He Y, Song J, Wang J, Basil P, Li W, Qian S, Saha P, Wang J, Cui C, Yang T, Zou K, Han Y, Amos CI, Xu Y, Chen L, Sun Z. REV-ERB in GABAergic neurons controls diurnal hepatic insulin sensitivity. Nature. 2021 Apr;592(7856):763-767. doi: 10.1038/s41586-021-03358-w. Epub 2021 Mar 24. PMID: 33762728

Defining the mammalian coactivation of hepatic 12-h clock and lipid metabolism

SRC-3 coactivation of 12-h clock regulates energy expenditure and links to metabolic diseases and cancer. **A)** The relative realtime energy expenditure (EE) values of the indicated mice (n = 4) housed under ad libitum feeding conditions. Averaged real-time EE values of the indicated mouse strains plotted against the indicated time points. **B)** The body composition of the indicated mice (n = 6) housed under ad libitum feeding conditions.



Unpaired Student's t test was performed with p value indicated. **C)** The integrated statistical analysis is shown for the indirect calorimetry experiments of the SRC-3 WT and KO mice (n = 4) housed under ad libitum feeding conditions. One-way ANOVA analysis with Tukey's post hoc analysis was performed with p value threshold 0.05. The ANOVA was normalized by lean mass at the indicated light, dark, and full-day conditions (n = 4). **D)** The relative real-time respiratory exchange ratio (RER) values of the indicated mice (n = 4) housed under ad libitum feeding conditions. Averaged real-time RER values of the indicated mouse strains plotted against the indicated time points. **E)** Raw, eigenvalue/pencil decompositions, and deconvolutions of the mean RER oscillations of 12-h (top, in red) and 24-h (bottom, in black) oscillations in the SRC-3 WT and KO mice. **F)** Food intake (top) and locomotor activity (bottom) in the SRC-3 WT and KO mice fed regular chow ad libitum (n = 4). Blue line,: SRC-3 WT; red line, SRC-3 KO. Mouse RER, EE, body mass, food intake, and locomotor activity data are graphed as means ± SEM. *p < 0.005, **p < 0.001, ****p < 0.0001.

Meng H, Gonzales NM, Jung SY, Lu Y, Putluri N, Zhu B, Dacso CC, Lonard DM, O'Malley BW. Defining the mammalian coactivation of hepatic 12-h clock and lipid metabolism. Cell Rep. 2022 Mar 8;38(10):110491. PMID: 35263593, PMCID: PMC8958721

NMR AND DRUG METABOLISM

The Nuclear Magnetic Resonance (NMR) and Drug Metabolism Core offers tools to support the discovery, synthesis, screening, identification, metabolism and pharmacokinetics of small molecules. Expert NMR services are available to determine small molecule identity or conformation, to identify and quantify metabolites that may serve as biomarkers, to determine macromolecular structure or detect structural perturbations upon ligand binding, or to screen small molecule compound libraries against purified macromolecular targets. The Core also investigates the metabolism and pharmacokinetics of small molecular weight compounds using liquid chromatography-mass spectrometry (LC-MS and MS/MS) and metabolic stability in liver microsomes by reaction phenotyping assays with CYP450s. Core personnel provide advice on the use of the supported methods for a wide variety of applications, and assistance is available in project experimental design and data analysis. NMR spectrometers are available for unassisted use by trained and qualified users, and user training in simple 1D and 2D NMR data acquisition and analysis is available.

MAJOR EQUIPMENT

- 800 MHz Bruker Avance HD III spectrometer
- 800 MHz Bruker QCI Cryoprobe
- SampleJet automated sample changer
- 600 MHz Bruker Avance HD III spectrometer
- SampleXpress automated sample changer
- Thermo Q Exactive Hybrid Quadropole-Orbitrap LC-MS system
- Thermo Quantis Triple Quadrupole LC-MS/MS system

SERVICES

- Compound identification and quality control
- Small molecule conformational analysis
- Ligand/target screening
- Chemical shift perturbation mapping of protein/ ligand interactions
- Feasibility studies for NMR structure determination
- NMR user training
- Drug quantification in fluids or tissues
- Drug metabolite identification
- Drug stability assays in microsomes
- Reaction phenotyping
- Pharmacokinetic profiles

CORE LEADERSHIP



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Feng Li, Ph.D. Co-Director Associate Professor Department of Pathology and Immunology 713.798.3623 feng.li@bcm.edu **Figure 1.** Mapping ligand binding by NMR chemical shift perturbations. **(A)** Binding of the small molecule 2,6-lutidine to the tandem Tudor domain of UHRF1 induces NMR chemical shift perturbations of the backbone amide resonances in 2D ¹H/¹⁵N HSQC spectra. **(B)** Mapping the CSPs onto the sequence reveals that many residues spread throughout the sequence are affected. **(C)** The ten largest CSPs map onto the crystal structure shows at one binding site between



the tandem domains and another within a domain.

Chang L, Campbell J, Raji IO, Guduru SKR, Kandel P, Nguyen M, Liu S, Tran K, Venugopal NK, Taylor BC, Holt MV, Young NL, Samuel ELG, Jain P, Santini C, Sankaran B, MacKenzie KR, Young DW. Discovery of small molecules targeting the tandem tudor domain of the epigenetic factor UHRF1 using fragment-based ligand discovery. Scientific reports 11, 1121 (2021).



Figure 2. Identification of novel adducts in the metabolism of the anti-depressant drug duloxetine.
A) LC-MS resolves *N*-acetyl cysteine adduct M38. B) Structure of adduct M38 is confirmed by MS-MS fragmentation analysis.
C) inferred pathways for generating glutathione-adduct mediated metabolites of duloxetine in mice.

Qin X, Hakenjos JM, MacKenzie KR, Barzi M, Chavan H, Nyshadham P, Wang J, Jung SY, Guner JZ, Chen S, Guo L, Krishnamurthy P, Bissig KD, Palmer S, Matzuk MM, Li F. Metabolism of a Selective Serotonin and Norepinephrine Reuptake Inhibitor Duloxetine in Liver Microsomes and Mice. Drug Metab Dispos. 50, 128-139 (2022).

OPTICAL IMAGING AND VITAL MICROSCOPY (OIVM)

The Optical Imaging & Vital Microscopy Core (OiVM) is a light microscopy core facility specializing in 3D optical sectioning tools for fluorescence microscopy. We operate as a 24/7 independent-use core, providing expert training and support that gives our investigators the tools and technical expertise they need to obtain reliable, reproducible imaging data using state-of-the-art Confocal, 2-Photon and Lightsheet imaging modalities.

We have over 20 years of experience educating, training, and assisting scientists with experiments using the latest cutting-edge microscopy techniques in a variety of applications such as understanding cell migration, optimizing angiogenic therapies, how blood flow influences development and cancer, immune cell recruitment, stem cell-niche interactions and cancer metastasis.

MAJOR EQUIPMENT

- Zeiss LSM 880 with AiryScan FAST High Speed Super Resolution/Confocal point scanning microscope
- Zeiss LSM 780 Multi-Photon Visible/Multi-Photon Spectral Confocal point scanning microscope
- Zeiss Lightsheet Z.1 Light-sheet fluorescence microscope
- Bruker Skyscan 1272 X-ray μCT
- Leica TCS SP8 MP Confocal and Multi-Photon microscope
- Zeiss AxioObserver Widefield Fluorescence microscope
- Zeiss Axio Zoom.V16 Stereomicroscope
- Optical Projection Tomography (OPT) microscope
- Logos Biosystems X-Clarity Tissue Clearing System
- 2× High End Image Processing Workstations equipped with Imaris, Arivis Vision 4D, Bruker CT, Zeiss ZEN and Fiji.

SERVICES

- Independent or Assisted Imaging
- Expert training/instruction on core instrumentation
- 24/7 Core Access for Trained Users
- Tissue Clearing with CLARITY protocol provided on a per sample basis
- Super Resolution Microscopy with AiryScan detection system
- Confocal microscopy with spectral array detection for imaging multiple fluorophores (up to 8) simultaneously
- Automated 3D tile scanning of image large fields with high resolution
- LightSheet fluorescence microscopy for 3D imaging of thick tissues cleared with Scale, CLARITY, etc.
- Two-photon and second harmonic generation (SHG) intravital imaging
- Live imaging of tissue/organ development in embryos and live animals with environmental control of the stage and anesthesia support of live animals
- High speed imaging of blood and fluid flow such as fluorescent beads, fluorescently labeled erythroblasts, etc.
- Imaging and 3D rendering of embryos, organs, bioengineered gels, etc. using the X-ray microCT scanner
- Imaging and 3D rendering of optically cleared (BABB) embryos, mammary glands, etc. using the OPT microscope
- Quantitative analysis of cellular dynamics and cell tracking
- 3D/4D Image Rendering using high end workstations equipped with latest image visualization/analysis software

CORE LEADERSHIP



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EZ Clear is a simple, rapid, and efficient tissue clearing method developed by the Optical Imaging and Vital Microscopy core for clearing whole mount adult mouse organs in three simple steps. Samples processed with EZ Clear can retain endogenous fluorescence and allow 3D whole organ lightsheet imaging. Samples can also be cryosectioned for histology and immunofluorescence staining following processing with EZ Clear.

Learn more about EZ Clear in our preprint available on bioRxiv here: https://www.biorxiv.org/content/10.1101/2022.01.12.476113v1.full



Depth-coded projection of intact mouse eye illustrating blood vessels perfused with lectin-649. Cleared with EZ Clear and imaged on Zeiss Lightsheet Z.1.



Coronal cryosection (100 um) of mouse brain with GFP⁺ tumor cells (green), blood vessels perfused with lectin-649 (red), stained with GFAP (white) and Hoechst (blue). The section was imaged on our Zeiss LSM 880 Airyscan FAST confocal microscope at 20×.

PATIENT-DERIVED XENOGRAFT AND ADVANCED IN VIVO MODELS CORE

The Patient-Derived Xenograft and Advanced In Vivo Models (PDX-AIM) Core is divided into two independent but closely interacting units, PDX models using immunocompromised mice as the host species, and an Advanced In Vivo Models (AIM) Unit created to facilitate establishment and use of patient material grown on the chorioallantoic membrane (CAM) of the chicken egg. The AIM Unit also employs the CAM model to establish non-tumorigenic spheroids, 3-D tumors from existing cancer cell lines, and creation and optimization of custom bioassays to assess tumor neovascularization, invasion, and metastasis. I 3-D PDX-derived organoid models serve as tools for pre-clinical large-scale compound or genetic screens.

A primary focus of the Core is to develop, and provide to the Baylor PDX community, computational and bioinformatics infrastructure to support large-scale generation, characterization, and use of PDX, CAM-PDX, and organoid models for breast, head and neck, pediatric cancers, pancreas, brain, and other cancer types of interest. The core also provides expertise in transplantation and animal handling to BCM investigators wishing to generate PDX from various cancer types. The core coordinates and assists withthe evaluation of experimental therapeutics using the PDX, CAM-PDX, and organoid platforms in conjunction with those investigators maintaining PDX collections for each organ/disease type.

All PDX work involving animals is conducted in dedicated housing and surgical suites in the Transgenic Mouse Facility of BCM, a fully AAALAC-accredited animal care and housing facility. Work is supported by the Center for Comparative Medicine (CCM), which provides veterinary care, administrative and regulatory oversight, and assistance with animal husbandry.

MAJOR EQUIPMENT

- IVIS Lumina III luminescence/fluorescence imager
- gentleMACS Octo Dissociator with heaters
- Tissue Cassette Labeler
- Alabama Tissue Slicer and Coring Press
- Computation and Bioinformatics Infrastructure for managing clinical and PDX associated data elements

SERVICES

MOUSE PDX:

- Development of computational/bioinformatics infrastructure to support PDX-based research
- Assist with, or facilitate, the generation of PDX models
- Facilitate in vivo treatment experiments with investigational drugs with PDX models. Provide training for PDX related procedures upon request
- Coordinate provision of snap frozen tissue, viably frozen tissue, serum/ plasma, and FFPE blocks/slides from PDX models from PDX program leads
- Coordinate provision of molecular derivatives of PDX models from PDX
 program leads
- Provide excess immunocompromised SCID/Bg mice from our breeding colony to BCM investigators

CAM-PDX:

- Conversion of cancer cell lines into 3D vascularized tumors
- Patient Derived Xenografts (PDX) growth on the chicken egg chorioallantoic membrane (CAM-PDX)
- Custom bioassays including angiogenesis, invasion, and metastasis
- Drug sensitivity screening on 3D vascularized cell xenografts and PDX
 Assistance in end-point assays (Flow cytometry, DNA/RNA purification, IHC,
- Assistance in end-point assays (Flow Cytometry, DNA/ RNA purification, Inc bioluminescence)
- Investigator access to IVIS Lumina III instrument for in vitro and ex vivo experiment imaging
- Provision of cryopreserved PDX-derived organoid models to BCM investigators
- Development and expansion of PDX-derived organoids for investigator-led compound or genetic screens
- User training provided for IVIS Lumina III imaging and gentleMACS octo dissociator equipment

MOUSE PDX MODELS AVAILABLE

- 82 Breast Cancer Models
- 26 Pancreatic Cancer Models
- 23 Leukemia Cancer Models
- 21 Sarcoma Cancer Models
- 10 Pediatric Liver Cancer Models
- 7 Bladder Cancer Models
- 6 Head & Neck Cancer Models
- 6 Mesothelioma Cancer Models
- 4 Glioblastoma Multiforme Cancer Models
- 2 Ovarian Cancer Model
- 1 Lung Cancer Model
- 1 Esophageal Cancer Model

CORE LEADERSHIP



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Mouse PDX: The presence of PHGDH^{low} cancer cells in primary tumors indicates poor prognosis and PHGDH expression decreases in CTCs and early metastasis.

Figure 1. a) Metastasis-free and b) disease-free survival (days) of patients with TNBC divided by homogeneous and high (hom./ high) and heterogeneous or low (het./low) PHGDH expression in primary tumors c) PHGDH protein expression in CTCs compared with the respective PDX-3107 primary tumors d) PHGDH protein expression in lymph node metastases and matched primary breast tumorsfrom patients. e) Representative pictures of PHGDH protein expression in early and late lung metastases from BCM-3107, BCM-4272. f) PHGDH protein expression in the BCM-3107 PDX



model in low or high vascularized primary tumour areas. Figure modified from Rossi M et al. Nature. 2022.

Rossi M, Altea-Manzano P, Demicco M, Doglioni G, Bornes L, Fukano M, Vandekeere A, Cuadros AM, Fernández-García J, Riera-Domingo C, Jauset C, Planque M, Alkan HF, Nittner D, Zuo D, Broadfield LA, Parik S, Pane AA, Rizzollo F, Rinaldi G, Zhang T, Teoh ST, Aurora AB, Karras P, Vermeire I, Broekaert D, Elsen JV, Knott MML, Orth MF, Demeyer S, Eelen G, Dobrolecki LE, Bassez A, Brussel TV, Sotlar K, Lewis MT, Bartsch H, Wuhrer M, Veelen PV, Carmeliet P, Cools J, Morrison SJ, Marine JC, Lambrechts D, Mazzone M, Hannon GJ, Lunt SY, Grünewald TGP, Park M, Rheenen JV, Fendt SM. PHGDH heterogeneity potentiates cancer cell dissemination and metastasis. Nature . 2022 May;605(7911):747-753. doi: 10.1038/s41586-022-04758-2. Epub 2022 May 18. PMID: 35585241.

CAM PDX: Lysosomal inhibition sensitizes TMEM16A-expressing cancer cells to chemotherapy

Figure 2. Hydroxychloroquine (HCQ) synergizes with cisplatin (CDDP) to overcome chemotherapy resistance in *in vivo* models. B) Bioluminescence was measured in the chicken egg CAM model on day five using head and neck cancer cell line HN31. Combined bioluminescence flux quantification from two experiments is shown as a graph (**left panel**) and IVIS bioluminescence imaging on the CAM (**right panel**). H & E staining of tumors grown on the CAM and treated with vehicle, HCQ, CDDP, or the combination therapy are displayed on the bottom panels with gross tumor appearance represented in the insets. Figure was adapted from Vyas A et al. PNAS. 2022.

Vyas A, Gomez-Casal R, Cruz-Rangel S, Villanueva H, Sikora AG, Rajagopalan P, Basu D, Pacheco J, Hammond GRV, Kiselyov K, Duvvuri U. Lysosomal inhibition sensitizes TMEM16A-expressing cancer cells to chemotherapy. Proc Natl Acad Sci U S A. 2022 Mar 22;119(12) Epub 2022 Mar 14. PMID: 35286200; PMCID: PMC8944912.



POPULATION SCIENCES BIOREPOSITORY (PSB)

The Population Sciences Biorepository (PSB) serves as a resource for centralized cost-effective biospecimen processing and storage for epidemiological, translational, and clinical studies. The PSB also provides risk factor and clinical data collection. Services are available for individually funded investigators as well as for clinical centers that require prospective banking of specimens from patients for future research projects. The PSB team will consult with you to plan for data collection and specimen processing and storage needs for your projects. In addition, the PSB has a banked collection of annotated samples from a variety of cancer types that are available for individual investigator use. Contact the PSB to learn how to gain access to these important samples. Learn more about the PSB by viewing our video on the ATC core website (https://www.bcm.edu/research/atc-core-labs/population-sciences-biorepository)

MAJOR EQUIPMENT

- Hamilton Verso Automated -20°C freezer system
- CryoBioSystem MAPI high-security straw system
- QIAcube robotic workstation
- Chemagic Prepito-D extraction system
- Chemagic 360 exatraction system
- Perkin Elmer Janus automated workstation
- Nano-drop 1000
- MVE 1536P LN2 vapor freezers
- VWR -80°C mechanical freezers
- Thermo Scientific VisionMate scanner
- Thermo Scientific 8-channel decapper
- Barcode printers and scanner system

SERVICES

- Patient consenting, phlebotomy, sample and data collection
- Questionnaire development and administration
- Full fractionation and aliquoting for blood, bone marrow and urine samples
- Mononuclear cell isolation and cryopreservation
- DNA extraction from whole blood, buffy coat, plasma, or saliva
- RNA extraction from whole blood or buffy coat
- DNA Quantitation (absorbance and pico-green fluorescence)
- Whole Genome Amplification
- Long-term specimen archival



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Biomarkers for the Early Detection and Risk Assessment of Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the second most common cause of cancer mortality worldwide. HCC incidence has increased over the past years in Western Europe and the United States. HCC is often diagnosed at a late stage and 5-year survival rate for HCC patients in the United States is around 15. To reduce HCC-related mortality, early detection of small tumors that are amenable to curative treatments is urgently needed. The PSB-supported study identified a panel of 4 markers with strong performances that could have significant utility in HCC early detection for patients with cirrhosis under surveillance.



Figure Correlations between selected FA levels and the time between baseline sample collection and HCC diagnosis in 40 patients with cirrhosis and HCC diagnosis during surveillance. **(A)** correlation between 18:3n3 (top), 18:2n6 (bottom) and days to HCC diagnosis; **(B)** correlation between 22:5n3 (top), 22:4n6 (bottom) and days to HCC diagnosis; **(C)** correlation between the ratio 22:5n3/18:3n3 (top), the ratio 22:4n6/18:2n6 (bottom) and days to HCC diagnosis. r, Pearson's correlation coefficient

Khan IM, Gjuka D, Jiao J, Song X, Wang Y, Wang J, Wei P, El-Serag HB, Marrero JA, Beretta L. A Novel Biomarker Panel for the Early Detection and Risk Assessment of Hepatocellular Carcinoma in Patients with Cirrhosis. Cancer Prev Res (Phila) 2021;14:667-74.

PROTEIN AND MONOCLONAL ANTIBODY PRODUCTION (RE-ORGANIZED 2022)

The Protein and Monoclonal Antibody Production Core (PMAPC) provides investigators with high quality mouse monoclonal antibodies (MAbs) and purified recombinant proteins to facilitate their research programs. The Core has more than 30-years experience in generating MAbs that perform for a wide range of applications including, but not limited to immunoblotting, immunoprecipitation, ELISA, immunocytochemistry, and immunohistochemistry. Expression and purification of recombinant proteins

of interest is a parallel service that involves consultation on design and construction of expression vectors, protein overexpression in the baculovirus insect cell system, E. coli or mammalian cells, and purification of the expressed protein. Characterization of protein products for purity and other quality controls is provided to assure suitability for biochemical and structure analysis studies. The instrumentation and expertise for biophysical and molecular characterization of proteins has been added as a major new service this year.



- HAMILTON ClonaCell EasyPick for robotic hybridoma cell cloning
- GE Healthcare ÄKTA FPLC systems
- Bioreactors for large scale insect and mammalian cell cultures
- Microfluidizer LM20 High Shear Fluid Processor
- Thermo MaxQ HP incubator and refrigerated console shaker for multi-liter scale bacteria cultures
- Roche Thermal cycler
- Beckman Analytical Ultracentrifugation.
- Malvern Auto-Isothermal Titration Calorimetry (ITC).
- Forte Bío Octet Bio-Layer Interferometry

SERVICES

- Generation of mouse monoclonal antibodies (MAbs) using standard hybridoma technology.
- Production & purification of monoclonal antibodies from existing hybridomas (up to gram-scale)
- Generation of recombinant baculovirus expression vectors for protein production in insect cells
- Overexpression of recombinant proteins in any of three systems below:
 - Insect cells (using baculovirus vectors)
 - E. coli
 - Mammalian cells (HEK293)
- Purification of recombinant proteins of interest from overexpression systems.
- Analytical Ultracentrifugation for determination of protein molecular size, shape and oligomerization states
- Auto-Isothermal Titration Calorimetry (ITC) for determination of protein binding affinities and high throughput binding measurements
- Forte Bío Octet Bio-Layer Interferometry (BLI) for determination of binding kinetics and affinities
- Differential Scanning Fluorimetry (DSF) by high throughput PCR for protein stability buffer screens.
- Consultation and project design



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Location and expression kinetics of Tc24 in different life stages of Trypanosoma cruzi (Jeroen Pollet)

Tc24-C4, a modified recombinant flagellar calcium-binding protein of Trypanosoma cruzi, is under development as a therapeutic subunit vaccine candidate to prevent or delay progression of chronic Chagasic cardiomyopathy. When combined with Toll-like receptor agonists, Tc24-C4 immunization reduces parasitemia, parasites in cardiac tissue, and cardiac fibrosis and inflammation in animal models. To support further research on the vaccine candidate and its mechanism of action, the core assisted with generation of murine monoclonal antibodies (mAbs) against Tc24-C4. Here, we report new findings made with mAb Tc24-C4/884 that detects Tc24-WT and Tc24-C4, as well as native Tc24 in T. cruzi on ELISA, western blots, and different imaging techniques. Surprisingly, detection of Tc24 by Tc24-C/884 in fixed T. cruzi trypomastigotes required permeabilization of the parasite, revealing that Tc24 is not exposed on the surface of T. cruzi, making a direct role of antibodies in the induced protection after Tc24-C4 immunization less likely. It was further observed by immunostaining T. cruzi-infected cells with mAb Tc24-C4/884, that expression of Tc24 decreases significantly when T. cruzi trypomastigotes enter host cells and transform into amastigotes. However, Tc24 is then upregulated in association with parasite flagellar growth linked to re-transformation into the trypomastigote form, prior to host



cellular escape. These observations have provided insights into potential mechanisms of vaccine immunity.

Fig 1. A) The binding of mAb Tc24-C4/884 to Tc24-C4, Tc24-WT and native Tc24 by ELISA. **B)** Detection of reduced and non-reduced Tc24-C4, Tc24-WT and native Tc24 by Tc24-C4/884 by western blot. **C)** Fluorescent confocal microscopy images of mouse primary cardio fibroblasts infected with T. cruzi show the return of Tc24 expression.

Versteeg L, Adhikari R, Poveda C, Villar-Mondragon MJ, Jones KM, Hotez PJ, Bottazzi ME, Tijhaar E, Pollet J. Location and expression kinetics of Tc24 in different life stages of Trypanosoma cruzi. PLoS Negl Trop Dis. 2021 Sep 3;15(9):e0009689

Broadly cross-reactive human antibodies that inhibit genogroup I and II noroviruses (Venkataram Prasad)

The rational development of norovirus vaccine candidates requires a deep understanding of the antigenic diversity and mechanisms of neutralization of the virus. A panel of broadly cross-reactive naturally occurring human monoclonal IgMs,IgAs and IgGs reactive with human norovirus (HuNoV) genogroup I or II (GI or GII) was isolated and characterized in the study. Three binding patterns and identify monoclonal antibodies (mAbs) that neutralize at least one GI or GII HuNoV strain when using a histo-blood group antigen (HBGA) blocking assay. The HBGA blocking assay and a virus neutralization assay using human intestinal enteroids reveal that the GII-specific mAb NORO-320, mediates HBGA blocking and neutralization of multiple GII genotypes. The Fab form of NORO-320 neutralizes GII.4 infection more potently than the mAb, however, does not block HBGA binding. The crystal structure of NORO-

320 Fab incomplex with GII.4 P-domain shows that the antibody recognizes a highly conserved region in the P-domain distant from the HBGA binding site. Dynamic light scattering analysis of GII.4virus-like particles with mAb NORO-320 shows severe aggregation, suggesting neutralization is by steric hindrance caused by multivalent cross-linking. Aggregation was not observed with the Fab form of NORO-320, suggesting that this clone also has additional inhibitory features. Our core facility produced VLPs used in the study. Sf9 insect cells were co-transfected with a transfer vector corresponding to a specific strain and with a bacmid vector. Recombinant baculovirus was isolated and expanded. VLPs were purified from cell culture supernatants using a sucrose and cesium chloride gradient abd verified using electron microscopy.

Fig 2. The hydrodynamic diameters of treated or untreated GII.4 HuNoV VLPs were measured using dynamic light scattering (DLS) on a ZetaSizer Nano instrument. **A)** Complete dynamic light scattering profile of the four tested conditions: GII.4 HOV VLP alone or in complex with 22D2 IgG, NORO-320 IgA, or Fab at a molar ratio 1:1 or 1:10. **B)** Average diameters were calculated for each sample condition using Zetasizer software. Samples were diluted to a final concentration of 330 nM for each component in phosphate-buffered saline pH 6, and a 3,300 nM concentration of NORO-320 Fab was prepared for the condition labeled VLP:NORO-320 Fab (1:10). Each data bar represents the mean ± SD of n=3 independent experiments.

Alvarado G, Salmen W, Ettayebi K, Hu L, Sankaran B, Estes MK, Prasad BV. Broadly crossreactive human antibodies that inhibit genogroup I and II noroviruses. Nat Commun 12, 4320 (2021).



RNA IN SITU HYBRIDIZATION

The Core performs non-radioactive RNA in situ hybridization (ISH) on tissue sections. A unique high-throughput technology developed by the Core is used to determine gene expression patterns on sections, with an emphasis on tissues from rodent experimental models. The Core provides a full service that includes collection of rodent tissue specimens, preparation of frozen sections, preparation of RNA probes from customer templates, conducting high-throughput ISH and documentation and quantification of expression patterns by microscopy.

MAJOR EQUIPMENT

- Tecan EVO Genepaint robot (for automated RNA *in situ* hybridization)
- Three cryostats (Leica)
- Autostainer (Leica)
- Automated coverslipper
- Zeiss Axio Scan.Z1 slide scanner (brightfield and fluorescence)

SERVICES

- RNA ISH on tissue sections-brightfield or fluorescence development
- ISH for microRNA
- Tissue processing and embedding (frozen tissue)
- Sectioning (frozen tissue)
- Preparation of non-radioactive RNA *in situ* probes (DIG- or FITC-labeled)
- Imaging (slide scanner-automated tiled images)
- Automated quantification of *in situ* hybridization signals, brightfield only (gene expression levels and cell counts)





CORE LEADERSHIP



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Morphology and gene expression analysis of choroid plexus (CP) tumors driven by aberrant NOTCH and SHH signaling

A) H&E staining is shown of coronal sections of upper roof plate/CP (arrows) in the hindbrain at day E14.5 in wild type and *Lcre;Ptch^{cko}* animals, choroid plexus papilloma (CPP) and abnormal CP growth (arrowheads) in *Lcre;NICD1* and *Lcre;Ptchcko;NICD1* mice, respectively. Red lines mark the roof plate magnified in inset images. Boxed region of roof plate/CP in a *Lcre;Ptchcko;NICD1* animal is shown in higher magnification on the right. Scale bars,100 μm. Images represent at least three independent experiments.



Representative results of *in situ* hybridization of Mycn. Gli1 and Shh mRNAs **B**) and immunohistochemical staining of cytokeratins and TTR **C**) are shown in upper roof plate (**marked by dotted lines**) and CP (**arrows**) at day E14.5 in the hindbrain in wild type and *Lcre;Ptch^{cko}* animals, CPP and abnormal CP growth (**black arrowheads**) in *Lcre;NICD1* and *Lcre;Ptch^{cko};NICD1* animals, respectively. Cytokeratins-expressing and TTR+ epithelial cells (**C, red arrowheads**) are mixed in abnormal CP growth in *Lcre;NICD1* and *Lcre;Ptch^{cko};NICD1* animals, respectively. Scale bars, 50 µm. Data represent at least three independent experiments.



Li, Q., Han, Ż., Singh, N. et al. Disruption of GMNC-MCIDAS multiciliogenesis program is critical in choroid plexus carcinoma development. Cell Death Differ (2022). https://doi.org/10.1038/ s41418-022-00950-z

SINGLE CELL GENOMICS CORE

Single Cell Genomics Core (SCGC) provides services to conduct high throughput genome profiling, including DNA, RNA, and Epigenetics on a single cell. We provide cost-effective and time-efficient access to cutting-edge genomic technologies and expert assistance with experimental design and sample preparation for 10X genomics single cell library preparations. We work closely with the Genomic and RNA Profiling (GARP) core to enable our users to have access to Illumina next generation sequencing services.

MAJOR EQUIPMENT

- 10× Genomics Chromium: Droplet based system capable of profiling the transcriptome of up to 10,000 cells.
- 10× Genomics Chromium X : Advanced hardware for all single cell assays including high-throughput.
- 10× Genomics Connect : High performance liquid handler that makes single cell library from cell/nuclei.
- EVOS microscope: High resolution microscope for broad range of imaging application.
- Pala Cell sorter: Benchtop sorter for single cell isolation.

SERVICES

- **Single cell capture and 3' RNAseq**: Provide service for single cell capture and 3' RNAseq using chromium from 10× Genomics and ICEII8 from Takara.
- Single cell capture and V(D)J/5' RNAseq: Provide service for single cell capture, VDJ profiling and 5' RNAseq using 10× Genomics.
- **Single cell capture and ATAC-seq**: Provide service for single nuclei capture and ATACseq using 10× Genomics.
- **Single cell Multiome-seq:** Provide service for performing single cell RNA-seq and ATAC-seq on the same cell using 10× Genomics.



CORE LEADERSHIP



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Figure 1. Single-Cell RNA-sequencing of Fetal Cerebrospinal Fluid Reveals Functional Immune Programming In Utero. A) (Aagaard Lab) A) Fetal....Fetal CSF and amniotic fluid were collected during neural tube defect repair surgeries 22-26 weeks gestation and subjected to 10× Genomics 3'v31 scRNA-seq. **B)** Unique Manifold Approximation and Projection (UMAP) of 22,058 CELLS (n=10,395CSF and n=11,663 AF) annotated based on differential expression of known marker transcripts and site exclusivity. **C)** T cells (n=1,210) were subset and annotated based on canonical T cell markers and pseutotime trajectory analysis revealed distinct branchpoints in differentiation and activated T cells. **D)** T cells were further characterized with paired CSF and AF samples (n=3 patients) subject to 5' scRNA-seq+ hTCR VDJ sequencing resulting in 10,036 cells. hTCR clonotype expansions were analyzed by scRepertoire. Expansion of clonotypes shared between AF and CSF in a paired samples are shown, suggesting these T cells traversed between AF and CSF in a paired samples are shown, suggesting these T cells traversed between these sites.

Barrazo ER, Seferovic M.D., Z eitsman SM, Do T, Tillery E, Shamshirsaz aa, Belfort MA, Aagaard KM. Profiling in utero fetal cerebrospinal fluid (CSF) cell populations by single cell RNA-sequencing (scRNA-seq) AJOG. 2022 Jan 1. DOI:10.1016/j.ajog.2021.11.116.



Figure 2. Single cell RNA-Seq to identify human periosteal stem/progenitor cell population scRNAseq was performed on 10k single cells from human periosteum tissue. Rare skeletal stem/progenitor cell population with osteochondrogenic differentiation potential was identified. (unpublished) Youngjae Jeong, Lorenzo Deveza, Laura Ortinau, Dongsu Park.

ZEBRAFISH (NEW 2022)

The Zebrafish Core provides expertise and equipment to support your zebrafish experiments. This includes projects to knockdown or knockout gene expression in zebrafish, generation of transgenic zebrafish to monitor expression of a gene of interest or to mark a cell population of interest and watch cell proliferation and differentiation in live embryos. We can also help to analyze phenotypes of mutant zebrafish. We can assist in preparing zebrafish for experiments requiring live imaging, histology, next generation sequencing or mass spectrometry. We can also help design and execute a chemical screen to identify small molecules that cause or rescue a phenotype of interest in zebrafish embryos. For those who want a more hands-on experience, we provide basic training in zebrafish handling, breeding, embryo care, genotyping, anesthesia, euthanasia, and other techniques pertaining to zebrafish. If you are interested in using zebrafish as a model to answer your research question, but do not want to get your hands wet, then contact us.

MAJOR EQUIPMENT

- Harvard Instruments pressure injection system, Narishige micromanipulators, Flaming/Brown micropipette puller
- Leica brightfield and fluorescent dissecting microscopes
- DanioVision chamber for behavior analysis
- Loligo swim tunnel & respirometer for metabolic/ cardiac phenotyping
- BioRad CFX Opus Touch real-time PCR instrument for genotyping
- Liquid nitrogen freezer for storing cryopreserved sperm

SERVICES

- Transgenics
- Generation of transgenic zebrafish
- Screening transgenic zebrafish
- CRISPR Genome Editing
- Guide RNA testing in zebrafish embryos
- Generation of mutant zebrafish
- Genotyping using high-resolution melting curve analysis, Sanger sequencing
- Cryopreservation and Embryology
- Zebrafish sperm cryopreservation
- Zebrafish in vitro fertilization
- Strain rederivation
- Colony expansion
- Strain import and maintenance



CORE LEADERSHIP



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Using Zebrafish in Your Research

Zebrafish are a powerful model system to study human development and disease. Zebrafish have several advantages: ability to rapidly generate targeted mutations; external fertilization and development of embryos provide unparallel access to cells and tissues for live imaging; high fertility of adults provides a costeffective method to generate and test large numbers of embryos in experiments; cost-effective to perform unbiased genetic and chemical screens for a wide range of phenotypes including abnormal behavior, gene expression and anatomic development. In addition, zebrafish can be housed in high densities, maximizing animal housing space.



Zebrafish embryos



State-of-the-art zebrafish facility in Center for Comparative Medicine



CORE DIRECTORY

Antibody-Based Proteomics

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BioEngineering Core

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Biostatistics and Informatics

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Core for Advanced MR Imaging (CAMRI)

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Cytometry and Cell Sorting

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Genetically Engineered Rodent Models (GERM)

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Genomic and RNA Profiling (GARP)

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Human Tissue Acquisition and Pathology (HTAP)

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Integrated Microscopy

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Macromolecular X-Ray Crystallography Core

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Mass Spectrometry Proteomics

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Metabolomics

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MHC Tetramer

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Mouse Metabolic & Phenotyping Core

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