Table of Contents | Shared Resources Posters

1 Transgenic Mouse Facility Shared Resource UCI

Chao Family Comprehensive Cancer Center | UCI Health

Leadership

Grant MacGregor, DPhil
Scientific Director

Jon Neumann, BS
Managing Director

Shimako Kawauchi, PhD
Project Manager

Mission

Facilitate use of the mouse as a mammalian model genetic system to investigate mechanisms of oncogenesis and testing of cancer therapeutics. To fulfill this mission the **TMF:**

- Advises investigators wishing to use genetically engineered mouse models (GEMMs) on experimental design and analysis, helps write grant proposals & manuscripts and provides letters of support.
- Provides access to specialized expertise and equipment to develop GEMMs, provides technical support, and sources other reagents required to manipulate the mouse genome and analyze the effect thereof.
- Communicates awareness of novel mouse-related resources via workshops, seminars, e-mail or the **TMF** Shared Resource website, facilitates their acquisition for Cancer Center investigators, and provides practical assistance with their use.
- Assists researchers by importing, or helping to develop, new experimental approaches required to address specific experimental questions in their research.

Services

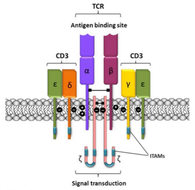
- Consultation for Cancer Center members about strategies to modify the mouse genome and use of genetically engineered mouse models in their research programs, at no cost.
- Genome engineering in mice and JM8 (C57BL/6N) ES cells via CRISPR / Cas9 and conventional targeting strategies.
- Production of mouse models using established genetically modified ES cells lines - e.g. KOMP, EuCOMM, etc.
- Targeted transgenesis at *Hipp11* (Ch 11) and *ROSA26* (Ch 6).
- Production of BAC random-integration transgenic mice.
- Southern analysis, including probe design and testing.
- Cryopreservation, import, export of mouse embryos and sperm.
- IVF and embryo transfer to re-animate, re-derive, expand or develop large cohorts of identical aged mice for experimental studies.
- Provision of oocytes or pre-implantation embryos for *in vitro* investigation.
- Sourcing of existing mouse models for UCI investigators.
- Molecular genotyping and breeding services.
- Customized services to accommodate investigator.
- Provision of commonly used CRE and FLP expression mice.
- Provision of letters of support for grant applications.
- Provision of language for manuscripts and grant applications.

Research Supported

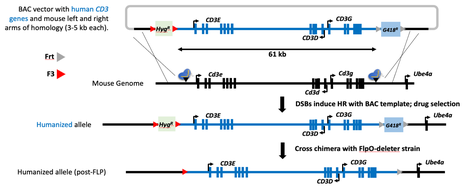
Example 1 | Humanization of mouse CD3 via CRISPR-mediated gene replacement in ES cells

- Michael Demetriou, MD, PhD (BIDD),
NCI - Biden Moonshot Program, NCI-NEXT Program, GlyTR

- PI requested development of humanized CD3 ε,δ,γ mouse for evaluation of CD3-mediated CAR-T therapeutics.
- Existing hCD3 mouse models have limitations or restrictive licensing agreements.
- **TMF** used novel CRISPR-based strategy in mouse ES cells to replace mouse CD3 ε,δ,γ loci with human counterparts.
- FACS analysis of splenic and thymic cells from chimeric mice indicate successful expression of hCD3 complex.
- **TMF** uses assisted reproduction to produce large cohorts of homozygous humanized CD3 ε,δ,γ mice for PI.



The TCR–CD3 complex is composed of a genetically-diverse αβ (or γδ) TCR heterodimer in noncovalent association with invariant CD3 dimers: CD3εγ, CD3εδ, and CD3ζζ. The TCR mediates recognition of peptide fragments bound to major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs).

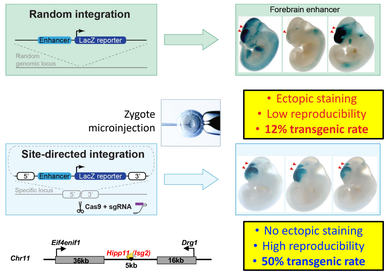


Strategy used to humanize the mouse CD3 locus comprised of three genes (*Cd3 d,e,g*). A 61kb region of the human genome taken from a BAC clone was used to generate a targeting vector with FRT'd mouse homology arms and dual drug selection cassettes. CRISPR-Cas9 was used to introduce double-strand breaks within the mouse genome close to the arms of homology. Correctly targeted clones were used to produce chimeric mice that were bread with FLPe mice to remove the selection cassettes. Homozygous hCD3e,d,g mice are being produced by the **TMF** for the Demetriou lab.

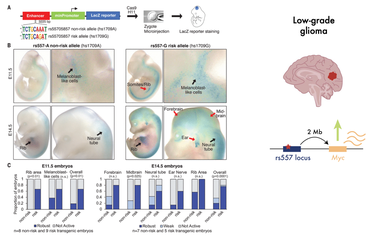
Example 2 | A noncoding single-nucleotide polymorphism at 8q24 drives *IDH1*-mutant glioma formation

- Evgeny Kvon, PhD (SPT), Collaboration with Schramek lab (Toronto)
Science (2022) (PMID 36201590)

- *rs55705857* risk-allele SNP - associated with 6x increased risk of IDH-mutant low-grade glioma.
- *rs55705857* within a highly conserved OCT2/4 brain-specific enhancer linked to *MYC*.
- PI wished to investigate relative activity of risk and non-risk *rs55705857* allele enhancer to drive brain-specific gene expression using transgenic mouse system.
- Random integration of transgenes produces significant variability that confounds analysis.
- Target transgenesis via CRISPR-Cas9 at *H11* locus allows reproducible assessment of enhancer activity - ~ 50% targeting efficiency.
- *rs55705857* risk-allele SNP mediates significant increase of brain specific gene expression *in vivo*.
- *rs55705857* non-coding region interacts with *MYC* locus and causes increased level of *MYC* RNA in presence of *rs55705857* risk-allele.



Cas9-mediated site-specific integration of transgenes enables efficient generation of transgenic animals with high reproducibility of transgene expression. From Kvon et al, (2020) *Cell* 180, 1262 (PMC7179509)

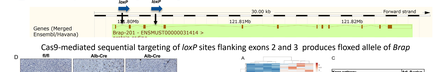


(**A**) Schematic of *rs55705857* LacZ enhancer reporter construct. (**B**) Representative whole-mount images of LacZ-stained *rs55705857* non-risk (left) and risk (right) enhancer reporter embryos. Black arrows denote LacZ staining found in both reporter mice, while red arrows indicate LacZ staining only found in risk reporter mice. (**C**) Summary for enhancer activity of the non-risk and risk allele.

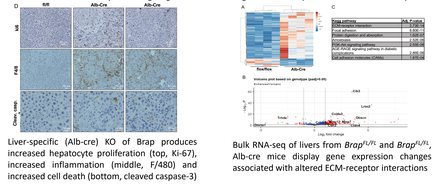
Example 3 | Loss of BRCA1 associated ubiquitin ligase (BRAP) depletes MST2 kinase resulting in increased YAP-target activity

- Peter Tontonoz MD, PhD (Jonsson CCC)
Proc Natl Acad Sci (USA) (2022) (PMC 9171358)

- Function of BRAP in liver homeostasis is unknown.
- Global KO of *Brap* produces lethality at e12, hence PI needed cKO allele of *Brap*.
- **TMF** used CRISPR-based sequential *loxP* insertion to flox exons 2 - 3 of *Brap* locus.
- Liver-specific KO of *Brap* in liver depletes MST2, allowing increased YAP activity, producing increased hepatocyte proliferation, cell death, inflammation and fibrosis.

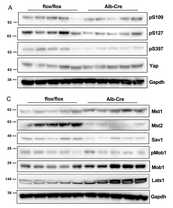


Cas9-mediated sequential targeting of *loxP* sites flanking exons 2 and 3 produces floxed allele of *Brap*

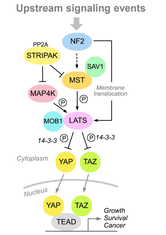


Liver-specific (Alb-cre) KO of Brap produces increased hepatocyte proliferation (top, Ki-67), increased inflammation (middle, F/480) and increased cell death (bottom, cleaved caspase-3)

Bulk RNA-seq of livers from $Brap^{fl/fl}$ and $Brap^{fl/fl}$, Alb-cre mice display gene expression changes associated with altered ECM-receptor interactions

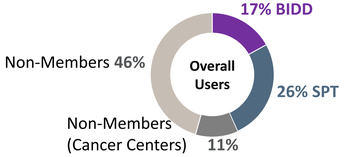


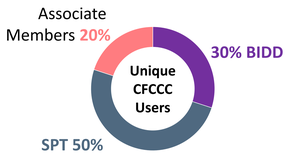
Livers from $Brap^{fl/fl}$, Alb-cre mice display normal level of YAP with decreased phosphorylation. Level of MST2 kinase is significantly reduced.



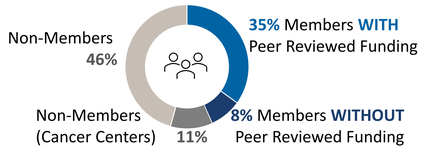
CCSG Metrics 1/1/22 – 12/31/22

Use by CFCCC Program

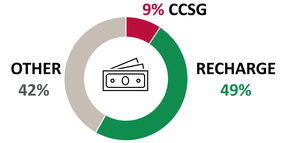




Peer Reviewed Use



Operational Fund Sources



Key Equipment & Technologies

- Bioinformatic analyses of mouse and human genomics to facilitate strategies for genome engineering.
- Apparatus for microinjection, electroporation and culture of zygotes / preimplantation embryos (Nikon, Narashige, BioRad, Planar).
- Culture and LN_2 cryogenic storage of sperm, embryos, mES cell lines.
- TaqMan, rhAMP based genotyping via Bio-Rad RT-PCR system.
- High-throughput (3 x 96-well tray) analysis of standard PCR reactions using Agilent capillary array Fragment Analyzer
- IVF-based mouse production using Planar incubators.
- PFGE and Southern analysis using Bio-Rad CHEF Mapper
- ICE-mediated deconvolution of Sanger-sequencing trace files, and Next-Gen amplicon sequencing.
- Multiple animal holding rooms with ventilated cage racks and sterile caging.
- Tissue culture suite with incubators, hoods and electroporation apparatus for ES cell culture (NuAire, BioRad).

Future Plans

- **Improve efficiency of production of CRISPR-engineered CRE-conditional (floxed allele) mice via sequential electroporation of pre-implantation embryos.** Electroporation of ssODN containing 1st loxP site at zygote stage, followed by electroporation of 2nd loxP site at 2-cell stage prior to transfer into reproductive tract of pseudo-pregnant female.
- **Faster throughput DNA sequence analysis of targeted ES cells and founder (G0) generation mice** generated by CRISPR via targeted sequencing using Oxford Nanopore MinION system.
- **Develop recorded video tutorials for TMF website** that provide background and information on technologies used to generate GEMM and their application for cancer studies.

Publications

CFCCC Investigator	Program	Journal	Year
Christina Sigurdson PhD	Moores (UCSD)	J Clin Invest	2020
Christopher Hughes, PhD	BIDD	Nat Commun	2020
David Fruman, PhD	SPT	iScience	2021
Mark Ginsberg, MD	Moores (UCSD)	J Exp Med	2022
Peter Tontonoz, MD, PhD	Jonsson (UCLA)	PNAS (USA)	2022
Evgeny Kvon, PhD	SPT (pending)	Science	2022

2 Optical Biology Core

UCI | Chao Family Comprehensive Cancer Center | UCI Health

Leadership

Rahul Warrior, PhD
Director

Jennifer Atwood, PhD
FCF Manager

Adeela Syed, PhD
OBC SUF Manager

Mihaela Balu, PhD
NLOM Manager

Mission

The OBC is a matrix of the 3 distinct cores that enable Chao Family Comprehensive Cancer Center members to utilize the most cutting-edge imaging and sorting capabilities in their cancer research

- **Self-Use Facility (SUF)** offers confocal, lightsheet two photon, and Super Resolution microscopes that allow everything from deep tissue, whole tissue, fluorescence lifetime (FLIM), and Super Resolution imaging
- **Non-Linear Optical Microscopy (NLOM) Laboratory** specializes in multiphoton microscopy-based imaging with large fields of view and rapid scanning for diagnosing skin cancers and other skin conditions and monitoring skin therapies. NLOM focuses on collaborative equipment use, development and protocol design
- **Flow Cytometry Facility (FCF)** operates a suite of multi-parameter flow cytometers equipped for fluorescence activated cell sorting and/or analysis.

Services

Self-Use Facility (SUF) *Walk-up use of suite of microscopes*

- 3 confocal microscopes with training on advanced imaging techniques, with a 4th to be added mid 2023
- 2-photon microscopy with fluorescence lifetime imaging microscopy (FLIM)
- Single plane illumination microscope (SPIM) able to perform both live sample and cleared tissue imaging.
- Super Resolution Lattice SIM with SMLM capabilities and down to 60nm resolution with SIM2 for live super resolution (255fps/60nm)

NLOM Laboratory

- Develops biophotonics technologies for basic research and pre-clinical/clinical applications via nonlinear optical microscopy (NLOM)
- NLOM for optical coherence tomography, diffuse optical spectroscopy and imaging, spatial frequency domain imaging, laser speckle imaging, Coherent anti-Stokes Raman Scattering (CARS) and FLIM to enable multi-photon deep tissue imaging

Flow Cytometry Facility (FCF):

- 5 multi-parameter flow cytometers each equipped for fluorescence activated cell sorting (FACS) including one in a BSL2 cabinet: Single cell preparations for scRNAseq
- Access to: High-end workstations for data analysis, including advanced 3D/4D analyses and cell sorting analysis

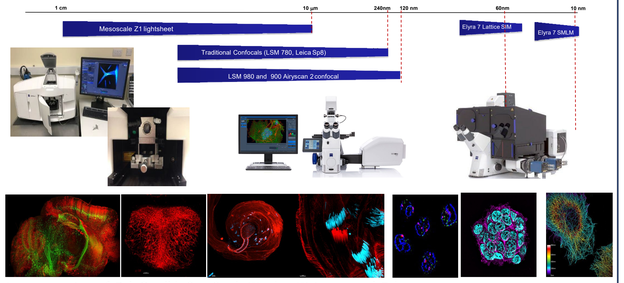
All three components of the OBC provide:

- Letters of support
- Assistance with study design, data processing and analysis strategies
- Imaging and Flow Cytometry Workshops offered multiple times throughout the year for Faculty, Students, and Staff

Research Supported

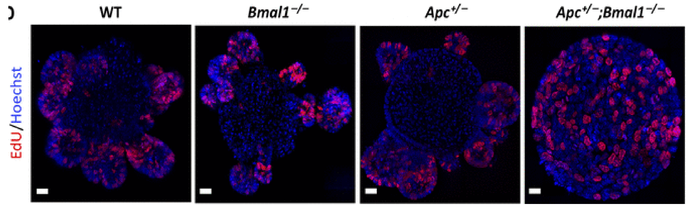
Example 1 | *SUF:* Disruption of the circadian clock drives *Apc* loss of heterozygosity to accelerate colorectal cancer

– Selma Masri, PhD (SPT) *Science Advances* 2022



Images courtesy of Dr's Gandhi, Sun, Syed, Warrior, Waterman and Zeiss

Suspected risk factors of young onset CRC include environmental aspects, such as lifestyle and dietary factors, which are also known to affect the circadian clock. Using Super Resolution Lattice SIM imaging of isolated intestinal crypts organoids, Masri et al demonstrate that clock disruption promotes transformation by driving *Apc* loss of heterozygosity, which hyperactivates Wnt signaling. This up-regulates *c-Myc*, a known Wnt target, which drives heightened glycolytic metabolism. This works show that circadian rhythms are lost in human tumors and demonstrate a previously unidentified mechanistic link between clock disruption and CRC, which has important implications for young onset cancer prevention.



Example 2 | *NLOM:* Label-free imaging of immune cells in human skin – Mihaela Balu, PhD (BIDD); Anand Ganesan, MD, PhD

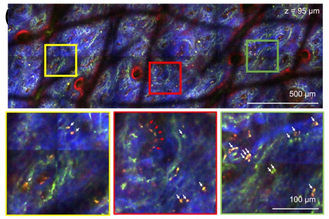


Figure 1. *In vivo* FLAME imaging of dermal cell populations in human skin.

In this study, we investigated the feasibility of a clinical home-built multiphoton microscope (fast, large area multiphoton exoscope: FLAME) to detect immune cells in human skin based on label-free molecular contrast by imaging the sun exposed forearm of a volunteer (Figure 1) and the non-sun exposed thigh area before and after an injury (Figure 2). The images show that FLAME has the ability to detect dermal cells, including resident immune cells in normal human skin, as well as an activation of a cellular immune response based on time-resolved single photon counting detection of the NADH fluorescence. The color coding of the cells is related to their temporal bin detection. The collagen fibers were captured through their second-harmonic generation signal detection. Different cell populations appear to be present based on their morphology and fluorescence lifetime. While these initial results show great promise, significant work remains to improve and validate the techniques we currently investigate. If successful this approach will provide a reliable tool for characterizing cytologic immune responses in human skin at the bedside, with broad applications ranging from detecting early immune reactions to developing improved cancer treatments.

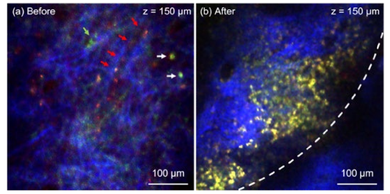
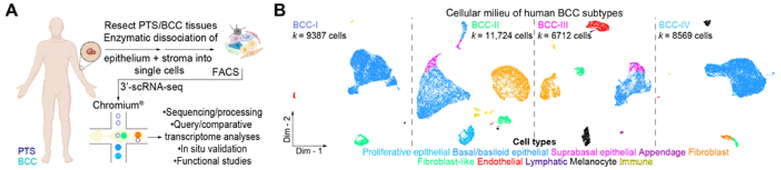


Figure 2. In vivo FLAME imaging captures immune cells recruited at an injury area in human skin.

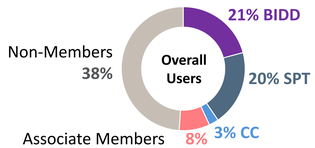
Example 3 | *FCF:* Single-cell analysis of human BCC reveals novel regulators of tumor growth and the tumor microenvironment

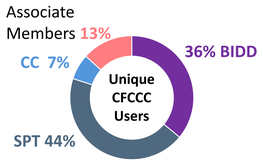


- Sort human BCCs, sc-RNAseq discriminates malignant and normal epithelial cells
- Analysis of tumor stroma indicates tumors respond to cancer-specific fibroblast inflammatory signals by producing heat-shock proteins
- Treatment with HSP70 inhibitor suppresses in vitro vismodegib-resistant BCC cell growth and in vivo tumor growth in a BCC mouse model

CCSG Metrics 1/1/22 – 12/31/22

Use by CFCCC Program





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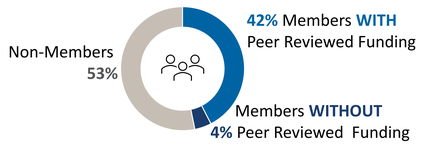
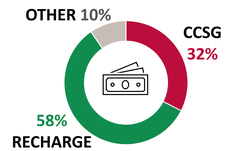


Operational Fund Sources



Key Equipment & Technologies

- **2020 (SUF)** - Elyra 7 Lattice SIM and SMLM localiztion based super resolution. Lattice SIM gives you super- resolution imaging at incredibly high frame rates. The new Apotome mode allows superfast optical sectioning of your 3D samples – with 255 fps. The new SIM² processing can resolve down to 60nm resolution!
- **2021 (FCF)** - ACEA NovoCyte Quanteon: Features 4 lasers and 25 Silicon photomultiplier detectors with 7.2 log dynamic range plus forward and side scatter parameters. Photodetectors and signal processing enable exceptional sensitivity and resolution down to 0.1 µm. Automated high throughput analysis of 24/48/96/384 well plates and traditional FACS 5ml tubes possible
- **2021 (NLOM)** - Leica Sp8 - photon counting capabilities, environmental chambers. 1PEF, 2PEF, SHG, CARS, FLIM imaging modalities
- **2021 (SUF)** - Zeiss LSM 900 Airyscan 2 is an area detector with 32 concentrically arranged detection elements that allows more photons to be collected. This produces much greater light efficiency and gives you a unique combination of super-resolution (120nm) imaging and high sensitivity

Future Plans

- **Installation of LSM 980 with Airyscan and NLO (SUF)**
  - The SUF was awarded an S10 for a Zeiss LSM 980 with Airyscan and NLO. This will have the added features of a two-photon laser and FLIM.
- **OBC renovations and expansion**
  - The SUF is undergoing renovations and expansion. The main microscope that houses 3 of our confocals is being upgraded to have independent HVAC, light control, 10Gb internet connectivity compressed air and curtains dividing the space.
  - Addition of 2 new rooms, each approximately 110 sf with sink, HVAC, both lab and compressed air, and 10Gb internet connectivity
- **Acquire BD Spectral 6-way sorter**
  - Spectral decomposition will allow for much higher parameter experiments
  - Bulk sorting of up to 6 populations simultaneously
  - Work with Schools/Departments for funding

Publications

CFCCC Investigator	Program	Published Journal	Year
Scott Atwood, PhD Robert Spitale, PhD	SPT BIDD	J. Am. Chem. Soc.	2022
Michelle Digman, PhD Robert Edwards, MD, PhD Nicholas Pannunzio, PhD Marcus Seldin, PhD Marian Waterman, PhD Selma Masri, PhD	BIDD SPT SPT SPT SPT SPT	Science Advances	2022
Xing Dai, PhD Maksim Plikus, PhD	SPT SPT	iScience	2022

3 Genomics Research and Technology Hub UCI

Chao Family Comprehensive Cancer Center | UCI Health

Leadership

Suzanne Sandmeyer, PhD
Director
Genomics Technologies

Jenny Wu, PhD
Director , Bioinformatics
Transcriptomic analysis

Melanie Oakes, PhD
Manager
Technical Operations

Ivan Chang, PhD
Bioinformatics Engineer
Data Sharing

Mission

To put emerging nucleic acid technologies into the hands of CFCCC investigators and enable bioinformatics analysis through consultation, training and collaboration.

To fulfill this mission **GRTH**:

- Provides guidance and education throughout the entire experimental process, including experimental design, data analysis and publication.
- Provides in-house staff with professional expertise in genome wide molecular technologies.
- Provides Bioinformatics Consulting Service for experimental design and data analysis staffed by PhD-level scientists experienced in bioinformatics.

Accomplishments & Services

- Outreach to clinical partners: presentations to all seven-cancer clinical Disease Oriented Teams (DOTS)
- Bioinformatics training: full day workshop series, beginner to advanced; guest workshops in CLC, IPA, Ensembl
- Bioinformatics tools: access and Jupyter notebook workflows on UCI High-Performance Computing Cluster, open- source software, and CLC Genomics Workbench Ingenuity Pathway Analysis commercial software
- Over 30 LOS and support for study design, data processing and analysis strategies

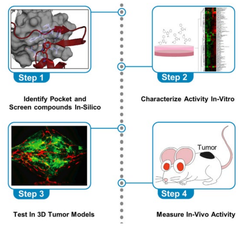
Technical Services provided:

- Illumina iScan beadarray: methylation, SNP and CNV analysis
- Illumina MiSeq, NovaSeq 6000: short read sequencing
- PacBio Sequel II: long-read sequencing
- BioNano-Saphyr 2: long-Range Mapping
- Library preparation: Illumina DNA-Seq, RNA-Seq, ChIP-Seq, Mate-Pair, Methyl-Seq; 16S amplicon; ATAC-seq; and PacBio SMRT bell sequencing
- NanoString: digital quantification of known nucleic acid targets
- 10X Genomics Chromium X: single cell gene (sc) RNA-seq; scATAC-seq; multiome; VD(J) typing
- Parse Biosciences: split-seq for high-throughput scRNA-seq
- Tapestri scDNA and protein typing: tumor lineage mapping
- Digital PCR: Bio-Rad ddPCR, ThermoFisher Quantstudio Q
- Nanostring GeoMx and 10x Visium: spatial transcriptomics

Research Supported

Example 1 | Structure-based design of CDC42 effector interaction Inhibitors for the treatment of cancer

– Jahid, Ortega, Vuong…., Hughes, De Vivo, Ganesan *Cell Reports, April 26, 2022*



In brief

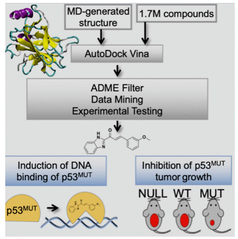
Jahid et al. report a multipronged approach to discover a drug candidate that can bind CDC42 family GTPases an can inhibit their interaction with downstream effectors. This molecule selectively targets CDC42 GTPases, inhibits MAPK and S6 signaling, and inhibits tumor growth and tumor angiogenesis.

Highlights

- Identified an allosteric pocket at the CDC42/RHOJ effector interaction interface
- Used *in silico* methods to identify and optimize ARN22089, which binds to CDC42
- ARN22089 has broad anti-cancer activity and inhibits MAPK and S6 signaling
- ARN22089 inhibits tumor angiogenesis *in vitro* and tumor growth *in vivo*

Example 2 | Discovery of compounds that reactivate p53 mutants *in vitro* and *in vivo*

– Durairaj, Demir, Lim…, Rychnovsky, Amaro, Kaiser *Cell Chemical Biology, September 15, 2022*



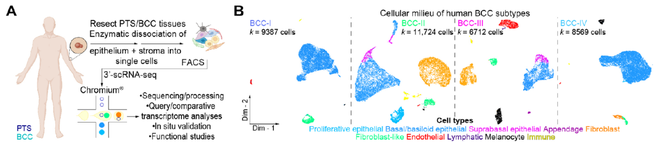
In brief

A large fraction of human tumors inactivate the major tumor suppressor p53 by mutations. Corrector drugs reactivating mutant p53 are of high clinical value for cancer therapy. Durairaj et al. identify small drug-like compounds that restore wild-type activity to mutant forms of p53 and exhibit anti-tumor effects.

Highlights

- Small molecules targeting a cryptic L1/S3 pocket of p53 are identified
- Experimental validation led to the identification of mutant p53 reactivators
- Compounds restore DNA binding of $p53^{MUT}$ *in vitro* and *in vivo*
- Compounds directly bind to $p53^{MUT}$ and exert anti-tumor effect in tumor models

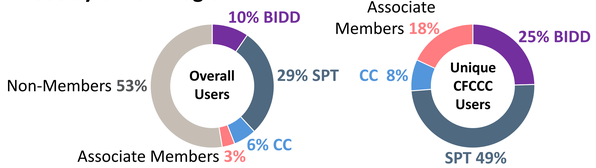
Example 2 | Single-cell analysis of human cell basal carcinoma reveals novel regulators of tumor growth and the tumor microenvironment – Guerrero-Juarez, Lee, Liu…Nie, Sarin, Atwood *Science Advances, June 10, 2022*



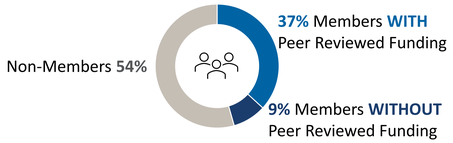
How basal cell carcinoma (BCC) interacts with its tumor microenvironment to promote growth is unclear. We use singe-cell RNA sequencing to define the human BCC ecosystem and discriminate between normal and malignant epithelial cells. We identify spatial biomarkers of tumors and their surrounding stroma that reinforce the heterogeneity of each tissue type. Combining pseudotime, RNA velocity–PAGA, cellular entropy, and regulon analysis in stromal cells reveals a cancer-specific rewiring of fibroblasts, where STAT1, TGF-β, and inflammatory signals induce a noncanonical WNT5A program that maintains the stromal inflammatory state. Cell-cell communication modeling suggests that tumors respond to the sudden burst of fibroblast-specific inflammatory signaling pathways by producing heat shock proteins, whose expression we validated in situ. Last, dose-dependent treatment with an HSP70 inhibitor suppresses in vitro vismodegib-resistant BCC cell growth, Hedgehog signaling, and in vivo tumor growth in a BCC mouse model, validating HSP70's essential role in tumor growth and reinforcing the critical nature of tumor microenvironment cross-talk in BCC progression.

CCSG Metrics 1/1/22 – 12/31/22

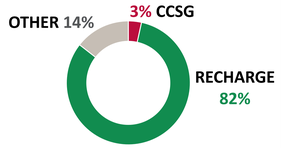
Use by CFCCC Program



Peer Reviewed Use



Operational Fund Sources



Key Equipment & Technologies

- **10X Chromium X: Single-Cell Assay.** 3' GEX, 5' GEX+VDJ, ATAC, Multiome (GEX+ATAC), Fixed RNA Profiling, and High-Throughput Assay Bullets.
- **Mission Bio Tapestri: Single-Cell DNA Assay.** Genomic DNA Panels, and Multi-omics (DNA+Protein)
- **Parse Biosciences: Single-Cell Assay.** Single-Cell Gene Expression Profiling. 10,000 – 1,000,000 Cells Library Kits.
- **Illumina Infinium assay: Beadchip Array iScan.** High-Throughput genotyping and DNA Methylation profiling.
- **ThermoFisher QuantStudio Absolute Q and Bio-Rad QX200: Digital PCR System.** High precision and sensitivity. Absolute quantification of targets.
- **TaKaRa Apollo and Eppendorf epMotion: Automated Library Prep System.** RNAseq and DNAseq - Up to 96 samples per run.
- **BioNano Saphyr 2: Long-Range Optical Mapper.** 1300 Gbp Human sample/flowcell. Detects large structural variants from 500bp to megabase pair lengths.
- **Nanostring GeoMx Digital Profiler**– spatial for proteins and RNA
- **10X Visium: Spatial Profiling.** Fresh Frozen and FFPE samples.
- **Illumina MiSeq and NovaSeq 6000: NGS System.** Flexible throughput from small targeted libraries to high-throughput, 20 billion reads in one run.
- **PacBio Sequel II: NGS Long-Read System.** Highly accurate long-read sequencing. Up to 25 kb.

Future Plans

- Expand clinically relevant genomics and epigenetics using iScan system;
- Maintain currency with acquisition of new technologies for short- and long-range sequencing and spatial multi-omics;
- Increase throughput, precision and accuracy with additional robotic systems (R24 application submitted);
- Formalize content in bioinformatics training with certificate program.

Publications

CFCCC Investigator	Program	Published Journal	Year
Nie, Qing, Atwood, Scott	SPT	Sciences Advances	2022
Digman, Michelle, Edwards, Robert Pannunzio, Nicholas, Seldin, Marcus Waterman, Marian, Masri, Selma	BIDD/SPT	Science Advances	2022
Shiu, Jessica, Waterman, Marian, Ganesan, Anand, Hedde, Per Niklas, Gratton, Enrico, Zhao, Weian	BIDD/SPT	Nature Communications	2022
Buisson, Remi	SPT	Star Protocols	2022
Tinoco, Roberto	BIDD	Cancer Immunology Research	2022
Jin, Rongsheng, Edwards, Robert Hughes, Christopher, Ganesan, Anand	BIDD/SPT	Cell Reports	2022
Kessenbrock, Kai, Dai, Xing	SPT	Cell Reports	2022
Jang, Cholsoon, Qiao, Feng, Huang Lan, Edwards, Robert, Rychnovsky, Scott, Kaiser, Peter	BIDD/SPT	Cell Chemical Biology	2022
Seldin, Marcus, Baldi, Pierre, Kaiser, Peter, Jang, Cholsoon	SPT	Molecular Metabolism	2022
Benavente, Claudia	SPT	Oncogenesis	2022
Edwards, Robert, Waterman, Marian	SPT	Molecular Cancer Research	2022
Dai, Xing	SPT	Scientific Reports	2022

4 In Vivo Functional Onco-Imaging

UCI | Chao Family Comprehensive Cancer Center | UCI Health

Leadership

Gultekin Gulsen, PhD
Co-Director

Zhuoli Zhang, MD, PhD
Co-Director

Farouk Nouizi, PhD
Facility Manager

Mission

Enhance and support basic and clinical cancer researchers by providing them with the necessary expertise, imaging instrumentation, and image analysis techniques.

To fulfill this mission the IVFOI –

- provides high-quality image acquisition and data analysis services for translational clinical studies;
- establishes several multi-modality imaging systems to support innovative imaging studies; and
- develops several cutting-edge technologies for quantitatively accurate high-resolution small animal imaging and translate them to clinical settings

Services

Existing systems (on Irvine campus):

- MR: 3.0 T (human)
- MR: 4.0 T (human and animal)
- MR: 9.4 T (animal)
- Combined MRI & Optical Tomography (animal)
- Combined X-ray micro CT & Fluorescence Tomography (animal)
- Hybrid MRI & SPECT (animal)

Existing Systems (located at UCI Medical Center):

- PET/CT (clinical scanner available at UCIMC)
- SPECT/CT (clinical scanner available at UCIMC)
- MR (1.5 & 3 T - clinical scanner available at UCIMC)

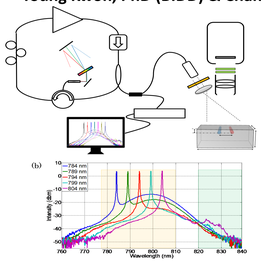
Systems currently under development or under acquisition:

- Micro SPECT/CT (Hitachi, animal)
- Micro PET/CT (Siemens, animal)
- MRI Sodium Imaging (brain cancer)
- Hybrid MRI/Scintimammography (breast cancer)
- Hybrid MRI/Positron Emission Mammography (PEM) (breast cancer)
- Temperature-modulated Fluorescence Tomography (animal)
- Photo-magnetic Imaging (animal)

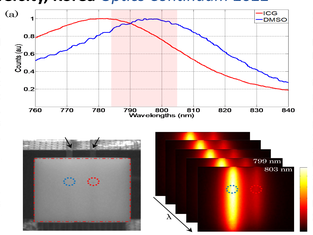
Research Supported

Example 1 | Swept-wavelength Laser for Spectral Fluorescence Imaging

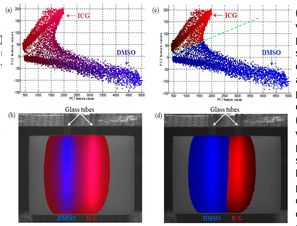
– Young Kwon, PhD (BIDD) & Chang-Seok Kim, Busan University, Korea *Optics Continuum 2022*



(a) A schematic of the wavelength-swept laser and our HER-NIRF system. SOA: semiconductor optical amplifier, P: polarization controller, CR: circulator, CL: collimator, GS: Galvo-scanner, G: grating, M: mirror, C: coupler, IS: isolator, Sp: splitter, F1: exciter bandpass filter, D: diffuser, F2: emitter bandpass filter, CCD: charge coupled camera, L: lens.
(b) Normalized output spectra of the wavelength-swept laser at representative tuning wavelengths. The passband of the filters is highlighted for the (orange) exciter and (green) fluorescence collection.



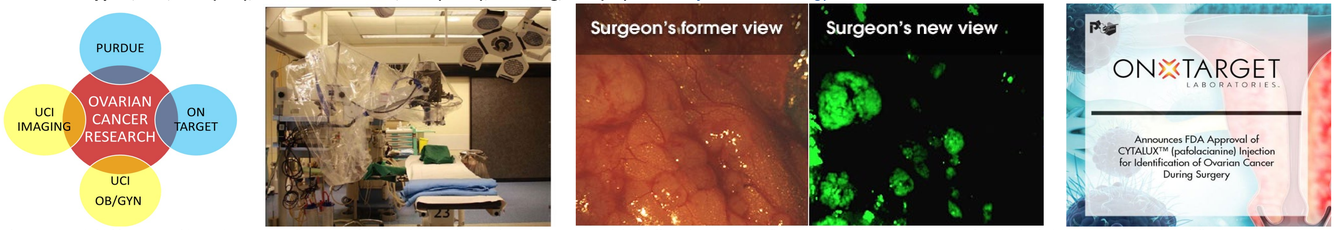
(a)Normalized absorption spectra of ICG dissolved in (blue) DMSO and (red) water. (b-left) Ambient-light top view of the phantom. The boundaries of the phantom are delineated with a red dash-dot line. Processing ROIs are delineated with dash line circles (blue) DMSO and (red) ICG. (b-right)



(a) Scatter plot of PC1 and PC2 feature values obtained on the pixels within the processing region. The color of each point is set as RGB = [PC2, 0, PC1]. (b) The pseudo color-coded image PC-RGB obtained by attributing each point color in (a) to its position in the image space superimposed on the ambient-light image of the phantom. (c) Scatter plot of PC1 and PC2 feature values obtained on the pixels within the processing region. The color of each point is set as blue for DMSO and red for ICG. The brightness of the points in each cluster is proportional to their distance from the center of their cluster. (d) The pseudo color-coded image k-means-RGB obtained by attributing each point color in (c) to its position in the image space superimposed on the ambient-light image of the phantom.

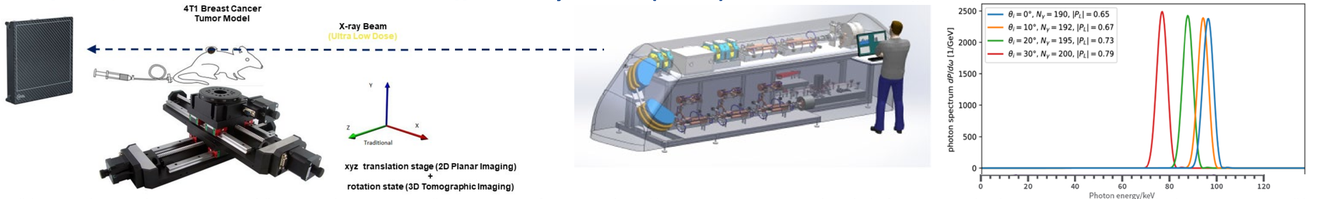
Example 2 | Folate Targeting Optical Probes for Fluorescence Imaging Guided Surgery

– Farshid Dayyani, MD, PhD (SPT); Maheswari Senthil, MD (BIDD); Jill Tseng, MD (CC) *Journal of Clinical Oncology 2022*



Folate Receptor is highly expressed in ovarian cancer. A fluorescent folate analog (Cytalux) binds to the receptor with high affinity (1nM). Cytalux fluorescence can be excited by near-infrared light during surgery to identify and illuminate ovarian cancer lesions. IVFOI played a key role in making UCI a site for Phase II and III clinical trials of Cytalux for ovarian cancer surgery. **FDA Approval** of this first-of-kind novel optical molecular probe was announced Nov. 29, 2021'. We are now working with PI Dr. Phil Low (faculty member of Purdue University (NCI) Center for Cancer Research) to develop a swept-wavelength laser based intra-operative surgery camera. Meanwhile OnTarget Laboratories has a second contrast agent for colon and gastric cancers. We are working on making UCI a Phase I clinical trial site for this agent.

Example 3 | LUMITRON Tunable Monoenergetic X-ray Source (TMXS) – Chris Barty, PhD (BIDD), Lumitron Technologies, *Irvine, CA*

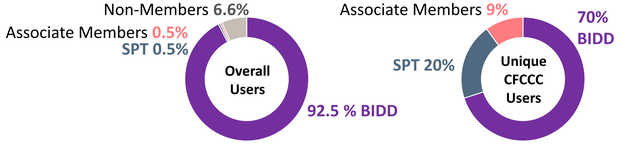


IVFOI collaborates with Dr. Barty's team to prepare a fully-automated, computer-controlled imaging set-up for LUMITRON's Tunable Monoenergetic X-ray Source (TMXS). The first step is to utilize this monoenergetic source for k-edge imaging. Images will be acquired just below and above K-edge of Gadolinium in tomographic fashion using mice bearing 4T1 breast cancer model. Imaging will be performed just before and after i.v. injection of Gd-DTPA and compared with MR images that will be acquired right after. Currently, the imaging interface is being developed together with a dedicated image reconstruction algorithm for this project to be able to achieve 3D tomographic imaging. The future plans include phase-contrast imaging as well as image guided therapy.

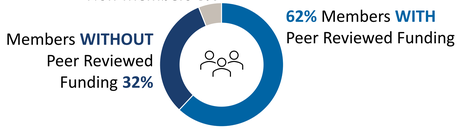
CCSG Metrics 1/1/22 – 12/31/22

Non-Members 38%

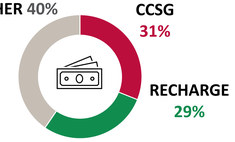
Use by CFCCC Program



Peer Reviewed Use

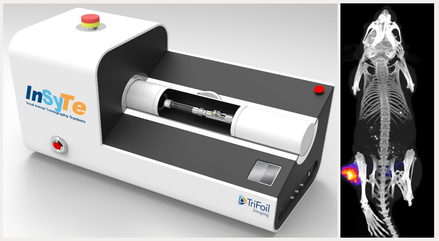


Operational Fund Sources



Key Equipment & Technologies

Our LA based industrial collaborator, TriFoil, Inc, installed one of their commercial X-ray CT/Fluorescence Tomography machine into **IVFOI**, which is now open to any cancer member user for free.



The TriFoil imaging platform and an example 3D fluorescence image of a 4T1 tumor bearing mice

Future Plans

- Planning an imaging workshop to show UCI researchers our imaging capabilities on site
- Establishing collaborations with two new companies, SMSBiotech (San Diego, CA) & ClearPointNeuro (San Diego, CA) to serve them with our fluorescence and MR imaging capabilities, respectively.
- Working with Farshid Dayyani, MD, PhD (**SPT**) & Maheswari Senthil, MD (**BIDD**) together with our industrial collaborators from OnTarget Laboratories to bring Phase I clinical trial of OTL410 to UCI. This is a fluorescent intra-operative contrast agent for colon and gastric cancers.
- Spending more effort on imaging with LUMITRON Tunable Monoenergetic X-ray Source to put UCI Cancer Center to the forefront of the innovative medical imaging research
- We are expanding our service area by helping/encouraging CFCCC members to utilize Artificial Intelligence (AI) in their research by collaborating with the UCI Center for Artificial Intelligence in Diagnostic Medicine (CAIDM).

Publications

CFCCC Investigator	Program	Published Journal	Year
Claudia Benavente, PhD	SPT	Oncogenesis	2022
Leslie Randall, MD	SPT	Journal of Clinical Oncology	2022
Nadine Abi-Jaoudeh,MD Vahid Yaghmai, MD Zhuoli Zhang, MD, PhD	BIDD BIDD BIDD	American Journal of Translational Research	2022
Min-Ying Su, PhD	BIDD	Frontiers in Oncology	2022

5 Experimental Tissue Resource

UCI | Chao Family Comprehensive Cancer Center | UCI Health

Leadership

Robert Edwards, MD, PhD
Co-Director

Wendy Cozen, DO, MPH
Co-Director

Delia Tifrea, MBA
Manager

Mission

To support the research mission across UC Irvine and the campus research community

- The goal of the **ETR** is to assist Chao Family Comprehensive Cancer Center investigators with tissue procurement, processing, and histopathology interpretation

Services

- Fresh and FFPE Tissue Procurement and Interpretive Histopathology Consultation
- Tissue Histology and IHC services
- Mouse Pathology services/consultation on mouse models of human disease
- Biorepository/tissue banking services, including a user-searchable de-identified database of archival tissue

INVENTORY (Available Samples)

TISSUE	SURGICAL RESECTIONS FFPE (# 5 years)	BIOPSIES FFPE (# 5 years)	FROZEN tissue
brain	589 (326)	3,006 (1297)	574
colon	2,204 (956)	23,352 (13,546)	189
pancreas	875 (266)	398 (211)	85
breast	5,808 (2,505)	1,211 (354)	110
uterus	899 (547)	237 (99)	209
ovary, adnexa	1,671 (713)	206 (77)	264
prostate	1,858 (602)	377 (239)	684
kidney	1,180 (422)	169 (78)	288
bladder	535 (288)	1,308 (513)	26
lung	285 (130)	177 (133)	9
TOTAL	328,987		4,583

SUPPORT PROVIDED (Annual)

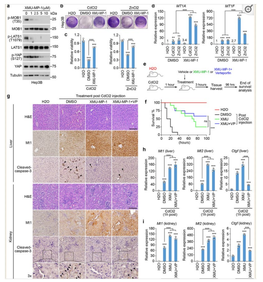
Clinical trials	87
Individual patients	850
Investigator-initiated trials	5
Basic research projects	43
ETR consultation for database, IRB, sample collection, protocol review	60

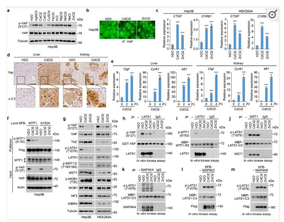
Cancer-related 65%

Research Supported

Example 1 | The Hippo pathway kinases LATS1 and LATS2 attenuate cellular responses to heavy metals through phosphorylating MTF1 – Wenqi Wang, PhD (SPT), *Nature Cell Biology* 2022

Hippo signaling deficiency promotes heavy metal response gene transcription and protects cells from heavy metal-induced toxicity, a process independent of its classic downstream effectors YAP and TAZ. The Hippo pathway kinase LATS phosphorylates and inhibits MTF1, an essential transcription factor in heavy metal response, with loss of heavy metal response gene transcription and cellular protection. LATS activity is inhibited upon heavy metal treatment, where accumulated zinc directly binds and inhibits LATS. The study reveals an interplay between the Hippo pathway and heavy metals, providing insights into this growth-related pathway in tissue homeostasis and stress response.

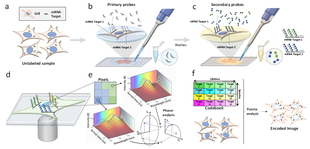




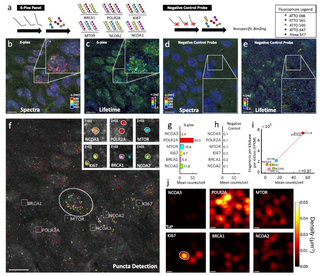
- Inhibition of the Hippo pathway promotes heavy metal detoxification
- The Hippo pathway is negatively regulated by heavy metals

Example 2 | Spatial transcriptomics using combinatorial fluorescence spectral and lifetime encoding imaging and analysis – Weian Zhao, PhD, *Nature Communications* 2022

A new spatialomics technology, termed Multi Omic Single-scan Assay with Integrated Combinatorial Analysis (MOSAICA), that integrates in situ labeling of mRNA and protein markers in cells or tissues with combinatorial fluorescence spectral and lifetime encoded probes, spectral and timeresolved fluorescence imaging, and machine learning-based decoding is described here. MOSAICA has multiplexing scalability in detecting 10-plex targets in fixed colorectal cancer cells using combinatorial labeling of five fluorophores with facile error-detection and removal of autofluorescence. MOSAICA's analysis is strongly correlated with sequencing data and was further benchmarked using RNAscopeTM and LGC StellarisTM. MOSAICA was applied for multiplexed analysis of clinical melanoma Formalin-Fixed Paraffin-Embedded (FFPE) tissues and simultaneous co-detection of protein and mRNA in cancer cells.



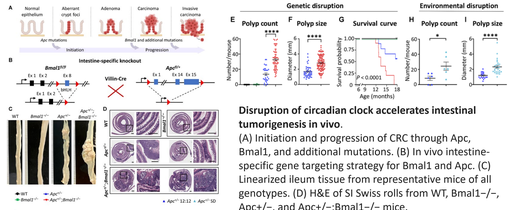
- Image and phasor analysis with spectrum and lifetime analysis in MOSAICA



- Multiplexed mRNA detection in epidermis region of human skin melanoma FFPE tissue.

Example 3 | Disruption of the circadian clock drives *Apc* loss of heterozygosity to accelerate colorectal cancer – Selma Masri, PhD (SPT), *Science Advances* 2022

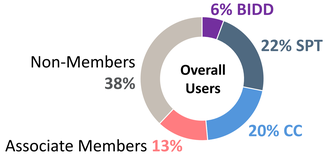
Suspected risk factors of young onset CRC include environmental aspects, is known to affect the circadian clock. Both genetic disruption and environmental disruption of the circadian clock accelerate *Apc*-driven CRC pathogenesis in vivo. Clock disruption promotes transformation by driving *Apc* loss of heterozygosity, which hyperactivates Wnt signaling. This up-regulates *c-Myc*, a known Wnt target, which drives heightened glycolytic metabolism. Using patient-derived organoids, this proves that circadian rhythms are lost in human tumors and that a variance between core clock and Wnt pathway genes significantly predicts the survival of patients with CRC.

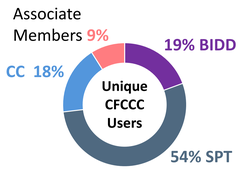


Disruption of circadian clock accelerates intestinal tumorigenesis in vivo.
(A) Initiation and progression of CRC through Apc, Bmal1, and additional mutations. (B) In vivo intestine-specific gene targeting strategy for Bmal1 and Apc. (C) Linearized ileum tissue from representative mice of all genotypes. (D) H&E of SI Swiss rolls from WT, Bmal1−/−, Apc+/−, and Apc+/−;Bmal1−/− mice.

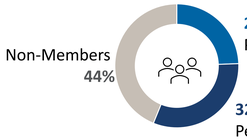
CCSG Metrics 1/1/22 – 12/31/22

Use by CFCCC Program

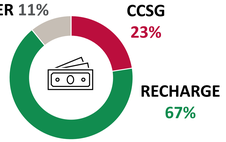




Peer Reviewed Use



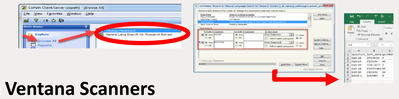
Operational Fund Sources



Key Equipment & Technologies

- **User-searchable database of FFPE archival tissue: 328,987 surgical cases and 2,255 autopsy cases**

  Queries of the database can be made back to 1984. The search platform returns Final Pathology Report, a research identifier that links back to the patient demographics (for retrieval by **ETR** staff operating as Honest Brokers), and other non-PHI-containing fields.



- **Whole Slide Ventana Scanners**
- **Ventana Discovery Ultra IHC/ISH Automated Slide Stainer**
- **Automated tissue microarray (TMA)** Cores from 0.5-2mm, up to 100 samples per block.

Future Plans

- To expand procurement of fresh specimens for clinical trialists and integration into clinical trials workflow.
- To include hospital-wide prospective informed consent for tissue donation for cancer patients undergoing surgical procedures and bone marrow aspirations for hematopoietic malignancies;
- Complete the dedicated **ETR** facility space for processing, annotation, and storage of high-quality solid organ and hematopoietic malignancy specimens, **with the goal of meeting CAP accreditation requirements for Biorepositories**
- Use the EMR-LIS integration platform Rhodes, to link surgical pathology specimen data with patient data to facilitate outcomes research
- Advertise the new services **automated tissue microarrays (TMA)** and **digital pathology analysis with VisioPharm software**

Publications

CFCCC Investigator	Program	Published Journal	Year
Anand Ganesan, MD, PhD	BIDD	Cell Reports	2022
Peter Kaiser, PhD	SPT	Cell Chemical Bio.	2022
Bogi Andersen, MD	SPT	J.Investig. Derm.	2022
Claudia Benavente, PhD	SPT	Oncogenesis	2022
Marian Waterman, PhD	SPT	Molecular Cancer Research	2022

6 Biostatistics Shared Resource

UCI | Chao Family Comprehensive Cancer Center | UCI Health

Leadership

Min Zhang, MD, PhD[2],
Director

Wen-Pin Chen, MS,
Facility Manager

Mission

The BSR provides a centralized resource of biostatistical expertise for the experimental design and analysis of basic, translational, clinical and population-based cancer research.

To fulfill this mission, the **BSR**:

- Initiates active participation during grant preparation in the areas of cancer etiology, genetics, detection, and prevention
- Partners on research design, qualitative and quantitative protocol features
- Incorporates existing and develops new statistical methods
- Provides guidance on sample size requirements

Services

Statistical Analysis
- Basic (manuscripts, grants)
- Advanced (project-oriented special study design and analysis)

Omics Data Analysis
- Genomic (SNP, WGS, WES) data analysis (including GWAS, PheWAS)
- Transcriptomic (microarray data, bulk RNA-seq) including eQTL
- Epigenetics (ChIP-seq; ATAC-seq)
- Single-cell omics
- Functional (pathway, GO)
- Metabolomics M
- Microbiome
- Radiomics and radiogenomics

Research Computing
- HIPAA-compliant computational needs, cloud computing technologies
- Setup and run computationally intensive jobs on Cloud
- Programming assistance
- Database design, creation and management

Training and Education (workshops, seminars, etc.)
- Annual NCI-funded workshop "*Big Data Training for Cancer Research*"

Consulting
- Bioinformatics & Biostatistics
- Database
- Machine learning
- Statistical/computational genetics & genomics

Research Supported

Example 1 | Adoptive T-Cell Therapy in Advanced Colorectal Cancer: A Systematic Review

Damie J. Juat , Stephanie J. Hachey , John Billimek , Michael P. Del Rosario, , Edward L. Nelson (BIDD) , Christopher C.W. Hughes (BIDD) , Jason A. Zell (CC)

Publication: *The Oncologist.* 2022; 27(3):210-219. PMC8914488
Funding: Supported by the Division of Hematology/Oncology, Department of Medicine, School of Medicine, University of California Irvine, and NIH/NCI (P30CA062203).

- For patients with advanced and unresectable CRC, treatment is palliative and typically involves chemotherapy, biologic therapy, and/or immune checkpoint inhibition. Adoptive T-cell therapy (ACT) could potentially be another option despite the concern of low immune infiltrate.
- BSR assisted in a systematic review of 15 published studies of ACT treatment of advanced CRC. The overall finding is that ACT shows favorable overall survival and progression-free survival estimates when compared to currently available agents in the second/third-line setting for metastatic CRC. However, while ACT is generally well tolerated in CRC patients, additional stage I/II clinical trials are needed to establish the efficacy and safety of ACT.

No. Paper	PFS median (range)	Censored, *n*	PFS rate, *n* (%)				Total patients, *n*
			3 months	6 months	1 year	2 years	
1	6.1 (0.6-35)	1	5 (83)	4 (67)	2 (33)	1 (20)	—
2	6.5 (3-29)	0	3 (75)	2 (50)	1 (25)	1 (25)	4
3	12 (6-36)	6	4 (80)	4 (80)	2 (40)	1 (20)	11
4	—	—	—	—	—	—	6
5	—	—	—	—	—	—	—
6	—	—	—	—	—	—	3
7	5.5 (5-6)	1	2 (100)	—	—	—	3
8	—	—	—	—	—	—	7
9	—	—	—	—	—	—	2
10	17.5 (7.5-28)	5	15 (100)	15 (100)	11 (79)	3 (25)	15
11	15 (8.3-21.6)	2	6 (100)	6 (100)	4 (67)	2 (33)	6
12	—	—	—	—	—	—	10
13	12 (5-24)	0	5 (100)	4 (80)	2(40)	—	5
14	—	—	—	—	—	—	—
15	—	1	—	—	– (89.5)	– (59.65)	21

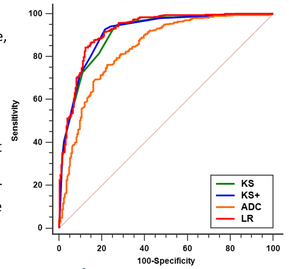
Example 2 | Assessment of breast lesions by the Kaiser score for differential diagnosis on MRI: the added value of ADC and machine learning modeling

Zhong-Wei Chen, You-Fan Zhao, Hui-Ru Liu, Jie-Jie Zhou, Hai-Wei Miao, Shu-Xin Ye, Yun He, Xin-Miao Liu, Min-Ying Su (BIDD), Mei-Hao Wang

Publication: *European Radiology.* 2022; 32(10):6608-6618. PMC9815725
Funding: Supported by the Key Laboratory of Intelligent Medical Imaging of Wenzhou (No. 2021HZSY0057), the Key Laboratory of Alzheimer's Disease of Zhejiang Province, Institute of Aging, Wenzhou Medical University, Wenzhou, Zhejiang, China, Wenzhou Science & Technology Bureau (No. Y20180185), the Medical Health Science and Technology Project of Zhejiang Province Health Commission (No. 2019KY102), the NIH/NCI (P30 CA062203, R01 CA127927, R21 CA208938), and the UC Irvine Comprehensive Cancer Center using UCI Anti-Cancer Challenge funds.

A Machine Learning approach was developed to train diagnostic models to differentiate between benign and malignant lesions. The approach compared four methods:
- **KS**: Kaiser Score (KS; a machine learning–derived clinical decision rule based on MRI BI-RADS)
- **KS⁺**: Kaiser Score + Apparent diffusion coefficient values (ADC; a quantitative measure of tissue diffusivity)
- **ADC** alone
- Logistic Regression (**LR**)

Conclusions:
- Using ADC to modify KS (KS+) can improve specificity, but it does so at the price of lowered sensitivity.
- Machine learning algorithms may be applied to consider the ADC as a continuous variable to build more accurate diagnostic models.

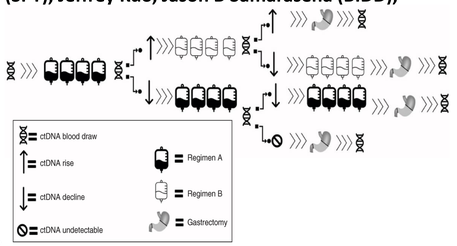


Example 3 | A phase Ib feasibility trial of response adapted neoadjuvant therapy in gastric cancer (RANT-GC)

Farshid Dayyani (SPT), Brian R Smith, Ninh T Nguyen, Shaun Daly, Marcelo W Hinojosa, Steven N Seyedin (SPT), Jeffrey Kuo, Jason B Samarasena (BIDD), John G Lee, Thomas H Taylor, May T Cho, Maheswari Senthil (BIDD)

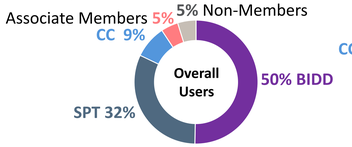
Publication: *Future Oncology.* 2022; 18(21):2615-2622. PMC9437768
Funding: Supported by the UC Irvine Comprehensive Cancer Center using Anti-Cancer Challenge funds and Natera, Inc.

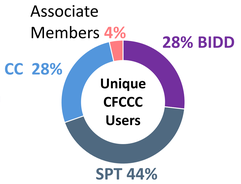
Current guidelines recommend neoadjuvant (NAC) and/or adjuvant chemotherapy for locally advanced gastric cancers (LAGCs). However, the choice and duration of NAC regimen is standardized, rather than personalized to biologic response, despite the availability of several different classes of agents for the treatment of gastric cancer (GC). The current trial will use a tumor-informed ctDNA assay (Signatera™) and monitor response to NAC. Based on ctDNA kinetics, the treatment regimen is modified. This is a prospective single center, single-arm, open-label study in clinical stage IB-III GC. ctDNA is measured at baseline and repeated every 8 weeks. Imaging is performed at the same intervals. The primary end point is the feasibility of this approach, defined as percentage of patients completing gastrectomy.



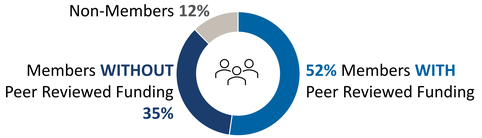
CCSG Metrics 1/1/22 – 12/31/22

Use by CFCCC Program

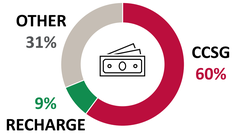




Peer Reviewed Use



Operational Fund Sources



Key Equipment & Technologies

- SAS® software Version of 9.4
- R package
- StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC.
- PASS 2021 Power Analysis and Sample Size Software (2018). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass.
- nQuery 8. Sample Size and Power Calculation. "Statsols" (Statistical Solutions Ltd), Cork, Ireland.

Future Plans

Community Engagement / Catchment Area
- Continue to support the development of manuscripts and grants that focus on the catchment area and result from partnerships developed through COE efforts;
- Continue to provide consulting services on bioinformatics, biostatistics, database access, data integration;
- Expand new services on machine learning, statistical genetics and genomics, research computing to facilitate interdisciplinary collaborations in catchment area.

Enhancing Diversity, Equity and Inclusion
- Offer scholarships for underrepresented trainees to attend the NCI-funded big data workshop;
- Develop new machine learning methods to improve the analysis of data from minority populations.

Education and Training
- Organize the annual NCI-funded summer workshop on "*Big Data Training for Cancer Research*";
- Offer regular need-based workshops on basic statistical analysis, workflow for sequencing data analysis, FAIR computational workflows on the cloud;
- Organize regular seminar series to provide education opportunities for trainees.

Publications

CFCCC Investigator	Program	Published Journal	Year
Claudia Benavente, PhD	SPT	Oncogenesis	2022
Edward Nelson, MD Christopher Hughes, PhD Jason Zell, DO, MPH	BIDD BIDD CC	The Oncologist	2022
Daniela Bota, MD, PhD Xiao-Tang Kong, MD	BIDD CC	Frontiers in Oncology	2022
Robert Edwards, MD, PhD Marian Waterman, PhD	SPT SPT	Molecular Cancer Research	2022