GREGORY FLEMING JAMES CYSTIC FIBROSIS RESEARCH CENTER

Director: Steven M. Rowe, MD, MSPH
https://www.uab.edu/medicine/cysticfibrosis/

The Gregory Fleming James Cystic Fibrosis (CF) Research Center was established in 1981 at UAB as a multidisciplinary Center to study CF basic research and therapy.

Also in 1981, the CF Foundation began an initiative for funding CF research known as the Research Development Program (RDP). The UAB Center was the first to receive RDP support from the Foundation, which now sponsors 10 such Centers in the United States. The Center has since maintained continuous CF Foundation and NIH funding, which has been supplemented by University Wide Interdisciplinary Research Center (UWIRC) and other important funding from the University and the State of Alabama.

Together, this support has fostered the Center's sustained legacy of advancing scientific success in CF, which was recognized through the recent renewal of its NIDDK P30 funding (1 of only 3 Centers selected nationwide) and RDP funding. Multidisciplinary collaborations across UAB and beyond--cultivated in part by the unique and cutting-edge facilities, techniques, and resources offered through the Center's 6 Cores--have been fundamental in driving these achievements and are a defining feature of our Center.

The CF Care Center located at the Children's Hospital of Alabama and UAB Hospital provides state-of-the-art care for approximately 500 CF patients, generating additional partnerships across departments and specialties.

Functional Assay Core

James F. Collawn, PhD

Monitoring of ion transport in CF and control cells and tissue

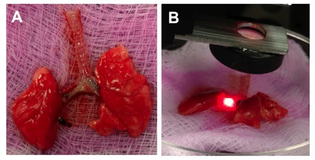
- Ussing chamber analyses
- Transepithelial chloride conductance (TECC) using robots that calculate changes in conductance or equivalent current in high-throughput formats

Measurements of mucociliary transport and fluid flux

- µOCT imaging of freshly harvested trachea with or without further experimental exposures ex vivo
- Quantitation of organoid swelling derived from airway cells, intestinal organoids, and iPS-derived spheroids

Patch clamp and single-channel analyses to study changes in CFTR channel gating and regulation

- Patch clamp unitary conductance tracings to monitor open channel probability and test potentiators (or correctors) on a wide spectrum of CF mutations. Mutagenesis evaluations can be coupled to uncover mechanisms
- Macropatch of cells or excised tissues or fluorescent dye-based halide efflux methods to evaluate CFTR activity in cells grown on coverslips (SPQ assays) or isolated from excised tissues



MCT measured in situ. A-B: CF rat lung dissected in situ (A) and under µOCT imaging (B). C: MCT rates after 2 weeks of nebulized treatment with the novel mucolytic PAAG, vehicle control, as compared to untreated WT control. *P<0.05, ***P<0.001, See Fernandez et al.

Gene Expression Core

Lianwu Fu, PhD

Cloning of new CFTR constructs and other CFTR regulatory proteins

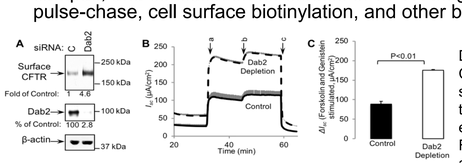
- Provided across widely used durable cell types (eg, FRT, Calu-3) and airway-specific cell expression systems emerging in their importance (eg, 16HBE14o-, CFBE41o-
- Viral and plasmid-based protocols

Creation of cell lines for investigating CFTR biology and therapy

- Large variety of plasmid-based CFTR vectors: CFTR-GFP fusions, individual CFTR domains, eukaryotic expression vectors, glycosylation mutants, epitope tagged CFTR, and over 50 clinical CFTR mutations or small deletions

Assistance with studies of precise detection of CFTR mRNA and protein expression

- Assays include PCR, Western blotting, digital PCR, RNAscope
- Maintains sh/siRNA and antisense oligonucleotide capabilities for knock-down and gene editing of CFTR and other cellular targets relevant to CF pathogenesis
- Provides a bridge to collaboration with single-cell RNA sequencing and analysis on campus, and consultation for studies of CFTR biogenesis (eg, immunoprecipitation, pulse-chase, cell surface biotinylation, and other biochemical means



Dab2 protein was depleted in CFTR- expressing CFBE41o- cells by siRNA oligos, and CFTR cell surface expression and function were measured through biotinylation (A) and Ussing chamber experiments (B and C), respectively. a, 10µM Forskolin; b, 10µM VX-770; c, CFTR Inh172 was added as indicated.

CF Clinical & Translational Assay Core

Steven Rowe, MD, MSPH and Amit Gaggar, MD, PhD

Designs and conducts in vivo measurements of CFTR activity in humans

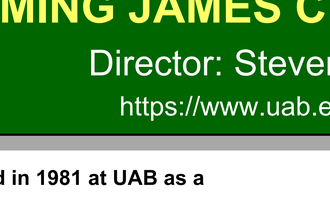
- Nasal potential difference (NPD)
- PD measurements of the lower airway and sinus tract (by the endoscopic approach)
- Sweat chloride analysis / sweat rate
- Rectal intestinal current measurements

Conducts cardinal measures of airway epithelial cell function in vivo

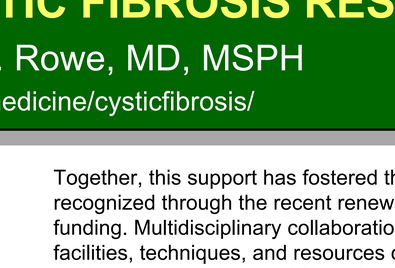
- µOCT imaging for in vivo use by endoscopic probes
- Whole lung MCC by Tc99 clearance approach
- Mucus rheology and solid content

Supports the execution of CF clinical studies

- Clinical trial design and regulatory support
- Collection and storage of biospecimens
- Supports key clinical outcome measures in infants, children, and adults with CF (nutritional outcomes, spirometry, lung clearance index (LCI), and infant PFTs)



µOCT functional anatomy in a live healthy vs. CF patient. µOCT provides simultaneous measurements of airway surface liquid and periciliary layer thickness, ciliary beat frequency, mucociliary transport rate, and mucus viscosity at 1-micron resolution without need for tissue manipulation.



Schematic of the nasal potential difference apparatus (*Chest 138:919, 2010*).

CFTR Rat Models Core

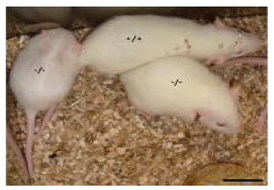
Susan E. Birket, PharmD, PhD

Breeds, genotypes, and distributes diverse CF rat models and their tissues

- CFTR+/-, hG551D, and G542X rats, in addition to maintenance of WT Sprague Dawley

Provides state-of-the-art endpoints in CF rat models with and without infection to elucidate disease mechanism, analyze pathways, or predict clinically relevant findings

- CTFR physiological outcome measures (NPD, sweat secretion)
- Assays of lung structure and function (micro-CT imaging, Flexivent)
- Collection and banking of biospecimen (survival bronchoscopy/BAL, blood, tissues)
- Systems to bridge studies from in vitro to in vivo use, including rat tracheal bronchial epithelial cell culture, nasal epithelial cell culture, and airway spheroid cultures



Sprague Dawley $Cftr^{+/+}$ (larger) and $Cftr^{-/-}$ (smaller) rats at post-natal day 24 characterized in the CF animal models core. This model displays several phenotypic traits characteristic of human CF disease.

Cell Model and Evaluation Core

George Solomon, MD and Bradford Woodworth, MD

Procures, grows, and distributes well-differentiated primary human airway epithelial cells from CF and non-CF donors

Conducts functional anatomic imaging of airway epithelia by µOCT in vitro and ex vivo

- Well-differentiated primary epithelial cells (of human or non-human origin)
- Intact full-thickness trachea or mainstem bronchi of human origin

Performs measures of CFTR activity and expression in primary cells

- Ussing chamber analyses, high-throughput evolution by equivalent and transepithelial conductance (Gt) and equivalent current (IEQ)
- Western blot, digital mRNA analysis utilizing primary cells on permeable supports

CF Animal and Preclinical Models Core

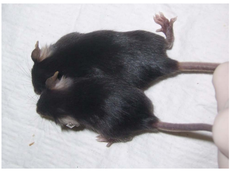
David M. Bedwell, PhD

Breeds, genotypes, and distributes CF knockout mice and CF mice harboring clinicallly relevant mutations

- $Cftr^{tm1Unc}$, $Cftr^{tm1Cam}$, $mCftr^{F508del\ Erasmus}$, $Cftr^{G551D}$, $Cftr^{G542X}$ and others

Generates and procures relevant CF animal models

- Includes analysis and procurement of pig, ferret (and very recently, CF rabbit tissues), and maintains a colony of ferrets to evaluate acquired CFTR dysfunction and therapeutic approaches



Cftr knock-out (smaller) and a wild type (larger) mice bred in the CF mouse models core.

Conducts endpoint measures to assess CFTR Function, epithelial physiology, preclinical endpoints, and biospecimen analysis in CF animal models

- Nasal and lower airway potential difference
- Short circuit current (Isc) measurements of excised trachea and intestine
- Measurement of lung function (Flexivent)
- In vivo and ex vivo µOCT imaging
- 6-Voxel resolution computed tomography
- Abdominal ultrasound
- Cough monitoring
- Glandular CFTR assay
- Electroporation-mediated gene manipulation
- Miniaturized bronchoscopy for longitudinal lung sampling
- Anesthesia, physiologic monitoring, intubation for exposure and assessment procedures

Other diseases related to CFTR defects studied in UAB CF cores

- COPD (chronic obstructive pulmonary disease)
- ABPA (allergic bronchopulmonary aspergillosis)
- IB (Idiopathic bronchiectasis)
- CRS (chronic rhinosinusitis)
- Primary Ciliary Dyskinesia
- Asthma
- Chronic Pancreatitis
- Cholera
- Other enterotoxigenic diarrheal diseases
- CBAVD (congenital absence of the vas deferens, male infertility)
- PKD (polycystic kidney disease)
- IPF (idiopathic pulmonary fibrosis)