Basic and Translational Research Division

Interim Scientific Director (since 3/2015): John Kappler, PhD
Distinguished Professor of Immunology & Microbiology

Head: Howard Davidson, PhD
Associate Professor of Pediatrics and Immunology & Microbiology

The BDC Research Division gains insight into the pathogenesis of type 1 diabetes (T1D) defined at the cellular and molecular level. A biomedical resource core services the basic and clinical research to monitor the effectiveness of therapeutic interventions and disease recurrence. These interventions are/will be based upon a mechanistic understanding of immune-based therapies and will work synergistically with the basic investigation of T-cell function incorporated into many of these studies. A major strength of the BDC is the breadth of work studying T-cell recognition and biology, genetics and the biology of inflammatory cytokine mediators. These studies are greatly strengthened by the unique clinical resource of ongoing studies of newborns from the general population and relatives of patients with T1D who are evaluated prospectively for the development of the antibodies associated with T1D and the development of clinical diabetes as well as the great number of new onset patients willing to participate in research (>300/yr). Ongoing trials implement antigen-specific therapies, including the use of small molecules aimed at restoration of immune tolerance.

Dr. Richard Bennigner Laboratory explores the dysfunction of the islet of Langerhans during the development of diabetes. The 4 main ongoing projects are; 1) Understanding emergent multicellular properties of the islet and how sub-populations of cells exert disproportionate control over the regulation of insulin secretion, including exacerbating the action of gene mutations that cause neonatal diabetes; 2) Understanding the interactions between islet function and islet autoimmunity during the development of T1D to protect the islet against immune-mediated decline; 3) Understanding the disruption to multicellular properties of the islet, with a focus on gap junction channels, during the development of T2D; 4) Developing new imaging approaches to non-invasively quantify the decline in islet function and beta cell mass during the development of T1D, allowing early clinical interventions and monitoring.

Dr. Howard Davidson Laboratory (now amalgamated with Dr. Hutton’s former Laboratory) has a long-standing interest in the molecular cell biology of insulin secretion and the biochemical composition and the process of biogenesis of the insulin granule. Many proteins involved in insulin secretion are also autoantigens in type 1 diabetes (eg IGRP, IA-2, phogrin, and ZnT8) and are being studied in both contexts. In particular the laboratory is interested in determining how the functional and cell biological properties of IA-2, IA-2δ (phogrin), IGRP and ZnT8, might contribute to their being targeted by the immune system, and whether post-translational modifications unique to the beta cell create “neo-antigens” from them that are not subject to central tolerance. There is also a long-standing research interest in the basic cell biology of antigen processing and presentation, particularly in B lymphocytes, and how this might be harnessed for the development of novel antigen-specific therapies that might be applicable to the treatment and/or prevention of T1D.

Dr. Peter Gottlieb Laboratory is focusing on improving our ability to detect and understand human immune responses in T1D. Central to this work has been the use of ELISPOT analysis to characterize the antigen specificity and inflammatory potential of these responses. He has been a member of the IDS T Cell Workshop Steering Committee and has collaborated to improve
detection of antigen-specific CD4 and CD8 T cell responses using ELISPOT, tetramers and other methodologies. His work focuses not only on using existing technology, but also collaborating with Drs. Love and Wucherpfennig to examine T cell response using unique microwell systems, which can detect single cell response and interrogate them in multiple assays simultaneously. Lastly, Dr. Gottlieb collaborates with Dr. Danny Zipris to understand the role of the microbiome in human T1D as well as of the innate immune system which appears to be activated early in disease and may be contributing to the world-wide rise in T1D. He also has initiated studies on the role of B lymphocytes in human T1D with Dr. Cambier which have detected a loss in anergic B cells during the development of autoimmune diabetes.

Dr. John Kappler Laboratory Dr. Kappler works closely with the Michels and Nakayama laboratories. In March 2015, upon accepting the position of the BDC interim Scientific Director, Dr. Kappler opened his own laboratory at the Barbara Davis Center that pursues studies of the trimolecular complex (MHC-TCR-autoantigen) in the pathogenesis of T1D in humans.

Dr. Aaron Michels Laboratory (previously Dr. George Eisenbarth Laboratory) develops technology to prevent or cure diabetes in humans through a precise knowledge of pathogenesis in animal models. Their studies indicate that insulin is the primary autoantigen whose targeting by the immune system leads to diabetes. Thus eliminating this abnormal response to insulin will be key for prevention. Trials with molecules such as the B:9-23 peptide require a more basic understanding of the cells mediating disease, as we now know that administration of B:9-23 dependent upon the route of administration can either prevent or inhibit development of diabetes.

Dr. Maki Nakayama Laboratory explores antigen specificity and function of autoreactive T cells. It also studies the role of T cells expressing specific TCRs in the development of T1D using an animal model and more recently human T cells isolated from pancreas and lymph nodes. It pursues the potential of TCR sequences to be used as T cell biomarkers to predict the development of T1D as well as recurrence of hyperglycemia after clinical therapeutic trials. They also explore the mechanism of transplantation failure in T1D patients.

Dr. Liping Yu Laboratory (previously Dr. Eisenbarth Laboratory) is the international reference laboratory for measurement of islet autoantibodies. It serves as the core laboratory for Type 1 Diabetes TrialNet, The Environmental Determinants of Diabetes in the Young (TEDDY) and the Immune Tolerance Network (ITN) consortia. TEDDY and TrialNet are described below (pgs. 37, 41). ITN solicits, develops, implements and assesses clinical strategies and biological assays for the purpose of inducing, maintaining, and monitoring tolerance for kidney and islet transplantation, and autoimmune diseases. Dr. Yu Laboratory also develops technology to predict diabetes in humans. In collaboration with the DAISY study, the laboratory has defined the genes associated with childhood diabetes. Type 1 diabetes is associated with other autoimmune diseases, mostly thyroid, celiac and Addison’s disease. This laboratory helps to routinely screen all BDC patients for the associated autoimmune conditions.

Dr. Danny Zipris Laboratory is focused on understanding how microbial infections and the innate immune system promote the development of T1D. The lab is testing the hypothesis that upregulation of proinflammatory pathways shortly after virus infection plays a crucial role in islet destruction. We have identified a number of innate immune modulators, such as steroids, antibiotics, and blockers of IL-1 and histone deacetylases that can protect animals from beta cell destruction. The laboratory also pursues studies of the influence of gut microbiome on the innate and adaptive immune mechanisms involved in the pathogenesis of T1D.
BASIC AND TRANSLATIONAL RESEARCH DIVISION

Richard Benninger, PhD
Assistant Professor of Bioengineering
Dr. Benninger joined the BDC faculty in 2011. Main goals of his research include understanding novel signaling pathways in the islet of Langerhans that enhance the regulation of hormone secretion; how disruptions to these signaling pathways cause islet dysfunction in diabetes; and how we can manipulate these signaling pathways to improve islet function towards developing new treatments for individuals with diabetes. He is utilizing state-of-the-art quantitative fluorescence microscopy, including two-photon microscopy, fluorescence lifetime imaging, polarization imaging and FRET in studying pancreatic islet dysfunction in diabetes. Dr. Benninger has developed an integrative model of how different cell-cell communication mechanisms dynamically interact within the islet. His lab has gained understanding into how this impacts in-vivo islet function and glucose homeostasis and is now demonstrating that gap junction channels can be modulated to improve islet function and insulin secretion in models of diabetes. Overall his work applies sophisticated quantitative techniques and predictive quantitative models to link emergent multi-cellular properties of the islet of Langerhans to in vivo physiology and diabetes, and test novel hypotheses regarding these properties that may be clinically important.

Howard Davidson, PhD
Associate Professor of Pediatrics and Immunology & Microbiology
Head of Basic & Translational Research Division
Dr. Davidson joined the BDC faculty in 2002. The ultimate goal of his research is to develop improved methods for measuring autoimmunity in type 1A diabetes, and to identify reagents that might have therapeutic utility for the prevention and/or treatment of this disease. Currently these studies focus principally on defining epitopes targeted by the cellular and humoral arms of the immune system in the major autoantigens zinc transporter 8 (ZnT8) and preproinsulin and developing improved bioassays for monitoring disease risk and therapeutic efficacy based upon this information. He also has a long-standing research interest in the basic cell biology of antigen processing and presentation, particularly in B lymphocytes, and is investigating how post-translational modifications to beta cell proteins influence how they are targeted by the immune system, and the roles that beta cell stress may play in the immunological events that eventually lead to the development of autoimmune diabetes.

Pamela Fain, PhD
Professor Emerita
Dr. Fain studied the genetics of type 1 diabetes, vitiligo and other autoimmune diseases with an emphasis on determining the relationship of these disorders with each other and with HLA, and other disease susceptibility genes. She served as the Director of Genotyping Mutation Screening Core Facility for the Human Medical Genetics Program. Dr. Fain has published more than 100 original articles in peer-review journals.

John Kappler, PhD
Interim Scientific Director (since 3/2015) of Basic and Translational Research Division
Distinguished Professor of Immunology & Microbiology
Dr. Kappler has collaborated with BDC investigators for the past six years and assumed the position of Interim Scientific Director of the Basic and Translational Research Division in March of 2015. He has been a Howard Hughes Medical Institute investigator since 1986 and a member of the National Academy of Sciences since 1989. The Kappler-Marrack Research Lab at National Jewish Health will remain his primary laboratory. He co-directs that lab and has shared multiple discoveries with his
wife, Philippa Marrack, PhD. Drs. Kappler and Marrack were the first to isolate the T cell receptor. Together they have contributed extensively to our understanding of the nature of antigen processing and major histocompatibility complex (MHC)-restricted peptide presentation. They have explored positive and negative selection of T cells in the thymus, T cell superantigens and the evolutionarily conserved structural relationship between T cell receptors and MHC molecules. At the Barbara Davis Center, Dr. Kappler will continue collaborations with investigators on the nature of the peptide/MHCII complexes that drive islet autoimmunity leading to type 1 diabetes.

**Maki Nakayama, MD, PhD**  
**Assistant Professor of Pediatrics**  
Dr. Nakayama joined the BDC faculty in 2009. Her research strives to understand the mechanism of initiation of anti-beta cell autoimmunity. She focuses on the tri-molecular complex consisting of antigen, major histocompatibility complex (MHC), and T cell receptor (TCR) that could be a key component for the development of T1D. Her laboratory explores antigen specificity of autoreactive T cells having different functions (i.e. pathogenic vs regulatory T cells) that target pancreatic beta cells; the role of T cells expressing specific TCRs in the development of T1D using an animal model; the potential of TCR sequences to be used as T cell biomarkers to predict the development of T1D as well as recurrence of hyperglycemia after clinical therapeutic trials; lastly, exploring the mechanism of transplantation failure in T1D patients. Dr. Nakayama has been part of the JDRF-Helmsley nPOD missions, which is an international network characterizing pancreata from cadaveric organ donors with T1D.

**Tomasz Sosinowski, PhD**  
**Instructor of Pediatrics**  
Dr. Sosinowski’s research focuses on understanding the role T cells play in the development and progression of type 1 diabetes (T1D). Dr. Sosinowski studies immune responses to proinsulin, and in particular on the regions of the beta chain (residues 9-23) and C-peptide (residues 41-62) previously shown by others to be presented by HLA-DQ2, DQ8, and the DQ8/DQ2 trans-dimer. Currently he has two projects: 1) Development of a humanized Mouse Model for preclinical testing of agents that target components of human tri-molecular complexes (e.g. small molecules and antibodies); and 2) Creation of an improved Biomarker Assays based on standardized artificial Antigen Presenting Cells (aAPCs) that selectively and specifically enumerates T cells recognizing proinsulin-MHC complexes. Such improved biomarker assays are urgently needed for more accurate monitoring of therapeutic efficacy during clinical interventions.

**Liping Yu, MD**  
**Research Assistant Professor of Pediatrics**  
Dr. Yu joined the BDC faculty in 2011. He directs a clinical immunology laboratory which is NIH/NIDDK designated North America Autoantibody/HLA Core laboratory. Dr. Yu developed several autoantibody assays that demonstrate high sensitivity and disease specificity with applications in national/international type 1 diabetes clinical trials and screening projects. Dr. Yu also has studied celiac disease through transglutaminase autoantibody testing and Addison’s disease using 21-hydroxylase autoantibodies.

**L Zhang, MD, PhD**  
**Instructor of Pediatrics**  
Dr. Zhang investigates insulin reactive T cell receptor repertoire to explore the role of insulin beta chain usage of pathogenic T cell receptors. She discovered insulin B:9-23 responsive gamma/delta cells in NOD mouse. In collaborating with Dr. John Kappler group, she developed a therapeutic way to
prevent diabetes with immunization of specific antigen-MHC complex. She is developing an antigen based therapeutic monoclonal antibody to prevent and stop the autoimmune progression of beta cell destruction by disturbing the process of autoantigen presentation relying on structural knowledge of how peptides are presented.

Danny Zipris, PhD
Associate Professor of Pediatrics
Dr. Zipris joined the BDC faculty in 2007. His laboratory investigates how microbial infections and the innate immune system promote the development of type 1 diabetes (T1D). Studies conducted in the BBDR and LEW1.WR1 rat models of Kilham Rat Virus (KRV)-induced diabetes have led to the hypothesis that the upregulation of proinflammatory pathways shortly after virus infection plays a crucial role in the course of islet destruction. A number of innate immune modulators have been identified, e.g., steroids, antibiotics, and blockers of IL-1 and histone deacetylases that can protect animals from beta cell destruction and mechanisms involved in disease amelioration are currently being investigated. Dr. Zipris also pursues the interactions between the gut microbiota and the innate immune system in islet autoimmunity. These studies are likely to advance the knowledge about early disease mechanisms and may lead to prevention of human diabetes.
<table>
<thead>
<tr>
<th>Project Name</th>
<th>PI Name</th>
<th>Direct Cost</th>
<th>Sponsor Name</th>
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<td>Multi-cellular interactions and dynamics underlying insulin secretion</td>
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<td>Multi-cellular interactions and dynamics underlying insulin secretion</td>
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<td>Zeiss 2-photon (2P) LSM780 laser scanning confocal microscope</td>
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<td>Mapping the histopathological landscape of type 1 diabetes: a pilot study</td>
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<td>Post-Translation Modification of B-cell Granule Autoantigens</td>
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<td>Characterization of Diabetes Subsets in Relation to Markers of Viral Infection</td>
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<td>nPOD-Autoantibody Core Activities</td>
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<td>36,364</td>
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<td>Impact of MHC Genotypes on ex vivo T Cell Function in Type 1 Diabetes</td>
<td>Gottlieb</td>
<td>548,061</td>
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<td>Trialnet: Diabetes Type 1 Prevention</td>
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<td>Aralast NP in the Treatment of Patients with Type 1 Diabetes</td>
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<td>AWD 123152-MODO3</td>
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<td>Phase 1 Study to evaluate multiple ascending doses of PF-06342674 (RN168)</td>
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<td>GLEEVEC</td>
<td>Gottlieb</td>
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<td>IDS_TCW HLA Class 1 Combinatorial Multimer Study</td>
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<td>Small Molecules Targeting Allele Specific MHC II Autoantigen</td>
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<td>Small Molecules Targeting Allele Specific MHC II Autoantigen</td>
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<td>Evaluation of Insulin-Specific T Cells and Autoantibody Isotypes in T1D</td>
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<td>The development of Cell Based Assay(s) …</td>
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<td>Effect of Alpha-Methyldopa on MHC Antigen Presentation in T1D</td>
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<td>Prevention of Type 1 Diabetes by targeting the Trimolecular Complex</td>
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<td>Randomized Control Trial</td>
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<td>Molecular Dissection of Antigen Targeting in Anti-Islet Autoimmunity</td>
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<td>Identification of Autoantigens for Islet-Specific Pathogenic and regulatory T cells</td>
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<td>Monoclonal antibody targeting Thered/Register-fixed primary peptide</td>
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<td>Monoclonal antibody targeting Thered/Register-fixed primary peptide</td>
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<td>The Role of the Microbiome in the Development of Autoimmune Diabetes</td>
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### Fellows 2013 - 2014

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<th>Faculty Mentors</th>
<th>Title of Research Project</th>
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<td>A. Piya, MD</td>
<td>A. Michels, MD</td>
<td>Small Molecules Targeting Insulin Specific T cell Receptors</td>
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<tr>
<td>K. Simmons, MD</td>
<td>A. Michels, MD</td>
<td>General population screening for type 1 diabetes risk</td>
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### Postdoctoral Trainees 2013 - 2014

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<td>N. Farnsworth, PhD</td>
<td>R. Benninger, PhD</td>
<td>Regulation of islet gap junction coupling and function under inflammatory conditions</td>
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<td>C. Kiekhaefer, MD, PhD</td>
<td>A. Michels, MD</td>
<td>Immune Stimulatory Small Molecules in Type 1 Diabetes</td>
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<td>B. Murphy, PhD</td>
<td>H. Davidson, PhD</td>
<td>Post-translation modification of Beta-cell granule autoantigens</td>
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<td>Z. Zhao, MD</td>
<td>L. Yu, MD</td>
<td>Expanding the existing ECL autoantibody technology</td>
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### Career Development Awards 2013-2014

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<td>Aaron Michels, MD</td>
<td>Structure-Based selection of small molecules targeting allele specific MHC II autoantigen presentation</td>
<td>NIH K08 DK095995</td>
<td>06/01/2012 - 05/31/2017</td>
</tr>
<tr>
<td>Maki Nakayama, MD</td>
<td>Structure Pepetide/Conserved T Cell Receptor Determining Diabetes</td>
<td>NIH R00 DK080885</td>
<td>04/01/2010 - 03/31/2013</td>
</tr>
<tr>
<td>Li Zhang, MD, PhD</td>
<td>Monoclonal Antibody targeting MHC Tethered/Register-Fixed Primary Pepetide</td>
<td>JDRF 10-20110138</td>
<td>03/01/2011 - 02/28/2014</td>
</tr>
</tbody>
</table>

### Junior Investigator Fellowships 2013-2014

The BDC offers two fellowships to support outstanding junior investigators as they establish independent research careers.

**O’Brien Fellow**
2012-2013  Melanie Green, MD, PhD

**Kovler Fellows**
2012-2013  Melanie Stumpf, PhD
2013-2014  Nikki Farnsworth, PhD
Excellence & Leadership 2013 - 2014

Li Zhang, MD, PhD
2013 The 13th Immunology of Diabetes Society (IDS) Travel award
sponsored by the Juvenile Diabetes Research Foundation

PATENTS
Yu, Liping, MD
Insulin Autoantibodies. Inventors: George Eisenbarth, Liping Yu.
Publication Date: 1/10/2013

Li Zhang, MD, PhD
2014 United States Patent Number: 8673300. Title: Therapeutic
Compositions and Methods for the Prevention of Autoimmune Diseases.
Inventors: George Eisenbarth, Li Zhang, John Kappler, Brian Stadinski.
Issue Date: 3/18/2014

Immunology/Autoimmunity Journal Club
In this Journal Club students and trainees, as well as more senior investigators, provide a
presentation of a new article or articles including a review of the topic and inclusions of pertinent
literature and how the reviewed article(s) fit into/advance current thought and where the field
might go from here. This popular program benefits participants who come from many areas of the
Anschutz Medical Campus, especially post-docs and junior faculty. The inclusion of many senior
faculty encourages exchange of ideas and provides knowledgeable comments and expertise on
the papers presented.

Research in Progress Series
The Research in Progress series fosters collaborative learning through weekly presentations by
BDC and affiliated investigators. Coordinated by Dr. Richard Benninger, the series provides over
20 presentations per year to interested participants.


38. Zhang L, Londono P, Yu L, Grimes S, Blackburn P, Gottlieb P, Eisenbarth GS. MAS-1 adjuvant immunotherapy generates robust Th2 type and regulatory immune responses providing long-term protection from diabetes in late-stage pre-


STAFF ROSTERS

Research Division

Aimon Alkanani
Professional Research Assistant

Taylor Armstrong
Sr. Professional Research Assistant

Sunanda Babu
Research Associate

Stephanie Case
Professional Research Assistant

Christine Collins
Professional Research Assistant

My Linh Dang
Professional Research Assistant

Nikki Farnsworth, PhD
Post-Doctoral Fellow

Lisa Fitzgerald-Miller
Professional Research Assistant

Tyler Fouts
Student Assistant

Kathryn Gray
Research Services Sr. Professional

Michelle Guyer
Professional Research Assistant

Naoko Hara
Professional Research Assistant

Anita Hohenstein
Sr. Professional Research Assistant

Rebekah Howison
Professional Research Assistant

Assistant
Ling Jiang
Professional Research Assistant
Carol Kiekhaefer, MD, PhD
Post-Doctoral Fellow
Laurie Landry
Professional Research Assistant
Yu Liu
Visiting Professor
Yalin Lu
Student Assistant
Kristen McDaniel
Professional Research Assistant
Dong Mei Miao
Sr. Research Associate
Brittany Murphy, PhD
Post-Doctoral Fellow
Kimanh Nguyen
Student Assistant
James Needell
Professional Research Assistant
Mallory O'Malley
Student Assistant
Bryan Pham
Student Assistant
Phillip Pratt
Professional Research Assistant
Reese Prussin
Professional Research Assistant
Marynette Rihanek
Professional Research Assistant

Joshua St Clair, PhD
Post-Doctoral Fellow
Melanie Stumpf
Hermreck, PhD
Post-Doctoral Fellow
James Tobin
Student Assistant
Thanh Tran
Professional Research Assistant
Jay Walters
Sr. Professional Research Assistant
Randy Wong
Sr. Professional Research Assistant
Zhiyuan Zhao, MD
Post-Doctoral Fellow