Core Research Services at the NCI FFRDC

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Animal Sciences and Modeling

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Animal Program Overview

The animal program supports multiple research and development efforts with flexible administrative & managerial support

- **Animal research support provided to:**
  - NCI Center for Cancer Research (CCR), Division of Cancer Treatment and Diagnosis (DCTD), Division of Cancer Biology (DCB), Other Institutes (NHGRI, NIAMS, NIDCD, NIDDK, NIMH, NIAID, NIA). Other federal agencies through interagency agreements and the National Lab's *AIDS and Cancer Virus Program* (ACVP)
- **Animal Care and Facility Management Support including:**
  - Management of 27 rodent and 1 non-human primate vivaria
  - Maintenance of ~350,000 animals occupying 60,000+ cages
  - Operates *Receiving and Quarantine*, coordinates animal shipments
  - Supports ~235 investigators with ~600 active animal study protocols
  - Provides support for the Frederick ACUC and Bethesda ACUC
  - Provides board-certified veterinary care for all research animals
  - Full AAALACi* accreditation at both campuses

*Association for Assessment and Accreditation of Laboratory Animal Care International

The animal program provides method development and technical expertise and refinement

- Rodent imaging facility equipped with multiple translational technologies
- Molecular histopathology provides method development and analytical studies
- Animal diagnostics and genotyping laboratories provide real-time animal health monitoring
- Gnotobiotics facility supports germ-free and defined flora studies
- Genome modification core
- Mouse modeling and cryopreservation core
- Support for NCI Mouse and miR ES Cell Repository
- Animal Research technology core provides technical expertise to NCI investigators
- Support to the Patient-Derived xenograft Models Repository

Small Animal Imaging Program

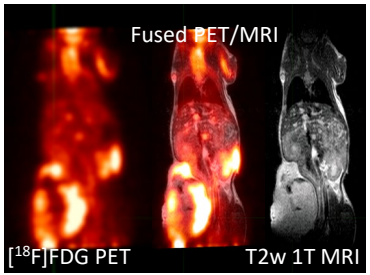
Collaborates with NCI investigators in:

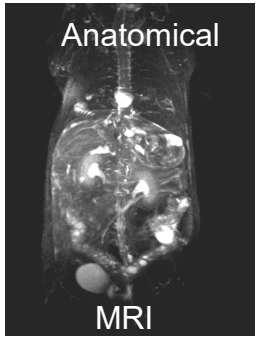
- **The monitoring of mouse models of cancer utilizing non-invasive, *in vivo* imaging techniques.**
- **The development of new molecular imaging probes.**

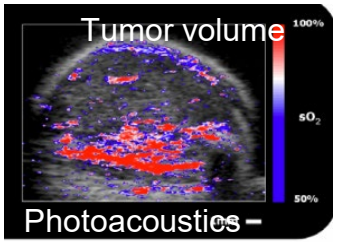
Capabilities/Assays:

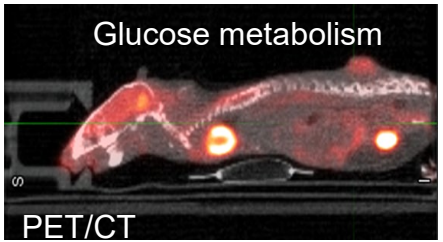
- **Ultrasound, MRI, PET/CT & SPECT/CT, BIOLUMINESCENCE**
  - **Perfusion (Ultrasound + dynamic susceptibility contrast MRI)**
  - **Anatomical volumes (Ultrasound and MRI)**
  - **Angiogenesis (Ultrasound using tagged microbubbles)**
  - **Glucose metabolism [^{18}F]FDG (PET/CT)**
  - **Permeability (Dynamic Contrast Enhanced DCE-MRI)**
  - **Hypoxia (Oxygen saturation) (Photo-acoustics)**
  - **Cell Proliferation [^{18}F]FLT (PET/CT)**
  - **Cell Trafficking (Fluorescence, Bioluminescence)**
  - **Metastasis (Bioluminescence and MRI)**
  - **Cardiac Function and Blood Flow (Ultrasound and Doppler)**
  - **Probe bio-distribution (Gamma-well counter)**

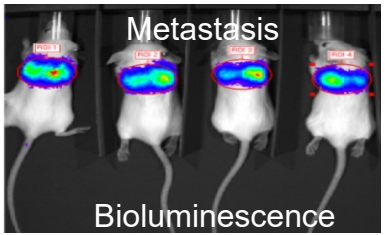
MRI/PET Fusion: enhancement for metabolic metastatic analysis





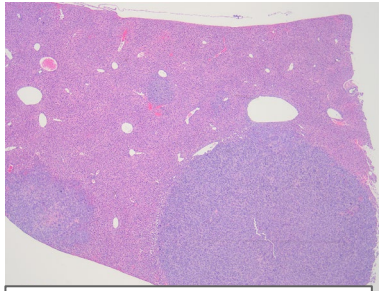






Molecular Histopathology Laboratory Services

- Rodent necropsy
- Rodent hematology and clinical chemistry
- Fixed and frozen tissue embedding
- Microtomy
- Histochemical and immunohistochemical stains
- Microarray (TMA) construction
- Laser micro-dissection (LCM)
- Pathology interpretation and reporting
- RNAscope® in situ hybridization (ISH)
- Target enrichment from frozen/fixed tissues, paraffin/OCT tissue blocks, or slides
- Digital imaging including:
  - Gross photography
  - Bright-field or fluorescent digital whole-slide imaging (WSI)
  - Leica Aperio eSlide Manager
  - Indica Labs HALO Link™



Liver H&E,
Multifocal metastatic lesions

PTAH: Special stains are utilized to evaluate suspected thrombi for the presence of fibrin. Thrombi observed in the lung (M888), Liver (M806), and head (M809) are evaluated and all are positive for fibrin

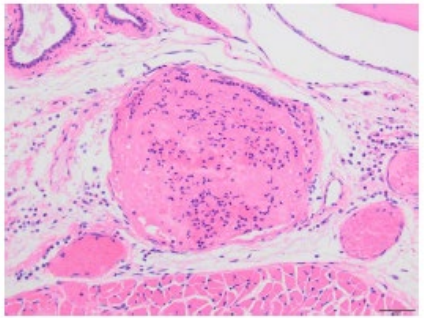


Fig 1. H&E of a thrombosed vessel from animal M809

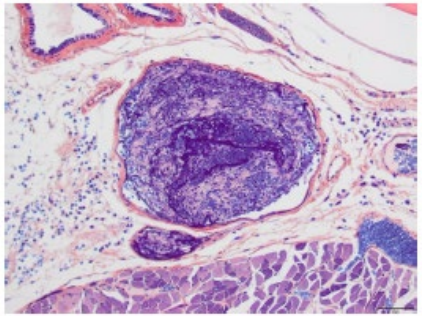


Fig 2. PTAH stain showing fibrin deposits (blue staining material) in a vessel from an animal with widespread intravascular thrombosis.

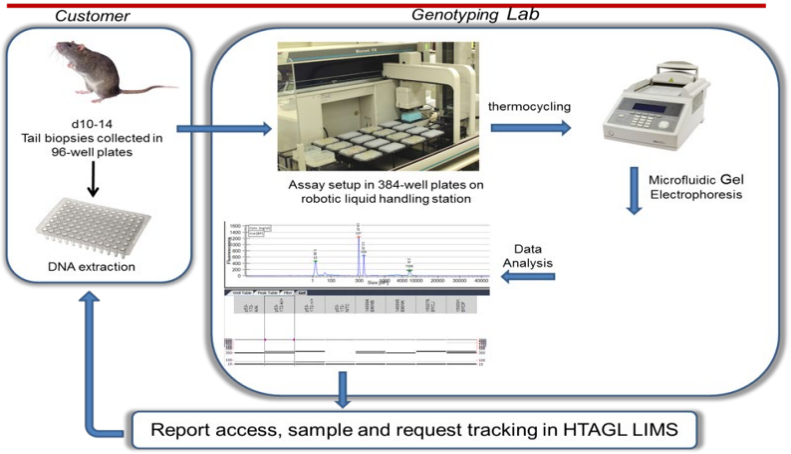
Animal diagnostics & genotyping laboratories provide real-time animal health monitoring and genotyping services

1. **Animal Health Diagnostics**

   Viral serology, bacteriology, and parasitology diagnostic services for rodents and non-human primates.

2. **Molecular-based Diagnostics and High-throughput Genotyping**

   Assists in health monitoring and provides many other advanced molecular services, including the monitoring of pathogens in cultured cells and tumor fragments, DNA fingerprinting technologies to evaluate genetic backgrounds, and high-throughput genotyping strategies and services.



The Gnotobiotic Facility (GF)

Technology Development Effort

The Gnotobiotic Facility was created and expanded to meet the growing demand for germ-free and defined flora mouse studies

- Axenic rederivation of multiple GEM strains
- Defined the safety requirements for performing ABSL-2 experimental procedures in bio-isolators, and implementation of the bio-containment racks
- Facilitated the use of micro-isolator racks and cages to undertake microbial and fecal transplants into germ-free mice
- GF services now include association studies of human (transplanted) microbiota with inflammation and cancer treatment responses

Four bio-containment racks (48 cages each)

The **Gnotobiotic Facility** works with NCI investigators to:

- Development and monitor germ-free mouse colonies
- Explore the role of the microbiota in inflammation and cancer
- Examine effects of microbiota on mouse models of human cancer

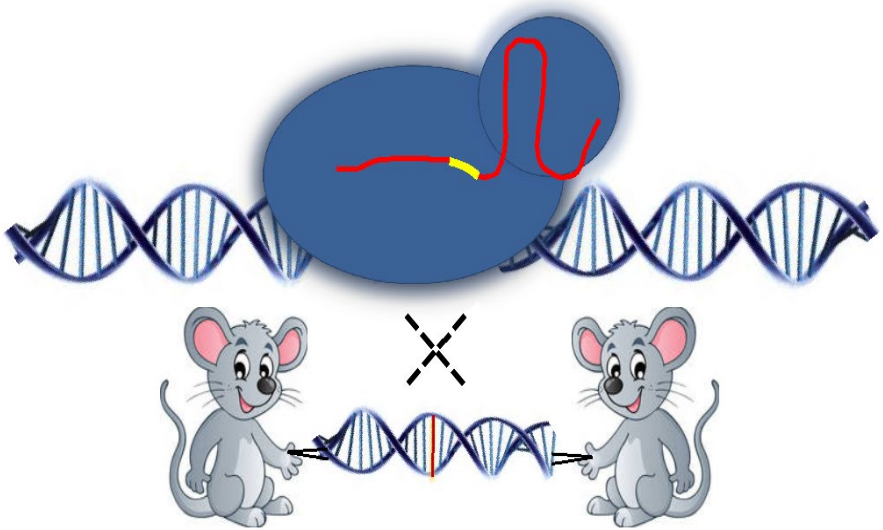
40 experimental isolators (3-5 cages)

10 breeder isolators (18 cages)

Large breeder isolator (50 cage)

- HEPA-filtered air in/out at the rack level.
- HEPA-filtered air in/out at the cage level.
- Suitable for association studies with bacteria and human fecal samples.
- ABSL2 compliant (negative pressure)

Genome Modification Core



(1)Expert guidance on editing procedures to create precise genetic and epigenetic modifications.

(2) The generation and validation of nuclease (Cas-9 and Cpf-1) reagents.

(3) Various pooled guide libraries useful in performing genetic screens.

The GMC offers technical advice and expertise for gene-editing in primary cells and cell lines and interacts closely with the LASP Mouse Modeling Core to facilitate the generation of CRISPR-mediated mouse models.

Mouse Modeling and cryopreservation support

Mouse Model Development

- Generation of transgenic and CRISPR-modified mice (pronuclear injection)
- Gene targeting in mouse ES cells and subsequent generation of mice (blastocyst or morulae injections)
- 50-100 models generated annually for NCI, NIAID, and other Institutes

Cryopreservation & Regeneration

- Cryopreservation of mouse strains and assisted reproduction (IVF)
- Regeneration of frozen mouse sperm or embryos to live mice

The NCI Mouse Repository

- Supported by **Division of Cancer Biology** (extramural research community)
- Cryopreservation and distribution of frozen sperm or embryos for >150 popular mouse models for cancer research
- Expansion and distribution of 1500+ validated ES cell clones with conditional-expression of miRNA constructs

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Assay Development and Execution

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Background

Assay Support

- Basic science
- Pre-clinical research
- Clinical trial support (non-CLIA and CLIA)

Assay Function

- Drug targets and mechanisms of action
- Identifying novel therapeutic agents
- Drug toxicity, metabolism and transport
- Detecting/diagnosing cancer/viral infections
- Monitoring disease progression
- Molecular signatures for response prediction
- Assignment to Personalized Medicine trials
- Biomarker assessment of Tx response
- Evaluating immune response to treatment

Assay Programs

- Mechanism of Action
- Pharmacodynamics
- Pharmacokinetics
- Toxicology
- Nanotechnology*
- Omics*
- High-throughput Screening*
- cGMP Product Stability*
- National Mission Programs* (Ras/ SeroNet, etc.)

Assay Biospecimens

- Tissue biopsy
- Saliva, urine, stool
- Buccal mucosa, nasal swabs
- Whole blood, plasma, PBMCs
- Circulating tumor cells (CTCs)
- Circulating nucleic acids
- Exosomes
- Antibodies

Assay Formats

PD Assays

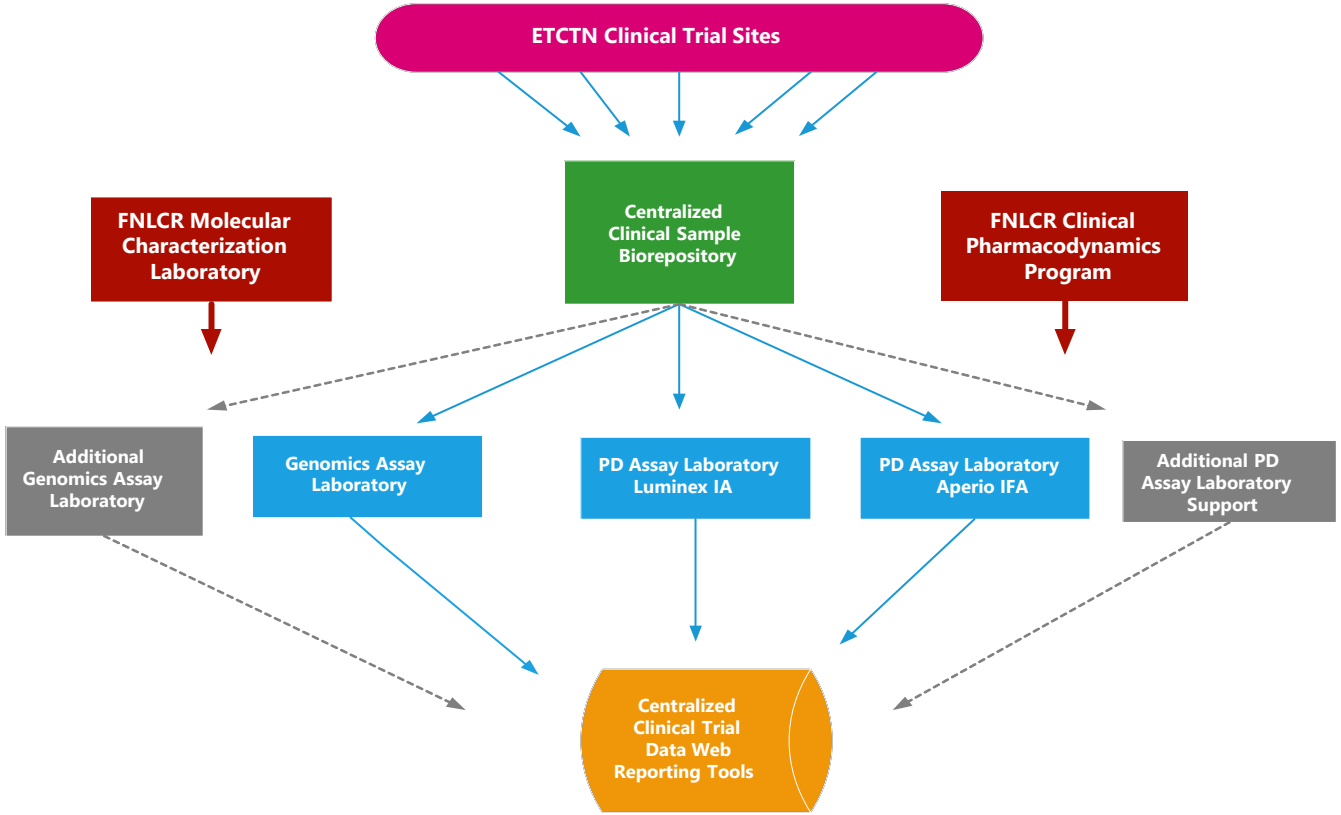
- Western Blot
- ICC/IHC/IFA
- Luminex
- meS-PCR
- Elisa
- Flow Cytometry

Omics Assays

- WGS / WES
- RNAseq
- NGS
- microRNA
- Targeted panels
- Taqman / ddPCR
- Ion Torrent
- Illumina
- Nanostring
- Sanger
- Single cell
- LC/MS

Example Support

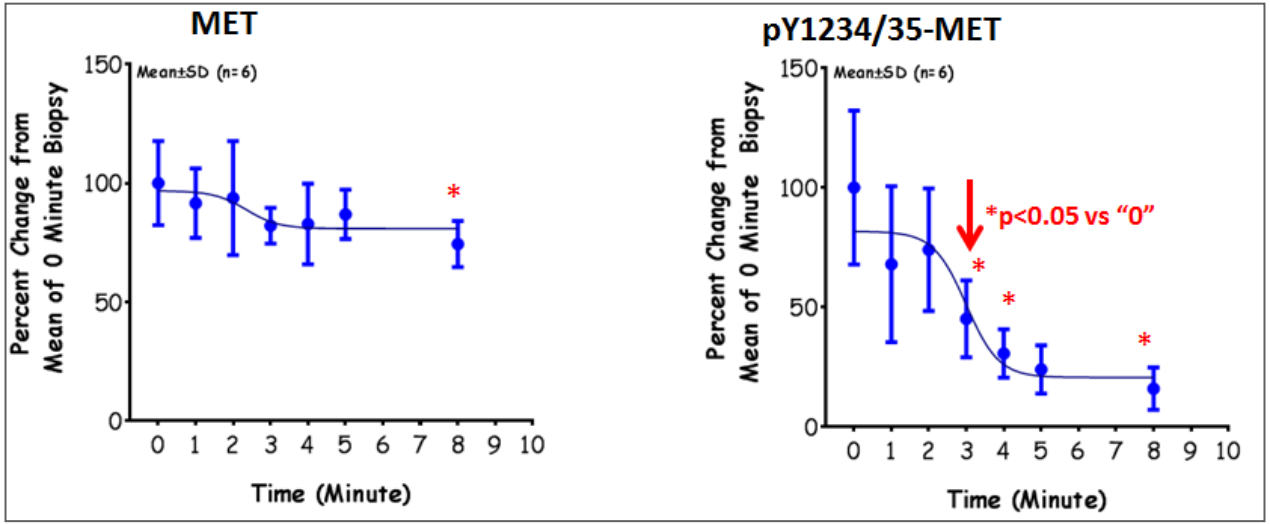
National Clinical Laboratory Network for Precision Medicine



Core genomic technologies supported by the NCLN

- Oncomine pan-cancer targeted gene panel (OCAv3)
- Whole Exome Sequencing (WES) & RNA Sequencing (RNA-Seq)
- TSO500 targeted gene panel for ctDNA

Rapid flash freezing to stabilize labile biomarkers (DCTD SOP340507)



- pMET signal is lost rapidly during ischemia
- Many proteins show significant alterations within 5 minutes
- Biopsies must be stabilized <2 minutes to measure drug-induced MET suppression and other critical drug response biomarkers

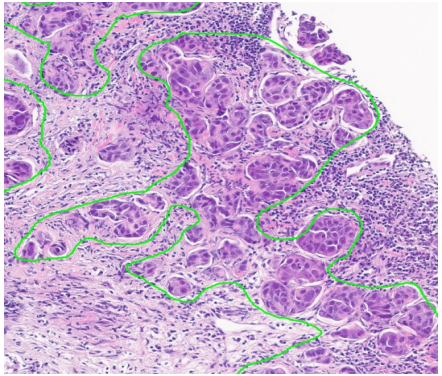
Srivastava AK, et. al., Clin Can Res, 2016

PD Assays

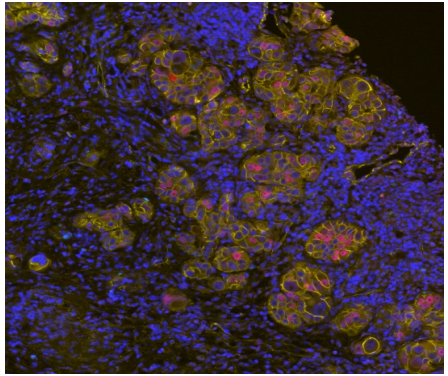
- Cell death
- Cancer Stem Cell (CSC) Factor
- DNA Damage Repair/Cell Cycle
- Epithelial Mesenchymal Transition (EMT)
- ER stress/oxidative damage assays
- Immuno-Oncology Multiplex IFA Panels
- Immuno-Oncology Phosflow Cytometry Panel
- Receptor and Other Kinase Biomarkers
- Signaling Pathways
- Tumor Segmentation Markers

Tumor segmentation is critical for accurate analysis of heterogeneous carcinoma biopsies

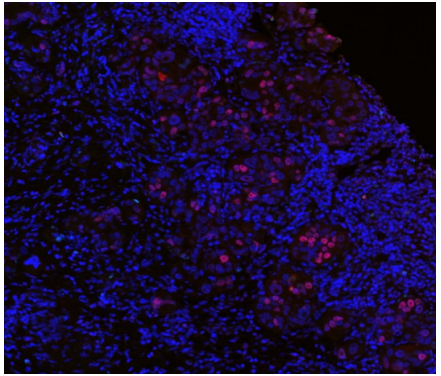
H&E annotation

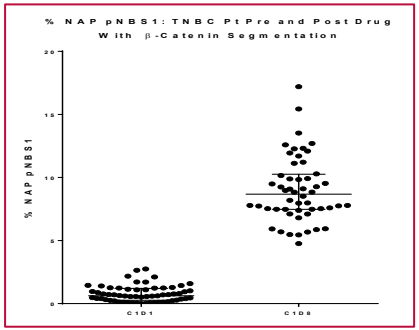


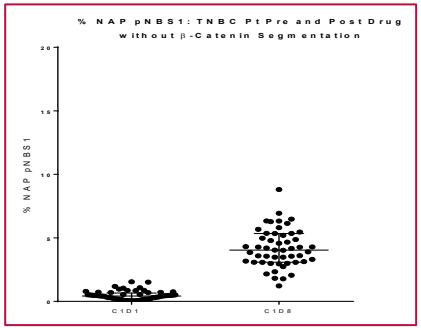
pNBS1, DAPI with β-Catenin

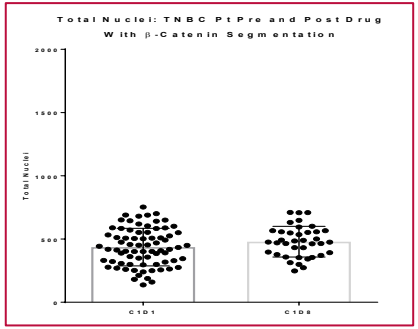


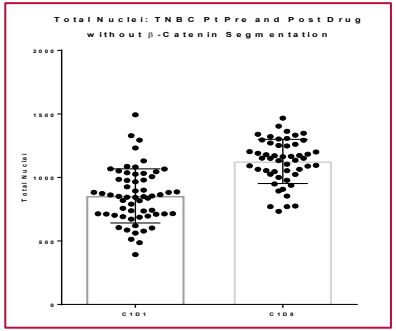
pNBS1, DAPI without β-Catenin



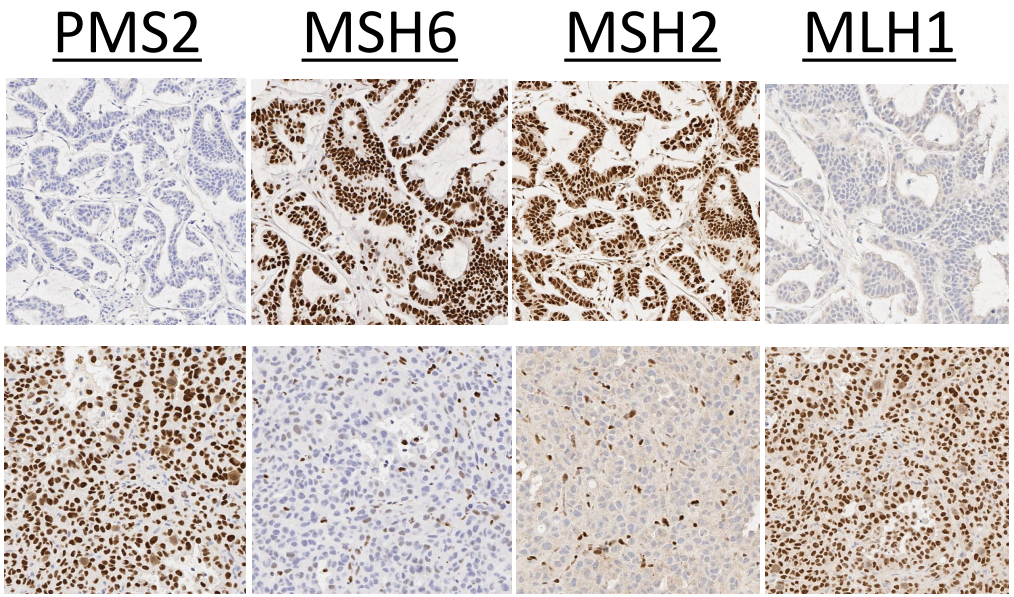








MMR expression assay with MSI-H Human PDX models

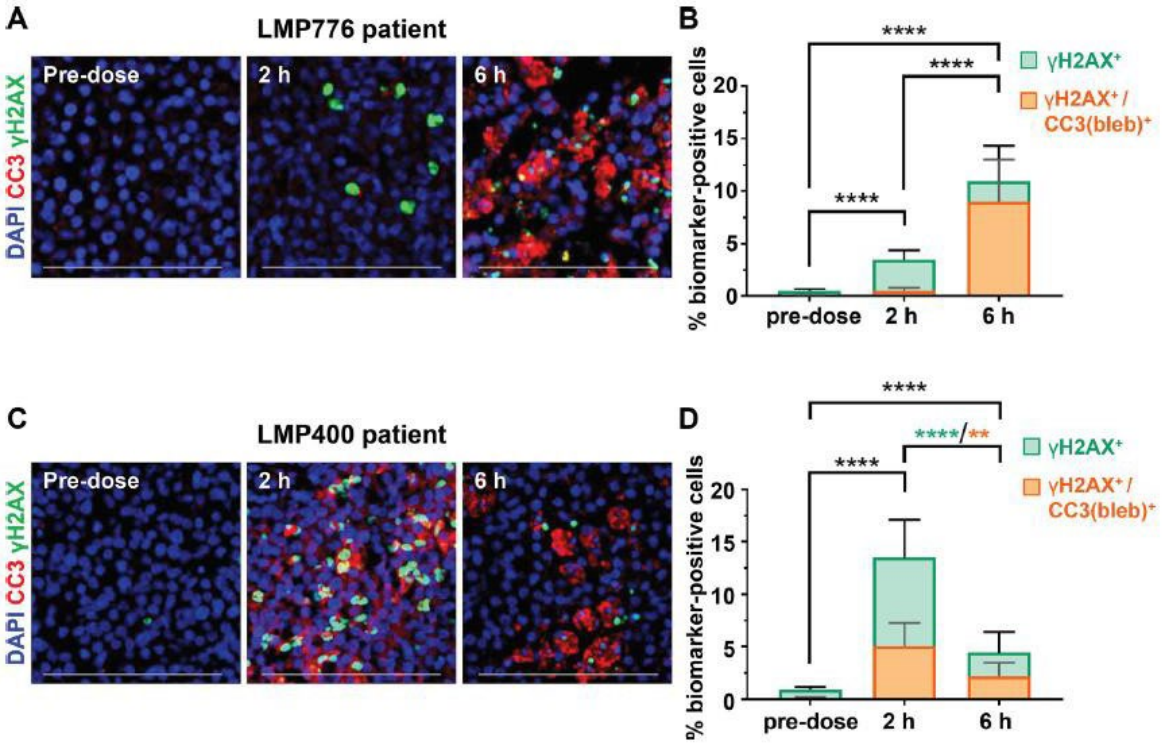


Colon Adenocarcinoma

Pancreatic Adenocarcinoma

Loss of MLH1 usually accompanied by loss of PMS2
Loss of MSH2 usually accompanied by loss of MSH6

Apoptosis in canine lymphoma patients corresponds with >60% tumor volume reduction



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Repository and Bioprocessing Activities at the FFRDC

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Regulations, Guidelines and Best Practices

- FFRDC contractor support Repository and Bioprocessing activities according to:
  - Department of Health and Human Services (DHHS), and HIPAA regulations (45 CFR 46.102 and 45 CFR part 160 and subparts A and E of part 164 – interactions with covered entities)
  - Good Laboratory Practices (GLP) guidelines, Good Manufacturing Practices (cGMP) guidelines
  - US Food and Drug Administration (FDA) requirements for Good Clinical Practice and Clinical Trials (21 CFR)
  - International Society for Biological and Environmental Repositories (ISBER) *Best Practices: Recommendations for Repositories 4th Edition* & ADDENDUM 1: *Liquid Nitrogen-Based Cryogenic Storage of Specimens*
  - NCI Best Practices for Biospecimen Resources *(2016)*
  - 61st/62nd editions of the IATA Dangerous Goods Regulations *(includes the provisions on competency-based training and assessment (CBTA) as agreed by the ICAO Dangerous Goods Panel in DGP/27 for shipping biological materials)*

Quality Control

- The Contractor shall maintain a Quality Control/Quality Assurance (QC/QA) program to monitor performance, including the development of standard operating procedures (SOPs) for each protocol or process, optimized for maintaining the integrity of human biological material; for monitoring the quality of products; for the operation of the laboratories; and for tracking specimen collection, input, processing, and dissemination.

FFRDC Repositories

FFRDC Repositories

- The Repositories are currently housed in 4 buildings; All three of these buildings are part of the NCI at Frederick. Three of these buildings are located on the NCI Campus at Frederick and one building is located off-site in leased space in the Frederick area.
- The Repositories supports the NCI intramural and some extramural projects, as well as NIAID intramural and specific small collections from other Institutes of the NIH.
- Approximately 95% of all material in the repositories is related to clinical or epidemiological studies
- Approximately 5% is support for ongoing basic research at the NCI at Frederick and storage of lab materials.

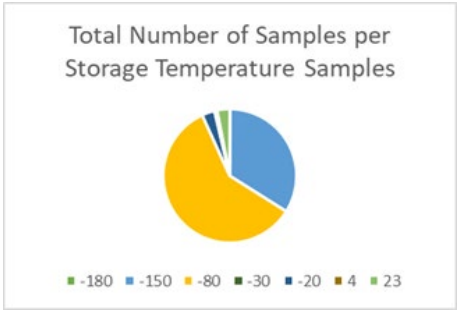
Repository Space and Existing Infrastructure

- The FFRDC Repositories occupy:
  - Area on NCI-Frederick campus is 25,000 RSF
  - Area 89,600 RSF at off-campus sites
  - Total FFRDC Repositories space – 114,600 RSF
- Existing infrastructure:
  - Power Redundancy
    - Backup generators and additional connection for temporary generator(s)
- The off-campus warehouse space is conditioned and secured with state-of-the-art monitoring for safety and security
- LN2 capacity
    - Regular delivery of LN2
    - Telemetry to alert FME and vendor
- BSI II is used for repository management of specimens for requisition, tracking and billing (2 instances cover collections)

Freezer counts housing current collections

Freezer Type	Total (physical units)
LN2 -150	228
LN2-fueled -80	125
Mechanical +4 and Bulk*	15
Mechanical -20 Bulk*, Upright and Upright Flammable	20
Mechanical -30 Upright	5
Mechanical -80 Bulk*, Chest and Upright	360
Grand Total	753

19M + biospecimens (humans and animal)



- **The -80 bulk unit counts as fourteen freezers in BSI, the bulk +4 as five and the bulk -20 as twenty-five freezers.*

Capabilities to support FFRDC Repositories

- State-of-the-art technical expertise in the operation of repositories at the NCI Frederick campus and offsite.
- Expertise in handling and maintaining stored clinical and non-clinical biological specimens, and reagents including, but not limited to, human and animal specimens, frozen tissue, whole blood, serum, plasma, peripheral blood cells, urine, stool, formalin-fixed paraffin-embedded (FFPE) blocks, slides, other bodily fluids and environmental samples. *(BSL-2 or lower; no select agents)*
- Expertise in repository management of ambient (RT and under gas/vacuum) and low/ultralow temperature [mechanical (4^{o}C, -20^{o}C, -80^{o}C), and liquid nitrogen (-80^{o}C, -150^{o}C, -196^{o}C)] storage capacity.

FFRDC contractor support repository operations including, but not be limited to:

- Curating specimens
- Technical personnel for managing the repository, including, but not limited to, receipt, inventory, storage, retrieval, distribution of specimens/samples, and monitoring of storage and transport units
- Manual and automated processes for the receipt, inventory, storage, retrieval and distribution of standard (1- and 2-D barcoded) and automation-formatted (barcoded on bottom of tube)
- Select packaging and training of IATA transport processes, domestically and internationally
- Preparation of inventory, monthly storage cost reports and other reports, as requested
- Maintenance of required storage units and facilities, as well as a documented and comprehensive Disaster Plan and Emergency Preparedness SOPs, to include pandemics
- Maintenance of QC and QA programs, SOPs for routine and emergency operations, and QA reporting

FFRDC contractor support repository operations including, but not be limited to (cont'd):

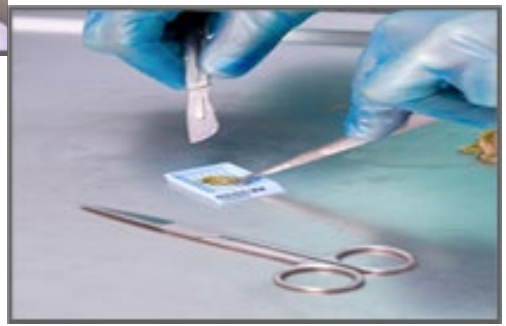
- Provision of adequate safeguards and emergency capabilities for physical repository and IT systems
- Maintenance and updates of specimen input, tracking storage and retrieval IT systems (Biological Specimen Inventory system), which feed into the contractor financial system(s) to support monthly vial and study level charging for activity and storage to Government sample custodians
- SOPs for daily, weekly, monthly and yearly preventive maintenance (e.g., temperature monitoring, gasket cleaning, professional maintenance, calibration) of storage units per manufacturer's recommendations.
- Complete record keeping for all maintenance procedures (e.g., work done, tests performed, results, etc.). Procedures for repairing storage equipment and validating all new or repaired equipment, including appropriate record keeping, should be in place.
- Highly skilled staff current on state-of-the-art technologies for manual and automated sample management, to serve as a resource to the government scientists.
- The adoption of new technologies, in sample management in support of biospecimen initiatives, is at the direction of the government.

Bioprocessing Activities

Human, Animal and Environmental Specimens

Bioprocessing at the FFRDC

- Bioprocessing is a generalized key activity across the basic, clinical and translational research programs supported by the contract. It underpins all of the molecular research supported at the FFRDC using human, non-human animal, microbial, viral and environmental specimens.
- The contract supports several centralized laboratories and additional dispersed capacity for specimen processing.



FFRDC facilities provide fresh and stored specimen processing including:

- Fresh and previously stored biospecimen processing and aliquoting
  - blood fractionation,
  - buccal cell processing,
  - RNA/DNA extraction,
  - PBMC cryopreservation;
  - post-storage processing, including aliquoting
- Freezing
- Lyophilization
- Flash-freezing of tissue in liquid nitrogen
- Administration and sample management of a Normal donor program (NDP)
  - NDP specimen processing of blood products, buccal cells, and urine for Quality Control (QC) material and specimen pre-analytical factor research

FFRDC facilities provide fresh and stored specimen processing including (cont'd):

- Separation and viable cryopreservation of cell lines, including routine assessment of viability
- Cryopreservation of red blood cells to maintain enzymatic activity
- Generation of primary cultures and established cell lines
- Large-scale propagation of cell lines
- Nucleic acid extraction (DNA and RNA)
- Distribution of specimens; shipping domestically and internationally
- Support to field centers in collection procedures, equipment, materials kit development and sample transportation
- Support for comprehensive approach to project planning, providing input on project design, laboratory activities, logistical plans, budgeting, activity timelines, including SOP generation, as needed

Examples of recent biospecimen processing activities at the FFRDC

- Rapid response for COVID-19 collection support and biospecimen processing
- Support for NCI Connect Cohort pilot studies and pre-launch of site collection activities.
- Preparation and receipt specimen-collection kits to NIEHS trial participants, processed and extracted clinical specimens
- Participation in network activities for NCI supported Cancer Prevention Clinical Trials Network's Data Management, Auditing, and Coordinating Center and the Early Detection Research Network.
- Processing, extracting and storage of 1000's of research samples across the FFRDC.
- Facilitation of sample and data management prior to and post- assay on biospecimens.

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Molecular and Cellular Biology

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Enabling NCI Science through Advanced Biotechnologies

- Centralized facilities available to specific NCI Divisions
- Mix of fee-for-service and collaborations depending on nature of project or technology
- All offer experimental design, sample qc, and primary data analysis
- Testing of emerging technologies and delivering the latest capabilities in consultation with NCI stake holders.
- Synergy across the labs to ensure seamless transitioning of project stages and pooling of expertise to deliver complete solutions

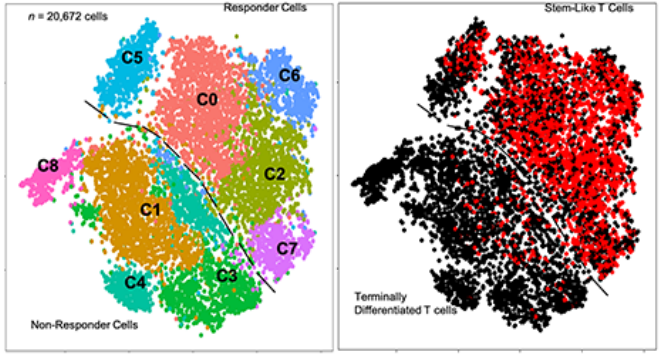
Genomic Technologies

- Next Generation Sequencing
  - Whole Exome, Transcriptome, and Genome Sequencing from fresh or FFPE tissue
  - ChIP-Seq, histone modifications, chromatin structure (*e.g.*, ATAC-seq) assays
  - Long-read sequencing platforms for genome assembly, identify mRNA isoforms & base modifications
  - Resequencing: SNP discovery/confirmation, CNVs, indels, structure variant discovery
  - Immune Repertoire Sequencing
  - Develop custom genomic assays (viral integration, CRISPR/Cas9 screening, CAR-T clonality analysis)
  - Shotgun metagenomic sequencing, 16S ribosomal RNA amplicon sequencing, metatranscriptomics
  - Whole genome methylation detection by bi-sulfite sequencing

Other Genomic Technologies

- Specialized Genomic Assays
  - Optical Genomic Mapping for analyzing structural variation
  - Genome wide methylation arrays for human and mouse (*e.g.,* Illumina 850k EPIC array, Capture EPIC library/NGS)
- Targeted Gene Panel Analysis
  - NanoString, HTG EdgeSeq, qPCR, and Droplet Digital PCR (miRNA, mRNA profiling, SNP detection)
  - Array-based methods for gene expression profiling, copy number, and SNP detection)
  - Pyromark DNA sequencing for specific CpG methylation

Single Cell Genomic Analysis

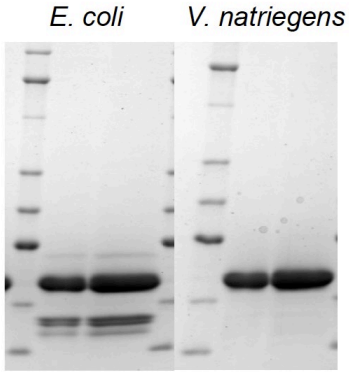


SC Analysis of 20,000 individual T cells divide into clusters with different genetic markers and functional properties. [Krishna, S. et al., 2020, Science]

- Single cell/nucleus Capture, Library prep, Sequencing
- Droplet, Plate, and Microwell-based platforms (*e.g.,* 3'/5', Smart-Seq2, *etc.)*
- Targeted, Whole Transcriptome, VDJ/TCR/BCR expression
- Cell surface protein measurements and cell hashing using bar-coded antibodies
- Chromatin accessibility assays (ATAC-seq) and Functional Genomics (CRISPR-based perturbations)
- Data quality control and primary analysis (including alignment, and counts matrix generation, and facilitating access to secondary bioinformatics analysis packages)
- Spatial Transcriptomics (from fresh frozen and FFPE tissue sections)

Protein Expression and Production

Optimizing protein production using novel expression systems

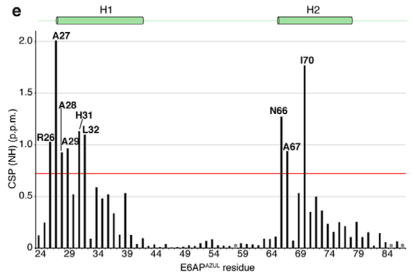


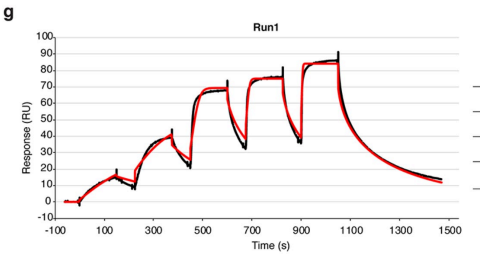
- Combinatorial cloning
  - Custom cloning and mutagenesis for protein expression
  - Gateway recombination-based cloning platform
  - Baculovirus genome production systems
- Protein Production
  - High-throughput expression/purification conditions at microscale
  - Expression in bacteria (*E.coli, Vibrio natriegens)*, insect cells, and mammalian cells
  - Large-scale purification from micrograms to grams
- Eukaryotic Expression
  - Baculovirus production
  - HEK293 expression using transient transfections

Molecular and Macromolecular Characterization

- Mass Spectrometry for proteins, metabolites, and small molecules
  - Identification from gel bands, tissues, and other biological matrices
  - Global Quantitative proteomics using TMT, label free, SILAC, and dimethyl labeling
  - Macromolecular interactions (protein/protein, protein/DNA, protein/peptide)
  - Post-translational modification (PTM) analysis: mapping phosphorylation, ubiquitination, etc. Global PTM analysis using quantitative methods
  - Intact mass analysis of antibody-drug conjugations and recombinant proteins
  - Targeted and global metabolite analysis
- Surface Plasmon Resonance (SPR) to characterize binding kinetics and affinities

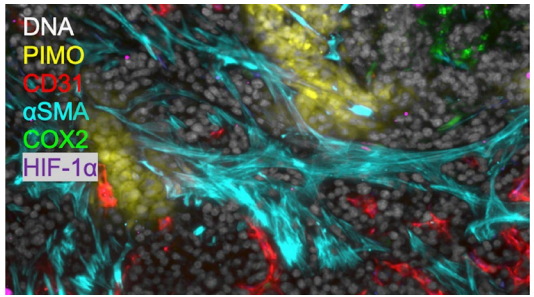
E6AP interacts with hRpn10305-377 through its AZUL domain with high affinity
Buel, G.R. et al., 2020, Nat. Comm.





Optical Microscopy and Image Analysis

- Provide expertise and training on advance imaging modalities
  - Wide-field fluorescence, confocal, 3D and live cell imaging
  - Super resolution of fixed samples, including PALM, TIRF, SIM, STORM, Light sheet, STED
  - High throughput cell imaging (plate format)
  - Atomic force microscopy
  - Multiplex imaging of proteins

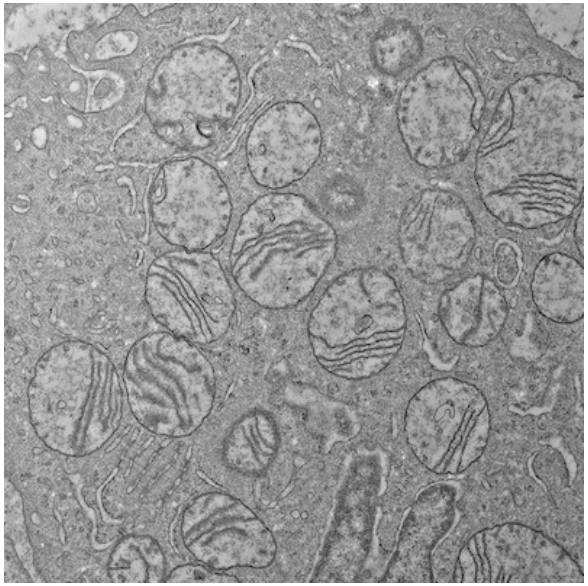


- Advise on sample preparation (tissue clearing/expansion, live cell, etc.)
- Support for Image Analysis for deconvolution, cell segmentation, cell and molecule tracking, co-localization, high content screens, etc.

Electron Microscopy

Technologies

- Transmission EM (TEM)
- Scanning EM (SEM)
- RT-Electron Microscopy
- Nanoparticle and liposome analysis
- Virus particle characterization
- Ultrastructure analysis
- Energy dispersive X-Ray spectroscopy (EDX)

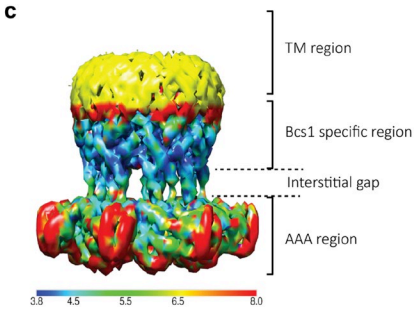


The ultrastructure of damaged mitochondria in a tumor from a patient with HLRCCC-associated kidney cancer. *Crooks et al., Science Signaling, 2021*

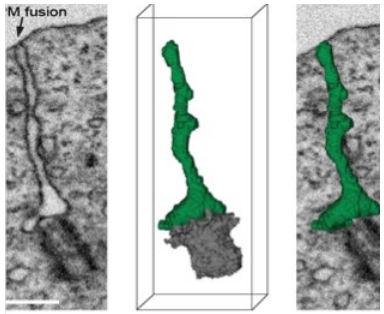
About 1,500-2000 samples processed annually for over 20 NCI PIs

Single Particle and Volume Imaging by EM

- Technical expertise and computational support for:
  - Single Particle Analysis (SPA): Negative stain (screen) and Cryo (high-resolution)
  - Electron tomography (ET): Negative stain and cryo for visualization of multiprotein complexes *in-situ*
  - Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) to enable volume (3D) imaging of cells and tissues.
  - Array Tomography followed by FIB-SEM for larger tissue samples.



Local resolution map for the apo mBcs1 structure at 2.5σ level. [Tang, W.K. et al., 2020, Nature Comm.]



F-BAR domain PACSIN proteins uncovers membrane tubulation function in cilia assembly and transport. [Insinna, C. et al., 2019, Nature Comm.]

Flow Cytometry

- Provides instrumentation access and technical expertise for CCR investigator cell analysis and separation needs
- High-parameter tools and latest fluorophores for cell sorting, maintenance, troubleshooting, and scientific consulting
- Provides materials for training and planning experiments

Benchtop analyzers

BD Symphony A5 (5B, 5G, 8V, 3R, 7UV)
BD LSRII-Fortessa (2B, 5G, 6V, 3R)
BD LSRII-SORP (2B, 5G, 6V, 3R)
BD LSRII (5B, 3V, 3R)
BD FACS Canto II (4B, 2V, 2R)
Miltenyi MACSQuant-16 (6B, 5V, 3R)
Cytek Aurora spectral analyzer (5 lasers, up to 40 colors)

Cell sorters

BD FACS Aria II (2B, 5G, 6V, 3R, 2UV)
BD FACS Aria II (2B, 5G, 6V, 3R)
BD FACS Aria II (2B, 5G, 6V, 3R)
BD Symphony S6 (5B, 8V, 8UV, 5YG, 3R)

www.cancer.gov

www.cancer.gov/espanol

CLIA-certified Lab Services

NIH NATIONAL CANCER INSTITUTE

CLIA Labs Overview

Develop and perform clinical assays on human specimens from clinical trials.

- For diagnosis/prevention/treatment of disease or assessment of health status.
- Obtain/maintain Clinical Laboratory Improvement Amendments (CLIA) certification.
- Provide molecular diagnostics, clinical diagnostics, and biomarker reference standards and others as required.

CLIA Labs Overview - continued

- Clinical trial support
  - Aid in development of comprehensive strategy for:
    - Sample collection
    - Processing
    - Stability
    - Testing (such as immunologic and labile analytes)
  - Collaborate with partners to:
    - Develop customized, validated, fit-for-purpose assays
    - Transfer existing assay technology with assay validation, data analysis and result interpretation.
- Biomarker Reference Standards
  - Establish/maintain biomarker references for standardizing assays/methodologies/quality control for reagents and technologies.
  - Support collaborative, consortia-directed studies to validate assays in multi-center settings.
  - Conduct studies to improve assay performance characteristics.
  - Provide biomarker reference libraries.

Pathology Overview

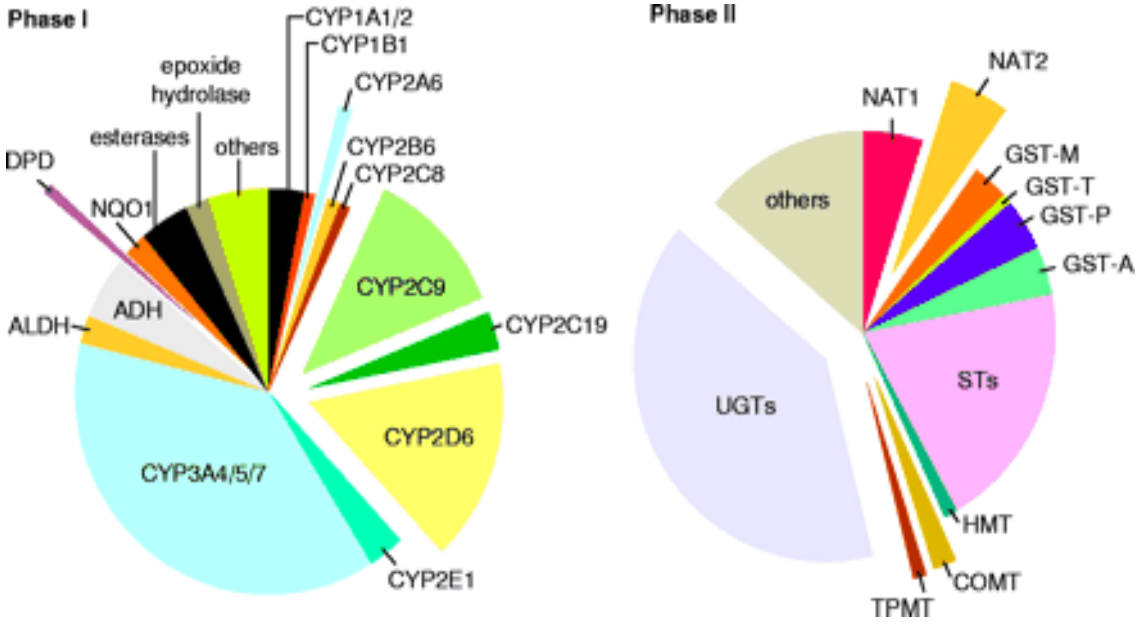
- Provide comprehensive pathology support for clinical studies.
  - CLIA approved
  - Including biomarker validation, diagnostic testing, etc.
  - Apply state-of-the-art technology as applicable
  - Trained/certified clinical/anatomic pathology staff
  - Support for assessment of molecularly targeted anti-cancer agents and genomic assessment for personalized medicine.
  - Conduct digital imaging studies.

CLIA Molecular Diagnostics Lab

CLIA Molecular Diagnostics Lab

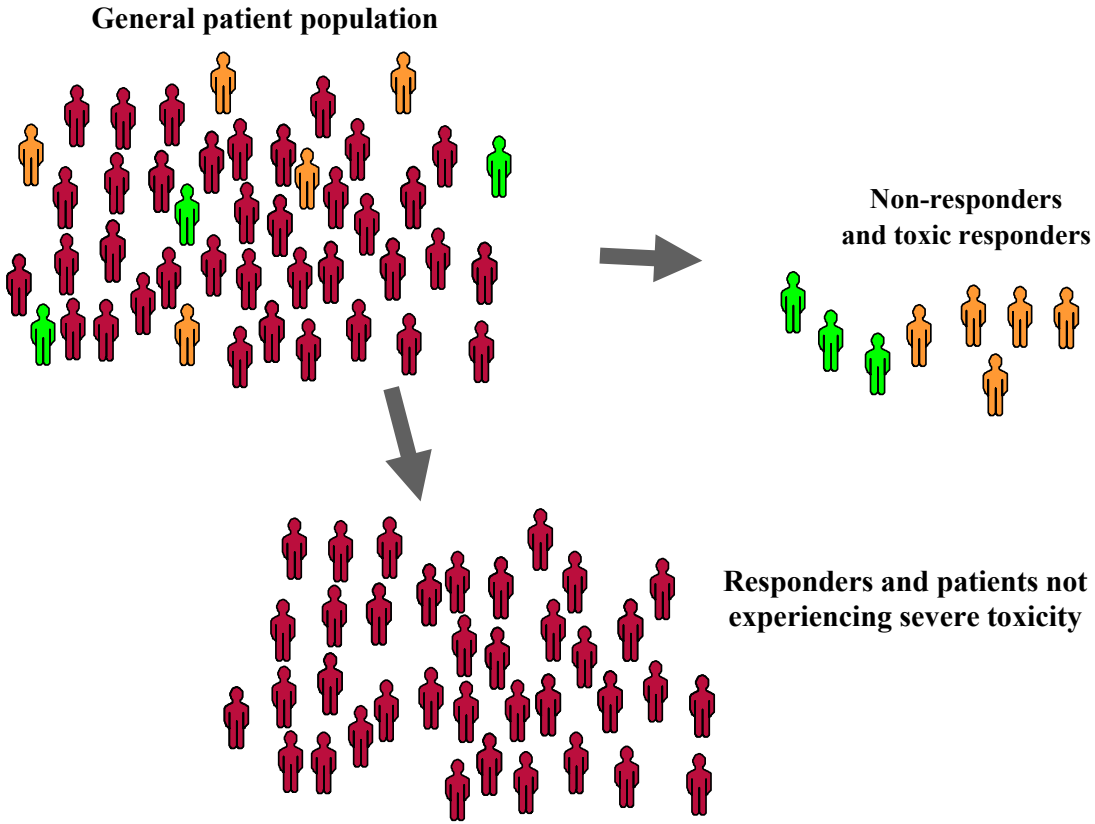
- The lab's long-term standing projects:
  - PCR and DNA sequencing-based genetic mutation detection for the Clinical Neutrophil Monitoring Laboratory/NIAID
  - Lymphoma/Leukemia Molecular Profiling Project, Nanostring
  - A core Sanger sequencing service to CCR and RAS investigators, as well as Hood College.
  - Pharmacogenetic testing (discussed below)

Drug metabolizing enzymes



(Evans and Relling, *Science* 286: 487-91, 1999)

The Goal of Pharmacogenetics



Pharmacogenetics

Implications of Polymorphisms on Pharmacokinetics

- Drug Absorption
- Drug Distribution
- Drug Metabolism
- Drug Elimination
- Drug Activation

Implications of Polymorphisms on Drug Effect (Response and Toxicity)

- Receptors
- Target Proteins
- Resistant
- Toxicity

Pharmacogenomics and Oncology

Pharmacogenomic Strategies Most Relevant When:

- **Narrow therapeutic indices**
- **High degree of inter-individual variability in response**
- **Little or no available methods to monitor safety or efficacy**
- **Few alternative treatment options**

Flowers and Veenstra 2004

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Flowers and Veenstra 2004

Anticancer agents meet all of these criteria

Genotyping Strategies in Medical Oncology

Example of Anticancer Drug Metabolism by Polymorphic Enzymes

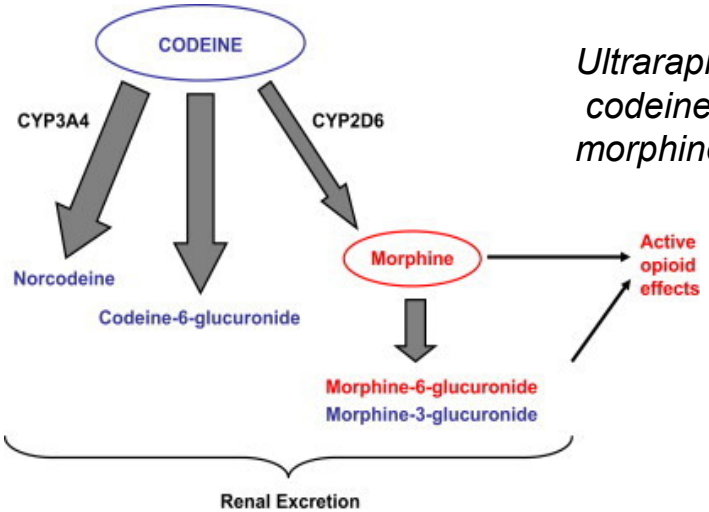
Drug	Elimination Pathway	Variability in CL
Amonafide	N-acetyl transferase (NAT)	>3-fold
Busulfan	Glutathione S-transferase (GST)	10-fold
Docetaxel	Cytochrome P-450 (CYP) 3A4 and 3A5	4 to 9-fold
5-Fluorouracil	Dihydropyrimidine dehydrogenase (DPD)	10-fold
Irinotecan	UDP glucuronosyltransferase (UGT)	50-fold
6-Mercaptopurine	Thiopurine methyltransferase (TPMT)	>30-fold

Evans and Relling, *Science* 286: 487-91, 1999

The NEW ENGLAND JOURNAL of MEDICINE
Volume 361:827-828; August 20, 2009

Case report: Codeine, Ultrarapid-Metabolism Genotype, and Postoperative Death

Healthy 2-yo boy, underwent outpatient elective adenotonsillectomy;
After surgery, instructions to take 10-12.5mg of codeine + 120 mg APAP q 4-6 hr prn; 2 days post surgery, child died
Autopsy results: Codeine (0.70 mg/L) & morphine (32 ng/ml) → toxic levels
CYP2D6 genotyping → 3 copies of *CYP2D6* allele → ultrarapid-metabolizer phenotype



Ultrarapid metabolizers may metabolize codeine too efficiently leading to morphine intoxication

In 2020 MDL changed from using the Affymetrix DMET platform to the Applied Biosystems PharmacoScan

Key features:

- 4,627 markers in 1,191 genes of known pharmacogenomic value including Phase I and Phase II enzymes, regulatory/modifier genes, drug target genes, and Phase III/transporter genes
- Genotyping of highly predictive markers in genes including GSTM1, CYP1A2, CYP2D6, CYP2B6, CYP2A6, SULT1A1, CYP2C19, and CYP2C9 that are in highly homologous regions
- Copy number states ranging from zero to three-plus for important ADME genes
- Star allele and translation for key genes

Table 2. Dose and administration of FOLFOX.

Cycle	Chemotherapy regimen	Notable adverse effects reported on D1 of the subsequent cycle
C1	• Oxaliplatin 85 mg/m^2 iv. infusion over 2 h • Leucovorin 400 mg/m^2 iv. infusion over 2 h • 5-FU bolus 400 mg/m^2 iv. bolus • 5-FU 2400 mg/m^2 (1200 mg/m^2/day) iv. continuous infusion over 46 h	Fatigue, mucositis, dysphagia, indigestion, mild nausea, oral candidiasis, temperature 37.8°C, ANC 0 K/μl, WBC 1.09 K/μl and platelets 18 K/μl
C2 (delayed)	• Oxaliplatin 50 mg/m^2 iv. infusion over 2 h • Leucovorin 400 mg/m^2 iv. infusion over 2 h • No 5-FU bolus • 5-FU 1200 mg/m^2 (600 mg/m^2/day) iv. continuous infusion over 46 h • Pegfilgrastim 6 mg subq 24 h after completion of chemotherapy	Tolerated well
C3	• Oxaliplatin 65 mg/m^2 iv. infusion over 2 h • Leucovorin 400 mg/m^2 iv. infusion over 2 h • No 5-FU bolus • 5-FU 1600 mg/m^2 (800 mg/m^2/day) iv. continuous infusion over 46 h • Pegfilgrastim 6 mg subq 24 h after completion of chemotherapy	Tolerated well
C4	• Oxaliplatin 65 mg/m^2 iv. infusion over 2 h • Leucovorin 400 mg/m^2 iv. infusion over 2 h • No 5-FU bolus • 5-FU 2000 mg/m^2 (1000 mg/m^2/day) iv. continuous infusion over 46 hours • Pegfilgrastim 6 mg subq 24 h after completion of chemotherapy	Lip ulcerations ANC 0 K/μl, WBC 1.84 K/μl and platelets 14 K/μl
C5 (delayed)	• Oxaliplatin 65 mg/m^2 iv. infusion over 2 h • Leucovorin 400 mg/m^2 iv. infusion over 2 h • No 5-FU bolus • 5-FU 1200 mg/m^2 (600 mg/m^2/day) iv. continuous infusion over 46 h • Pegfilgrastim 6 mg subq 24 h after completion of chemotherapy	Tolerated well overall This dose continued through C12.

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Sissung TM, (Figg WD) et al. (2021) Pharmacogenomics, 22(2):81-5.

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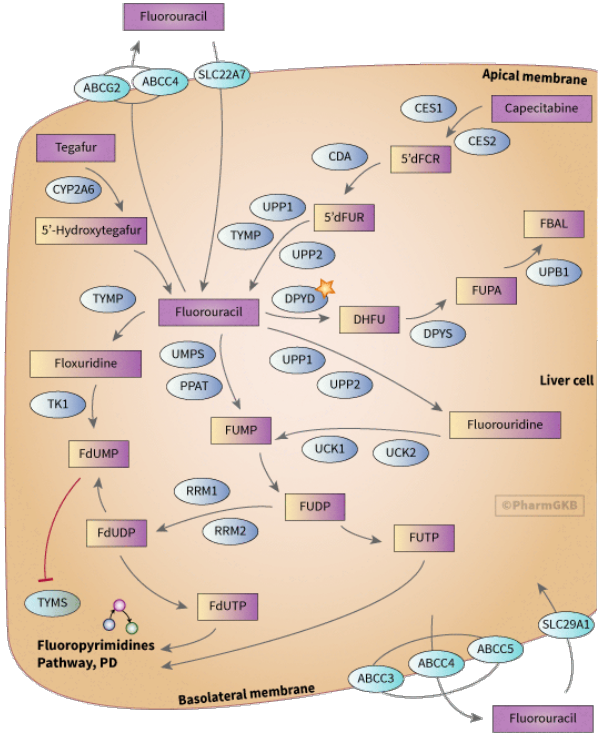
DPYD Genotyping



DMET array

- Used after first cycle toxicity
- 18 DPYD variants
- Patient wild-type at all tested loci

Pharmacoscan array

- 53 DPYD variants
- Heterozygous for 2 variants:
  - Y186C: ~3% of Af. Americans and .6% of admixed Americans
  - 21528C>T (3'-UTR): ~0.6% of Af. Americans and not observed in admixed American population
- Estimated ~1/2 DPYD activity observed in normal metabolizers*

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* Offer SM, et al. (2013) Clin Pharmacol. Ther., 94(1):131-6.

The ultimate goal of pharmacogenetics is to provide a patient with individualized therapy (“getting the dose right”)

Using candidate gene approach - It will be virtually impossible to assign a patient to an unequivocal phenotype and especially to an unequivocal genotype

PharmacoScan: 4,627 SNPs in 1,191 Genes

- ***Phase I enzymes:*** *CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2A7, CYP2A13, CYP2B6, CYP2B7, CYP2B7P1, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2S1, CYP3A4, CYP3A5, CYP3A7, CYP3A43, CYP4A11, CYP4B1, CYP4F2, CYP4F3, CYP4F8, CYP4F11, CYP4F12, CYP4Z1, CYP7A1, CYP7B1, CYP8B1, CYP11A1, CYP11B1, CYP11B2, CYP17A1, CYP19A1, CYP20A1, CYP21A2, CYP24A1, CYP26A1, CYP27A1, CYP27B1, CYP39A1, CYP46A1, CYP51A1*
- ***Phase II enzymes:*** *ADH1A, ADH1B, ADH1C, ADH4, ADH5, ADH6, ADH7, ALDH1A1, ALDH2, ALDH3A1, ALDH3A2, CHST1, CHST2, CHST3, CHST4, CHST5, CHST6, CHST7, CHST8, CHST9, CHST10, CHST11, CHST13, COMT, DPYD, FMO1, FMO2, FMO3, FMO4, FMO5, FMO6, GSTA1, GSTA2, GSTA3, GSTA4, GSTA5, GSTM1, GSTM2, GSTM3, GSTM4, GSTM5, GSTO1, GSTP1, GSTT1, GSTT2, GSTZ1, MAOA, MAOB, NAT1, NAT2, NNMT, NQO1, SULT1A1, SULT1A2, SULT1A3, SULT1B1, SULT1C1, SULT1C2, SULT1E1, SULT2A1, SULT2B1, SULT4A1, TPMT, UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2A1, UGT2B4, UGT2B7, UGT2B11, UGT2B15, UGT2B17, UGT2B28, UGT8*
- ***Transporters:*** *ABCB1, ABCB4, ABCB7, ABCB11, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, ABCC8, ABCC9, ABCG1, ABCG2, ATP7A, ATP7B, SLCA13, SLC10A1, SLC10A2, SLC13A1, SLC15A1, SLC15A2, SLC16A1, SLC19A1, SLC22A1, SLC22A11, SLC22A12, SLC22A14, SLC22A2, SLC22A3, SLC22A4, SLC22A5, SLC22A6, SLC22A7, SLC22A8, SLC28A1, SLC28A2, SLC28A3, SLC29A1, SLC29A2, SLC5A6, SLC6A6, SLC7A5, SLC7A7, SLC7A8, SLCO1A2, SLCO1B1, SLCO1B3, SLCO2B1, SLCO3A1, SLCO4A1, SLCO5A1*
- ***Other:*** *ABP1, AHR, AKAP9, ALB, AOX1, ARNT, ARSA, CBR1, CBR3, CDA, CES2, CROT, DCK, EPHX1, EPHX2, FAAH, G6PD, HMGCR, HNMT, MAT1A, METTL1, NR1I2, NR1I3, NR3C1, ORM1, ORM2, PNMT, PON1, PON2, PON3, POR, PPARD, PPARG, PTGIS, RALBP1, RPL13, RXRA, SEC15L1, SERPINA7, SETD4, SPG7, TBXAS1, TPSG1, TYMS, VKORC1, XDH*

UGT1A1 Genotype-Dependent Dose Adjustment of Belinostat in Patients With Advanced Cancers Using Population Pharmacokinetic Modeling and Simulation

Simulation
EM: Extensive Metabolizer
IM: Intermediate Metabolizer

Cody J. Peer, MS, PhD[1], Andrew K. L. Goey, PharmD, PhD[1], Tristan M. Sissung, MS, PhD[1], Sheryl Erlich, MA[1], Min-Jung Lee, PhD[2], Yusuke Tomita, MD[2], Jane B. Trepel[2], Richard Piekarz, MD, PhD[3], Sanjeeve Balasubramaniam, MD[2], Susan E. Bates, MD[2], and William D. Figg, PharmD, FCP[1,4]

The Journal of Clinical Pharmacology
2016;56:450-460

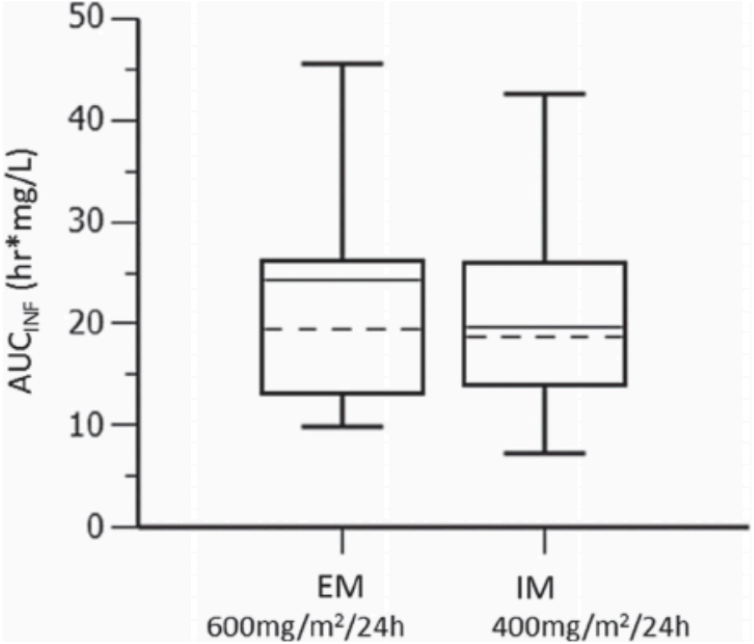

Figure 4. Simulations for optimal belinostat doses to predict equivalent exposures. Simulated AUC_{inf} from EM given 600 mg/m^2/24 h versus IM given 400 mg/mg^2/24 h from a single 48-hour intravenous infusion.

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use BELEODAQ safely and effectively. See full prescribing information for BELEODAQ.

BELEODAQ® (belinostat) for injection, for intravenous administration
Initial U.S. Approval

----------INDICATIONS AND USAGE----------
Beleodaq is a histone deacetylase inhibitor indicated for the treatment of patients with relapsed or refractory peripheral T-cell lymphoma (PTCL). This indication is approved under accelerated approval based on tumor response rate and duration of response. An improvement in survival or disease-related symptoms has not been established. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trial. (1)

Reduce the starting dose of Beleodaq in patients known to be homozygous for the UGT1A1*28 allele to minimize dose limiting toxicities is recommended.

Study Design (B + C + E)

- Parallel design in which the starting dose of belinostat is administered at two possible doses based on genotype:

  1) 400 mg/m^2/day for *UGT1A1*28/*28* or at least one *UGT1A1*60* allele *UGT1A1*28* and *UGT1A1*60* genotypes

  or

  2) 600 mg/m^2/day for wild-type patients and those carrying *UGT1A1*1/*28* in the absence of other variant alleles

- All patients will also receive cisplatin at 60 mg/m^2 IV on day 2, and etoposide at 80 mg/m^2 IV daily x 3 on days 2 - 4

Pharmacogenetic Guided Trials (Since the 2015)

- Mithramycin
- Gemcitabine
- PEN-866 (i.e., HSP90 targeting ligand linked to SN-38)
- MM-398 (i.e., nanoliposomal irinotecan)
- Belinostat

Molecular Diagnostics Laboratory Requirements

- ✓ All routine clinical center genotyping samples in a CLIA setting
- ✓ Handles prospective PG testing for all clinical trials in a CLIA setting
- ✓ Transmits data to patients' electronic health record (CRIS)
- ✓ Generates reports for physician use in concert with PG subcommittee under the P&T
- ✓ Handles retrospective PG testing for clinical studies (non-CLIA)
- ✓ Develops and optimizes other assays with advanced technological tools (e.g., ddPCR, Illumina Hi-Seq, microarrays, etc.)

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Questions?

All questions must be entered into the WebEx chat