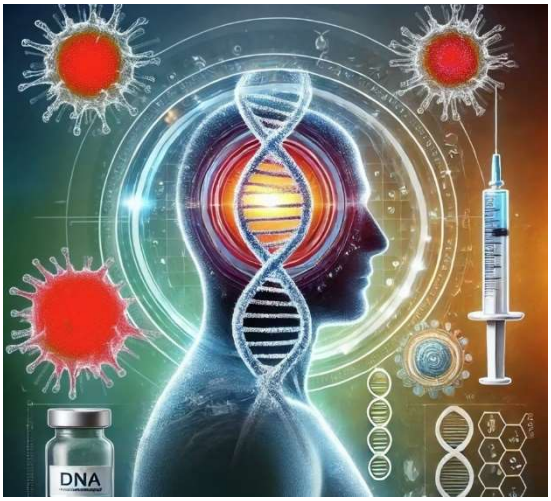
HBIGS
Heidelberg Biosciences International Graduate School

Summer School for PhD students

Sept 15th – Sept 18th, 2024 in Boppard am Rhein

"THE ERA OF HUMAN GENE THERAPY – PROSPECTS AND CHALLENGES"

Our aim is to train and educate a new generation of young scientists in the exciting and rapidly growing field of human gene therapy, and to foster their interaction by establishing world-wide networks and critical infrastructure. The 2024 HBIGS Summer school addresses this need by bringing together international leaders in relevant research fields and by enabling the participating PhD students to directly and actively interact with these experts as well as with each other. The invited speakers not only comprise leaders in biological sciences, such as the two organizers who have collaborated and contributed to gene therapy technologies for over a decade, but also experts in virotherapy, vaccine development, disease models, regulatory aspects & clinical translation.



Location:

Hotel „Das Ebertor"
Heerstraße 172
56154 Boppard
https://www.das-ebertor.de/

Dirk Grimm

Dominik Niopek

Program overview

Sun. Sept 15th	Mo. Sept 16th	Tue. Sept 17th	Wed. Sept 18th
16:00 – 18:00 Registration (Hotel Ebertor)	**09:00 – 10:30 Session 1**	**09:00 – 10:30 Session 5**	**09:00 – 11:15 Session 7**
	10:30 Coffee break	10:30 Coffee break	11:15 Coffee break & farewell
	10:45 – 12:45 Session 2	**10:45 – 12:45 Session 6**	
	12:45 Lunch	12:45 Lunch	
	14:30 – 16:30 Session 3	16:00 Boat cruise on the Rhine	
	16:30 Coffee break		
	16:45 – 18:15 Session 4		
19:30 Dinner	19:30 Dinner	19:30 Dinner	

Scientific Program

Monday September 16th

Session 1:		**Chair:**
09:00 – 09:30	WELCOME & INTRODUCTION	Prof. Dr. Dirk Grimm & Prof. Dr. Dominique Niopek
09:30 – 10:00	(A) Kühne, Patrizia Deprt. of Cardiology	"Investigating the metabolic role of free PRAS40 in the Heart Failure with preserved Ejection Fraction (HFpEF) mouse model"
10:00 – 10:30	B) Hildebrand, Heinz-Georg Dept. of Infectious Diseases	"Engineering of myotropic AAV vectors for expression of broadly neutralizing anti-HIV-1 antibodies"
10:30 – 10:45	Coffee break	

Session 2:		**Chair:**
10:45 – 11:15	(C) Holzleitner, Noah TU Munich	"Application of Deep Learning for Engineering of Miniature CRISPR-Cas Proteins"
11:15 – 11:45	(D) Fischer, Nico Dept. of Infectious Diseases	"Assessment and progression of AAV-based CRISPR/Cas Type VI-D or RNAi-based effector systems to combat CHB-triggered CD8+ T cell exhaustion"
11:45 – 12:15	(E) Lashkari, Ali Biochemistry Center (BZH)	"A Possible Role for Plasma Membrane Nano-domains in in Unconventional Secretion of Fibroblast Growth Factor 2"
12:15 – 12:45	(F) Brenker, Luca Institute of Pharmacy and Molecular Biotechnology	"A multimodal anti-CRISPR effector regulation for opto- and chemogenetic control of CRISPR-Cas9 and Cas12 inhibition across a wide range of orthologs"

12:45 – 14:30	Lunch break incl. Poster Session	
Session 3:		**Chair:**
14:30 – 15:00	(G) Bergamino, Mariano Clinical pharmacology and pharmacoepidemiology	"Study design of the LipOra Trial"
15:00 – 17:15	Poster Session incl. Coffee break	
	(1) Bhunia, Sayari Pharmacological Institute HD	"Optimisation of advanced gene editing techniques and their application in the treatment of cardiovascular diseases"
	(2) Southern, Nicholas Institute of Pharmacy and Molecular Biotechnology	"Phage-assisted evolution of chimeric chemogenetic and optogenetically switchable proteins"
Session 4:		**Chair:**
	(3) Lakkaraju, Sricharan Institute for Experimental Cardiology	"NT-HDAC4 Gene therapy : cardioprotection"
	(4) Gupta, Lavanya Dept. of Vascular Dysfunction Mannheim	"Identifying novel cysteinolytic regulators regulating stemmness "
17:15 – 18:00	Blank, Antje Clinical pharmacology and pharmacoepidemiology	"From bench to bedside: clinical translation in practical terms"
19:30	Dinner	
Tuesday September 17th		
Session 5:		**Chair:**
09:00 – 09:45	Kunz, Joachim Pediatric Oncology, Hematology and Immunology Heidelberg	"Gene therapy of hemoglobinopathies"
09:45 – 10:30	Grünewald, Julian Technical University of Munich	"Engineering and clinical application of CRISPR technologies in cardiovascular medicine"
10:30 – 10:45	Coffee break	
Session 6:		**Chair:**
10:30 – 11:15	VandenDriessche, Thierry	"Turbocharging gene therapy by boosting tissue-

	University of Brussels	targeted transcription”
11:15 – 12:00	Lächelt, Ulrich University of Vienna	"Non-viral delivery of nucleic acids"
12:00 – 12:45	Ungerechts, Guy NCT Heidelberg	“Immunovirotherapy – Clinical Translation”
12:45 – 14:00	Lunch break & Foto of all participants	
14:45 – 15:30	Haar, Janina – ONLINE Revvity Germany	“A scientific journey at the forefront of next-generation gene therapy vector development”
16:00	Boat cruise on the Rhine	
19:30	Dinner	

Wednesday September 18th		
Session 7:		**Chair:**
09:00 – 09:45	Fakhiri, Fakhiri - ONLINE Hoffmann-La-Roche	“Mission: possible – How science and coffee bridged and fueled my journey to big biotech”
09:45 – 10:30	Vandenberghe, Luk Harvard Medical School	“Where the shoe fits (and doesn´t) – translating biomedical innovation in genetic medicine“
10:30 – 11:15	Backs, Johannes Cardiology Heidelberg	“Cardiometabolic Disease – from bench to bed”
11:15 – 12:00	Coffee break and farewell	

Short Biosketch of the external speakers:

Prof. Dr. Antje Blank – University Hospital Heidelberg

Antje Blank MD leads the Pharmacological Early Clinical Trial Unit at Heidelberg University Hospital, Internal Medicine IX, Dep of Clinical Pharmacology and Pharmacoepidemiology. She studied medicine at the University of Freiburg and trained at hospitals affiliated with the University of San Francisco. Beginning her career in cardiology, she has since gained extensive experience in internal medicine and clinical research, including roles at Roche Pharmaceuticals and as a Clinical Research Consultant in Singapore.

For over 15 years, Antje Blank has focused on translational medicine, planning and conducting early-phase clinical trials. She has overseen the development of various investigational drugs, leading 17 first-in-human and phase I trials and participating in over 100 clinical drug trials. Her work spans oncology, infectious diseases, and cardiovascular disease. Additionally, she teaches clinical pharmacology at Heidelberg University and is involved in developing electronic clinical decision support systems.

Prof. Dr. Joachim Kunz – University Hospital Heidelberg

Prof. Dr. med. Joachim Kunz is a Senior Consultant in Pediatric Hematology and Oncology at the University Hospital Heidelberg, within the Center for Pediatric and Adolescent Medicine and the Hopp Children's Cancer Center. Prof. Kunz began his academic journey with medical studies at the Universities of Tübingen and Heidelberg, completing his medical degree and earning his doctorate (Dr. med.) from the University of Tübingen in 2002. In parallel, he pursued a degree in Biochemistry, obtaining his diploma from the University of Tübingen in 1999. His professional career commenced in 2003 as a Resident at Heidelberg University Hospital in the Department of Pediatric Hematology and Oncology. His expertise was further recognized with board certifications in Pediatrics (2009) and Pediatric Hematology and Oncology (2010). Over the years, he advanced through various roles, becoming a Consultant in 2011 and later a Senior Consultant in 2013. In addition to his clinical work, Prof. Kunz has contributed to academia through his postdoctoral lecture qualification (Habilitation) in Pediatrics in 2020 and was appointed as an associate professor (apl. Prof.) by the Heidelberg University Medical Faculty in 2023. His research interests include innovative therapies for pediatric cancers and the genetic underpinnings of hematological diseases.

Prof. Dr. Julian Grünewald – Technical University of Munich

Julian Grünewald is a physician-scientist, Emmy Noether group leader, and Assistant Professor of Gene Editing at the Technical University of Munich (TUM). His lab is focused on engineering new CRISPR technologies for research and therapeutic application in cardiovascular medicine. After medical school and training as a resident of internal medicine, Julian trained as a postdoc in the laboratory of J. Keith Joung at Massachusetts General Hospital and Harvard Medical School.

Prof. Dr. Thierry VandenDriessche – University of Brussel

Dr. VandenDriessche is tenured Full Professor at the Vrije Universiteit Brussel (VUB, Belgium), Faculty of Medicine & Pharmacy, where he is Founding Director of the Department of Gene Therapy and Regenerative Medicine. He obtained his PhD at the VUB in 1992 in the field of gene therapy and was a visiting fellow at the Weizmann Institute for Science (Israel). He continued his research in gene therapy as a post-doctoral fellow at the National Institutes of Health (USA) in the labs of Dr. Rick Morgan, Dr. Mike Blaese, and Dr. French

Anderson and later worked with Dr. Désiré Collen at the University of Leuven in Belgium. He previously served as President of the European Society of Gene & Cell Therapy and is the recipient of the EGSCT Outstanding Achievement Award.

Prof. Dr. Ulrich Lächelt – University of Vienna

Ulrich Lächelt studied pharmaceutics at the University of Heidelberg. He received license as pharmacist in 2011 and a doctoral degree for his work on non-viral gene vectors at the LMU Munich in 2014. He continued research on different therapeutic nucleic acid classes as junior research group leader and obtained habilitation (venia legendi) in 2021. Currently, he is assistant professor for Preclinical Medicines Development at the University of Vienna and focusses on non-viral delivery of mRNA, antisense oligonucleotides and genome editing components. He has published 65 articles in peer-reviewed journals, 3 book chapters and received a prize for excellent exam, the AbbVie Doctoral Thesis Award and the Galenus Technology Prize. He is extraordinary member of the Center for NanoScience at the LMU Munich, editorial board member of the European Journal of Pharmaceutics and Biopharmaceutics and Pharmaceutical Nanotechnology.
Research interests

- Nanopharmaceuticals for intracellular delivery of biomacromolecules
- Delivery of nucleic acids and Cas9/sgRNA complexes
- Metal-organic hybrid materials for pharmaceutical applications

Prof. Dr. Guy Ungerechts – NCT Heidelberg

Guy Ungerechts MD, PhD is a medical oncologist trained in the field of oncolytic viruses at Mayo Clinic, Rochester, USA and at the Ottawa Hospital Research Institute (OHRI), Ottawa, Canada. He is Deputy Director of the Medical Oncology Department at the National Center for Tumor Diseases (NCT) Heidelberg, and heads the Clinical Cooperation Unit Virotherapy at DKFZ. His clinical focus is GI oncology with particular interests in translational medicine and early clinical trials of immuno(viro)therapy. Guy is heading the Early Clinical Trial Team at NCT Heidelberg. Furthermore, within the German network of NCT Clinical Trial Centers (CTCs), he is co-responsible for the local Heidelberg NCT-CTC. He has been deeply involved (mostly as PI) in the development and conduction of 14 phase I/II and III trials with different oncolytic viral vectors.

Dr. Janina Haar – Revvity Gene Delivery GmbH

Janina Haar is a virologist and molecular biologist by training who holds a PhD from the German Cancer Research Center (DKFZ) and Ruperto Carola University of Heidelberg. During her Postdoc between 2016 and 2019, Janina was the leading scientist within an industry collaboration between Shire and the laboratory of Dirk Grimm aiming at the development of improved liver-tropic AAV capsids for hemophilia treatment. She transitioned to industry for a second Postdoc at Boehringer Ingelheim between 2019 and 2022, where she focused on the optimization of inducible gene expression cassettes for AAV-based gene therapy applications and joined Vector BioPharma AG in Basel as Senior Scientist in 2022 working with adenoviral vectors for immuno-oncology applications and exploring regulatory elements and genetic circuits for regulatable gene therapies. Since April 2024, Janina took over the interim leadership of the laboratory team at the Heidelberg site of Revvity Gene Delivery GmbH where she currently supports AAV production for collaboration projects with the Grimm lab. With nearly 10 years of experience working in gene therapy research at the intersection of pharmaceutical industry, biotech and academia, Janina acquired a broad range of skills and know-how needed for the development of next-generation virus-derived gene therapy vectors.

Dr. Julia Fakhiri – Hoffmann-La-Roche

Julia Fakhiri is a dedicated scientist with a strong foundation in pharmacy, pharmaceutical chemistry, and molecular biology. She earned her Ph.D. in Biology from the University of Heidelberg in 2019, where she developed bocaviral vectors and self-inactivating CRISPR systems for applications in human gene therapy. After completing her Ph.D., she continued her research at the University of Heidelberg as a postdoctoral researcher. During this time, she focused on the in vivo delivery and screening of Adeno-associated viral (AAV) vectors in mice and non-human primates (NHPs). Her research significantly contributed to the expanding knowledge base in gene therapy, particularly in optimizing viral vector delivery systems. Later, she transitioned into the pharmaceutical industry through a prestigious postdoctoral fellowship at Roche, a global leader in healthcare. There, she worked as a scientist on the production of recombinant AAV vectors, playing a key role in advancing the company's vector production capabilities. Currently, Julia serves as a Senior Scientist and bioanalytical lead within Roche's early research and development (pRED) division. In this role, she oversees bioanalytical efforts across multiple portfolio projects, both in preclinical and clinical stages. Her work ensures the accuracy and reliability of bioanalytical data, which is essential for the development and validation of new therapies. Julia's career exemplifies a seamless blend of academic rigor and industry expertise, making her a valuable contributor to the advancement of gene therapies to human applications.

Dr. Luk Vandenberghe - Harvard Medical School

Dr. Vandenberghe is the Grousbeck Family Professor in Gene Therapy at Harvard within the Mass General Brigham hospital system. His research pursues broadening opportunities for gene transfer in medicine. He seeks to address questions of mechanism, solve for technological limitations, and pursues translational programs into the clinic. Previously, Dr. Vandenberghe's team elucidated entry mechanisms for AAV, discovered and developed novel AAV technologies (e.g. AAV9 and Anc-AAVs), and contributed to translation for gene therapies for e.g. wetAMD (retinal disorders), OTOF (hearing disorders), MPS1 (liver), SMA (neuromuscular), and infectious disease (COVID). Dr. Vandenberghe is further co-founder of GenSight Biologics, Akouos (now Eli Lilly), Albamunity/ciendias bio, and Affinia Therapeutics. He started Odylia Therapeutics is a non-profit that works on the plight of gene therapy development for ultra-rare disorders. Dr. Vandenberghe currently also serves as the Chairman of the Board of Directors of Addgene, a global organization with scientific reagent sharing as its mission.

Prof. Dr. Johannes Backs – Cardiology Heidelberg

Johannes studied Medicine in Giessen, Heidelberg and Freiburg and received his MD in 1998. He obtained his doctoral degree (Dr. med.) in Molecular Cardiology in 2002 from the Medical Faculty Heidelberg of Heidelberg University. After his clinical residency in the Department of Cardiology at Heidelberg University Hospital from 1998-2003, he conducted his postdoctoral fellowship work at UT Southwestern Medical Center at Dallas under the supervision of Eric N. Olson. Johannes started then his independent career as an Emmy Noether Junior Research Group Leader in 2007 in the Department of Cardiology at the Medical Faculty Heidelberg, and he became there a tenured faculty member as an associated W3 DZHK (German Center for Cardiovascular research) Professor in 2013. In 2015 he was promoted to a full professor, and he became the Director of the Department of Molecular Cardiology and Epigenetics, and in 2018 he moved into a new position as the inaugural Director of the Institute of Experimental Cardiology at the Medical Faculty Heidelberg of Heidelberg University. Johannes received several awards from the American Heart Association, European Society of Cardiology, International Society of Heart Research (ISHR) and the Germany Cardiac Society (DGK) and he served on the Board of Directors of the ISHR, ESC and DGK. From 2019-2024 he was the

spokesperson of the DZHK partner site Heidelberg/Mannheim. Johannes is since 2022 the spokesperson of the Collaborative Research Center 'Molecular Circuits of Heart Disease' (SFB-1550) and since 2024 one of three Directors (interim) of the Helmholtz Institute for Translational AngioCardioscience (HI-TAC). Johannes serves currently also as one of the spokespersons of the Review Board Medicine of the German Research Foundation (DFG). He is the main founder of Revier Therapeutics that develops therapies to treat cardiometabolic disease. His research is focused on a better understanding of regulatory epigenetic mechanisms underlying heart disease and the translation towards drug development.

Abstracts PhD students:

ORAL PRESENTATIONS:

(A) Kühne, Patrizia

The kinase mechanistic target of Rapamycin (mTOR) is a strictly regulated master regulator of cell growth and metabolism. One of these regulators is proline rich AKT substrate 40kDa (PRAS40) which acts as a specific mTOR complex 1 (mTORC1) inhibitor that binds to the kinase region of mTOR. It is released from mTORC1 in response to growth factors, insulin, glucose and other nutrients. However, the function of free/mTOR unbound PRAS40 in the context of Heart Failure with preserved Ejection Fraction (HFpEF)is still largely unknown. In recent unpublished data, we could show that PRAS40 KO mice with induced HFpEF gained less weight compared to wildtype animals. Further on they seemed to be more protected against the cardiac phenotype of HFpEF induction. However, it remains unclear which molecular mechanisms lie behind these observations. Therefore, investigating different cell type specific Pras40KO mouse models undergoing HFpEF are planned for the near future.

(B) Hildebrand, Heinz-Georg

This research introduces an innovative strategy in the fight against HIV, one that harnesses the power of specially engineered viruses called adeno-associated viruses (AAVs). Unlike traditional HIV vaccines, which have struggled to overcome hurdles, this approach draws inspiration from individuals known as long-term non-progressors (LTNP), who naturally resist HIV's progression. By capitalizing on the harmless nature and ability of AAVs to persistently produce antibodies within the body, researchers aim to deliver potent therapeutic agents effectively. Through meticulous design and advanced techniques, these recombinant AAVs are optimized to minimize side effects while maximizing their effectiveness. Encouraging results from initial experiments in mice, with anti-HIV-1 antibody levels >1mg/ml, even 84 days post administration, have spurred further exploration in models infected with HIV. This pioneering research represents a beacon of hope for the development of a safe and long-lasting solution to HIV, with the potential to move closer to a functional cure.

(C) Holzleitner, Noah

CRISPR-Cas9 and Cas12 proteins are essential tools for RNA-guided DNA binding and cleavage, as well as for advanced applications like epigenetic modification, base editing, and prime editing. Despite their utility, the large size of these proteins poses significant challenges for in vivo delivery. In this work, we present a strategy that integrates structure-guided techniques with AI-driven protein design to enhance the gene editing functions of a smaller type V CRISPR system in human cells. We explore and validate various computational approaches for protein engineering in the CRISPR field, ultimately establishing new scalable pipelines and providing a detailed analysis of these innovative methods.

(D) Fischer, Nico

Chronic infections with the hepatitis B virus (HBV; CHB) remain a major global health burden, especially since only preventive but no curative treatments are available. Fortunately, our growing knowledge of HBV biology allows to envision complementary strategies to combat viral infection and to implement a cure for CHB.

One challenge towards a cure for CHB is posed by the insufficient immune response against HBV. This is evidenced by an enhanced expression of inhibitory immune checkpoints (ICP) on HBV-specific CD8+ T cells. Knocking down these ICP by CasRx may help to reconstitute T cell responses. We therefore screened gRNAs targeting Gal9 or PD-L1 mRNAs and tested them in vitro. Surprisingly, knockdown efficiencies were moderate compared to a non-treated (NT) control, or even increased in target expression. Still, we identified two gRNAs in THP-1 cells that reduced Gal9 (hGal9) expression compared to the scrgRNA control and one gRNA in NIH/3T3 cells. To assess whether AAV transduction had triggered a reverse effect on ICP protein expression, gRNA knockdown efficiencies were validated by Luciferase reporter assay. There, highly efficient reductions in Renilla luciferase signal were observed.

Possibly, AAV transduction triggers overexpression of ICPs and may involve TLR9-mediated recognition of the transgene, which we aim to evaluate.

(E) Lashkari, Ali

Fibroblast Growth Factor 2 (FGF2) is essential for various biological processes, including tumor cell survival. My research explores the unconventional secretion (UPS) of FGF2, focusing on the role of nanodomains in the plasma membrane. Specifically, I investigate the alpha-1 subunit of Na/K-ATPase, examining its palmitoylation and interaction with sphingolipids, which may influence its localization within Lo domains and impact FGF2 secretion. Preliminary data suggest that palmitoylation is crucial for the partitioning of Na/K-ATPase into these domains, affecting FGF2 translocation across the membrane. This project aims to shed light on the spatiotemporal dynamics of FGF2 secretion, providing insights into the regulation of this vital process.

(F) Brenker, Luca

CRISPR-Cas technologies have revolutionized life-sciences by enabling precise genome editing. While CRISPR-Cas effectors are naturally constitutive, the ability to control their activity in time and space using exogenous triggers would enhance dynamic and safe genome interventions. Harnessing the full potential of dynamic CRISPR control remains challenging, however, as most available tools are limited to a few selected class-II CRISPR-Cas orthologs. Anti-CRISPR (Acr) proteins are phage-derived inhibitors of CRISPR-Cas systems, with some members showing activity across a wide range of CRISPR-Cas orthologs. Here, we introduce a switchable anti-CRISPR platform based on two broad-spectrum Acrs, engineered for optogenetic and chemogenetic control of Cas9s from type II-A, -B, and -C clades, as well as various type V effectors. We demonstrate conditional genome editing in human cells using blue light and clinically approved drugs. Our toolbox offers multidimensional control for diverse CRISPR-Cas systems, facilitating finely regulated genome interventions for research and therapeutic applications.

(G) Bergamino, Mariano

Octreotide (OTT) is a synthetic octapeptide analogue of somatostatin. It is indicated as therapy for a diversity of diseases. Chronic treatment with OTT, as indicated in acromegaly, usually consists of two to three daily, subcutaneous (s.c.) doses. This can be associated with site of injection complications such as erythema, pain and pruritus. Patients´ adherence to the treatment could be affected as a consequence. Moreover, even in the absence of local reactions, the (self-)administration of s.c. injections per se can be uncomfortable, especially for patients with aversion to needles. LipOra peptide, a cyclic cell-penetrating peptide- lipid conjugate, is a novel functional excipient without pharmacological activity developed by the Department of Clinical Pharmacology and Pharmacoepidemiology of Heidelberg University Hospital which shall serve as a platform technology for enhancing intestinal absorption of oral (p.o.) administered peptides and macromolecules. In Beagle dogs´ experiments OTT´s oral bioavailability was increased more than 4 times and up to 7.1 %. Further GLP toxicological studies reported good tolerability and no relevant toxicity signs.

This is the first-in-human application of an oral OTT / LipOra peptide formulation intended to evaluate oral bioavailability and, thus, to explore a new formulation alternative. The current trial is designed to assess the safety, tolerability and pharmacokinetics of ascending single oral doses of OTT / LipOra peptide in healthy volunteers. This trial is divided into 2 consecutive phases A and B as a phase 1, open-label, safety, tolerability, and PK, first-in-human trial in healthy volunteers with an adaptative design, and into 2 comparative cohorts as an open-label, randomized bioavailability, cross-over trial.

POSTER PRESENTATIONS:

(1) Bhunia, Sayari

Cardiovascular diseases (CVD) remain a predominant cause of global mortality. Research has shown that cardiomyocytes (CMs) experience localized calcium ion (Ca2+) concentration increases at the junction between lysosomes and the sarcoplasmic reticulum, facilitated by two-pore channels (TPCs) in the acidic lysosomal stores, which contribute to ventricular arrhythmia and pathological cardiac remodeling.

Cytosine base editing (CBE) facilitates the introduction of precise C-to-T transition mutations without inducing double-strand DNA breaks, opening new avenues for cardiac gene therapy. Our study aims to optimize a CBE toolset with high editing efficiency, enhanced targeting precision, minimized indel formation, and reduced off-target effects by screening a state-of-the-art CBE library using HEK293 cells in vitro and postnatal day one (P0) mice in vivo. A significant challenge in the in vivo application of CBEs is their size (>5 Kb), which impedes delivery into animal models using Adeno-associated viruses (AAVs) with a packaging capacity of ~4.7 Kb. Thus, we aim to engineer miniCBEs to facilitate efficient delivery via AAVs. Next, we want to apply these CBE tools in understanding cardiac dysfunctions linked to disrupted nicotinic acid adenine dinucleotide phosphate (NAADP)- and TPC-dependent Ca2+ release from lysosomes in CMs and the regulatory effect of Organellar Calcium Regulatory Protein 2 (OCaR2) over this process in mice models by delivering the editors via AAV targeting specific organs.

This comprehensive exploration of cardiac calcium signaling mechanisms, utilizing cutting-edge gene editing techniques, aims to provide essential insights into cardiomyopathies, potentially facilitating the development of novel and high-precision therapeutic strategies in cardiac disorders to overcome current limitations.

(2) Southern, Nicholas

Proteins play vital roles in all cellular functions, from metabolism, signaling, and gene expression. Allostery, where a protein's activity is regulated by conformational changes at a site distant from its active center, is crucial for enabling rapid responses to various stimuli. Engineering single-component protein switches based on inducible allostery, triggered by chemicals or light, offers powerful tools for controlling cellular pathways in synthetic biology. However, this process often requires extensive optimization to address challenges like disrupted enzymatic activity and off-target effects. To overcome these issues, we developed a directed evolution approach using Phage Assisted Non-Continuous Evolution (PANCE) to optimize allosteric protein switches. By subjecting M13 phage encoding engineered switches to cycles of mutagenesis and selection, we enhanced the performance of the arabinose-responsive E. coli transcription factor AraC and its derivatives. Our method not only improves protein switch engineering but also deepens our understanding of allostery in cellular functions.

(3) Lakkaraju, Sricharan

My research explores the role of NT-HDAC4 in repressing Myocyte Enhancer Factor 2 (Mef2) in cardiac muscle. Under stress, HDAC4's de-repression of Mef2 can lead to adverse effects, but NT-HDAC4(201aminoacids), generated through proteolysis by Protein Kinase A (PKA), inhibits Mef2 and offers cardioprotective effects. First question I'm trying to understand is mode of function for NT-HDAC4 that can compensate full length HDAC4 (1084aminoacid) function. Building on this, I am investigating NT-HDAC4's therapeutic potential in various heart failure models, including HFpEF, RBM20 cardiomyopathy, and pulmonary hypertension. My goal is to assess NT-HDAC4's viability as a gene therapy treatment for these cardiac disorders, offering new avenues for therapeutic intervention.

(4) Gupta, Lavanya

Stemness is characterized by a tightly regulated transcriptional and metabolic program. Preliminary findings from Wittig et al., revealed that the consumption of cysteine-related metabolites was high in human induced pluripotent stem cells (iPSCs) and reduced during specification. Cysteinolysis is regulated by two enzymes named cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CSE). In this study, we aim to identify novel regulators of CSE and CBS expression.

To address our questions, we performed an unbiased screening of genes responsible for the regulation of CSE and CBS expression. RNA seq analysis of human induced pluripotent stem cells (iPSCs) revealed that transcriptional factors (TFs) and Deubiquitinases (DUBs) are differentially expressed across different lineages. Therefore, we decided to use genome wide CRISPR libraries containing gRNAs against transcriptional factors and Deubiquitinases. Human induced pluripotent stem cells (iPSCs) were transduced with CBS and CSE reporters expressing GFP and RFP under the EF1α promoter linked via P2A and T2A peptide to human CBS and CSE enzymes to select the transduced cells with Puromycin. Subsequently the iPSCs were transduced with Cas9 lentivirus and selected with Blasticidine for 7 days, which was followed by CRISPR library transduction containing gRNAs against TFs and DUBs. Now, the cells were FACS sorted according to GFP high/low and RFP high/low. We then isolated the genomic DNA and sent our samples for NGS sequencing. Next, we will perform a MAGeCK analysis to identify critical regulators and pathways involved in CSE and CBS

expression. Our best targets will be further evaluated with gain and loss of function experiments and the mechanisms by which they regulate transcriptional levels of CSE and CBS will be found. The project aims to evaluate the effects of genetic manipulation of CSE on human induced pluripotent stem cells (iPSCs) through traditional in vitro assays.