इनस्टेम
inStem

inStem

INSTITUTE FOR
STEM CELL SCIENCE AND
REGENERATIVE MEDICINE

ANNUAL REPORT

2022-2023

Annual Report-2022-2023

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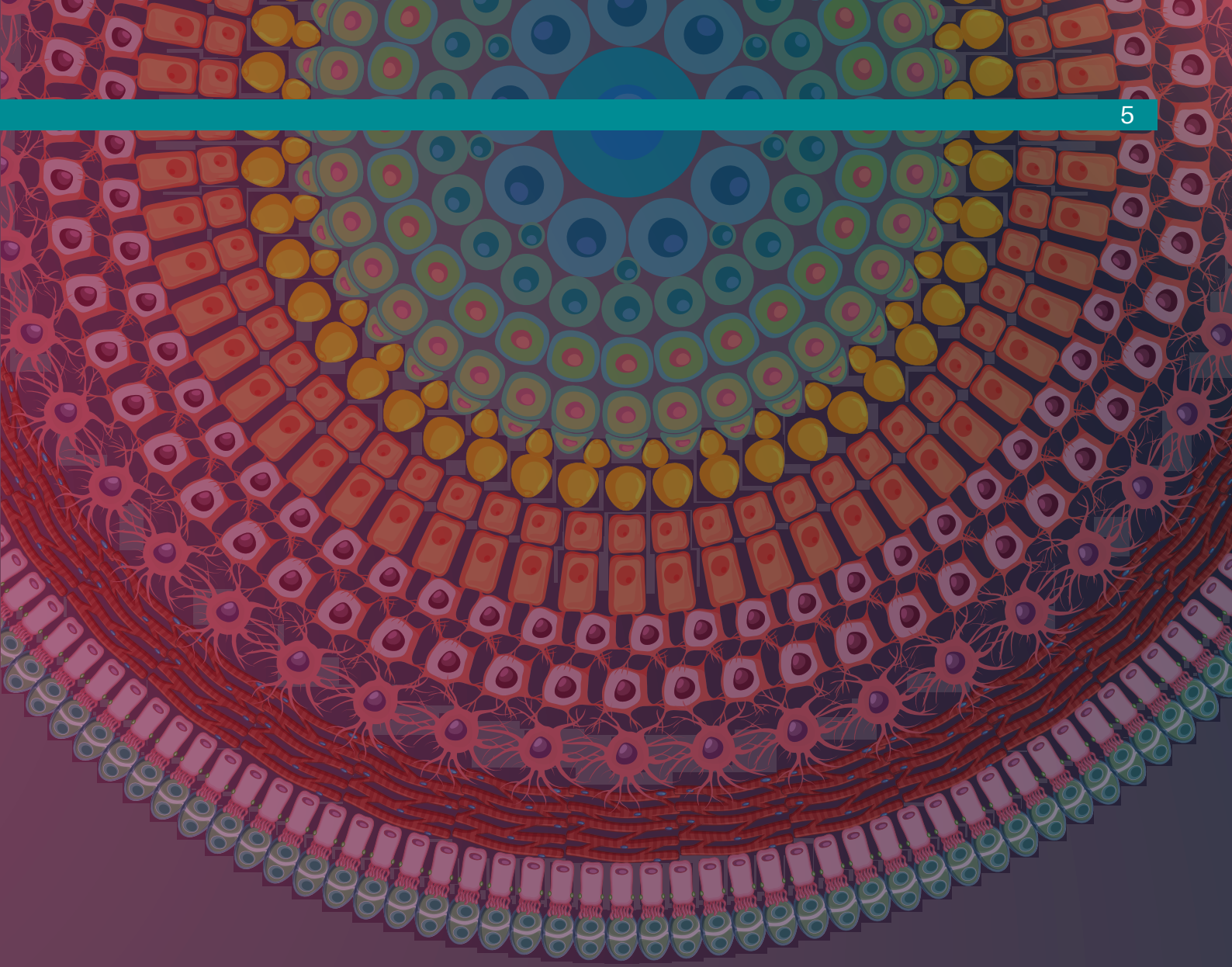
Annual Report-2022-2023



01

Director’s Note

01.
Director's Note

The Institute for Stem Cell Science and Regenerative Medicine (DBT-inStem) has entered its 'teen' years. Unlike the troublesome and often dreaded teen years of human life, institutions gain momentum and maturity in their adolescence and it has been an absolute pleasure to be actively engaged in this transition of DBT-inStem. I took charge as the Director of DBT-inStem in August 2022, as we were ushering in the post-COVID era. I gratefully acknowledge the leadership before me for setting a strong foundation for inStem. Especially, my immediate predecessors Professor Apurva Sarin, who steered DBT-inStem through the most challenging pandemic phase, and Dr. K. Thangaraj for keeping the ship steady as in-charge, during the transition period.

What a tremendous year of activities and achievements we had! Our operations at DBT-inStem are in full swing on all fronts, including ambitious new research directions in stem cell science, fostering new collaborations, recruiting faculty and staff, establishing impactful training programs, and engaging with the BLiSc and wider Bangalore research community.

But first, let us reflect and thank the DBT-inStem researchers and staff for their unwavering efforts in our fight against COVID-19 during the pandemic. Our COVID testing facility has conducted more than 4 lakh tests since the pandemic began. Kudos to the team for this remarkable feat and service to our nation. Another major ongoing activity is our effort to sequence the emerging COVID-19 variants and store them in our biorepository, under the INSACOG program, with DBT support. These activities highlight our capability and underscore inStem's ability to pivot during times of need, along with its campus partners, to meet any new challenge.

DBT-inStem's mandate is to undertake world-class research in the area of stem cells and regenerative medicine for the benefit of humankind and society. Building on the strong foundation of infrastructure and capabilities established thus far, we now focus on fulfilling our mandate in the best possible manner. Intense brainstorming sessions over the past year helped us enhance our existing research programs and initiate new programs at the forefront of regenerative science. Capitalizing on the diverse and cross-disciplinary expertise we have, new approaches to regenerative biology have been initiated. Our focus is on developing out-of-the-box solutions for stem cell research and its application.

An important emphasis is also to "be local, go global". *India's genetic diversity has been neglected in stem cell research and undervalued. InStem has embarked on an ambitious plan to be a resource for quality Indian stem cell lines. With this goal, we set up the unique ESCORT program: A Platform Enabling Stem Cell and Organoid Research and Training. This aims to equip academia and industry with the skills to generate stem cells and organoids, through extensive hands-on training and hybrid workshops. We have already had two phenomenally successful international workshops this year, on pluripotent stem cell and organoid models for use in basic research, human disease modeling, and drug development. To ensure quality is maintained at an international level, we are pleased to partner with the International Stem Cell Banking Initiative (ISCBI). Stem cell ethics, regulation, and policy have context-specific aspects and are extremely important facets of stem cell research. Taking advantage of our newly established Research Integrity Office (RIO) and Regulatory Compliance Office (RCO), we developed unique courses tailored to local needs but internationally relevant. It is heartening to note that these courses were found beneficial by scientists all over the country and are in great demand. We are proud to announce that the ESCORT program, India's*

first state-of-the-art stem cell and organoid facility, will be funded by the Department of Biotechnology, Government of India, under the aegis of SAHAJ.

In the fast-paced world of stem cell science, discoveries, and inventions are being made each day and progressing very rapidly to translation and the clinic. InStem is proud to be at the forefront of such efforts. While research using cardiac and brain organoid models was already ongoing at inStem, the addition of three faculty members with core stem cell expertise has boosted our efforts. Many of my faculty colleagues have pivoted to include stem cell-derived models in their research programs, aiming for retinal, bladder, and lung organoids or developing better polymers and matrices for stem cell culture. Our efforts are expanding to include the nascent field of developing models of early human development. Thanks to these ambitious and forward-looking steps by DBT-inStem, we are very pleased to announce the initiation of a major program funded by the Bill & Melinda Gates Foundation, focused on developing organoid models of early human development, for de-risking drug discovery.

At the Centre for Stem Cell Research (CSCR), DBT-inStem's translation unit in CMC, Vellore, research on gene editing and prime editing to treat inherited blood disorders has gained momentum. It is indeed a matter of national pride that the CSCR has entered the critical clinical trial phase for the treatment of hemophilia.

Our on-campus engagement with the BLiSc community was further bolstered with the joining of Prof. L. S. Shashidhara as Center Director of NCBS-TIFR. We warmly welcome him and are working with him on several fronts to enhance our ecosystem. In line with several shared infrastructures, I am happy to announce the establishment of the Animal BSL3 facility, a shared facility among BLiSc researchers that will be open to scientists across the nation.

In recognition of their outstanding fundamental research and translation to application, our faculty and researchers have also been conferred with several prestigious grants, fellowships, and awards, including from HFSP, EMBO, and India Alliance. We are also deeply grateful for ongoing support from the Department of Biotechnology, SERB, DST, and our philanthropic funders T. T. Narasimhan Grant, Pratiksha Trust, and the Kiran Mazumdar Shaw Foundation that have helped sustain our ambitions.

Collaborative work has been the mantra for DBT-inStem, and in addition to our publications and research grants, we also lead in entrepreneurship and translational efforts. DBT-inStem has signed a research collaboration agreement with Sun Pharma Advanced Research Company (SPARC) to establish industry-academia partnerships and a license agreement with Artus Therapeutics. DBT-inStem faculty have also been very proactive in launching new startups, with many more in the pipeline, that are spin-offs of research done in-house. I invite you to go through our extensive list of achievements in the "Year at a Glance" section of this report. It is heartening to note that our accomplishments have been duly recognized by the inclusion of DBT-inStem in the "Circle of Stem Cell Institutes and Centres" of the International Society of Stem Cell Research (ISSCR).

DBT-inStem also actively participated in the India International Science Festival – 2023 Bhopal edition and National Technology Week – New Delhi, during which we showcased our research. We also hosted the DBT, India- NSF, USA roundtable discussion on "Collaborative Opportunities for Investment and Innovation in Biomanufacturing Sector", organized by BIRAC and ABLE, which has kick-started discussions on industry and academic needs.

While we are extremely excited about the ambitious plans we have made in this most exciting area, we are cognizant of our small numbers. However, being in the wonderful growth phase we made energetic efforts to recruit new faculty and continue to do so. I am happy to say that in the first round, we could attract principal investigators Dr. Reena Singh and Dr. Srinivas Repudi, spearheading investigations into cardiovascular and brain development, respectively, and using organoids. At CSCR, Vellore, Dr. Srujan Marepally joined as an assistant investigator to embark on lipid-mediated delivery systems. We strongly believe that their inclusion will further enhance our efforts in the field of stem cell science and regenerative medicine. We look forward to adding principal investigators at various career levels who share our vision and goals and will benefit from our excellent ecosystem. We also welcome recruits across different administrative and

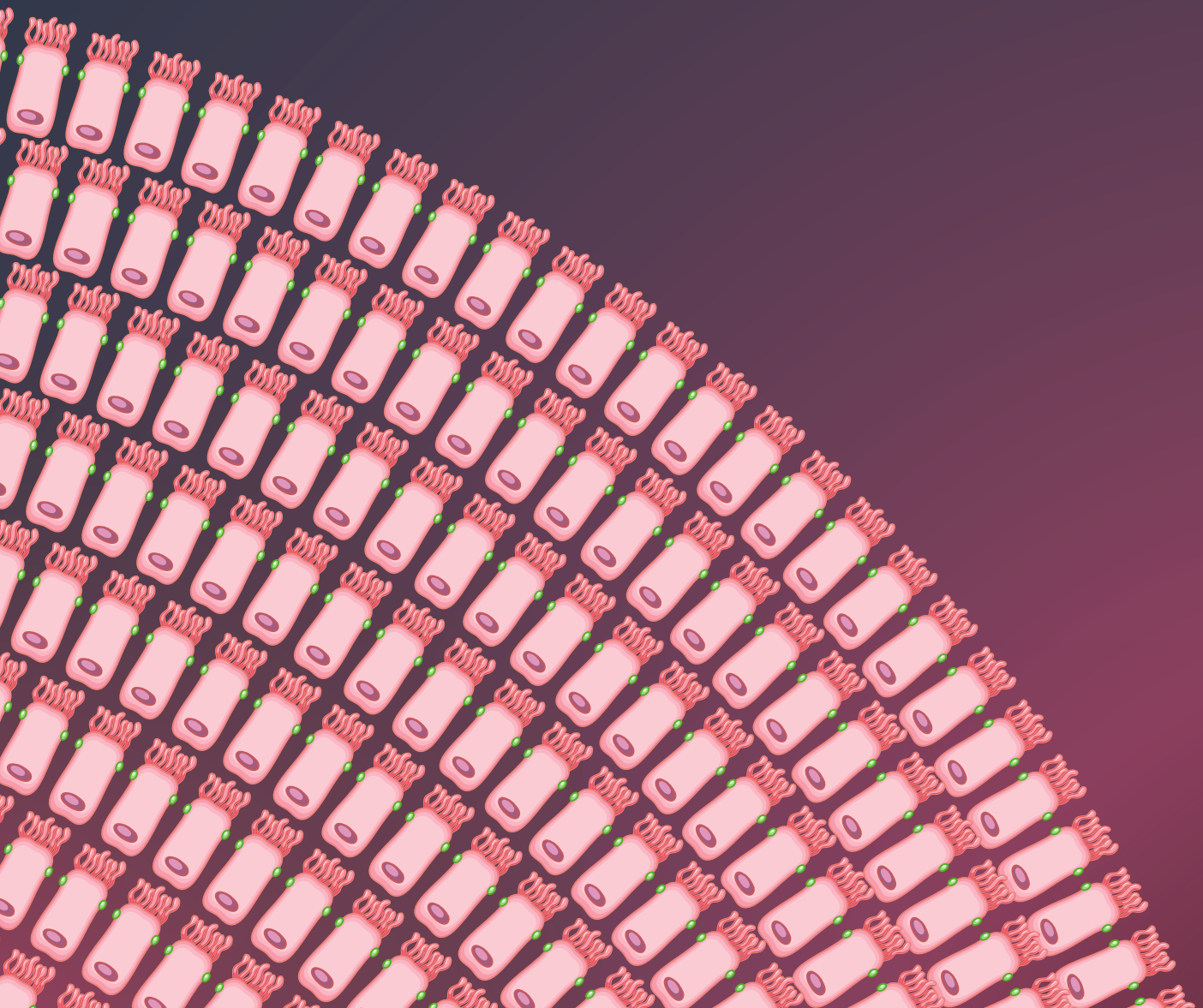
technical sections. It is a relief to have an administrative head to oversee our operations. Mr. A K Prakash, joined us as Registrar, in August 2023, a year after me, and I look forward to spending less time on routine administrative matters. Lastly, we warmly welcome back Prof. K. VijayRaghavan as "inStem Honorary Professor of Eminence" and look forward to his renewed engagement with us.

I am also glad to report that DBT-inStem has enthusiastically been at the center of vibrant interactions with the community in the form of several international and national meetings, workshops, training, and outreach activities, as detailed in the Science Outreach and Communications section. It was enriching and exciting to resume our Annual Review of Research and Scientific Advisory Board (SAB) meetings in-person, after a brief hiatus during the pandemic. We thank our SAB members for their enthusiastic interaction and continued support. A major highlight of the year was the visit of Secretary, DBT, Dr. Rajesh Gokhale to DBT-inStem. His passion for science and his dedication to streamlining and resolving administrative matters were inspiring to all. What a happy coincidence it was that we could witness the historic moon landing of Chandrayaan alongside Dr. Gokhale. We further look forward to working together with other DBT institutes under the enabling umbrella of BRIC (Biotechnology Research and Innovation Council).

Maneesha Inamdar
Director, DBT-inStem

02

Year at a Glance at inStem



02.
Year at a Glance at inStem

inStem ranked 15th in the Life Sciences category in India according to the Nature Index 2022 rankings.

The project *"Novel Approaches To Haematological Diseases (NAHD)"* funded by DBT, New Delhi at CSCR (a unit of inStem) and CMC Vellore, Tamil Nadu, India accomplished a major milestone: Phase 1 clinical trial of lentiviral vector-based gene therapy of haemophilia A recruited its first subject in May 2022. For the first time in the world, a novel therapy using cutting-edge biotechnology has been performed in India.

Dr. Srujan Marepally, CSCR (a unit of DBT-inStem), developed self-skin permeable nano-lithocholic-lipidoid technology as a potential therapeutics for psoriasis.

Dr. Minhaj Sirajuddin has been selected in the 3-year Catalysts program of *EMBO Journal*.

Dr. Bhavana Muralidharan received a travel award from the Japan Neuroscience Society to attend their Annual meeting NEURO2022 in Okinawa, Japan, where she was invited to present her work on *"Chromatin regulation of nervous system development in health and diseases"*.

Dr. Diya Binoy Joseph was awarded the DBT-Wellcome Trust India Alliance Early Career Fellowship to study the *"Regulation of immune defences and homeostasis at the urethral barrier"*.

Edriss Yousuf, a Ph.D. student from Prof. Colin Jamora's lab, won the Rapid-Fire Talk prize at the Singapore International Skin Conference held in March 2022, organised by the Skin Research Society & the Skin Research Institute of Singapore. Prof. Colin Jamora is an Investigator at the Centre for Inflammation and Tissue Homeostasis (CITH), DBT-inStem, and works on wound healing and related diseases.

Dr. Kruttika Phalnikar, a postdoctoral fellow in Dr. Bhavana Muralidharan's Lab at DBT-inStem, was awarded the DBT-Wellcome Trust India Alliance Early Career Fellowship to study the *"Neuropathological mechanisms of bipolar disorder"*.

DBT-inStem celebrated Stem Cell Awareness Week 2022 through lecture series, workshops, podcasts, and hands-on training.

inStem participated in IISF 2022 (21st– 24th January 2023) held at Maulana Azad National Institute of Technology, Bhopal.

Students and faculty delegation from the Department of Biotechnology, Tamil Nadu Dr. J Jayalalitha Fisheries University (TNJFU) visited the campus on 6th February 2023.

DBT-inStem organised a Panel discussion on the topic *"Celebrating Women in Different Avenues of Science"* to celebrate the International Day of Women and Girls in Science on 11th February 2023.

National Science Day and Rare Disease Day was celebrated on 28th February 2023, with 40 M.Sc. first-year students (Biotechnology) visiting from St. Joseph's College accompanied by few faculty members.

03

Faculty Reports

03.
Faculty Reports

3.1 Dhandapany Perundurai

Title of the research program:

Cardiogenomics, stem cells, and precision medicine

Summary:

Cardiomyopathies are a group of life-threatening heart muscle diseases representing a significant proportion of heart failure (HF) and sudden cardiac death. Among cardiomyopathies, hypertrophic cardiomyopathy (HCM) is a hypertrophied heart without other cardiac and systemic diseases. Pathogenic gene variants encoding the sarcomere and signalling proteins cause the disease. However, for around 50% of cases, novel disease-associated genes remain to be discovered. In our gene discovery phase, applying exome sequencing of the Indian HCM cohort, we identified novel disease-associated variants in TTL and PRKCA genes. We performed functional characterisation using iPSC-derived cardiomyocytes and a transgenic mouse model and confirmed its pathogenicity.

Report:

1. Discovery of South Asian-specific heart failure gene variants: Using our established in-house pipeline, we expanded our analysis of the whole exome sequencing data obtained from patients with unrelated idiopathic HF. We identified various HF-associated gene variants, including *TTL* and *PRKCA*. In replication analysis using exomes from UK Biobank, Estonia, and Tommo Biobanks, additional variants in the *PRKCA* gene were identified.

2. Modelling patient iPSC-derived cardiomyocytes for understanding novel HF-related genes:

   a. Generation and characterisation of *TTL* variant-specific iPSC line – We reprogrammed blood into iPSC by transducing non-integrative Sendai virus vectors expressing c-Myc, Klf4, Oct3/4, and Sox2. The iPSC colonies obtained showed human embryonic stem cell morphology and high-level expression of pluripotency markers (OCT3/4, NANOG, TRA-1-60, and SSEA4) using immunofluorescence staining. Upon embryoid body formation, they showed successful differentiation of ectoderm (NES), mesoderm (NODAL and SOX2), and endoderm (GATA4), analysed by expression of respective markers using quantitative reverse transcriptase PCR.

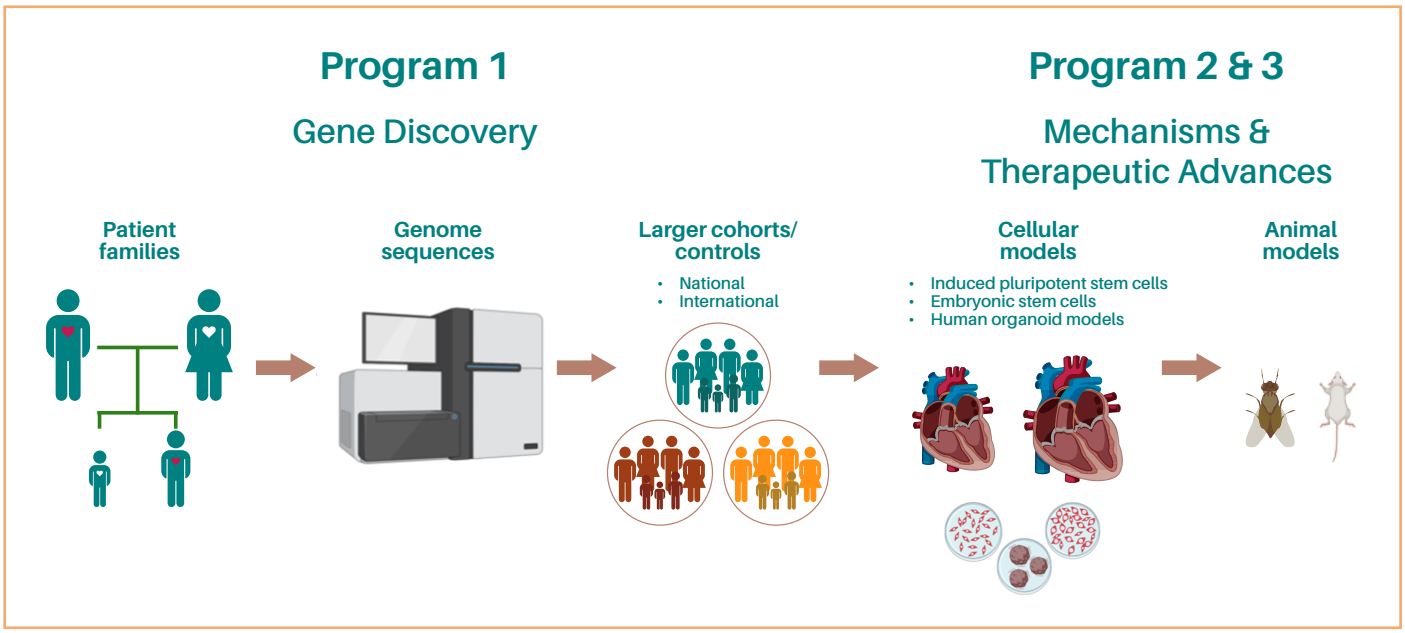
   b. Generation of iPSC-derived cardiomyocytes – We performed cardiomyocyte differentiation from healthy control and *TTL* variant-specific iPSC lines by suitable sequential application of a glycogen synthase kinase 3 (GSK3) inhibitor followed by chemical inhibition of canonical Wnt signalling. Subsequently, we confirmed the expression of cardiac-specific markers such as sarcomeric α-actinin by immunocytochemistry. We quantified the mRNA levels of various bonafide hypertrophic markers and observed a significant upregulation of *ANP, BNP, ACTA1,* and *MYH7* in *TTL*-variant cardiomyocytes compared with that in control. Immunoblot analysis of lysates obtained from the *TTL*-variant cardiomyocytes showed significantly increased detyrosinated tubulin and phosphorylation of ERK1/2 and desmin, with no alterations in the tyrosinated tubulin levels compared

with that in wildtype cardiomyocytes. Global RNA sequencing showed upregulation of hypertrophic markers and downregulation of redox metabolism markers in the *TTL*-variant cardiomyocytes compared with that in the wildtype. Further, immunoblot analysis of lysates obtained from *TTL*-variant cardiomyocytes showed significantly increased NRF2 and phosphorylation of AKT compared with that in the wildtype cardiomyocytes.

c. Functional characterisation of gene variants in iPSC-derived cardiomyocytes – Similarly, iPSC-derived cardiomyocytes expressing *PRKCA* variants (p.E207G and p.V566L) exhibited multiple hallmarks of hypertrophy, including cellular enlargement with foetal gene re-expression, including *ANP, BNP, b-MHC*, and *ACTA1*. Immunoblot analysis showed increased ERK phosphorylation in the *PRKCA*-variant cardiomyocytes than in the control.

3. Testing of therapeutic drug in a pre-clinical model: To test whether newly identified gene variants can induce cardiomyopathy in *in vivo* models, we generated a representative transgenic mouse model that specifically expressed a representative green fluorescent-tagged human *PRKCA*-E207G in the heart. At the age of eight to twelve weeks, transgenic mice displayed an increase in the heart-to-body weight (HW/BW) ratio, the myocyte cross-sectional area, the expression of foetal genes (*Anf, Bnp,* and *Ska*), and myocardial fibrosis compared to those in the controls. Echocardiographic analysis showed alterations in ventricular chamber dimensions with increased ejection fraction and fractional shortening percentage in *PRKCA*-E207G transgenic mice compared to that in the wildtype. Immunoblot analysis of heart lysate obtained from *PRKCA*-E207G mice indicated the activation of ERK1/2 pathways in comparison with that in the wildtype mice. Intra-peritoneal administration of MEK-inhibitor rescued hypertrophy phenotypes of *PRKCA*-E207G mice.

Graphical summary:



Publications:

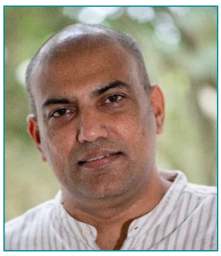
1. Dileep, D., Syed, T. A., Sloan, T. F., **Dhandapany, P. S.**, Siddiqi, K., & Sirajuddin, M. (2023). Cardiomyocyte orientation recovery at micrometer scale reveals long-axis fiber continuum in heart walls. The *EMBO journal*, e113288. Advance online publication. https://doi.org/10.15252/embj.2022113288
2. Rana, I., Kataria, S., Tan, T. L., Hajam, E. Y., Kashyap, D. K., Saha, D., Ajnabi, J., Paul, S., Jayappa, S., Ananthan, A. S. H. P., Kumar, P., ... **Dhandapany, P. S.**, He, Y. W., Varga, J., Varghese, S., Jamora, C. (2023). Mindin (SPON2) Is Essential for Cutaneous Fibrogenesis in a Mouse Model of Systemic Sclerosis. The *Journal of investigative dermatology*, 143(5), 699–710.e10. https://doi.org/10.1016/j.jid.2022.10.011
3. Chimata, P., Kashyap, D. K., Sairam, T., Ganesh, A., Thangaraj, K., Purushottam, M., Viswanath, B., Jain, S., & **Dhandapany, P. S.** (2022). Generation of a new human induced pluripotent stem cell (hiPSC) line from a South Asian Indian with a MYBPC3Δ25bp variant. *Stem cell research*, 65, 102978. https://doi.org/10.1016/j.scr.2022.102978
4. Desai, D. A., Rao, V. J., Jegga, A. G., **Dhandapany, P. S.**, & Sadayappan, S. (2022). Heterogeneous Distribution of Genetic Mutations in Myosin Binding Protein-C Paralogs. *Frontiers in genetics*, 13, 896117. https://doi.org/10.3389/fgene.2022.896117

Awards and grants (2022/2023):

- Grant from Indian Council of Medical Research (ICMR) for project titled *"Repurposing anti-diabetic drug and related rescue mechanisms for glycogen storage cardiomyopathy"*
- Grant from DBT for project titled *"Functional genomics of congenital heart disease"*

Outreach and other activities:

- Co-organised a workshop on human pluripotent stem cell research (Part I) in coordination with the International Stem Cell Banking Initiative (ISCBI, www.iscbi.org) at inStem from 22nd– 23rd May 2023
- Co-organised a workshop on human pluripotent stem cell research (Part II) in coordination with the International Stem Cell Banking Initiative (ISCBI, www.iscbi.org) at inStem from 29th August to 1st September 2023
- Delivered an invited talk titled *"Adiponectin receptor 1 variants contribute to hypertrophic cardiomyopathy that can be reversed by rapamycin"* at Heart Failure Conflux 2023 held from 4th– 5th February 2023 and organised by Heart Failure Association of India (HFAI), Sree Chitra Tirunal Institute for Medical Sciences & Technology (SCTIMST), and ICMR Centre for Advanced Research and Excellence in Heart Failure
- Delivered an invited talk titled *"Interorgan cross-talks: Heart vs Gut"* at Advances in Cardiovascular Medicine and Research (ACMR) 2023, held from 16th–18th February 2023 and organised by the International Society for Heart Research (ISHR) and International Academy of Cardiovascular Sciences (IACS)
- Delivered an invited talk titled *"Cardiogenomics, stem cells, and precision medicine approach"* at the international conference on 'Translating Human Evolutionary History to Precision Medicine' held at Banaras Hindu University, Varanasi, Uttar Pradesh, from 10th– 12th March 2023



3.2 Minhaj Sirajuddin

Title of the research program:

Structure and function of cytoskeleton and motility systems across scale dimension

Summary:

Eukaryotic biological motions across orders of magnitude scale involve cytoskeleton and motility elements. Inherited genetic mutations and perturbations in these elements are frequently associated with human pathology, e.g., cardiomyopathies and neurological disorders. My research program delineates physiological and pathological mechanisms related to cytoskeleton and motility systems from molecular and cellular to organ scale.

Report:

In the past years, our lab has demonstrated technical prowess in researching cytoskeleton and motility systems at each scale dimension and uncovered new biology, which has opened new avenues for further research. In this report, I am highlighting our recent work on micron-scale imaging of the whole heart and modelling myofiber organisation.

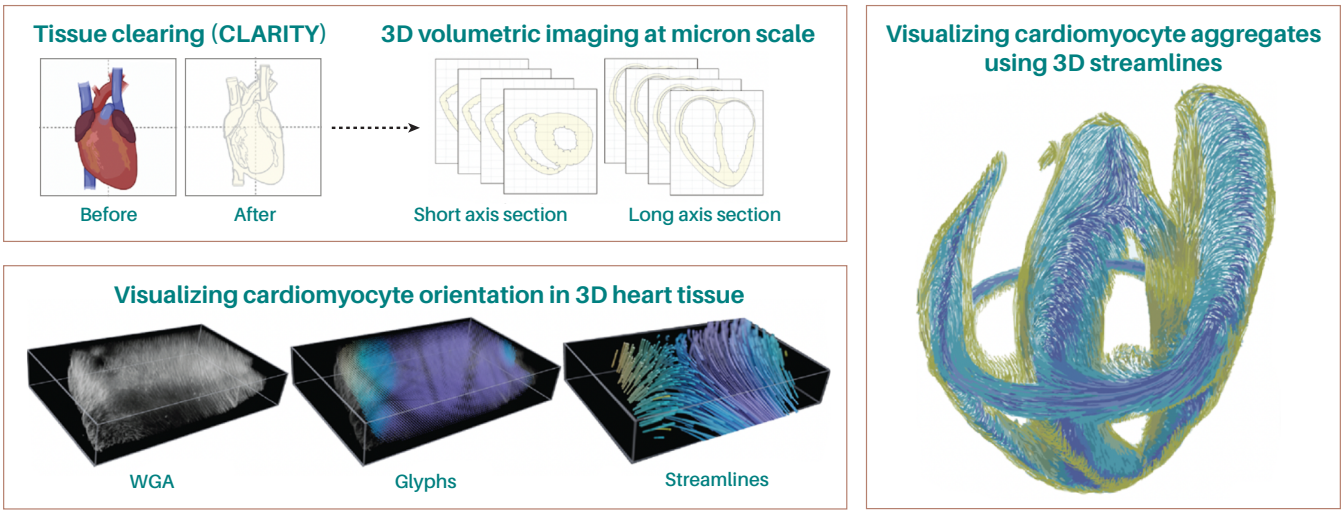
Heart ventricular walls are composed of specialised muscle cells called cardiomyocytes. These cardiomyocytes are densely packed and are aligned both end-on-end and laterally at the longest- and shortest-axis respectively to form fibre-like organisation that are abstractly called myofibers. At a coarse spatial scale, the geometric organisation of myofibers has been described as a helical continuum, wrapping around the chambers of the heart. However, these models are derived from millimetre-resolution diffusion-tensor magnetic resonance imaging (DT-MRI), and at the millimetre scale, hundreds of cardiomyocytes can occupy a single voxel. Understanding the geometry and orientation of cardiomyocytes across entire heart walls is an open question, and will advance present knowledge of heart physiology and associated disease pathologies.

To solve this fundamental problem, we developed tissue-clearing methods that enabled us to employ confocal light microscopy-based deep and wide imaging of heart tissues at a micron-scale. The microscopy images were analysed using the structure tensor method to estimate cardiomyocyte orientations across the entire heart and displayed as glyphs (Figure). We also used bi-directional streamlines as a proxy for visualising the myofiber orientations across the heart wall (Figure). Our three-dimensional reconstructions at unprecedented spatial resolution revealed, for the first time, the long-axis fibres extending along the entire length of the ventricular walls, orthogonal to the circumferential myofibers. Our findings represent a conceptual breakthrough in understanding heart myofiber geometry, thus opening new opportunities to investigate the structure and physiology of orthogonal fibre systems in the heart and diseases affecting its function.

Previous studies of heart contraction and electrical wave propagation models have not accounted for the long-axis myofibers continuum or the orthogonal fibre system described here. It remains to be seen whether the longitudinal fibres play roles in contraction, electrical wave propagation, or other unidentified specialised roles.

Our findings also provide a framework to study the development process of orthogonal fibre systems in hearts and remodelling in response to diseases that alter myofiber geometry. Because of its muscular nature, heart muscle-related diseases are broadly defined as cardiomyopathies. However, there is no uniformity in reporting the morphological changes during cardiomyopathy disease condition. For example, hypertrophic cardiomyopathy hearts are frequently reported to have excessive fibrosis, hypertrophied cells, and/or myofiber and sarcomere disarray. This could largely be due to the lack of methods to study heart myofiber organisation at a micron scale. Our recent 3D model of myofiber organisation for healthy/normal hearts will serve as a gold standard to study and measure the changes that occur in the ventricular wall of cardiomyopathy hearts. Another aspect of our recently reconstructed myofiber model is that it will help us understand the origin of the orthogonal fibre system during heart development, which is an open unaddressed question in the heart field.

Graphical summary:



Publications:

1. Mendon N, Ganie RA, Kesarwani S, Dileep D, Sasi S, Lama P, Chandra A, **Sirajuddin M.** 2023. Nanobody derived using a peptide epitope from the Spike protein receptor-binding motif inhibits entry of SARS-CoV-2 variants. *The Journal of Biological Chemistry, 299*(1), 102732. doi: 10.1016/j.jbc.2022.102732

2. Syed TA, Wang Y, Dileep D, **Sirajuddin M**, Siddiqi K. 2023. Ultrastructure Analysis of Cardiomyocytes and Their Nuclei. In: Bernard, O., Clarysse, P., Duchateau, N., Ohayon, J., Viallon, M. (eds) Functional Imaging and Modeling of the Heart. FIMH 2023. Lecture Notes in Computer Science, vol 13958. *Springer,* Cham. (doi: 10.1007/978-3-031-35302-4_2)

3. Dileep D, Syed TA, Sloan FWT, Dhandapany PS, Siddiqi K, **Sirajuddin M.** 2023. Cardiomyocyte orientation recovery at micron scale reveals long-axis fiber continuum in heart walls. *The EMBO Journal, 42*(19), e113288. doi: 10.15252/embj.2022113288

Awards and grants (2022/2023):

- DBT/Wellcome Trust India Alliance Senior Fellowship

Outreach and other activities:

- Delivered an invited talk on *"Live cell markers for tubulin PTMs"* at the EMBO/EMBL Microtubule meeting, held at Heidelberg, Germany on 11th June 2022
- Delivered an invited talk on *"Structure, function and pathologies of contractile assemblies across scale dimensions"* at DBT-NCCS, Pune, on 8th November 2022
- Delivered a virtual talk on *"Science and scientists' storytelling through artistic media"* at the ASCB-EMBO Cell Bio Meet, *"Subgroup: Science and Art: Bridging Two Creative Universes"* on 3rd December 2022
- Conducted a public engagement talk on *"Colors in Nature"* at the Bangalore Design Week – Cubbon Park Metro Station on 3rd December 2022
- Publicised a popular science book titled *"Actually, Colors Speak – by Minhaj Sirajuddin and Ipsa Jain"*. The book, suitable for all ages, explores why and how animals change their colour in nature. The book is available at online retail stores.
- Acted as the Executive Producer and Scientific Advisor for the documentary film Written Out of History – Forgotten Indian Scientists. The film, funded by DBT/Wellcome Trust India Alliance, highlights the forgotten scientists and their discoveries. It can be accessed at: https://www.youtube.com/watch?v=pj676wJx1g8

3.3 Maneesha Inamdar

Title of the research program:

Understanding cytoskeletal regulation of early development using stem cell models

Summary:

We aim to unravel mechanisms that operate in the early development of the human cardiovascular system. Towards this, we are generating new tools to understand stem cell maintenance and differentiation. We also model aspects of early human development and disease by generating embryoids and gastruloids using pluripotent stem cells. For mechanistic insight, we focus on understanding the cytoskeletal regulation of cardiovascular development using human stem cells and knockout mouse models.

Report:

To investigate the role of the cytoskeleton in active regulation of early development, we examined how cytoskeletal components remodel in response to changes in the cell cycle state. We use Rudhira/BCAS3, a cytoskeletal protein that crosslinks microtubules and intermediate filaments, as a tool to understand this regulation. In response to migratory cues, Rudhira relocalizes to the leading edge and promotes Cdc42 activation. Rudhira is essential for TGFb signalling and tubulin stability. To track the dynamic location of Rudhira and its interactors in the cell cycle, we generated CRISPR/Cas9-mediated knockout human stem cell lines and knock-in reporters for cytoskeletal elements such as tubulin and actin. We also studied the interdependence of the cytoskeletal elements on Rudhira during cell cycle progression using human cell lines.

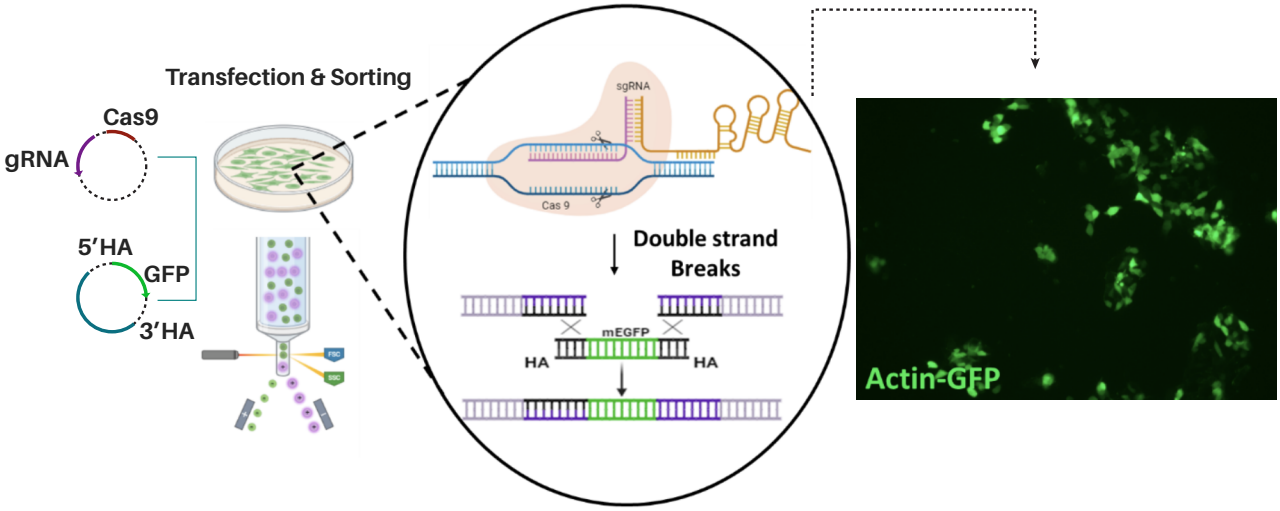
To understand the contribution of cytoskeletal rearrangements in actively directing early development *in vitro*, we differentiated human pluripotent stem cells to generate gastruloids that model aspects of early human development. These are being analysed further.

To understand how cytoskeletal reorganization contributes to cardiomyocyte orientation, in collaboration with Sirajuddin's research group at inStem, we are developing protocols for whole-mount 3D reconstruction of the developing mouse heart at micron-scale resolution, ensuring native heart morphology.

Graphical summary:

Generation of CRISPR/Cas9-mediated knockout human stem cell lines and knock-in reporters for cytoskeletal elements

Work-flow for gene editing



Investigation of the cytoskeleton's role in regulation of early development and cardiac patterning

In vitro

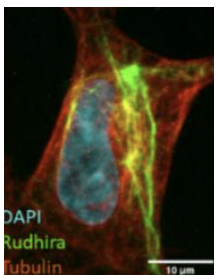
interdependence of the cytoskeletal elements on Rudhira during cell cycle progression, examined using human cell lines

generation of gastruloids that model aspects of early human development

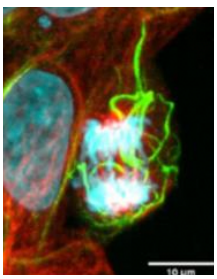
In-vivo

protocols for whole-mount 3D reconstruction of the developing mouse heart at micron-scale resolution, ensuring native heart morphology

Interphase



Dividing



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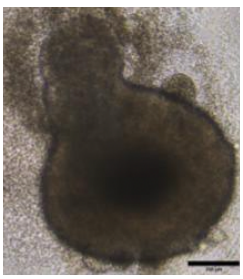
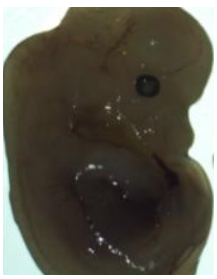


Scale bar - 200um

Mouse embryos (E13.5 dpc)



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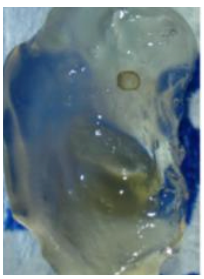


after CLARITY

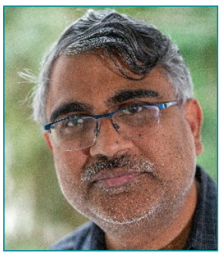
Publications:

1. Kamat K, **Inamdar MS.** 2023. Generation of OCIAD2 homozygous knockout (BJNhem20-OCIAD2-CRISPR-33) and heterozygous knockout (BJNhem20-OCIAD2-CRISPR-40) human embryonic stem cell lines using CRISPR-Cas9 mediated targeting. *Stem Cell Research,* 67, 103026. doi: 10.1016/j.scr.2023.103026
2. Prakash A, Kamat K, **Inamdar, MS.** 2023. Generation of an OCIAD2 overexpressing transgenic human embryonic stem cell line, BJNhem20-OCIAD2-OV. *Stem Cell Research,* 67, 103027. doi: 10.1016/j.scr.2023.103027
3. Ludwig TE., Andrews PW, Barbaric I, Benvenisty N, Bhattacharyya A, Crook JM, Daheron LM, Draper JS, Healy LE, Huch M, **Inamdar, MS**, *et al.* 2023. ISSCR standards for the use of human stem cells in basic research. *Stem Cell Reports,* 18(9), 1744–1752. doi: 10.1016/j.stemcr.2023.08.003

Outreach and other activities:

- Delivered an invited research seminar at the *"Drosophila Blood Cell Biology Symposium"* held at the University of Strasbourg, France, from 21st–24th September 2022
- Visited Cambridge Stem Cell Institute on 26th September 2023, for discussions on stem cells
- Panellist and committee member for the ISSCR Standards initiative task force meeting to develop quality standards for pluripotent stem cell research to improve rigor and reproducibility. The meeting was organised by the International Society for Stem Cell Research in London from 27th–30th September 2022
- Delivered an invited talk at the Cancer Grand Challenges consultation workshop organised by Cancer Grand Challenges, UK in Singapore from 23rd –25th November 2022
- Invited as an expert for the Workshop on scientific, ethical, legal, and societal implications of developing technologies for the creation of large genomes by Wellcome Trust London, UK from 7th–9th December 2022
- Acted as an expert on Panel discussion on Ethics of human brain organoids at the EMBO Organoid meeting from 6th–9th February 2023 at inStem, Bangalore
- Invited speaker at the Mito Metab Network meeting, which was held at IISER Pune from 13th–15th February 2023
- Delivered an invited talk and overview on inStem at the Young Investigator Meet 2023, held at IIT Gandhinagar from 15th–17th February 2023
- Participated in the Panel discussion on the occasion of International Day of Women and Girls in STEM at inStem, Bengaluru on 8th March 2023
- Distinguished speaker at the International Women's Day Celebration of Women in Science, Technology, Engineering, Mathematics, Medicine, Education, Entrepreneurship at the Indian Institute of Science, Bengaluru on 8th March 2023
- Invited speaker at the Frontiers in Biology Symposia at IISER Thiruvananthapuram, Kerala from 17th–18th March 2023
- Invited speaker at the Sun Pharma Science Foundation's National Conference organised by the National Academy of Medical Sciences, New Delhi on 18th March 2023
- Organised the INSA Award lecture and Prof. V. V. Narlikar Lecture of Prof. B. V. Rajarama Bhat, jointly with the Department of Mathematics, Indian Institute of Science, Bangalore on 20th March 2023

- Panellist at a discussion on *"Biotechnology & Biosecurity Cooperation in the Indo-Pacific"* at the UK and Indo-Pacific Takshashila Institution in partnership with the British Deputy High Commission on 27th March 2023
- Organised the INSA Award lecture of Prof. Tushar Kanti Chakraborty, jointly with Mount Carmel College (autonomous), Bengaluru on 3rd April 2023
- Organised the INSA Award lecture and Prof. Darshan Ranganathan Memorial Lecture of Prof. R. Sowdhamini, jointly with Ramaiah University of Applied Sciences, Bengaluru on 21st April 2023
- Participated in the Stem Cell workshop on Human Pluripotent Stem Cell Research, organised by inStem-ISCBI from 22nd–23rd May 2023
- Delivered a talk at the Annual Review of Research (ARR) under *"Drosophila Blood Cell Biology Symposium"*, held from 23rd–25th May 2023
- Participated in the DBT AI Director's meeting at the National Institute of Animal Biotechnology (NIAB), Hyderabad and delivered a talk titled 'Institutional Research Strategy for inStem, Bangalore' from 26th–27th May 2023



3.4 Dasaradhi Palakodeti

Title of the research program:

Elucidating the mechanism of protein synthesis critical for stem cell function and regeneration

Summary:

The dynamic change in cellular protein repertoire is critical for biological processes, including stem cell state transitions and regeneration. Translational regulation modulates protein repertoire during cell state transition and organogenesis. Our lab studies *in vivo* regenerative models like planarian *Schmidtea mediterranea* and *in vitro* stem cell models like mouse embryonic stem cells (mESCs) to identify novel mechanisms and regulators of translation. We identified non-coding RNAs and RNA binding proteins that interact with mRNA's 3' UTR/polyA tail, regulating stem cell proliferation and differentiation. Our work also demonstrated that mitochondrial state changes are critical for stem cell maintenance and differentiation; however, these mechanisms are not well understood. Our unpublished data indicate that nuclear-encoded transcripts associated with RNP granules containing Tudor protein are translationally repressed and are essential for mitochondrial fusion and oxidative phosphorylation. We aim to understand how state changes in mitochondria are regulated during stem cell differentiation by deciphering the mechanism of translation regulation.

Report:

Mitochondria, the bioenergetic hub of a cell, play an important role in stem cell function. Our recent research on planaria showed how the mitochondrial state is a defining feature of the self-renewal and differentiation of pluripotent stem cells. Pluripotent stem cells were found to have low mitochondrial mass, which increased as the cells transitioned towards differentiation. Inhibition of mitochondrial activity led to increased stemness, indicating that changes in mitochondrial state are essential for differentiation.

By using mitochondrial state changes to enrich pluripotent stem cells, we were able to identify pluripotent stem cell populations of planaria. Our single-cell transcriptome analysis revealed the enrichment of ribosomal proteins and translation regulators in these cells. However, we still need to address several questions, such as why ribosomal proteins, which are core components of ribosomes, are enriched in pluripotent stem cells, and what role these proteins play in the maintenance of stemness. Knockdown of these ribosomal proteins led to specific defects during cell cycle progression and differentiation of neoblast to specific lineages. Our work highlights the heterogeneity in the composition of the ribosomal subunits critical for the maintenance and differentiation of stem cells. We are currently investigating the mechanisms essential for the biogenesis of ribosomal heterogeneity and how this heterogeneity could regulate protein synthesis critical for stem cell function and regeneration.

Our work also showed high levels of Tudor domain-containing proteins TDRD9 and TDRD12 in the pluripotent stem cell population of planaria. In mammals, these proteins are part of the RNP granule essential for piRNA biogenesis and regulation of transposable elements. Knockdown of TDRD9 in planaria led to defects in regeneration and a reduction in the stem cell population. Our data also showed the expression of these proteins in mammalian embryonic stem cells, suggesting that these proteins might have a functionally conserved role in the maintenance of pluripotency. Our recent study showed that mitochondrial state changes from an inactive to an active state of oxidative phosphorylation

are critical for the differentiation of the neoblast. We hypothesise that Tudor proteins, through their association with mitochondria in the neoblast, might regulate the function and activity of the mitochondria critical for the maintenance of pluripotency.

In summary, our work at inStem has helped to unravel novel mechanisms and factors that regulate protein synthesis essential for stem cell function. We are also working towards understanding ribosome assembly and its function in translational regulation, which has strong therapeutic implications in stem cell biology and regenerative medicine.

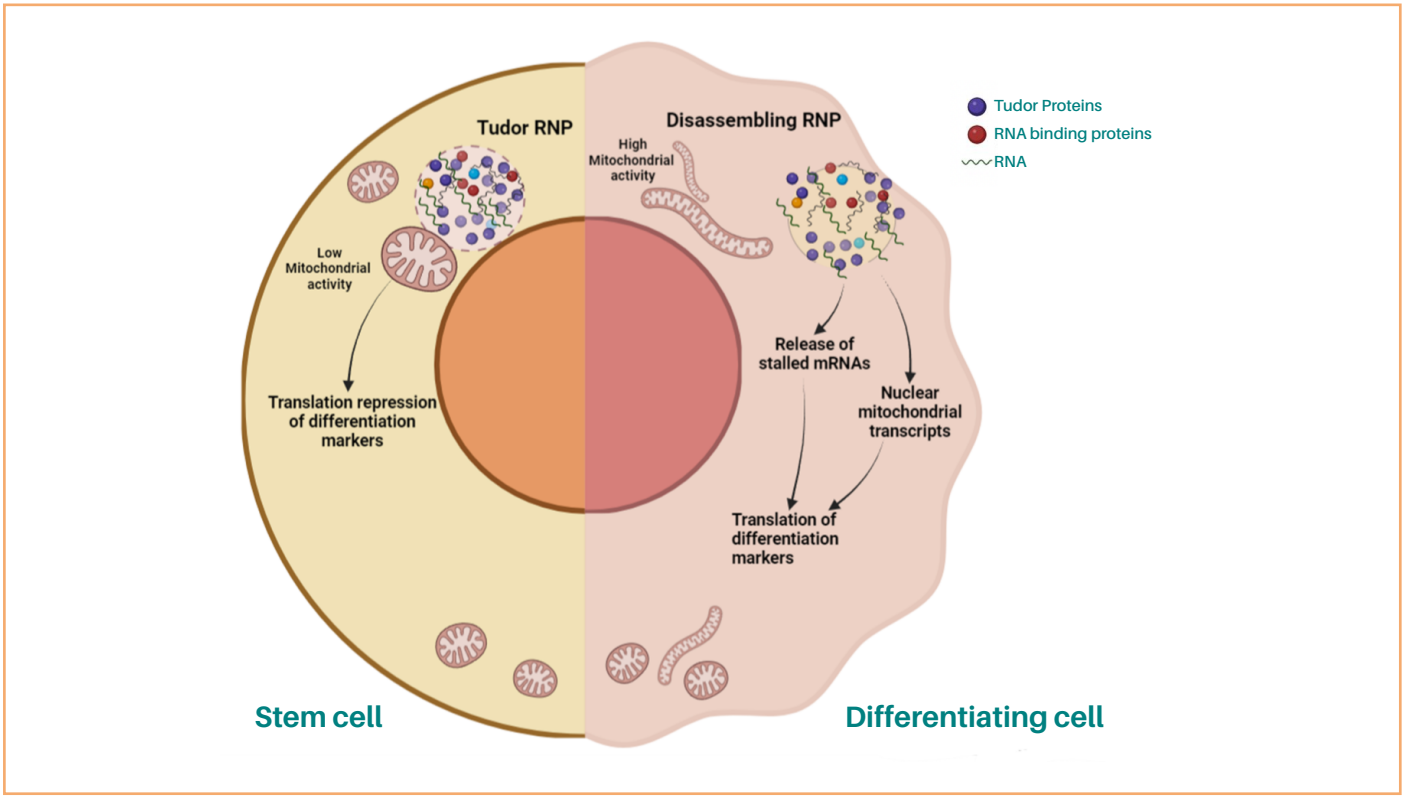
Key findings in the past year:

1. Using single-cell transcriptomic approaches, we identified the metabolic states that could potentially define the pluripotent state of the neoblast.
2. We functionally characterised the ribosomal proteins enriched in the pluripotent stem cell population of planaria.
3. We dissected the functional role of Tudor-containing RNP granules in stem cell maintenance and differentiation.

Projects/Research Questions for the following year:

- Decipher the ribosomal code critical for stem cell maintenance and differentiation
- Establish the role of Tudor domain proteins (TDRD9, TDRD12, and TDRD1) in the regulation of mitochondrial states critical for stem cell function
- Dissection role of the poly A tail and polyadenylation machinery in stem cell function and regeneration

Graphical summary:



Publications:

1. Corsi GI, Gadekar VP, Haukedal H, Doncheva NT, Anthon C, Ambardar S, **Palakodeti D**, Hyttel P, Freude K, Seemann SE, Gorodkin J. 2023. The transcriptomic landscape of neurons carrying PSEN1 mutations reveals changes in extracellular matrix components and non-coding gene expression. *Neurobiology of Disease,* 178, 105980. doi: 10.1016/j.nbd.2022.105980
2. Dubey VK, Sarkar SR, Lakshmanan V, Dalmeida R, Gulyani A, **Palakodeti D**. 2022. S. mediterranea ETS-1 regulates the function of cathepsin-positive cells and the epidermal lineage landscape via basement membrane remodeling. *Journal of Cell Science, 135*(20), jcs259900. doi: 10.1242/jcs.259900
3. D'Souza MN, Ramakrishna S, Radhakrishna BK, Jhaveri V, Ravindran S, Yeramala L, Nair D, **Palakodeti D**, Muddashetty RS. 2022. Function of FMRP domains in regulating distinct roles of neuronal protein synthesis. *Molecular Neurobiology, 59*(12), 7370–7392. doi: 10.1007/s12035-022-03049-1
4. Sarkar SR, Dubey VK, Jahagirdar A, Lakshmanan V, Haroon MM, Sowndarya S, Sowdhamini R, **Palakodeti D**. 2022. DDX24 is required for muscle fiber organization and the suppression of wound-induced Wnt activity necessary for pole re-establishment during planarian regeneration. *Developmental Biology,* 488, 11–29. doi: 10.1016/j.ydbio.2022.04.011
5. Hariharan N, Ghosh S, **Palakodeti D**. 2023. The story of rRNA expansion segments: Finding functionality amidst diversity. *Wiley Interdisciplinary Reviews RNA, 14*(1), e1732. doi: 10.1002/wrna.1732
6. Haroon MM, Vemula PK, **Palakodeti D**. 2022. Flow cytometry analysis of planarian stem cells using DNA and mitochondrial dyes. *Bio-Protocol, 12*(2), e4299. doi: 10.21769/BioProtoc

Outreach and other activities:

- Delivered an invited lecture titled *"Sneek peek into mechanisms that regulate stem cell function and regeneration in planarians"* at ShivNadar hosted by Prof. Sanjeev Galanade and Dr. Ashish on 8th December 2022
- Delivered an invited lecture titled *"Sneek peek into mechanisms that regulate stem cell function and regeneration in planarians"* at Ashoka University hosted by Dr. Kasturi Mitra on 9th December 2022.
- Co-organised International Genomic Analysis and Technology Conference, GATC-2023 from 6–9th February 2023 at inStem.
- Hosted students from the Centre for Human Genetics, Electronic City on 15th October 2022, to demonstrate how planarian models can be used for stem cell and regeneration studies.

3.5 Praveen Kumar Vemula

Title of the research program:

Developing biomedical technologies for unmet clinical needs

Summary:

The lab's primary focus is to develop a wide range of technologies to solve unmet clinical needs and translate those technologies through entrepreneurial efforts. During the reporting period, our lab has developed a novel medical device, a microneedle-based adopter, for painless injections. Repeated insulin injections with conventional insulin syringes cause microinjury to tissues and exert mechanical forces, which leads to fibrotic tissue and lipohypertrophy (adipose tissue formation). Therefore, our device eliminates injection-induced skin tension and prevents mechanical forces-induced tissue fibrosis and lipohypertrophy. Insulin could be intradermally administered using this device without causing pain and has been shown to control hyperglycaemia in diabetic rats.

Report:

Millions of people worldwide require daily insulin injections to control blood sugar levels, known as glycaemic control. Insulin administration is carried out with conventional 27-31 Gauge hypodermic needle-based syringes and insulin pens. These intramuscular and subcutaneous injections cause pain, and repeated injections can cause micro-injury that elicits inflammation. The injection-induced inflammation can lead to fibrotic tissue formation. In addition, needle insertion can cause tension on the skin. It is known that needle-induced mechanical forces can activate fibroblasts and adipocytes to induce fibrosis and adipogenesis, respectively. Therefore, developing a microneedle-based medical device to administer insulin intradermally and eliminate the mechanical forces on the skin can have a huge impact on diabetic management.

The main challenge in achieving successful intradermal drug delivery involves strategies to overcome resistance by the top layer of the skin called the stratum corneum (SC). A drug molecule that successfully crosses SC can be transported through the epidermis to the dermis, where it can readily achieve systemic circulation or trigger immunogenic reactions through specialised carrier cells (called dendritic cells). Micron-sized needles, called "*microneedles*", are very effective in breaking the SC barrier to make epidermal and dermal layers accessible for drug delivery. We developed a microneedle device for intradermal delivery and proved its functionality in Sprague–Dawley (SD) rats. We also observed similar efficacy in glycaemic control between conventional subcutaneous injections and microneedle-device-assisted intradermal injections in SD rats. The novel features of this device include a unique needle tip profile and special device-skin interfacing assisted with a passive vacuum mechanism. The absence of any active system, such as pumps, allowed the development of syringe retrofit, a disposable device that can be readily used on a large scale. This device has been made using a stainless steel needle with a unique tip profile glued to an adapter made up of biocompatible plastic. Within needle heights of just 700 μm, the device can accomplish leakage-free drug delivery in the SD rat model.

Through this device, insulin can be administered without causing pain, and we demonstrated that the same dose of insulin administered through our device and conventional hypodermic syringes have similar efficacy in controlling hyperglycaemia in diabetic SD rats. Repeated daily injections for six months showed that conventional syringes activated fibroblasts and adipocytes to form fibrotic and lipohypertropic tissues, respectively. In contrast, repeated injections with the microneedle-device prevented fibrotic tissue formation and lipohypertrophy in rats.

Key findings in the past year:

We developed a novel microneedle device that can administer insulin without causing pain, with comparable efficacy to control hyperglycaemia in diabetic animals. The novel device with a passive vacuum mechanism eliminated the tension caused by needle insertion, which prevented repeated injection-induced fibrosis and lipohypertrophy.

Graphical summary:

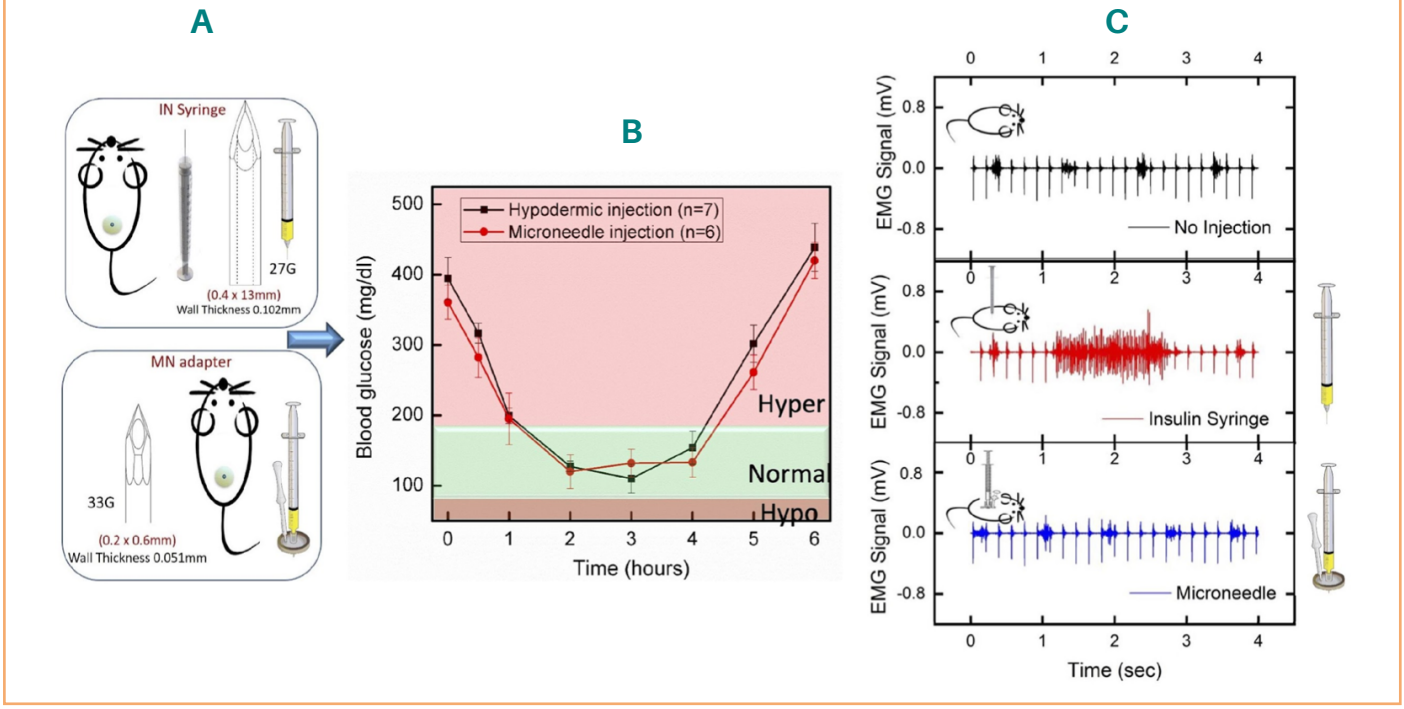


Figure: A) Schematic representation of needle-tips and devices of the conventional hypodermic syringe and microneedle-adopter-based syringe. B) Glycaemic control in streptozotocin-induced diabetic SD rats. A single-dose insulin administration through conventional hypodermic injection or a microneedle device shows identical efficacy in controlling hyperglycaemia. C) Electromyogram demonstrating that conventional insulin syringes cause muscle twitch (surrogate to pain), whereas microneedle device eliminates the muscle twitch, suggesting that microneedle device is painless.

Publications:

1. Kotla NG, Isa ILM, Larranaga A, Maddiboyina B, Swamy SK, Sivaraman G, **Vemula PK**. 2023. Hyaluronic acid-based bioconjugate systems, scaffolds, and their therapeutic potential. *Advanced Healthcare Materials, 12*(20), e2203104. doi: 10.1002/adhm.202203104

2. Pandey S, Mahato M, Srinath P, Bhutani U, Goap TJ, Ravipati P, **Vemula PK**. 2022. Intermittent scavenging of storage lesion from stored red blood cells by electrospun nanofibrous sheets enhances their quality and shelf-life. *Nature Communications,* 13, 7394. doi: 10.1038/s41467-022-35269-3

3. Ghate V, Renjith A, Badnikar K, Pahal S, Jayadevi SN, Nayak MM, **Vemula PK**, Subramanyam DN. 2022. Single step fabrication of hollow Microneedles and an experimental package for controlled drug delivery. *International Journal of Pharmaceutics,* 632, 122546. doi: 10.1016/j.ijpharm.2022.122546

4. Pahal S, Boranna R, Tripathy A, Goudar VS, Veetil VT, Kurapati R, Prashant GR, **Vemula PK**. 2022. Nanoarchitechtonics for free-standing poly electrolyte multilayers films: Exploring the flipped surfaces. *ChemNanoMat* , 8, e202200462. doi: 10.1002/cnma.202200462
5. Sunnapu O, Subramanian S, **Vemula PK**, Karuppannan S. 2022. Zingerone-encapsulated solid lipid nanoparticles as oral drug delivery systems to potentially target inflammatory diseases. *ChemNanoMat*, 8, e202200388. doi: 10.1002/cnma.202200388
6. Sunnapu O, Khader R, Dhanka M, **Vemula PK**, Karuppannan S. 2022. Enzyme-responsive hydrogel for delivery of an anti-inflammatory agent zingerone. *ChemNanoMat*, 8, e202200334. doi: 10.1002/cnma.202200334
7. Ghosh S, Singh R, Vanwinkle ZM, Guo H, **Vemula PK**, Goel A, Haribabu B, Jala VR. 2022. Microbial metabolite restricts 5-fluorouracil-resistant colonic tumor progression by sensitizing drug transporters via regulation of FOXO3-FOXM1 axis. *Theranostics*, 12, 5574–5595. doi: 10.7150/thno.70754
8. Dhayani A, Bej S, Mudnakudu-Nagaraju KK, Chakraborty S, Srinath P, Kumar AH, Ann Maria PS, Khristi A, Ramakrishnan S, **Vemula PK**. 2022. An amphiphilic double-brush polymer hydrogel for sustained release of small molecules and biologics: Insulin-delivering hydrogel to control hyperglycemia. *ChemNanoMat*, e202200184. doi: 10.1002/cnma.202200184

Patents (2022-2023):

- *"Scaffolds for selective scavenging of storage lesion from biological material and methods thereof"* Praveen Kumar Vemula, Manohar Mahato, Subhashini Pandey, Preethem Srinath, Utkarsh Bhutani. PCT Application: PCT/IB2023/052628 (Filed: 2023)
- *"Therapeutic agents for enhancing epithelial and/or endothelial barrier function"* Praveen Kumar Vemula, Nicholas Kenneth Terrett, Sakthimala Jagadeesan, Venkatesh Ravula. USA Provisional Patent Application: 63/419,015 (Filed: 2022)
- *"Compositions and methods to enhance the quality and shelf-life of biological materials"* Praveen Kumar Vemula, Manohar Mahato, Subhashini Pandey, Preethem Srinath, Utkarsh Bhutani. India Provisional Patent Application Number: 202241014827 (Filed: 2022)

Awards and grants (2022/2023):

- Received the National Biotechnology Innovation Award 2023
- Received the SERB-SUPRA (Scientific and Useful Profound Research Advancement) grant for the period of 2023–2026 for the project entitled *"Development of machine for rapid fabrication of the biocompatible microneedles, followed by clinical testing"*

Outreach and other activities:

- Delivered ~20 invited talks on science and entrepreneurship across many colleges and university campuses to encourage and inspire students

3.6 Colin Jamora

Title of the research program:

Wound healing and allied diseases

Summary:

The overall objective of our laboratory is to decipher the regulation of tissue regeneration and repair and apply these insights to biomedical applications. With a major focus on understanding the cutaneous wound healing response, our efforts are centred on achieving a mechanistic understanding of the crosstalk between different cell types in the skin, such as stem cells, immune cells, and the vasculature that collectively function to repair the tissue when it is damaged. These activities in the basic sciences are complemented and enhanced with translational studies on wound healing-related diseases that pose a substantial public health burden such as diabetes, cancer, and tissue fibrosis.

Report:

With the goal of advancing the field of tissue regeneration and repair, there are currently three major areas of focus in the laboratory:

- Dissecting how stem cell decisions are made to regenerate a barrier tissue such as the skin
- Understanding how these decisions are skewed either during disease (e.g., cancer, diabetes, and inflammatory diseases) or during physiological processes (such as wound healing)
- Elucidating the complex network of signals exchanged between cells within the tissue to produce an efficient wound-healing response

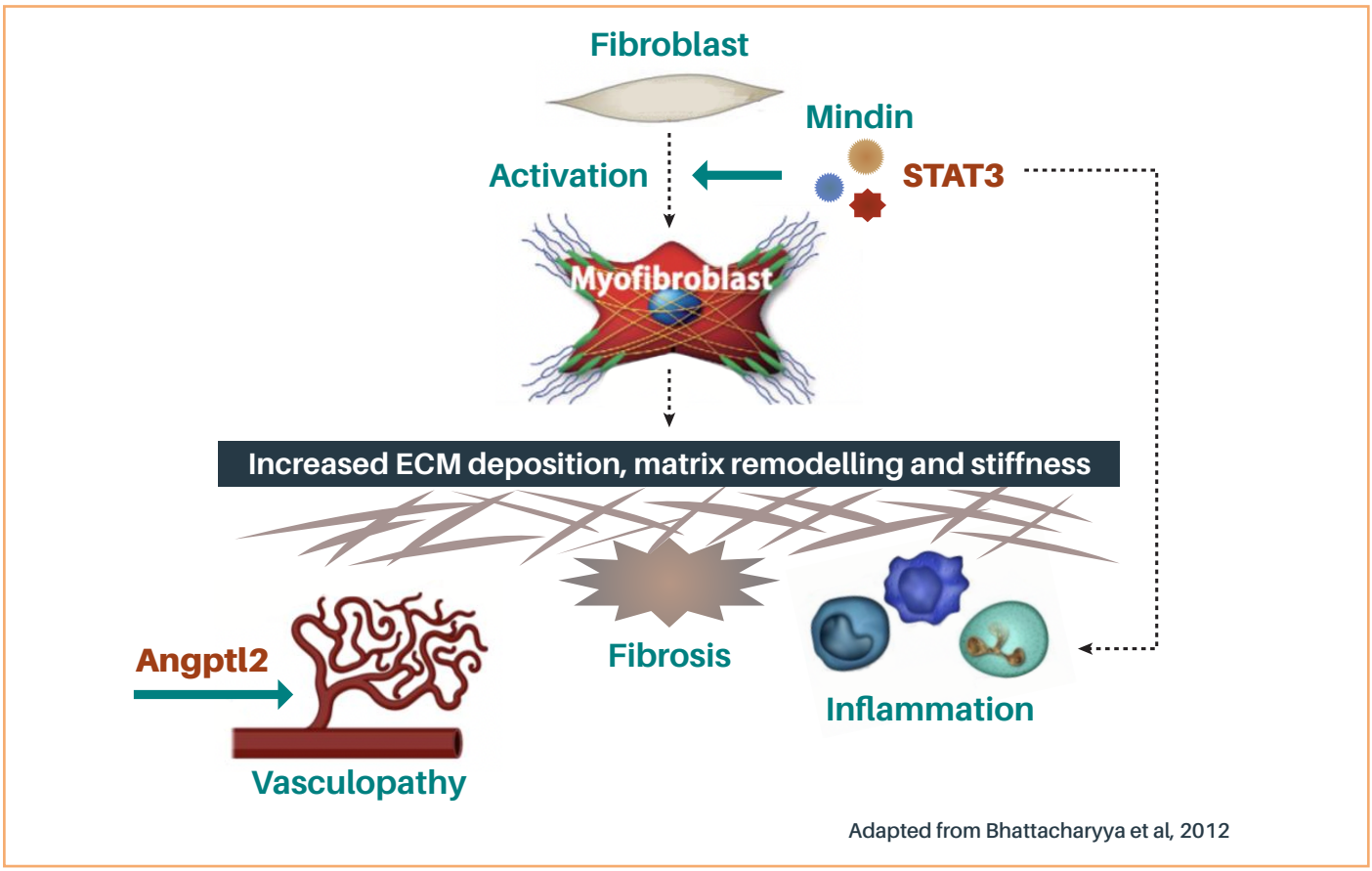
Key findings in the past year:

- Developed a novel mouse model that recapitulates the diagnostic features of the human fibrotic skin disease called scleroderma
- Identified the protein Mindin (Spondin-2) as a critical player in the process of fibrogenesis
- Elucidated a role for Mindin in maintaining the stemness of cancer stem cells, and thus uncovered a novel target to cripple these cells and potentially control metastasis and relapse following chemotherapy
- Discovered that the initiation of the wound healing program in the skin is the product of both mechanical and epigenetic cues

Research directions for the following year:

1. Continue to decipher the intracellular crosstalk in the skin that mediates physiological conditions such as wound healing and pathological scenarios such as fibrosis and cancer
2. Unravel the epigenetic and mechanical regulation of the initiation of the wound healing response
3. Determine whether identified proteins from the fibrosis studies can be used as diagnostic biomarkers and/or therapeutic targets

Graphical summary:



Publications:

1. Rana I, Kataria S, Tan TL, Hajam EY, Kashyap DK, Saha D, Ajnabi J, Paul S, Jayappa S, Ananthan AS, Kumar P, Zaarour RF, Haarshaadri J, Kansagara G, Rizvi A, Zirmire RK, Badarinath K, Khedkar SU, Chandra Y, Samuel R, George R, Danda, D, Jacob PM, Dey R, Dhandapany PS, He YW, Varga J, Varghese S, **Jamora C.** 2023. Mindin is essential for cutaneous fibrogenesis in a mouse model of systemic sclerosis. *Journal of Investigative Dermatology, 143*(5), 699-710.e10. doi: 10.1016/j.jid.2022.10.011

2. Zaarour RF, Saha D, Dey R, Dutta A, Kumar P, Rana I, Pulianmackal A, Rizvi A, Misra N, Breton L, **Jamora C.** 2022. The neuropeptide Substance P facilitates the transition from an inflammatory to proliferation phase-associated responses in dermal fibroblasts. *Experimental Dermatology,* 31, 1188–1201. doi: 10.1111/exd.14573

3. Sobecki M, Chen J, Krzywinska E, Nagarajan S, Fan Z, Nelius E, Monne Rodriguez JM, Seehusen F, Hussein A, Moschini G, Hajam EY, Kiran R, Gotthardt D, Debbache, J, Badoual C, Sato T, Isagawa T, Takeda N, Tanchot C, Tartour E, Weber A, Werner S, Loffing J, Sommer L, Sexl V, Munz C, Feghali-Bostwick C, Pachera E, Distler O, Snedeker J, **Jamora C**, Stockmann C. 2022 Vaccination-based immunotherapy to target profibrotic cells in liver and lung. *Cell Stem Cell,* 29, 1459–1474.e9. doi: 10.1016/j.stem.2022.08.012

4. Hajam EY, Panikulam P, Chu CC, Jayaprakash H, Majumdar A, **Jamora C.** 2022. The expanding impact of T-regs in the skin. *Frontiers in Immunology,* 13, 983700. doi: 10.3389/fimmu.2022.983700

5. Bhatt T, Dey R, Hegde A, Ketkar AA, Pulianmackal AJ, Deb AP, Rampalli S, **Jamora C**. 2022. Initiation of wound healing is regulated by the convergence of mechanical and epigenetic cues. *PLoS Biology* **20**, e3001777. doi: 10.1371/journal.pbio.3001777

6. Badarinath K, Dam B, Kataria S, Zirmire RK, Dey R, Kansagara G, Ajnabi J, Hegde A, Singh R, Masudi T, Sambath J, Sachithanandan SP, Kumar P, Gulyani A, He YW, Krishna S, **Jamora C**. 2022. Snail maintains the stem/progenitor state of skin epithelial cells and carcinomas through the autocrine effect of matricellular protein Mindin. *Cell Reports* 40, 111390. doi: 10.1016/j.celrep.2022.111390

Awards and grants (2022/2023):

- Selected as *PLoS Biology* Academic Editor from 2021–2024
- Selected as a member of the Scientific Advisory Committee, Therapy Resistance and Stem Cell Biology Group, Advanced Centre for Treatment, Research, and Education in Cancer (ACTREC), Mumbai India from 2021–present
- Received DBT grant of INR 180 lakhs (January 2021–January 2024) for the project entitled "*Epigenetic regulation of the wound healing program*"
- Received DBT grant of INR 168.75 lakhs (November 2020–May 2023) for the project entitled "*COVID bioresource*"
- Received DBT grant of INR 334.35 lakhs (November 2021–October 2024) for the project entitled "*Translational platforms for discovery, repurposing, and clinical development for COVID-19 therapeutics*"
- Received BIRAC grant of INR 1245 lakhs (November 2021–September 2023) for the project entitled "*Enabling preclinical studies for COVID-19 research, diagnostics, and drug development*"
- Received BIRAC grant of INR 674.12 lakhs (September 2022–September 2023) for the project entitled "*Enabling COVID-19 vaccine and therapeutics development using animal models*"
- Received DBT grant of INR 23.70 lakhs (December 2022–December 2023) for the project entitled "*Genomic surveillance for SARS-CoV-2 in India: Indian SARS-CoV-2 Genomics Consortium (INSACOG)-Phase II*"
- Received CCAMP-InDx (Rockefeller Foundation) grant of INR 129.50 lakhs (September 2020–May 2023) for the project entitled "*InDx Centre of Excellence for Clinical Studies*"

Patents:

- Provisional Application (Joint with Unilever): Products and compositions for the treatment of viral infections (Application No: IN 202211048425, filed on 25.08.2022)

Four Spin-off Companies:

- [ ]

Outreach and other activities:

- Gave a seminar on "*Stem Cells and Wound Healing*" at the Indian Academy Degree College in May 2022
- Gave a seminar on "*Stem Cells and Wound Healing*" at Rajiv Gandhi Centre for Biotechnology (RGCB) in February 2022 as part of National Science Day celebration
- Delivered a talk titled "*Regulation of epithelial stem cells in wounded skin*" at the University of Hyderabad in October 2022
- Delivered a talk titled *"Regulation and deployment of stem cells in the wounded skin"* at the Stem Cell and Regenerative Medicine Conference, SPGIMS, Centre for Stem Cell Research in November 2022
- Delivered a talk titled "*The matricellular protein Mindin drives fibrogenesis in a mouse model of scleroderma*" at the University of Michigan, Skin Biology and Disease Research Centre in December 2022
- Delivered a talk titled "*Regulation of the wound healing response*" at Shiv Nadar University in May 2023
- Gave a seminar on "*A multidisciplinary approach towards understanding the wound healing program*" at the Bioanalytic Workshop, held in IISER Kolkata in June 2023

3.7 Arjun Guha

Title of the research program:

Mechanisms of lung injury-repair

Summary:

My laboratory is broadly interested in the mechanisms that protect the lungs from environmental toxicants and repair the lungs in the aftermath of injury. Our research in the 2023–2024 period, based predominantly on the mouse model and cell lines of murine and human origin, has led to advances in both these areas. Looking ahead, we are invested in expanding the repertoire of environmental challenges that we work with to include common infectious agents and to extend our findings to the human lung.

Report:

We have made significant advances in our understanding of how stress responses in the lung are regulated, how the plasticity of epithelial cells during injury-repair is regulated, and how senescent cells, a pathological feature of aging and fibrotic lungs, are regulated. A central idea that motivates our research is that the ability of the lung to respond effectively to environmental challenges is critically dependent on efficient cellular remodelling and plasticity.

Key findings in the past year:

First, we have advanced our understanding of how the integrated stress response (ISR) pathway is regulated in the lung. We found that the fragile X mental retardation protein (FMRP) specifically regulates induction of the protein kinase R (PKR)-dependent arm of the ISR by facilitating the interaction of PKR and its substrate eif2alpha. Our studies to date strongly suggest that individuals with fragile X syndrome living in areas of higher pollutant load may be more susceptible to lung damage/disease.

Second, we established that Notch signalling has a pervasive role in maintaining the fate of the mucociliary epithelium. Loss of Notch signalling leads to remodelling of multiciliated cells and the transdifferentiation/dedifferentiation of non-ciliated secretory cells. We surmise that the epithelial cell remodelling and plasticity evidenced upon Notch inhibition are essential for the maintenance and restoration of airway homeostasis.

Third, we showed that an autocrine TGF-beta signalling pathway actively controls the senescent state in lung-derived cell lines. Our study implicates senescent cells as potent drivers of lung fibrosis.

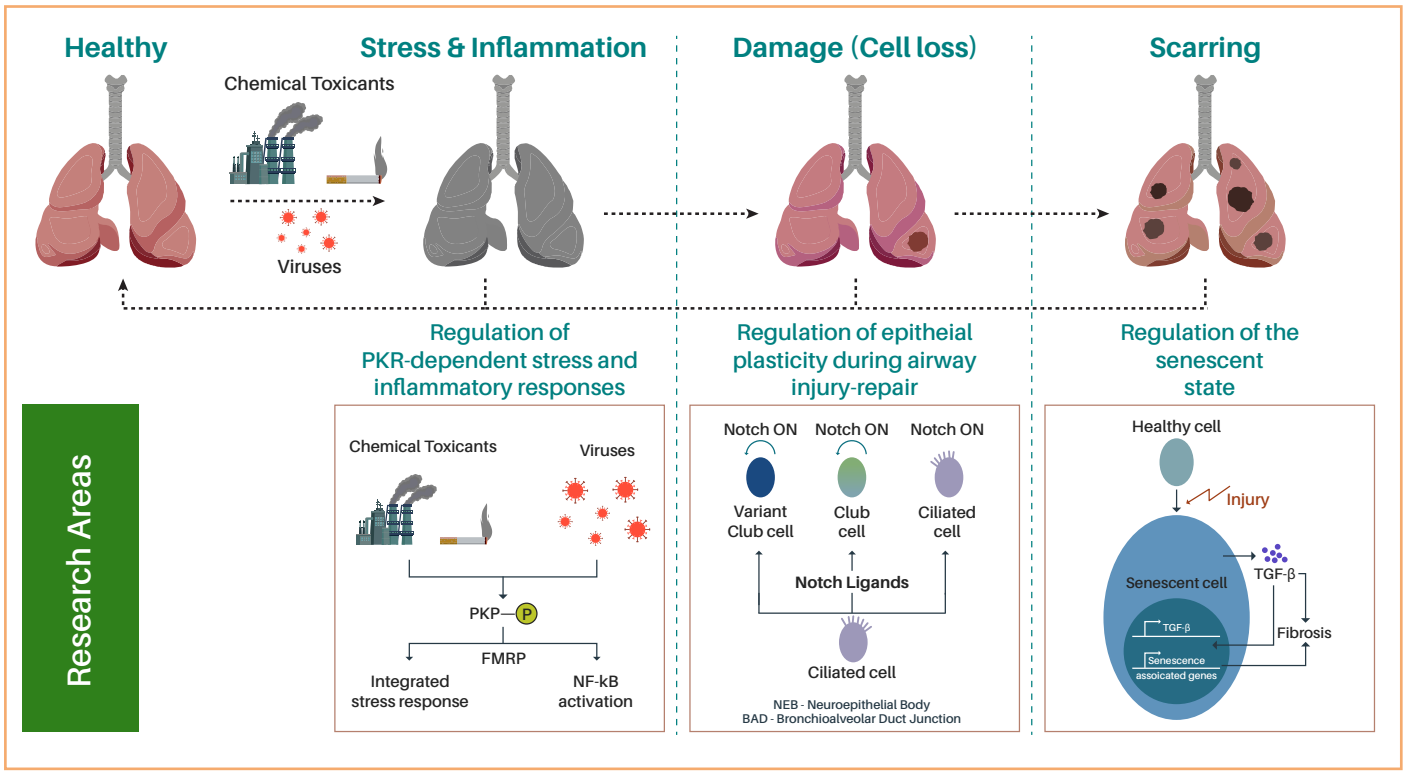
Projects/Research Questions for the following year:

First, we would like to probe the role of FMRP in the human lung by developing iPS-derived lung organoids. We will expose normal and FMRP-deficient lung organoids to both chemical (diesel exhaust) and viral (SARS-CoV-2) challenges.

Second, we would like to probe the signalling systems that act in concert with Notch signalling to regulate remodelling and plasticity of airway epithelial cells. We would also like to extend our findings on the pervasive role of Notch signalling in the mouse lung to the human lung using iPS/adult stem cell-derived airway organoids.

Third, we are interested in probing the role of senescent cells as regulators of cellular remodelling and plasticity in ageing and fibrotic lungs.

Graphical summary:



Publications:

Three manuscripts are in preparation and will be submitted by the end of 2023.

Outreach and other activities:

- Presented at various online (India Investigator Network, India, 2nd Subhash Mukhopadhyay Symposium, India, Marsico Lung Institute, USA) and in-person (Simons Foundation Cell Lineages and Development Meeting, India, and IISER-Pune, India) research seminars
- Attended the Chan Zuckerberg Initiative Annual Single Cell Biology Meeting, USA
- Co-organised and taught a basic course titled *"Introduction to Cell Biology"* to incoming students. Additionally, taught a basic course on Developmental Biology
- Served as the Head/Chair of inStem Institutional Technical Services, inStem Building Committee, and a team that is designing and constructing an animal BSL3 facility. Served on several other campus committees
- Organised and acted as an instructor in the Bangalore Microscopy Course

3.8 Diya Binoy Joseph

Title of the research program:

Epithelial homeostasis and innate immune defences in the genitourinary tract

Summary:

The reproductive and urinary systems, collectively known as the genitourinary tract, are lined by specialised epithelial linings tailor-made to perform essential physiological functions in their respective milieus as well as to protect from infection and other environmental insults. Epithelial patterns in the genitourinary tract are established early in the development of this system. During postnatal and adult life, these epithelial linings act as robust biochemical and physical barriers against pathogens and xenobiotics. My program aims to understand how epithelial linings in the genitourinary tract are patterned and how these epithelial linings acquire innate immune defences to protect against diverse insults.

Report:

The establishment of epithelial differentiation patterns in the lower urinary tract is of particular interest to my lab. Early patterning in the embryo results in the differentiation of bladder and urethral epithelia from a common precursor. This foreshadows later differences in epithelial gene content between the bladder and urethra that also impact epithelial defences and the resident immune niche in these organs. My doctoral work showed that the embryonic bladder mesenchyme possessed the capacity to reprogram non-bladder epithelial cells into bladder-like cells with important implications for bladder regeneration. We focused on identifying early genes patterning the embryonic bladder and urethra.

Urinary tract infections are common worldwide and represent a significant healthcare burden. Infections disproportionately affect women and are a common cause of morbidity in older patients who are hospitalised or undergoing repeated catheterisation. Urinary tract infections are majorly caused by uropathogenic *Escherichia coli* (UPEC) originating from the gut. Although bacteria need to ascend the urethra to seed infection in the bladder, the role of the urethra during infection is largely unexplored. My postdoctoral work identified that the urethral lining constitutively expresses genes involved in antimicrobial defence. We aimed to address a significant gap in the field i.e. the role of the urethra during the course of urinary tract infections.

Key findings in the past year:

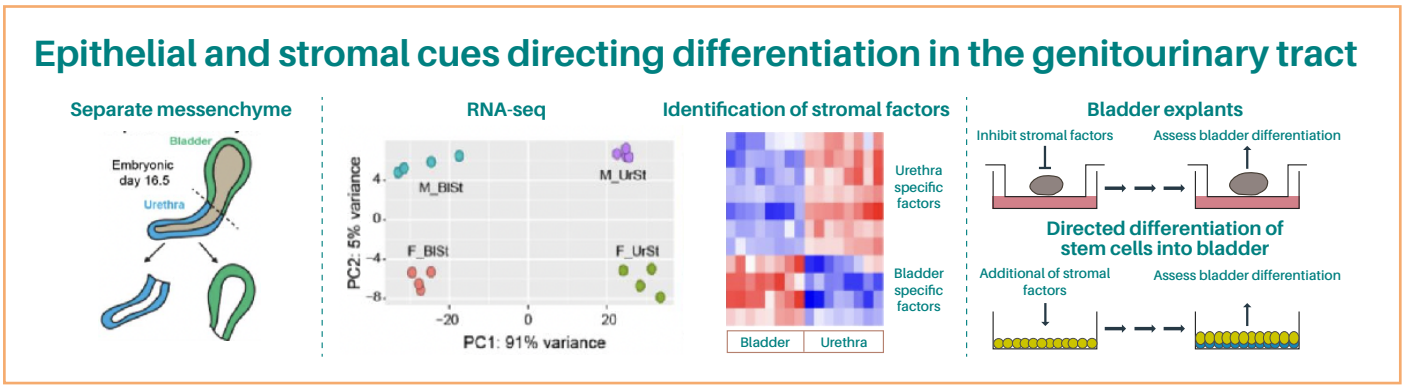
1. Distinct stromal genes pattern the developing lower urinary tract: To identify stromal factors patterning the lower urinary tract, we performed gentle enzymatic digestion of embryonic day 16.5 lower urinary tracts from mice and performed mechanical separation of the epithelial and mesenchymal layers. Using RNA sequencing, we identified a plethora of stromal factors that showed preferential distribution in the urethral or bladder mesenchyme. These factors cover the gamut of growth factors, morphogens, and inhibitors of key signalling pathways like Wnt and Bmp/Smad signalling. Currently, we are validating the spatial localisation of these genes in the developing lower urinary tract by in-situ hybridisation.

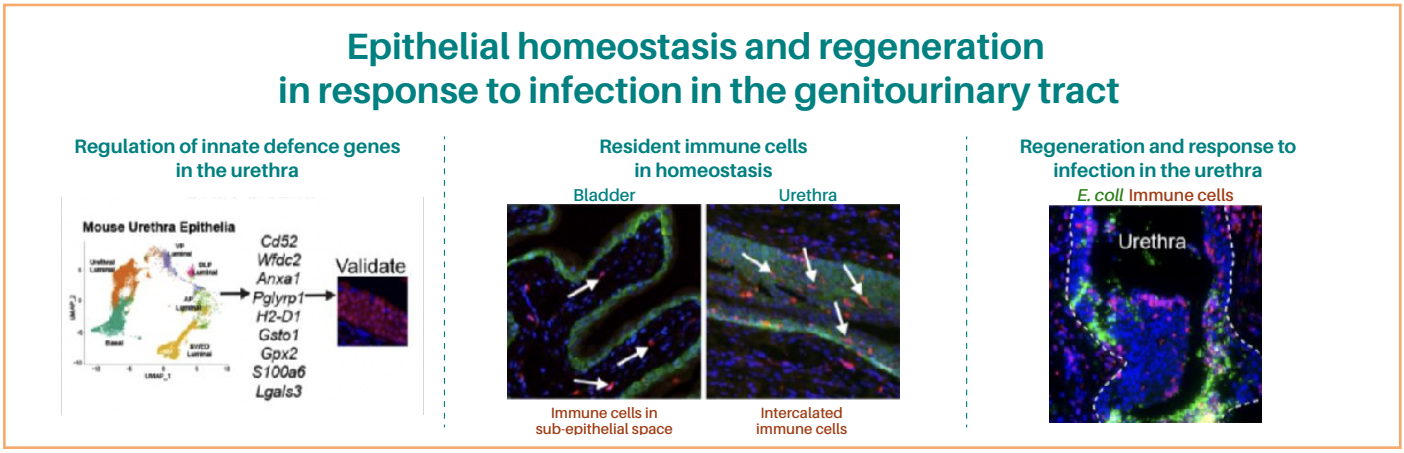
2. **Intercalation of macrophages in the urethral lining:** Urethral epithelial cells express heightened levels of antimicrobial and immunomodulatory genes compared to non-barrier epithelium. Coupled with the presence of chemosensory and neuroendocrine cells interspersed in the urethral lining, this suggests a sensory role for the urethra in pre-empting uropathogenic bacteria from infecting the bladder. Tissue-resident immune cells play an important role in restoring homeostasis after an injury or infection. We found that the macrophages are the predominant immune population in the urethra, comprising approximately 50% of immune cells with a greater number of macrophages in the female urethra than in the male urethra under resting conditions. Additionally, we observed that macrophages in the male and female urethra are intercalated in the epithelial layer compared to their sub-epithelial localisation in the bladder.
3. **The urethra can harbour UPEC during an infection:** We demonstrated that primary human urethral cells isolated from the proximal prostate region of male organ donors can support adhesion and invasion by UPEC. Bacterial infection induces an NF-KB-mediated response in these cells, which show increased expression of interleukin-6 and tumour necrosis factor. Additionally, we performed transurethral instillation of fluorescently-labelled *E. coli* in wildtype male and female mice and observed consistent infection of the urethra. We observed significant infiltration of circulating macrophages into the urethra during infection. Further studies are being conducted to understand the inflammatory response in the urethra and how it differs from that in the bladder.

Projects/Research Questions for the following year:

1. **The role of Galectin-3 in urinary tract infections:** Galectin 3, encoded by the *Lgals3* gene, is an immunomodulatory lectin with a proposed role in infectious diseases and inflammation. We traced the expression of this gene to the epithelial lining of the lower urinary tract, immune cells, selected tubules in the kidney, and the parietal epithelial cells lining the Bowman's capsule. Preliminary studies with uropathogenic infection in wildtype and knockout mice suggest that loss of *Lgals3* results in an increased infection burden in the urethra, bladder, and kidney at 24 hours post-infection.
2. **Urinary tract microbiota:** We hypothesised that exposure of the lower urinary tract epithelium to its native microbiota allows for immune education of the epithelial lining and the constitutive expression of innate immune genes in the urethral lining, which serves as the first line of defence against ascending infections into the bladder. As a first step, we assessed bacterial burden in the lower urinary tract and successfully identified low levels of bacterial DNA by PCR for 16S rRNA in the urine, urethra, and bladder. We are currently optimising DNA extraction protocols to isolate bacterial genomic DNA for 16S rRNA gene amplicon sequencing.

Graphical summary:





Publications:

Joseph DB, Henry GH, Malewska A, Reese JC, Mauck RJ, Gahan JC, Hutchinson RC, Mohler JL, Roehrborn CG, Strand JDW. 2022. 5-Alpha reductase inhibitors induce a prostate luminal to club cell transition in human benign prostatic hyperplasia. *The Journal of Pathology, 256*(4), 427–441. doi: 10.1002/path.5857 (from postdoctoral work).

Awards and grants (2022/2023):

- DST SERB Start-up research grant (Start date: September 2022)
- DST-Inspire Faculty Fellowship (declined in favour of the India Alliance Early Career Fellowship)
- India Alliance Early Career Fellowship (Start date: February 2023)

Outreach and other activities:

1. Part of the screening committee for graduate program applications from May–June 2022
2. Part of the planning committee for the 3rd floor terrace renovation of inStem in June 2022
3. Ad hoc reviewer for the *European Journal of Pharmacology, Journal of Experimental Pharmacology,* and *International Journal of Molecular Sciences*
4. Co-coordinator, with Dr. Sudarshan Gadadhar, for inStem Foundation Day Program, held on 27th August 2022
5. Delivered three lectures on genitourinary tract development as part of the Developmental Biology course in December 2022
6. Part of the selection panel for inStem Mid-term interviews in December 2022
7. Part of the Stem cell facility advisory committee and Tissue culture facility advisory committee from December 2022–present
8. Part of the selection committee for interview for Senior Technical Officer (Electrical)-08/22-UR at DBT-inStem-reg in February 2023
9. Moderator, Panel for International Day of Women and Girls in Science at inStem in February 2023
10. Instructor at the EMBO Workshop in February 2023 at IISc, which included a soft-skills workshop for new investigators
11. Co-editor, *Frontiers in Immunology* special issue (ongoing since March 2023)

3.9 Sudarshan Gadadhar

Title of the research program:

Role of tubulin posttranslational modifications in regulating primary cilia functions that control tissue homeostasis

Summary:

Primary cilia are a hub of signalling and sensory activities through which they regulate development, organ function, homeostasis, and regeneration. These microtubule-based structures undergo diverse tubulin posttranslational modifications (PTMs), two of which, polyglutamylation and polyglycylation, generated by a variety of enzymes, are highly enriched. While loss of specific enzymes can lead to sterility, respiratory defects, retinal degeneration, and colorectal cancer, the underlying pathophysiological mechanisms are unclear. Our lab is understanding how these PTMs control primary cilia functions and thus, homeostasis, regeneration, and organ function in mammals. We are also establishing iPSC-based human models in a dish to identify the molecular processes affected due to clinical mutations of the PTM enzymes in known ciliopathies.

Report:

I started my lab in April 2022 and it took me a few months to procure the necessary clearances from the IBSC and IAEC to start the work, as well as equip the lab with personnel and consumables. Once I procured the cell lines, antibodies, and plasmids from my previous lab and the consumables, we started the preparatory work by the end of June. During the past year, we have prepared stable cell lines of hTERT-Rpe1 and NIH/3T3 cells overexpressing the active or dead versions of the tubulin glycylase TTLL3 with a ciliary localisation signal (CLS), to study the effect of hyperglycylation of cilia on different ciliary functions like intraflagellar transport and ciliary signalling, as well as cellular functions like migration, orientation, and directionality. We have also generated the lentiviral constructs for known clinical mutations of tubulin glutamylase, TTLL5, that lead to retinal dystrophy, both with and without the CLS. This will help us determine whether the disease manifestation due to the mutation is a cilia-dependent or -independent mechanism.

We have also prepared stable HEK293 cell lines transduced with either the tubulin glycylase TTLL3, tubulin glutamylases TTLL4, TTLL5, or TTLL6 to grow them in suspension and purify custom-modified tubulin to be used for *in vitro* studies. Simultaneously, we have purified some kinesin motors from bacteria for the *in vitro* analyses. We have also standardised the microtubule gliding assays to determine the effect of specific tubulin PTMs on microtubule-motor interactions.

Key findings in the past year:

Tubulin glutamylation and glycylation compete for the same sites on the C-terminal tails of both α- and β-tubulin. It is known that, in certain conditions, loss of glycylation leads to hyperglutamylation, and we hypothesise that loss of glutamylation can lead to increased glycylation. In this regard, we began understanding the effect of hyperglycylation on cilia using Rpe-1 cells overexpressing TTLL3 in the cilia. Our preliminary observations indicated that in cells with increased glycylation, there is decreased polyglutamylation. More interestingly, we observed an increase in ciliary length in cells with excessive glycylation, although there was a decrease in trafficking within the cilia. Moreover, when cells stable for CLS-TTLL3 were treated with smoothened agonist (SAG), which activates the sonic hedgehog signal, we observed a marked reduction in the localisation of the smoothened receptor within the cilia. These observations are specific to hyperglycylation as the cells stable with CLS-TTLL3mut (inactive) do not show any such defects, suggesting

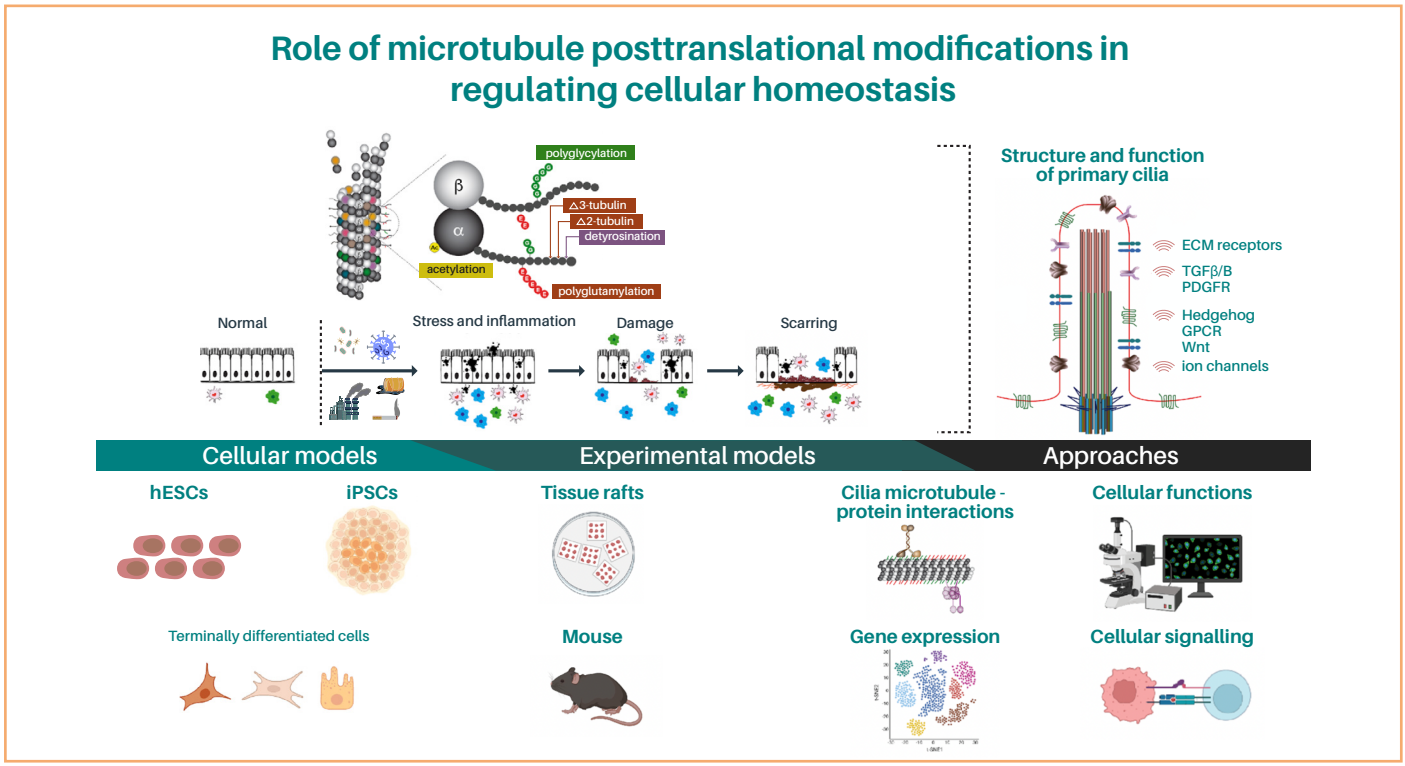
that too much glycylation affects ciliary trafficking, and thus signalling through cilia.

Our observations with the different clinical mutants of the tubulin glutamylase, TTLL5, revealed that all the mutations result in loss of activity. This loss in activity is observed for one of the mutants even within the cilia in Rpe-1 cells where the TTLL5 is localised to the cilia using a CLS. The loss of glutamylation is coupled to concomitant increase in tubulin glycylation in these cells, indicating that loss of glutamylation does increase glycylation. The functional implications behind this increase are being investigated.

Projects/Research Questions for the following year:

1. Decipher how tubulin glycylation regulates intraflagellar transport in cilia *in cellulo* and *in vitro*
2. Determine the role of tubulin glycylation in regulating the activity of diverse motors and microtubule-associated proteins present within the cilia
3. Identify how altered tubulin glycylation impacts cilia-dependent signalling pathways including sonic hedgehog, PDGF, and TGFβ
4. Generate retinal pigment epithelium from human ESCs and iPSCs for the molecular understanding of the role of cilia and its tubulin PTMs in Rpe development functions
5. Understand the molecular mechanisms leading to retinal ciliopathies due to mutations of tubulin glutamylase using ESC- and iPSC-derived Rpe cells
6. Identify the non-tubulin substrates of the different tubulin glutamylases and glycylases to understand to the role of these PTMs beyond microtubules

Graphical summary:



Awards and grants (2022/2023):

- DBT/IA Intermediate Fellowship (February 2023 – December 2027)
- SERB Start-up Research Grant (Approved September 2023)

Outreach and other activities:

- Involved in refurbishing the stem cell facility at DBT-inStem
- Part of the organising committee of a 2-part Workshop on Human pluripotent stem cell research, jointly organised by DBT-inStem and International Stem Cell Banking Initiative (ISCBI), in May (Part-I) and August (Part-II), 2023
- Member of the Board of Studies at Jain University, with active involvement in designing the Curriculum Matrix, Syllabus, and related matters for the programmes under the Department of Microbiology and Botany
- Co-Coordinator of the core course on Fundamentals of Science Communication
- Instructor in the Human Anatomy course starting in 2023 – three lectures on the cytoskeleton and their role in different cellular functions
- Discussed, along with Dr. Sunil Laxman, about stem cells, their functions and their use during the Stem Cell Awareness week. The video of the talk, which is in the local language, is available on the inStem YouTube channel.
- Gave scientific talks about the work we do in our lab at the Centre for Human Genetics, Bangalore and the Department of Life Sciences, Jain University, Bangalore

3.10 Arvind Ramanathan

Title of the research program:

Skeletal muscle homeostasis in ageing and disease conditions

Summary:

Skeletal muscle is a central metabolic and regenerative organ important for mechanical work and physiological regulation. Loss of muscle mass and function and diminished regeneration are important drivers of ageing and related diseases such as diabetes. Our broad research goals are to: (i) Enhance the metabolism and differentiation of muscle stem cells; (ii) Understand the underlying signalling mechanisms by which senescent cells affect satellite cells during aging; (iii) Develop clinical metabolomics to identify biomarkers of ageing-mediated skeletal muscle loss; and (iv) Develop functional human 3D organoids for modelling myopathies and sarcopenia and test therapeutics.

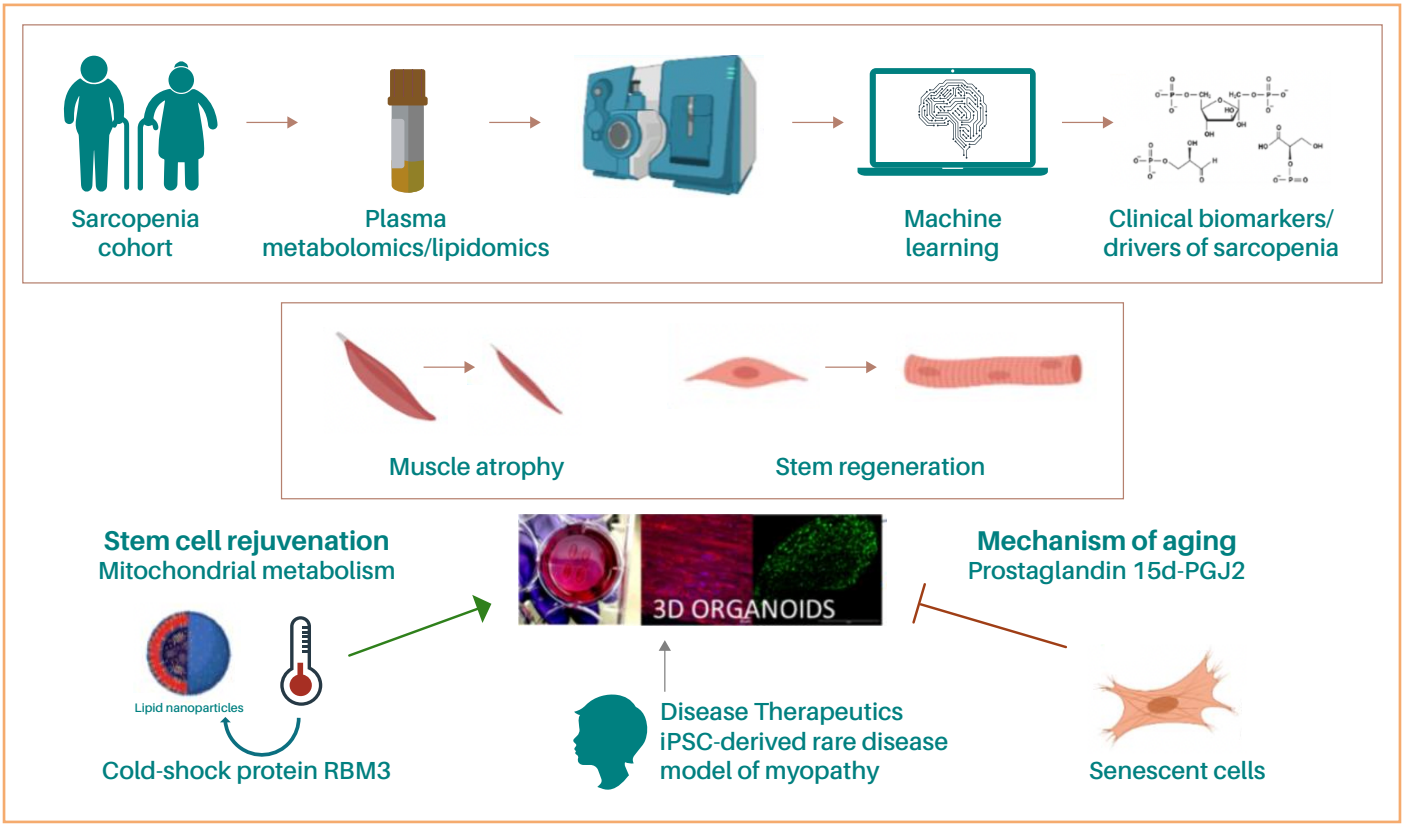
Report:

Following the mandate of inStem, my laboratory is applying regenerative medicine to target musculoskeletal defects in ageing-related sarcopenia and rare myopathies. In this context, my research program is developing two technology platforms: (i) Clinical metabolomics/lipidomics to identify drivers of sarcopenia in an older Indian cohort, and (ii) 3D human skeletal muscle organoids for modelling human diseases and testing therapeutic interventions.

Key findings in the past year:

1. We found that hypothermic adaptation can rejuvenate skeletal muscle stem cells via the upregulation of a cold-shock RNA binding protein RBM3. We showed that overexpression of RBM3 can improve mitochondrial metabolism and differentiation in C2C12 and primary satellite cells. Interestingly, RBM3 and hypothermia can partially restore muscle stem cell markers and proliferation in satellite cells obtained from aged mice. This work is published on *bioRxiv* and is currently under review for publication in a peer-reviewed journal.
2. It is known that aged/senescent cells inhibit muscle stem cell proliferation and differentiation. We demonstrated that senescence-secreted prostaglandin lipid 15d-PGJ2 controls myoblast proliferation and differentiation by directly modifying HRas at C' terminal cysteines. This finding provides a novel approach to inhibit HRas and target ageing-related muscle loss.
3. We implemented the country's first Sarcopenia Clinical Metabolomics study between inStem and Baptist Hospital. We are in the process of using Machine Learning (and AI in the future) to identify plasma lipids and metabolites that may drive ageing-related muscle loss.
4. We successfully developed 3D human organoids, which are being used, in collaboration with TIGS, to model human myopathies, specifically the mitochondrial very long chain fatty acid oxidation disorder.

Graphical summary:



Publications:

1. **Arvind Ramanathan.** Book Chapter Title: Ageing at the Cellular Level: Advances in Research. Publisher: Think Mines Media, ISBN 8196259050 (2023)
2. **Arvind Ramanathan.** Book Chapter Title: Tissue Engineering in the Health Sector. Publisher: Think Mines Media, ISBN 9395264225 (2022)
3. Dey P, Rajalaxmi S, Thakur PS, Hashmi MA, Lal H, Saini N, Muralidharan S, Math RGH, Saha P, Pundlik SS, Singh N, **Ramanathan A.** 2023. Proteomics of hypothermic adaptation reveals that RBM3 enhances mitochondrial metabolism and muscle stem-cell differentiation. https://biorxiv.org/cgi/content/short/2023.05.05.539524v1

Outreach and other activities:

- Delivered an invited talk on *"Metabolic Drivers of Aging"* at National Health Conclave, Bengaluru held in May 2023
- Delivered an invited talk on *"Lipidomics of human disease"* at Pathfinders Conferences, Mumbai held in March 2023
- Delivered an invited talk on *"Targeting Aging"* at Synergia Foundation, Bengaluru in February 2023

3.11 Tina Mukherjee

Title of the research program:

Metabolic control of immune development and function

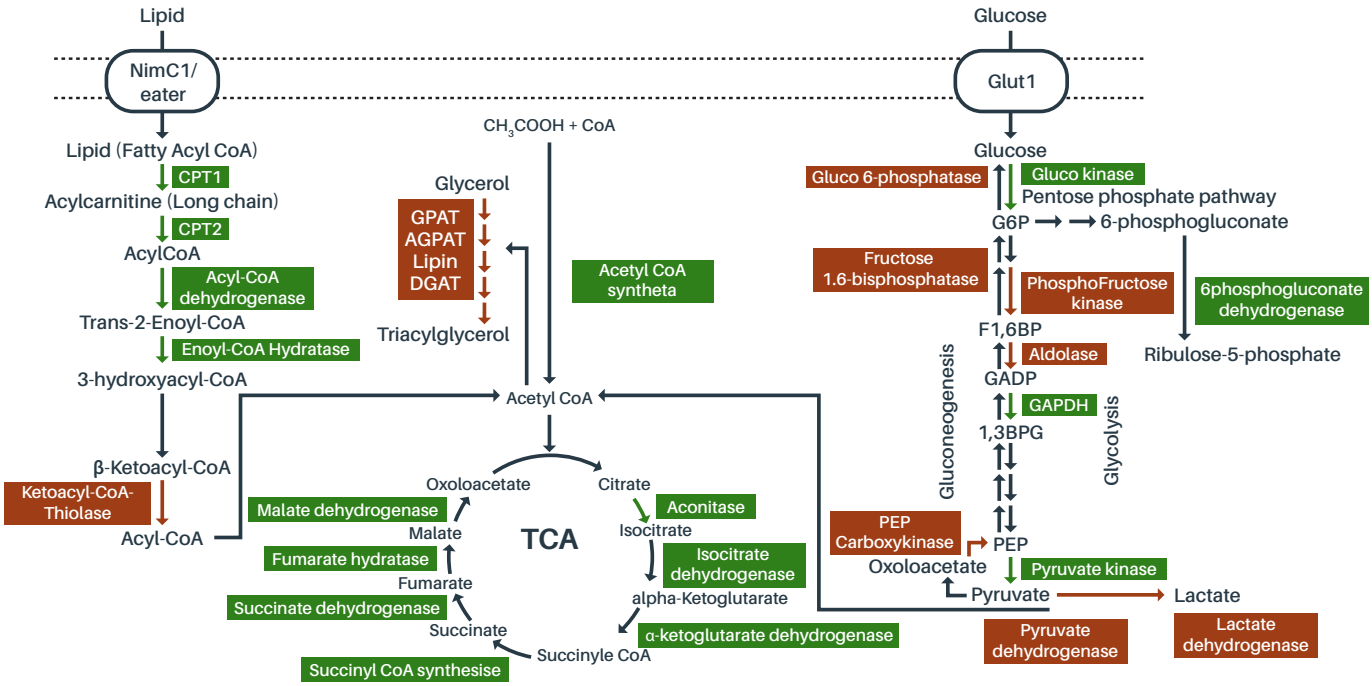
Summary:

As immune cells survey and engage in cross-talk both locally and systemically, we propose that immune-metabolic states are influenced by internal changes within the animal. This allows immune cells to operate as internal sensors and coordinate systemic homeostasis. Consequently, any alteration in immune-metabolism alters their homeostatic roles and leads to pathological outcomes. Moving forward, a guiding principle of our investigations will be the exploration of *"myeloid cells as mobile sensors of animal physiology"*.

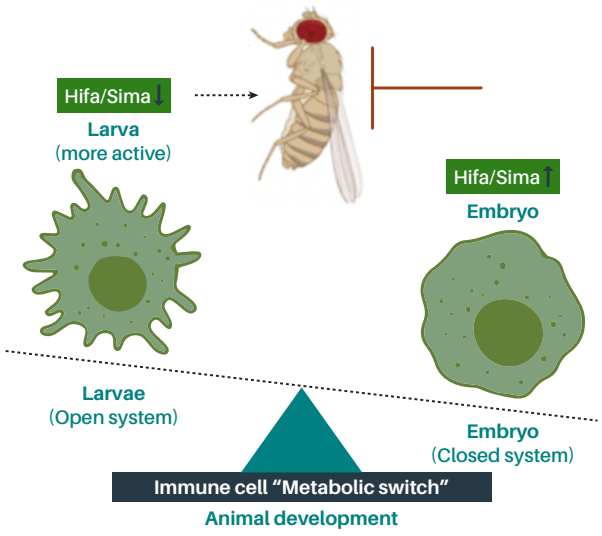
Report:

Our recent findings in *Drosophila* have revealed distinct metabolic states of immune cells and their importance in immune development and immunity, with further implications in non-immune contexts such as coordinators of organismal physiology and stress tolerance. In this regard, the direct utilization of neurotransmitters as metabolites in blood development has emerged as a key finding. The use of dopamine as a proliferative cue by the immune progenitor cells *(Kapoor et.al., 2022)* and *GABA (Goyal et.al. 2022)* for moderating blood progenitor ROS homeostasis and immunity strengthens this notion. As part of our ongoing collaborative initiatives with Prof. Giangrande's laboratory, our work has revealed distinct metabolic and functional states of embryonic and larval immune cells. The work reveals embryonic immune cells to be metabolically glycolytic and lipogenic and functionally immature, which is unlike larval immune cells that are functionally more active and metabolically oxidative and lipolytic *(Cattenoz et.al., 2020)*. This has led us to uncover the relevance of such state changes in animal growth control and dietary stress tolerance *(Mahanta et.al., 2022 in submission)*. Based on these data, we propose that the metabolic state of immune cells is influenced by the internal state changes of the animal and this is important to maintain systemic metabolic homeostasis and overall organismal physiology (Figure 1). Moving forward, our investigations are directed towards exploring *"immune cells as mobile sensors of animal physiology"*. We aim to develop a comprehensive framework that will define novel non-immune functions of innate immune cells. The role of innate immune cells as key sensors of the animal's internal state and its relevance in organismal development, metabolism, behaviour, and predisposition to metabolic disorders will be some of the key findings that are expected to emerge from our ongoing research program.

Graphical summary:



Immune cell metabolism and animal growth



Publications:

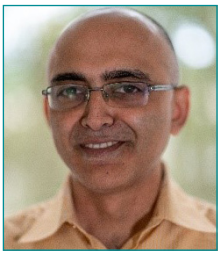
1. Kapoor A, Padmavathi A, Madhwal S, **Mukherjee T.** 2022. Dual control of dopamine in *Drosophila* myeloid-like progenitor cell proliferation and regulation of lymph gland growth. *EMBO Reports,* 27, e52951. doi: 10.15252/embr.202152951
2. Goyal M, Tomar A, Madhwal S, **Mukherjee T.** 2022. Blood progenitor redox homeostasis through olfaction-derived systemic GABA in hematopoietic growth control in *Drosophila*. Development, 149 (8), dev199550. doi:10.1242/dev.199550
3. Kapoor A*, Kumar A*, **Mukherjee T.** 2023. Pumping up the blood progenitors by Piezo. *Proceedings of the Academy of Sciences of the United States of America,* 120(23), e2306004120. doi: 10.1073/pnas.2306004120. (*equal contribution)

Awards and grants (2022/2023):

- Welcome Senior Research Fellowship, awarded by the DBT-Wellcome Trust India Alliance, India (2022)
- Awarded the Human Frontier Science Program Research Grant (2023)

Outreach and other activities:

- Delivered a talk titled *"Sniffing your enemy: Role of odors in immune priming"* as part of the Science Setu event conducted by inStem on 7th May 2022
- As an invited Plenary Speaker, delivered a talk titled *"Understanding blood progenitor development: Through the lenses of sensory perception"* at the 20th ISSCR Meeting, San Francisco, on 17th June 2022
- Delivered an invited talk titled *"Sensory control of blood progenitor development"* at the University of Purdue, on 21st June 2022
- Delivered an invited talk titled *"Immune metabolic states as developmental sensors of animal physiology"* at the GRC Developmental Biology meeting on 26th June 2023



3.12 Sunil Laxman

Title of the research program:

Organisational principles of metabolic networks and resource sharing in cells

Summary:

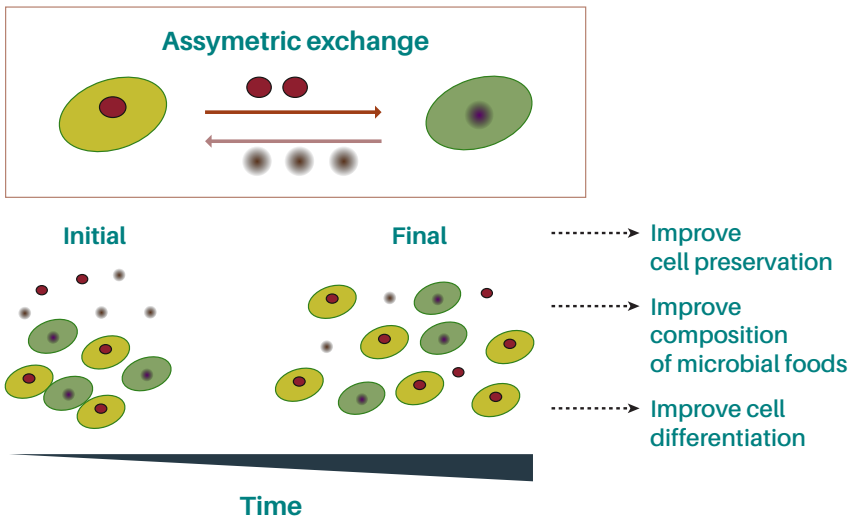
The goal of our research is to identify the rules, chemical logic, and organizational principles of metabolic networks in cells. For this, we study how some metabolites are formed and sensed within and between cells. We also study system-level principles of metabolic network organisation and build frameworks to understand metabolic exchange between cells. An extension of these studies is to understand extreme biology in model and non-model organisms. We are integrating these studies using synthetic-systems approaches for regenerative biology, food, synthetic biology, and environmental biotechnology.

Report:

We aim to holistically understand the biochemical logic for how cell fates are regulated by metabolic states, and translate this basic understanding to address key problems in human health. From studying metabolic signalling mechanisms, our lab now addresses questions on metabolic organisation from molecules to systems and networks. In collaboration with Prof. Apurva Sarin, we identified a key metabolic determinant of T cell fate, where Treg cells have a critical requirement for methionine in their survival, via a novel function of an amino acid transporter in a Notch1 signalling pathway-dependent manner (Naaz et al, *Life Science Alliance 2023*). In distinct studies, in collaboration with Dr. Bakthavachalu (IIT-Mandi), we identified a metabolic basis for the survival and 'regeneration' of the embryos of the most important disease vector in India, the Aedes mosquito. This is a striking example of making discoveries in basic biology by studying extreme biology and regeneration, which could lead to a unique avenue for intervening in a major public health problem.

In other ongoing studies, we expanded our research directions towards understanding how mitochondrial organisation, outputs, and metabolic states are coupled to regulate cell states. Here, we have made exciting findings about how mitochondrial repression is achieved, and the mechanisms of how the mitochondrial network is organized. In work intended to lead to translational outcomes, we are using our systems-level understanding of cell states to develop synthetic circuits for optimised metabolic cellular engineering. Finally, we anticipate exciting collaborations across multiple themes in inStem, to collectively address common problems in cell fate regulation.

Graphical summary:



Publications:

1. Singh G, George G, Raja SO, Kandaswamy P, Kumar M, Thutupalli S, **Laxman S**, Gulyani A. 2023. A molecular rotor FLIM probe reveals dynamic coupling between mitochondrial inner membrane fluidity and cellular respiration. *Proceedings of the Academy of Sciences of the United States of America, 120*(24), e2213241120. doi: 10.1073/pnas.2213241120

2. Naaz A, Saini N, Metur S, Gahlot P, Walvekar A, Dutta A, Davathamizhan U, Sarin S, **Laxman S.** 2022. Methionine uptake via SLC43A2 transporter is essential for regulatory T lymphocyte survival. *Life Science Alliance, 5*(12), e202201663. doi: 10.26508/lsa.202201663

3. Bandyopadhyay P, Pramanick I, Biswas R, Sabarinath PS, Sreedharan S, Singh S, Rajmani RS, **Laxman S**, Dutta S, Singh A. 2022. S-Adenosylmethionine-responsive cystathionine β-synthase modulates sulfur metabolism and redox balance in Mycobacterium tuberculosis. *Science Advances, 8*(25), eabo0097. doi: 10.1126/sciadv.abo0097

4. Tripathi A, Anand K, Das M, O'Niel RA, Sabarinath PS, Thakur C, Reddy RRL, Rajmani RS, Chandra N, **Laxman S**, Singh A. 2022. Mycobacterium tuberculosis requires SufT for Fe-S cluster maturation, metabolism, and survival in vivo. *PLoS Pathogens, 18*(4), e1010475. doi: 10.1371/journal.ppat.1010475

5. Prasad A, Sreedharan S, Bakthavachalu B, **Laxman S**. Aedes aegypti eggs use rewired polyamine and lipid metabolism to survive extreme desiccation. *Plos Biology* (in press). Preprint: *BioRxiv:* https://www.biorxiv.org/content/10.1101/2022.12.30.522323v1

6. Vengayil V, Niphadkar S, Adhikary S, Varahan S, **Laxman S.** Phosphate budgeting to mitochondria controls glucose-mediated mitochondrial repression. *eLIFE* (Accepted). Preprint: *BioRxiv:* https://www.biorxiv.org/content/10.1101/2022.12.29.522272v2

Awards and grants (2022/2023):

- DBT Wellcome India Alliance Senior Research Fellowship (Initiated January 2023)

Outreach and other activities:

- Delivered a talk on extreme biology as part of DBT-inStem Science Setu event
- Popular science interview in Kannada on stem cells (https://www.youtube.com/watch?v=o6W2qjcE7bQ&t=4s)
- Delivered a popular science talk to students in the Indian National Junior Science Olympiad (INJSO) programme (IJSO 2023), Mumbai

3.13 Bhavana Muralidharan

Title of the research program:

Probing the mechanisms of chromatinopathies in brain development

Summary:

Neurodevelopmental disorders (NDDs) affect critical periods of brain development and plasticity. Chromatin-associated genes are frequently mutated in NDDs. The mutations affect chromatin structure and function, leading to disorders called chromatinopathies. Yet the mechanisms by which chromatinopathies disrupt brain assembly remain unclear. My lab utilises an integrative approach, combining functional genomics with iPSC-derived cerebral organoid biology, to unravel the molecular and cellular processes underlying chromatinopathies and NDDs.

Report:

Neurogenesis begins with neural stem cells undergoing symmetric proliferative divisions to expand and then switching to asymmetric differentiative divisions to generate neurons in the developing brain. Chromatin regulation plays a critical role in this switch. Histone lysine-specific demethylase LSD1 demethylates H3K4me1/2 and H3K9me1/2 but the mechanisms of its global regulatory functions in human neuronal development remain unclear.

We performed genome-wide ChiP-seq of LSD1 occupancy, RNA-seq, and Histone ChiP-seq upon LSD1 inhibition to identify its repressive role in human neural stem cells. Novel downstream effectors of LSD1 were identified, including the Notch signalling pathway genes and human-neural progenitor-enriched extracellular matrix (ECM) pathway/cell adhesion genes, which were upregulated upon LSD1 inhibition. LSD1 inhibition led to decreased neurogenesis, and overexpression of downstream effectors mimicked this effect. Histone ChIP-seq analysis revealed that active and enhancer markers H3K4me2, H3K4me1, and H3K9me1 were upregulated upon LSD1 inhibition, while the repressive H3K9me2 mark remained mostly unchanged. Our work identifies human-neural progenitor-enriched ECM pathway/cell adhesion genes and Notch signalling pathway genes as novel downstream effectors of LSD1, regulating neuronal differentiation in human neural stem cells. Mutations in the conserved lysine-specific demethylase LSD1 are implicated in intellectual disability. We have recently identified a human-specific, genomic control mechanism of LSD1 function in neuronal development. We now aim to investigate how pathogenic mutations in LSD1 disrupt this regulatory process and its downstream cellular effects.

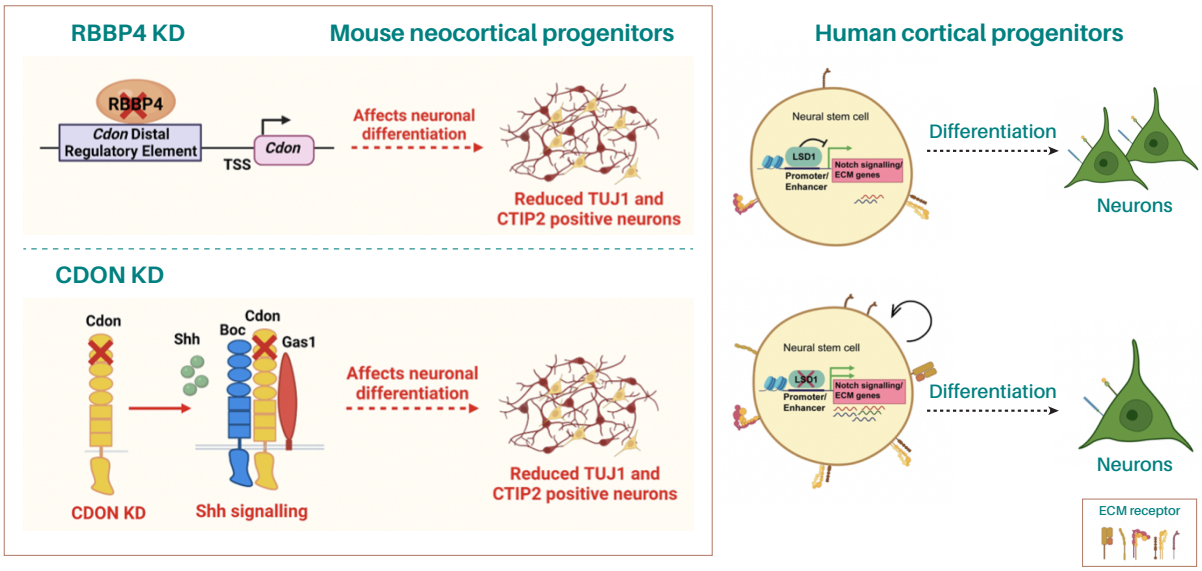
Neocortical glutamatergic neurons are generated from progenitors in the ventricular zone lining the ventricle in a timed inside-out manner. Chromatin regulation plays a crucial role in neurogenesis in the neocortex. RBBP4 is a core subunit of several chromatin-modifying complexes; however, its functional role and genome-wide occupancy profile in the neocortical primordium are unknown. To address this, we performed RBBP4 knockdown using the CRISPR/Cas9 approach on neocortical progenitors at embryonic age 12.5, during deep layer neurogenesis, which involves the production of Layer VI and layer V neurons. Our study found that downregulation of RBBP4 in E12.5 neocortical primordium results in reduced neuronal output from progenitors, specifically decreasing CTIP2-expressing layer V neuronal numbers while having no significant impact on TLE4-expressing layer VI neurons.

Genome-wide occupancy analysis revealed that RBBP4 primarily binds to distal regulatory elements and that neuron differentiation is a significant GO biological pathway of RBBP4-bound genes. Interestingly, we found that RBBP4 binds to *Cdon*, a receptor protein in the Shh signalling pathway, at the transcription start site (TSS) and distal regulatory

elements. Knockdown of *Cdon* resulted in a significant reduction in the generation of neurons, particularly CTIP2-positive layer V neurons, phenocopying RBBP4 function. Our results shed light on the cellular role of RBBP4 and identify CDON as a novel regulator of deep-layer neurogenesis in the developing neocortex.

Graphical summary:

Deciphering molecular mechanisms of chromatin regulation inmurine and human neocortical progenitors



Awards and grants (2022/2023):

- Interviewed as part of 'Transitions in Development' series of the journal *Development*, which aims to promote prominent, early-to-mid career-level developmental biologists (https://doi.org/10.1242/dev.201836)
- Received the Japanese Neuroscience Society Travel Award to attend JNS 2022, July 2022 in Okinawa, Japan
- The DBT MK Bhan Postdoctoral fellowship of INR 100 lakhs was awarded in January 2023 to Dr. S. K. Dhanya, a postdoctoral fellow in the lab
- DBT/Wellcome Trust India Alliance Early career postdoctoral fellowship of INR 170 lakhs was awarded to Dr. Kruttika Phalnikar, a postdoctoral fellow in the lab

Outreach and other activities:

- The lab hosted undergraduate and Masters' students on various occasions to discuss and showcase research work on studying brain development
- Co-organiser and instructor at the two-part Human Stem Cell workshop, jointly organised by inStem and ISCBI, in May and August 2023
- Organiser of the Stem Cell Awareness Week at inStem, featuring talks and video podcasts by leading stem cell biologists and experts
- Organiser of an online one-day symposium entitled *"Brain Functions: From Basic Understanding to Translational Approaches"* to commemorate Brain Awareness Week, featuring talks from neuroscientists across institutes in India

3.14 Mohankumar K. Murugesan

Title of the research program:

Therapeutic genome editing for haematological disorders

Summary:

The major goal of my research is to develop preclinical genome editing strategies for the treatment of monogenic haematological disorders. To this end, we utilise advanced genome engineering platforms based on CRISPR/Cas9 system such as base editor as well as prime editor to correct or create specific mutations in hematopoietic stem cells. Genome engineering for beta hemoglobinopathies is aimed at either the precise correction of disease-specific mutations or the creation of beneficial mutations for reactivating developmentally-silenced foetal haemoglobin. To overcome the undesired effects in other cell lineages, we use a lineage-specific approach for disruption of major transcriptional factors or regulatory elements in erythroid cells to elevate foetal haemoglobin. For haemophilia, we aim to develop a strategy for lineage-specific expression of FVIII in haematopoietic stem cells using CRISPR/Cas9-mediated homology-dependent repair.

Report:

Through the use of advanced genome engineering platforms such as base editor and prime editor, we identified novel targets that are more efficient than the current clinical trial targets for the treatment of inherited haematological disorders.

Key findings in the past year:

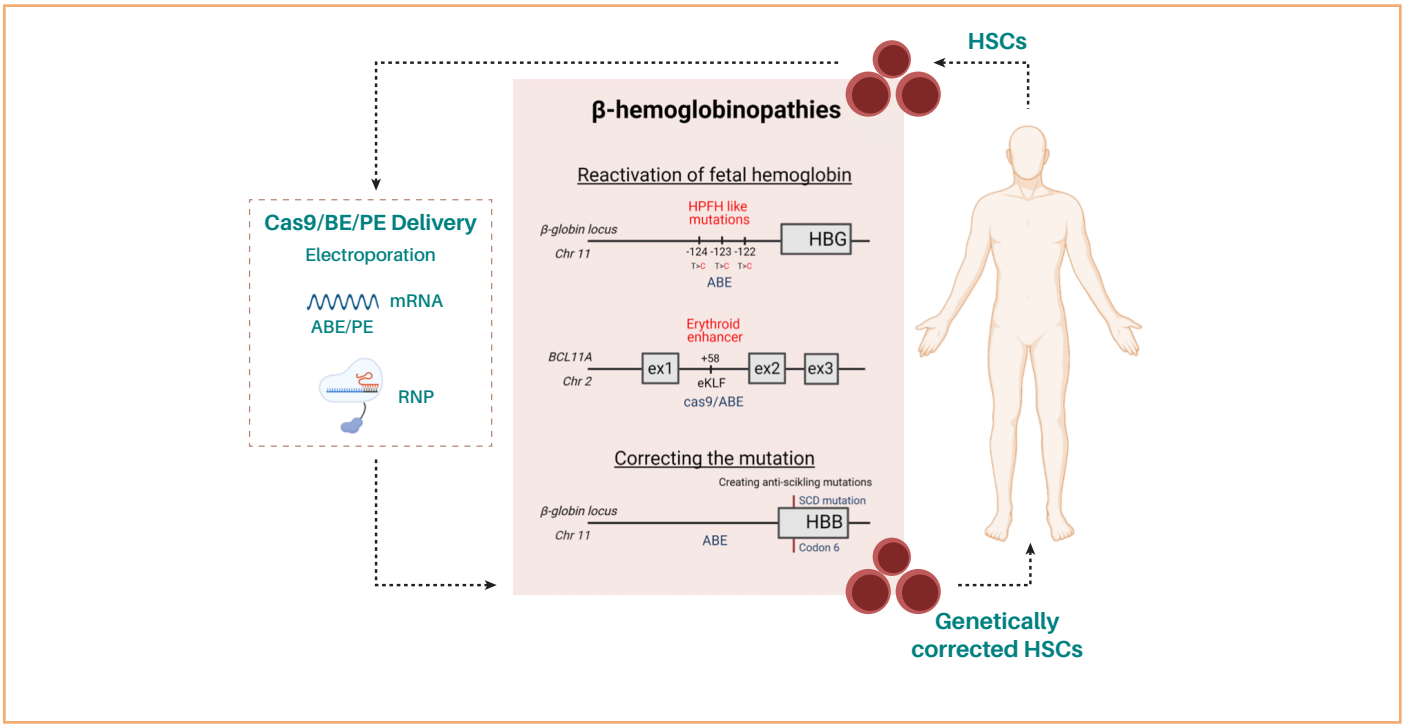
We developed six different major approaches to overcome the current limitations for the genome editing of sickle cell disease and beta-thalassemia. (1) Base editing at the novel targets identified from the screen in the *HBG* promoter up-regulates HbF level than the disruption of well-known BCL11A binding site in erythroblasts derived from human CD34+ hematopoietic stem and progenitor cells without any detrimental effects on erythroid differentiation. Editing at the highly homologous *HBG1* and *HBG2* promoter using base editor showed a very minimal level of off-targets both at the DNA and RNA levels. (2) Targeting specific regions of BCL11A enhancer at the functional core of +58 DHS in human $CD34^+$ HSPCs showed better enucleation and comparative induction of foetal haemoglobin levels than the current clinical trial targets. (3) Key nucleotide substitution at the zinc finger domains present uniquely in the BCL11A-XL isoform provides novel approaches for the treatment of beta-haemoglobinopathies. Specifically compared to the current clinical trial targets based on BCL11A, alteration of the zinc finger domain dramatically up-regulated foetal haemoglobin levels with no effect on erythroid maturation and minimal transcriptomic changes. (4) Lineage-specific knockdown of another major gamma globin repressor (LRF/ZBTB7A) resulted in a therapeutic induction of foetal haemoglobin in human erythroid cells. Currently, we are working towards understanding the molecular mechanism of LRF/ZBTB7A regulation on erythropoiesis. (5) We precisely corrected and modelled the spectrum of β-thalassemia mutations at the HBB promoter, exon, and intronic regions in human erythroid cells using base editors. (6) We demonstrated the use of prime editor for the efficient creation as well as correction of sickle cell mutations in human erythroid cells. These results demonstrated the reconstitution of functional beta-globin chain at the DNA, mRNA, and protein levels, highlighting the potential use of prime editing as a valuable therapeutic gene editing approach for sickle cell disease.

For haemophilia, we aim to develop a strategy for targeted integration of FVIII/FIX under the endogenous locus in HSPCs using the CRISPR/Cas9 system. Towards this end, we optimised the AAV production and HDR-mediated target integration of GFP in CD34+ HSPCs and successfully expressed the transgene under the endogenous locus.

Emerging directions:

The novel targets identified from our current studies will be validated in β thalassemia and SCD patient-derived HSPCs at a pilot scale using base editor and prime editor. After validation, the HSPCs obtained from healthy donors will be edited at a clinical scale in pre-GMP conditions to confirm reproducibility during scaling up.

Graphical summary:



Publications:

1. Rajendiran V, Devaraju N, Ravi NS, Panigrahi L, Paul J, Gopalakrishnan C, Wyman S, Ariudainambi K, Mahalingam G, Periyasami Y, Prasad K, George A, Sukumaran D, Gopinathan S, Pai AA, Nakamura Y, Balasubramanian P, Ramalingam R, Thangavel S, Velayudhan SR, Corn JE, Crossley M, Marepally S, Srivastava A, **Mohankumar KM.** 2023. Base editing of key residues in the BCL11A-XL-specific zinc finger domains de-represses fetal globin expression. *Molecular Therapy* (in revision)

2. Prasad K, Devaraju N, George A, Ravi NS, Paul JP, Mahalingam G, Rajendiran V, Panigrahi L, Venkatesan V, Lakhotiya K, Periyasami Y, Pai AA, Nakamura Y, Kurita R, Balasubramanian P, Thangavel S, Velayudhan SR, Marepally S, Srivastava A, **Mohankumar KM**. *Molecular Therapy - Nucleic Acids* (in revision)

3. Ravi NS, George A, **Mohankumar KM**. 2023. Arrayed gRNA screening by base editors in mammalian cell lines using lentiviral system, *Star Protocol, 4*(4), 102668. doi: 10.1016/j.xpro.2023.102668.

4. Venkatesan V, Christopher AC, Rhiel M, Kumar K Azhagiri M, Babu P, Walavalkar K, Saravanan B, Andrieux G, Rangaraj S, Srinivasan S, Karuppusamy KV, Jacob A, Bagchi A, Pai AA, Nakamura Y, Kurita R, Balasubramanian P, Pai R, Marepally SK, **Mohankumar KM**, Velayudhan SR, Boerries M, Notani D, Cathomen T, Srivastava A, Thangavel S. 2023. Editing the core region in HPFH deletions alters fetal and adult globin expression for treatment of β-hemoglobinopathies. *Molecular Therapy - Nucleic Acids*, 32, 671–688. doi: 10.1016/j.omtn.2023.04.024

5. Mahalingam G, Arjunan P, Periyasami Y, Dhyani AK, Devaraju N, Rajendiran V, Christopher AC, Kt RD, Dhanasingh I, Thangavel S, **Mohankumar KM**. 2023. Correlating the differences in the receptor binding domain of SARS-CoV-2 spike variants on their interactions with human ACE2 receptor. *Scientific Reports, 13*(1), 8743. doi: 10.1038/s41598-023-35070-2

6. George A, Ravi NS, Prasad K, Panigrahi L, Koikkara S, Rajendiran V, Devaraju N, Paul J, Pai AA, Nakamura Y, Kurita R, Balasubramanian P, Thangavel S, Marepally S, Velayudhan SR, Srivastava A, **Mohankumar KM**. 2022. Efficient and error-free correction of sickle mutation in human erythroid cells using prime editor-2. *Frontiers in Genome Editing,* 4, 1085111. doi: 10.3389/fgeed.2022.1085111

7. Bagchi A, Devaraju N, Chambayil K, Rajendiran V, Venkatesan V, Sayed N, Pai AA, Nath A, David E, Nakamura Y, Balasubramanian P, Srivastava A, Thangavel S, **Mohankumar KM**, Velayudhan SR. 2022. Erythroid lineage-specific lentiviral RNAi vectors suitable for molecular functional studies and therapeutic applications. *Scientific Reports, 12*(1), 14033. doi: 10.1038/s41598-022-13783-0

8. Devaraju N, Rajendiran V, Ravi NS, Mohankumar KM. 2022. Genome Engineering of Hematopoietic Stem Cells Using CRISPR/Cas9 System. *Methods in Molecular Biology* (pp. 307–331). New York, NY: Springer US

9. Ravi NS, Wienert B, Wyman SK, Bell H, Vu J, Pai AA, Balasubramanian P, Nakamura Y, Kurita R, Marepally S, Thangavel S, Shaji RV, Srivastava A, DeWitt MA, Crossley M, Corn JE, **Mohankumar KM**. 2022. Identification of novel HPFH-like mutations by CRISPR base editing that elevates the expression of fetal hemoglobin. *eLife,* 11, e65421. doi: 10.7554/eLife.65421

10. Lohchania B, Christopher AC, Arjunan P, Mahalingam G, Kathirvelu D, Prasannan A, Venkatesan V, Taneja P, **Mohan Kumar KM**, Thangavel S, Marepally S. 2022. Diosgenin enhances liposome-enabled nucleic acid delivery and CRISPR/Cas9-mediated gene editing by modulating endocytic pathways. *Frontiers in Bioengineering and Biotechnology,* 10, 103104. doi: 10.3389/fbioe.2022.1031049

11. Mahalingam G, Rachamalla HK, Arjunan P, Periyasami Y, Salma M, Thangavel S, **Mohankumar KM**, Moorthy M, Velayudhan SR, Srivastava A, Marepally S. 2022. Optimization of SARS-CoV2-pseudovirion production in lentivirus backbone with a novel liposomal system. *Frontiers in Pharmacology,* 13, 840727. doi: 10.3389/fphar.2022.840727

Awards and grants (2022/2023):

- Ben Barres Spotlight Award from eLife (2022)
- Grant from Wellcome Trust India Alliance for the sanctioned project entitled *"Functional assessment of genetic variants associated with fetal hemoglobin levels using base editor mediated saturated mutagenesis for the treatment of beta hemoglobinopathies"*
- Grant from SERB for the sanctioned project entitled *"Precise correction of HbE and major β-thalassemia mutations using Base editor"*
- Grant from ICMR for the sanctioned project entitled *"Therapeutic rescue of neutrophil maturation arrest by base editing of ELANE in severe congenital neutropenia"*

- Grant from DBT for the sanctioned project entitled *"Targeting the gamma globin regulatory elements using base editors for clinical application in patients with β-hemoglobinopathies"*
- Grant from DBT for the ongoing project *"Recreating HPFH-associated point mutation to elevate the fetal hemoglobin levels for the treatment of beta-hemoglobinopathies using CRISPR-based tools"*

Outreach and other activities:

- Delivered lecture on *"Genome Engineering for Hemoglobinopathies: Strategies of Stem Cell Therapy"* at Indira Gandhi Medical College and Research Institute, Puducherry (2023)
- Gave a presentation on *"Genome-editing to treat hematological diseases"* at the Sun Pharma Science Foundation's National Annual Conference (2023)
- Gave a presentation on *"Applications of gene editing in medical science"* at National Hemophilia Foundation (2022)
- Gave a presentation on *"Therapeutic genome editing for Hematological disorders"* at Sri Ramachandra Institute of Higher Education and Research (2022)
- Delivered lecture on *"Gene targeting and editing strategies"* at RUSA 2.0, University of Madras (2022)
- Gave a presentation on *"Cell and Gene Therapy"* at the Bengaluru Tech Summit (2022)
- Gave a presentation at the 7th Annual Cell and Gene Therapy Symposium, held at Centre for Stem Cell Research, Vellore (2022)

3.15 Saravanabhavan Thangavel

Title of the research program:

Gene-edited hematopoietic stem and progenitor cells (HSPCs) for gene therapy applications

Summary:

Gene-edited autologous HSPCs have great potential to cure several inherited and acquired haematological disorders. By gene editing autologous HSPCs, we aim to develop a cure for the most common monogenic-inherited disorder, beta hemoglobinopathies, as well as the infectious disease HIV. We also aim to develop technologies that simplify the generation of gene-edited stem cells.

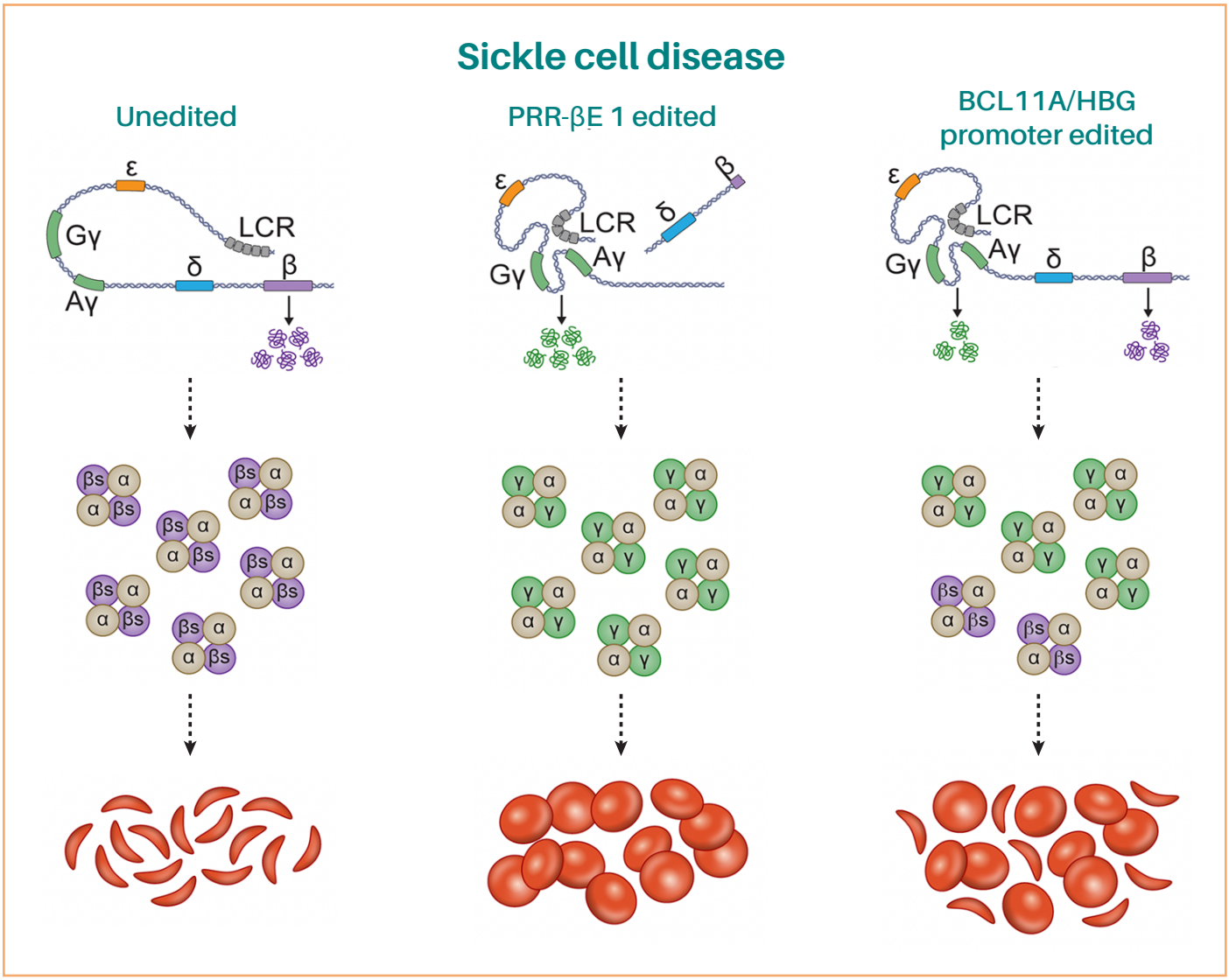
Report:

Key findings in the past year:

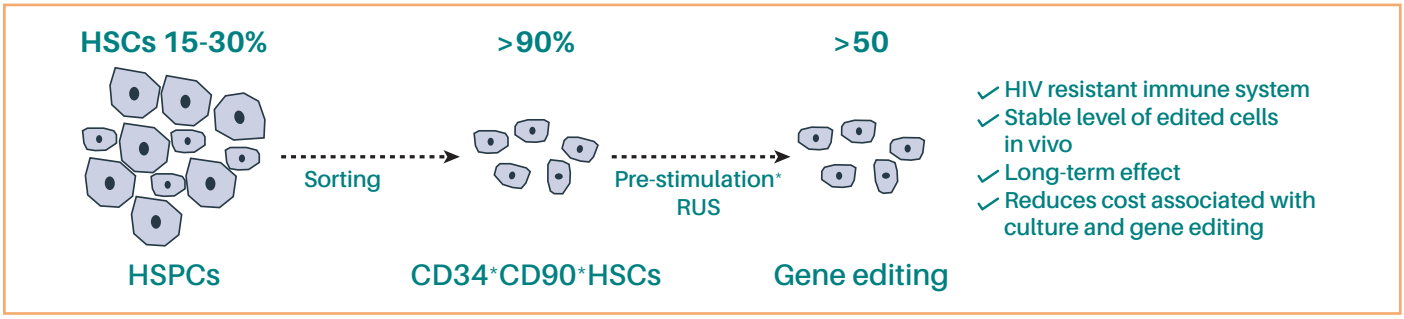
β-hemoglobinopathies gene therapy: Reactivation of foetal haemoglobin (HbF) is a commonly adopted strategy to ameliorate β-hemoglobinopathies. However, the continued production of defective adult haemoglobin (HbA) limits HbF tetramer production, affecting the therapeutic benefits. We evaluated deletional hereditary persistence of foetal haemoglobin (HPFH) mutations and identified an 11 kb sequence, encompassing putative repressor region (PRR) to β-globin exon-1 (βE1), as the core deletion that ablates HbA and exhibits superior HbF production compared with HPFH or other well-established targets. PRR-βE1-edited hematopoietic stem and progenitor cells (HSPCs) retained their genome integrity and their engraftment potential to repopulate for long-term haematopoiesis in immunocompromised mice producing HbF-positive cells *in vivo*. Furthermore, PRR-βE1 gene editing is feasible without *ex vivo* HSPC culture. Importantly, the editing induced therapeutically significant levels of HbF to reverse the phenotypes of both sickle cell disease and β-thalassemia major. These findings imply that PRR-βE1 gene editing of patient HSPCs could lead to improved therapeutic outcomes for β-hemoglobinopathy gene therapy.

HIV gene therapy: *CCR5* gene-edited autologous HSPCs can be a potential alternative to haematopoietic stem cell transplantation (HSCT) from HLA-matched *CCR5* null donor. However, the clinical application of gene-edited autologous HSPCs is critically limited by the quality of the graft, as HIV also infects HSPCs. By using mobilised HSPCs from healthy donors, we showed that the $CD34^+CD90^+$ hematopoietic stem cells (HSCs) express 7-fold lower levels of CD4/*CCR5* HIV receptors, higher levels of SAMHD1 anti-viral restriction factor, and possess lower susceptibility to HIV infection than the CD34+CD90- hematopoietic progenitor cells. To demonstrate that $CD34^+CD90^+$ HSC population is an ideal graft for HIV gene therapy, we sort purified $CD34^+CD90^+$ HSCs, and edited the *CCR5* gene with a single sgRNA. On transplantation, 100,000 $CD34^+CD90^+$ HSCs were sufficient for long-term repopulation of the entire bone marrow of NBSGW mice. Importantly, the gene editing efficiency of ~90% in the infused product was maintained *in vivo*, facilitating the generation of *CCR5* null immune cells resistant to HIV infection. Altogether, *CCR5* gene editing of $CD34^+CD90^+$ HSCs provides an ideal gene manipulation strategy for autologous HSCT-based gene therapy for HIV infection.

Graphical summary:



Venkatesan et al., *Molecular Therapy Nucleic Acids* 2023. Cover page article
Graphical abstract showing the mechanism behind gamma globin activation and beta-globin downregulation in PRR-BE1 edited cells.



Karuppusamy K *et al., Frontiers in Immunology* 2022. Graphical abstract showing the approach for HIV gene editing therapy.

Publications:

1. Venkatesan V, Christopher AC, Rhiel M, Azhagiri MKK, Babu P, Walavalkar K, Saravanan B, Andrieux G, Rangaraj S, Srinivasan S, Karuppusamy KV, Jacob A, Bagchi A, Pai AA, Nakamura Y, Kurita R, Balasubramanian P, Pai R, Marepally SK, Mohankumar KM, Velayudhan SR, Boerries M, Notani D, Cathomen T, Srivastava A, **Thangavel S.** 2023. Editing the core region in HPFH deletions alters fetal and adult globin expression for treatment of β-hemoglobinopathies. *Molecular Therapy Nucleic Acids,* 32, 671–688. doi: 10.1016/j.omtn.2023.04.024
2. Lohchania B, Christopher A, Arjunan P, Mahalingam G, Kathirvelu D, Prasannan A, Venkatesan V, Taneja P, Kumar Km M, **Thangavel S**, Marepally S. 2022. Diosgenin enhances liposome-enabled nucleic acid delivery and CRISPR/Cas9-mediated gene editing by modulating endocytic pathways. *Frontiers in Bioengineering and Biotechnology,* 10, 1031049. doi: 10.3389/fbioe.2022.1031049
3. Christopher AC, Venkatesan V, Karuppusamy K V, Babu P, Alagiri M, **Thangavel S**. 2022. CRISPR/Cas9 gene editing of hematopoietic stem and progenitor cells for gene therapy applications. *Journal of Visualized Experiments,* 186. doi: 10.3791/64064
4. Karuppusamy KV, Demosthenes JP, Venkatesan V, **Thangavel S**. 2022. The CCR5 gene edited CD34+CD90+ hematopoietic stem cell population serves as an optimal graft source for HIV gene therapy. *Frontiers in Immunology,* 13, 792684. doi: 10.3389/fimmu.2022.792684
5. Christopher AC, Venkatesan V, Karuppusamy KV, Srinivasan S, Babu P, Azhagiri MKK, Chambayil K, Bagchi A, Rajendiran V, Ravi NS, Kumar S, Marepally SK, Mohankumar KM, Srivastava A, Velayudhan SR, **Thangavel S**. 2022. Preferential expansion of human $CD34^{+}CD133^{+}CD90^{+}$ hematopoietic stem cells enhances gene-modified cell frequency for gene therapy. *Human Gene Therapy,* 33, 188–201. doi: 10.1089/hum.2021.089
6. George A, Ravi NS, Prasad K, Panigrahi L, Koikkara S, Rajendiran V, Devaraju N, Paul J, Pai AA, Nakamura Y, Kurita R, Balasubramanian P, **Thangavel S**, Marepally S, Velayudhan SR, Srivastava A, Mohankumar KM. Efficient and error-free correction of sickle mutation in human erythroid cells using prime editor-2, *Frontiers in Genome Editing,* 4, 1085111. doi: 10.3389/fgeed.2022.1085111
7. Ravi NS, Wienert B, Wyman SK, Bell HW, George A, Mahalingam G, Vu JT, Prasad K, Bandlamudi BP, Devaraju N, Rajendiran V, Syedbasha N, Pai AA, Nakamura Y, Kurita R, Narayanasamy M, Balasubramanian P, **Thangavel S**, Marepally S, Velayudhan SR, Srivastava A, DeWitt MA, Crossley M, Corn JE, Mohankumar KM. 2022. Identification of novel HPFH-like mutations by CRISPR base editing that elevate the expression of fetal hemoglobin. *Elife,* 11, e65421. doi: 10.7554/eLife.65421
8. Bagchi A, Devaraju N, Chambayil K, Rajendiran V, Venkatesan V, Sayed N, Pai AA, Nath A, David E, Nakamura Y, Balasubramanian P, Srivastava A, **Thangavel S**, Mohankumar KM, Velayudhan SR. 2022. Erythroid lineage-specific lentiviral RNAi vectors suitable for molecular functional studies and therapeutic applications. *Scientific Reports,* 12(1), 14033. doi: 10.1038/s41598-022-13783-0
9. Precilla S, Kuduvalli S, Praveena E, **Thangavel S**, Anitha TS. 2022. Integration of synthetic and natural derivatives revives the therapeutic potential of temozolomide against glioma- an *in vitro* and *in vivo* perspective. *Life Sciences,* 301, 120609. doi: 10.1016/j.lfs.2022.120609
10. Prasad K, Devaraju N, George A, Ravi NS, Mahalingam G, Rajendiran V, Panigrahi L, Venkatesan V, Lakhotiya K, Moorthy Y, Pai AA, Nakamura Y, Kurita R, Balasubramanian B, **Thangavel S**, Velayudhan SR, Marepally S, Srivastava A, Mohankumar KM. 2022. Precise modelling and correction of a spectrum of β-thalassemic mutations in human erythroid cells by base editors. *bioRxiv* [cited 2022 Jun 13]. doi: 10.1101/2022.06.01.494256

Patents:

- *A Method for Reactivation of Fetal Hemoglobin and A Composition Thereof* (application no: 202241030465), dated 27th May, 2022. Inventor(s): **Saravanabhavan Thangavel**, Alok Srivastava
- *Compositions and methods for treating a ß-thalassemia disease.* U.S. Pat. App. No. 63/251,229: David I. K. Martin, Mark DeWitt, Mark C. Walters, Wendy J. Magis, S**aravanabhavan Thangavel**, and Dario Boffelli, filed on 1st October, 2022

Awards and grants (2022/2023):

- DBT grant for preclinical gene editing studies for the treatment of HIV infection (2022–2025); amount: 69,62,120 INR
- Grant for the safety and scale-up studies with gene-edited hematopoietic stem and progenitor cells for the treatment of β-hemoglobinopathies (2023–2026); amount: 1,65,84,240 INR

Outreach and other activities:

- Conducted a theory and demo class on stem cells for the students of Thiruvalluvar University from March–April, 2023
- Delivered an invited talk titled *"Genome edited stem cells for therapeutic applications"* at Sri Balaji Vidyapeeth, Puducherry (ADHR-sponsored training programme) on 10th February, 2023
- Delivered an invited talk titled *"Career in science"* at Vellammal Bodhi campus, Vellore on 12th December, 2022
- Delivered an invited talk titled *"Genome engineering for therapeutic applications"* at VIT, Vellore on 16th November, 2022
- Delivered an invited talk titled *"Genome Engineering of human hematopoietic stem and progenitor cells for gene therapy applications"* at Bharath Institute of Higher Education and Research, Chennai on 6th August, 2022
- Delivered an invited talk titled *"Genome engineering of human hematopoietic stem and progenitor cells for gene therapy applications"* as part of RUSA 2.0, Genome Editing Seminar Series at University of Madras, on 12th April, 2022
- Delivered an invited talk titled *"Genome engineering for therapeutic applications"* at VIT Vellore (special seminar series) on 11th April, 2022
- Delivered an invited talk titled *"Preferential expansion of primitive hematopoietic stem cells for gene therapy applications"* at the International Bone Marrow Failure Syndrome symposium held at CMC Vellore on 8th April, 2022.

3.16 Shaji R Velayudhan

Title of the research program:

iPSC and pre-clinical approach in Haematological diseases

Report:

Our laboratory is focussed on two projects; 1. Disease modelling of haematological diseases and 2. Pre-clinical lentiviral gene therapy vectors for haematological diseases.

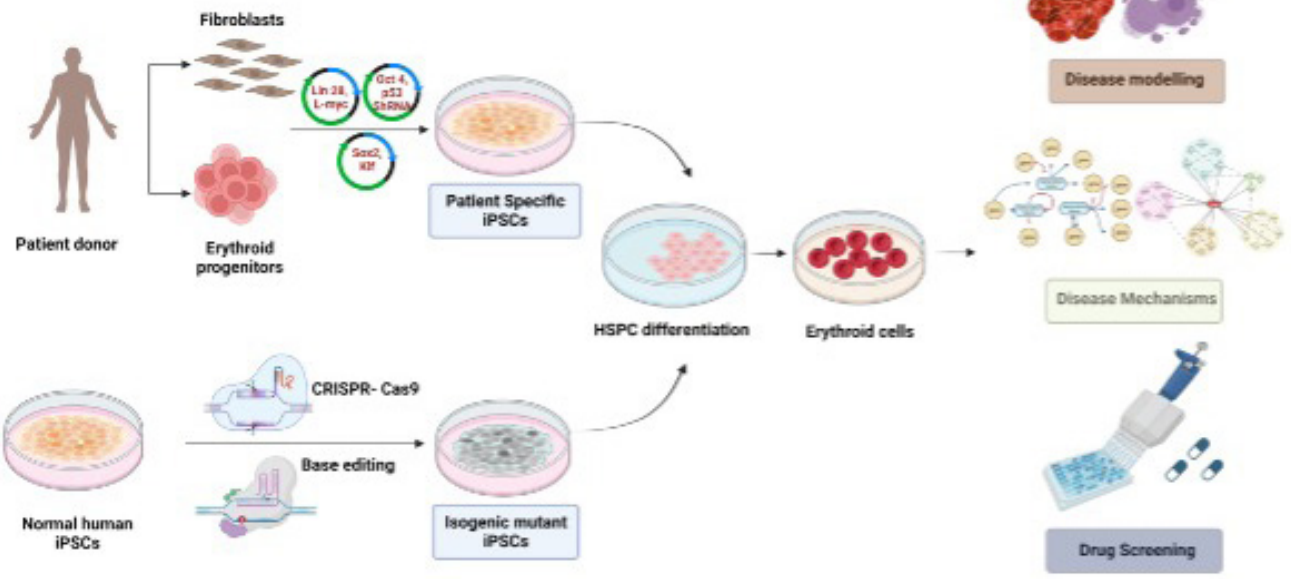
iPSC-based disease modelling of haematological diseases:

We employ induced pluripotent stem cells (iPSCs) to model four haematological diseases: Fanconi anaemia (FA), Diamond-Blackfan anaemia (DBA), congenital dyserythropoietic anaemia (CDA), and chronic myeloid leukaemia (CML). Patient-specific iPSC lines were generated from individuals afflicted with each disease, enabling a comprehensive investigation of disease pathogenesis in vitro. Additionally, utilizing CRISPR-Cas9 and base editing techniques, we introduced targeted mutations into the disease-associated genes within the iPSCs, creating isogenic lines that served as valuable controls for comparative analyses. Through rigorous characterization, we successfully recapitulated disease-specific phenotypes in the iPSC-derived hematopoietic cells, providing critical insights into the molecular basis of FA, DBA, and CML. These disease models demonstrated impaired haematopoiesis, aberrant cell differentiation, and altered cell signalling pathways, faithfully mirroring the in vivo pathology observed in patients. We plan to use these cellular models for drug screening and CRISPR-Cas9 screening to identify the factors that can restore the normal phenotypes in the iPSC-derived hematopoietic progenitors and erythroid cells.

Pre-clinical lentiviral gene therapy vectors for haematological diseases:

We developed specialized RNA interference (RNAi) lentiviral vectors, H23B-Ery-Lin-shRNA, and H234B-Ery-Lin-shRNA, to investigate gene functions in erythroid cells exclusively, leaving other hematopoietic lineages unaffected. Their lineage specificity was confirmed through fluorescent protein expression in various hematopoietic cells. Unlike previous vectors, ours enable the cloning of short hairpin RNAs (shRNAs) for any gene and offer efficient knockdown with a single shRNA integration per cell. Notably, the downregulation of BCL11A, a known gamma-globin gene transcriptional repressor, significantly increased foetal haemoglobin production using these vectors. Transducing healthy donor $CD34^+$ cells resulted in >80% target protein reduction and up to 40% elevation in γ-chain levels. These novel vectors hold potential for gene therapy in hemoglobinopathies and high-throughput RNAi screening for erythropoiesis research. We further modified these vectors to decrease the length of the core transcriptional regulatory elements to increase the transgene expression.

Graphical summary:



Publications:

1. Ijee S, Chembayil K, Chaudhury AD, Das S, Bagchi A, Modak K, Benjamin ES, Rani S, Nath A, Palani D, Priyanka S, Ravichandran R, Babu D, Thamodaran V, **Velayudhan SR**. Efficient Deletion of microRNAs using CRISPR/Cas9 with Dual Guide RNAs (Under Review).
2. Krittika N, Rani S, Babu D, Palani D, Premkumar C, **Velayudhan S.** Efficient base editing of induced pluripotent stem cells for disease modeling. *Scientific Reports* (In Press).
3. Rani S, Thamodaran V, Fouzia NA, **Velayudhan SR**. A DBA iPSC line for disease modeling. *Human Cell* (In Press).
4. Benjamin ES, Babu B, Joshi G, Rajamani BM, Nandy K, Rani S, Anandhan S, PremKumar C, Maddali M, Abraham A, **Velayudhan SR**⁎, Balasubramanian P⁎ (⁎Corresponding Authors) Inhibition of BCR::ABL1 tyrosine kinase activity Aids in the Generation of Stable Chronic Myeloid Leukemia Induced Pluripotent Stem Cells. *bioRxiv*. (https://www.biorxiv.org/content/10.1101/2023.06.01.543015v1).
5. Raina K, Joshi G, Modak K, Premkumar C, Priyanka S, Rajesh P, **Velayudhan SR**,Thummer RP. Generation and characterization of induced pluripotent stem cell line IITGi001-A derived from adult human primary dermal fibroblasts. *Stem Cell Research*. 2023 Jun 28;71:103159. doi: 10.1016/j.scr.2023.103159.

6. Rajamani BM, Illangeswaran RSS, Benjamin ESB, Balakrishnan B, Jebanesan DZP, Das S, Pai AA, Vidhyadharan RT, Mohan A, Karathedath S, Abraham A, Mathews V, **Velayudhan SR**, Balasubramanian P. Modulating retinoid-X-receptor alpha (RXRA)expression sensitizes chronic myeloid leukemia cells to imatinib in vitro and reduces disease burden in vivo. *Frontiers in Pharmacology*. 2023 May 31;14:1187066.

7. Venkatesan V, Christopher AC, Rhiel M, Azhagiri MKK, Babu P, Walavalkar K, Saravanan B, Andrieux G, Rangaraj S, Srinivasan S, Karuppusamy KV, Jacob A, Bagchi A, Pai AA, Nakamura Y, Kurita R, Balasubramanian P, Pai R, Marepally SK, Mohankumar KM, **Velayudhan SR**, Boerries M, Notani D, Cathomen T, Srivastava A, Thangavel S. Editing the core region in HPFH deletions alters fetal and adult globin expression for the treatment of β-hemoglobinopathies. *Molecular Therapy Nucleic Acids*. 2023 Apr 26;32:671-688. doi: 10.1016/j.omtn.2023.04.024. PMID: 37215154; PMCID: PMC10197010.

8. Illangeswaran RSS, Jebanesan DZP, Sivakumar KK, Vidhyadharan RT, Rajamani BM, Janet NB, David E, **Velayudhan SR**, Mathews V, Balasubramanian P. Chemotherapeutic drugs elicit stemness and metabolic alteration to mediate acquired drug-resistant phenotype in acute myeloid leukemia cell lines. *Leukemia Research*. 2023 May;128:107054.

9. Joshi G, Arthur NBJ, Geetha TS, Datari PVR, Modak K, Roy D, Chaudhury AD,Sundaraganesan P, Priyanka S, Fouzia NA, Ramprasad V, Abraham A, Srivastava VM, Srivastava A, Kulkarni UP, George B, **Velayudhan SR**. Comprehensive laboratory diagnosis of Fanconi anemia: comparison of cellular and molecular analysis. *Journal of Medical Genetics*. 2023 Mar 9:jmedgenet-2022-108714.

10. George A, Ravi NS, Prasad K, Panigrahi L, Koikkara S, Rajendiran V, Devaraju N, Paul J, Pai AA, Nakamura Y, Kurita R, Balasubramanian P, Thangavel S, Marepally S, **Velayudhan SR**, Srivastava A, Mohankumar KM. Efficient and error-free correction of sickle mutation in human erythroid cells using prime editor-2. *Frontiers in Genome Editing*. 2022 Dec 20;4:1085111.

11. Dahariya S, Raghuwanshi S, Thamodaran V, **Velayudhan SR**, Gutti RK. Role of Long Non-Coding RNAs in Human-Induced Pluripotent Stem Cells Derived Megakaryocytes: A p53, HOX Antisense Intergenic RNA Myeloid 1, and miR-125b Interaction Study. *Journal of Pharmacology and Experimental Therapeutics*. 2023 Jan;384(1):92-101.

12. Bagchi A, Devaraju N, Chambayil K, Rajendiran V, Venkatesan V, Sayed N, Pai AA, Nath A, David E, Nakamura Y, Balasubramanian P, Srivastava A, Thangavel S,Mohankumar KM, **Velayudhan SR**. Erythroid lineage-specific lentiviral RNAi vectors suitable for molecular functional studies and therapeutic applications. *Scientific Reports*. 2022 Aug 18;12(1):14033.

13. Das S, Stallon Illangeswaran RS, Ijee S, Kumar S, **Velayudhan SR**, Balasubramanian P. Pooled shRNA Library Screening to Identify Factors that Modulate a Drug Resistance Phenotype. *Journal of Visualized Experiments*. 2022 Jun 17;(184).

14. Mahalingam G, Rachamalla HK, Arjunan P, Periyasami Y, M S, Thangavel S, Mohankumar KM, Moorthy M, **Velayudhan SR**, Srivastava A, Marepally S. Optimization of SARS-CoV-2 Pseudovirion Production in Lentivirus Backbone With a Novel Liposomal System. *Frontiers in Pharmacology*. 2022 Mar 25;13:840727.

15. Ravi NS, Wienert B, Wyman SK, Bell HW, George A, Mahalingam G, Vu JT, Prasad K, Bandlamudi BP, Devaraju N, Rajendiran V, Syedbasha N, Pai AA, Nakamura Y,Kurita R, Narayanasamy M, Balasubramanian P, Thangavel S, Marepally S, **Velayudhan SR**, Srivastava A, DeWitt MA, Crossley M, Corn JE, Mohankumar KM.Identification of novel HPFH-like mutations by CRISPR base editing that elevate the expression of fetal hemoglobin. *Elife*. 2022 Feb 11;11:e65421.

16. Nath A, Rayabaram J, Ijee S, Bagchi A, Chaudhury AD, Roy D, Chambayil K,Singh J, Nakamura Y, **Velayudhan SR**. Comprehensive Analysis of microRNAs in Human Adult Erythropoiesis. *Cells*. 2021 Nov 4;10(11):3018.

17. Christopher AC, Venkatesan V, Karuppusamy KV, Srinivasan S, Babu P, Azhagiri MKK, Chambayil K, Bagchi A, Rajendiran V, Ravi NS, Kumar S, Marepally SK, Mohankumar KM, Srivastava A, **Velayudhan SR**, Thangavel S. Preferential Expansion of Human CD34+CD133+CD90+ Hematopoietic Stem Cells Enhances Gene-Modified Cell Frequency for Gene Therapy. *Human Gene Therapy*. 2022 Feb;33(3-4):188-201.

18. Singh G, Manian KV, Premkumar C, Srivastava A, Daniel D, **Velayudhan SR**. Derivation of Clinical-Grade Induced Pluripotent Stem Cell Lines from Erythroid Progenitor Cells in Xenofree Conditions. *Methods in Molecular Biology*. 2022;2454:775-789.

19. Thamodaran V, Rani S, **Velayudhan SR**. Gene Editing in Human Induced Pluripotent Stem Cells Using Doxycycline-Inducible CRISPR-Cas9 System. *Methods in Molecular Biology*. 2022;2454:755-773.

04

Multi-Institutional Programs

04.
Multi-Institutional Programs

4.1 Joint program of Centre for Stem Cell Research (CSCR), a unit of inStem, Bengaluru and Christian Medical College, Vellore

Summary of Research (2022–23)

The Centre for Stem Cell Research (https://www.cscr.res.in/) continues to focus on translational research in cell and gene therapy towards regenerative medicine to bring stem cell science and other novel therapies to the management of patients with unmet needs. It is the goal of scientists at CSCR to work in teams directed at particular themes to find solutions for current medical needs in the country. Three thematic multi-individual, multi-disciplinary, and multi-institutional research programs are described below.

1. Musculoskeletal regeneration

This is a strong thematic program at CSCR and involves two teams.

The first team is led by Vrisha Madhuri and includes Srujan Marepally, Mohan Kumar, Nihal Thomas, Vikram Mathews, Dolly Daniel, Lilly Verghese, and Alok Srivastava. The major focus of the team is clinical and preclinical translation related to physis, articular cartilage, bone, and muscle regeneration. Towards this, the team focuses on two major areas. The first is cell-based therapy for bone, cartilage, and muscle regeneration. In collaboration with Karolinska Institute, Sweden, they have an ongoing phase I/II clinical trial for the treatment of osteogenesis imperfecta using foetal liver-derived mesenchymal stem cells. In parallel, they are also exploring the paracrine and immunogenic effects of multiple infusions of MSCs via intraosseous and intravenous routes. In another phase I/II trial, culture-expanded muscle-derived stem cells are used for the treatment of urinary sphincter incontinence. The second research area is cell-free therapy for cartilage and bone regeneration using biomolecules. In collaboration with multidisciplinary groups from SCTIMST, Trivandrum, Kerala and CSCR, the team has identified suitable biomaterials with kinetics for the sustained release of therapeutic biomolecules. A new initiative includes the use of extracellular vesicles for the treatment of osteoporosis in genetic defect animal and cellular models. They are also generating *in vitro* data to convert autologous chondrocyte therapy for physis or articular repair to a single-step procedure bypassing the cell expansion step.

The second team is led by Elizabeth Vinod and includes Solomon Sathishkumar, Alfred Job Daniel, Abel Livingston, and Viju Daniel Varghese. The primary focus of the team is to create and validate osteoarthritic animal models, characterise cartilage-derived progenitors, and assess their potential implications for cartilage regeneration under *in vitro* and *in vivo* conditions. The team hypothesises that a deeper understanding of these progenitors, in comparison to other cell types, will enable the establishment of a detailed biological profile and devise better approaches for treating cartilage pathologies. In the fields of cartilage tissue engineering and regenerative medicine, there is a need to enhance biological and functional outcomes, both in terms of refining existing treatments and developing novel therapeutic strategies.

More details on musculoskeletal regeneration program can be found at: https://www.cscr.res.in/annual-reports/

2. Gene therapy - thematic program

A major focus of research at CSCR is gene therapy. The goal is to capitalize on the recent advances in the world towards gene therapy of monogenic haematological disorders and make them possible for patients in India. Several scientists and physicians are involved in this work, which is coordinated by Alok Srivastava and includes R. V. Shaji, Saravanabhavan Thangavel, Mohankumar Murugesan, Srujan Marepally, and Gurbind Singh at CSCR and several other faculties from CMC, Vellore as well as many external collaborators.

A.Haemophilia:

At present, this program involves two major components:

I. First is a clinical trial for AAV vector-based gene therapy for haemophilia B in collaboration with Emory University, Atlanta, USA and the University of Florida, Gainesville, USA. Given the success of AAV-based gene therapy reported in recent years, they have developed a novel transgene and vector combination for gene therapy of haemophilia B. Due to difficulties in producing cGMP grade AAV vector in USA and unsurmountable challenges with trying to establish that technology at CSCR, they have now shifted this effort to facilitating a collaboration between an industry partner in India and the University of Florida to take this forward. The completed proof of concept data and the experience gained in product development will aid this collaboration as well.

II. The second is a lentiviral vector-mediated transduced autologous hematopoietic stem cell-based gene therapy for haemophilia A. Based on strong proof of concept studies and after appropriate regulatory approvals in India, this novel first-in-human clinical trial of gene therapy for haemophilia A (factor VIII deficiency) was initiated in June 2022. Three subjects have been treated so far with no major unexpected safety concerns. It is highly gratifying that there has also been a clinically significant therapeutic response in all of them. This is also the first example of a gene therapy product being taken to clinical trials with academic funding outside of the scope of major pharmaceuticals. The long-term support of the Department of Biotechnology of the Ministry of Science and Technology of the Government of India made this possible along with the pioneering work of the scientists at CSCR and our collaborators.

With these programs, several widely used technologies for gene therapy of many diseases will get established in India along with its regulatory processes and will aid the development of more gene therapy products in the future.

B. Major haemoglobin disorders:

i. ***Lentiviral vector-based gene therapy***: This project aims to evaluate lentiviral vectors for developing gene therapy for major haemoglobin disorders. This is coordinated by R. V. Shaji. In collaboration with Emory University, globin gene addition lentiviral vectors have been generated for gene therapy of haemoglobinopathies. In addition, the team has generated novel lentiviral shRNA vectors for the knockdown of BCL11A in human erythroid cells. They have generated eight different lentiviral vectors with modified regulatory sequences for a high level knockdown of BCL11A and upregulation of foetal haemoglobin (HBF) in erythroid cells. The efficacy of these vectors has been tested in mouse transplantation studies.

ii. ***Gene Editing Approach I***: The first gene editing approach is led by Saravanabhavan Thangavel. The primary aim is to recapitulate HPFH-like deletions in the hematopoietic stem and progenitor cells (HSPCs) of patients with sickle cell disease (SCD) and thalassemia. The target includes a region that is conserved among many HPFH deletions. The team successfully introduced these deletions in the HSPCs with an efficiency of >70% and observed that when these edited HSPCs are differentiated into erythrocytes they express high foetal haemoglobin. They have also transplanted the gene-edited cells into NSG mice and NBSG-W mice and observed that the gene-edited cells engraft and re-populate in mouse bone marrow.

iii. ***Gene Editing Approach II:*** The second gene editing approach is led by Mohankumar Murugesan. The team has developed six different major approaches to overcome the current limitations for the genome editing of SCD and beta-thalassemia.

(1) Base editing at the novel targets identified from the screen in the HBG promoter up-regulates HbF level than the disruption of well-known BCL11A binding site in erythroblasts derived from human CD34+ hematopoietic stem and progenitor cells without any detrimental effects on erythroid differentiation. Editing at the highly homologous HBG1 and HBG2 promoter by base editor showed a very minimal level of off-targets at both the DNA and RNA levels.

(2) Targeting specific regions of BCL11A enhancer at the functional core of +58 DHS in human $CD34^{+}$ HSPCs showed better enucleation and comparative induction of foetal haemoglobin levels than the current clinical trial targets.

(3) Key nucleotide substitution at the zinc finger domains present uniquely in the BCL11A-XL isoform provides novel approaches for the treatment of beta-hemoglobinopathies. Specifically compared to the current clinical trial targets based on BCL11A, alteration of the zinc finger domain dramatically up-regulated foetal haemoglobin levels with no effect on erythroid maturation and minimal transcriptomic changes.

(4) Lineage-specific knockdown of another major gamma globin repressor (LRF/ZBTB7A) resulted in a therapeutic induction of foetal haemoglobin in human erythroid cells. Currently, the team is working on understanding the molecular mechanism of LRF/ZBTB7A regulation on erythropoiesis.

(5) Precisely corrected and modelled the spectrum of β-thalassemia mutations at the HBB promoter, exon, and intronic regions in human erythroid cells by base editors.

(6) The team demonstrated the use of prime editor for the efficient creation as well as correction of sickle cell mutations in human erythroid cells.

These results demonstrated the reconstitution of functional beta-globin chain at the DNA, mRNA, and protein levels, highlighting the potential use of prime editing as a valuable therapeutic gene editing approach for SCD.

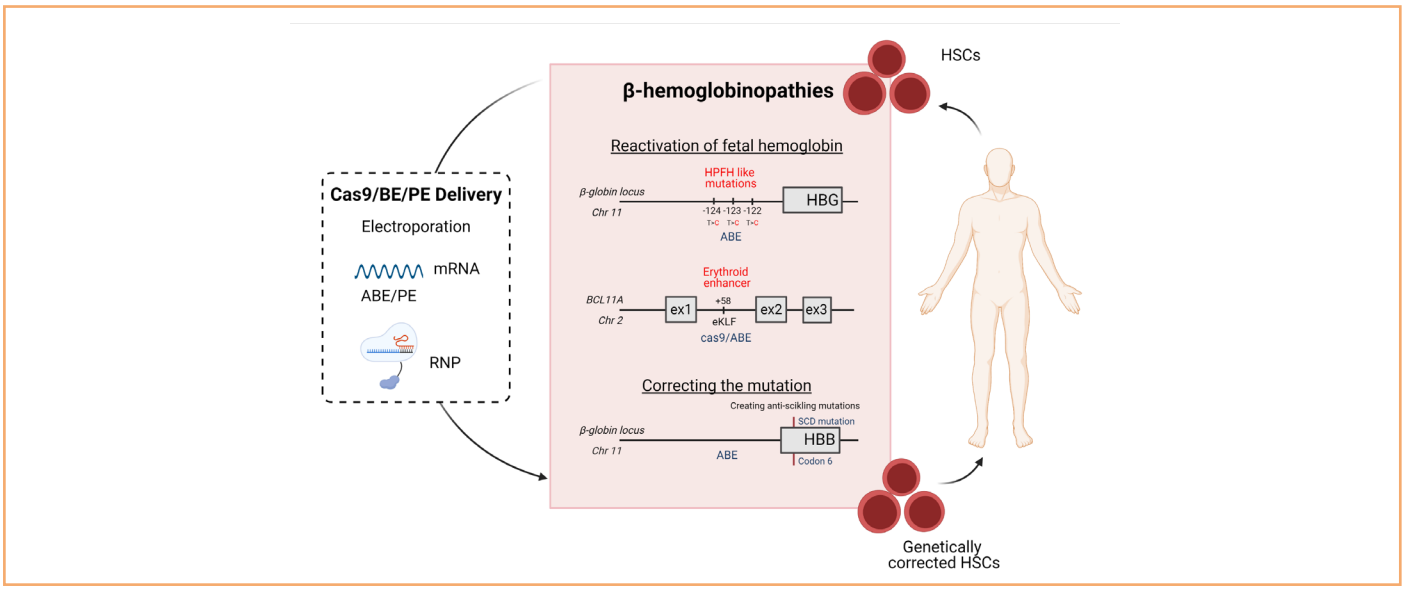


Figure: Therapeutic genome editing for Hematological disorders

More details can be found at: https://www.cscr.res.in/annual-reports/

3. Other gene therapy projects

A. Gene Therapy – Pre-clinical for HIV infection: The project is led by Saravanabhavan Thangavel and his team. They are developing CCR5 gene-edited autologous HSPCs as an alternative to haematopoietic stem cell transplantation (HSCT) from an HLA-matched CCR5 null donor. However, the clinical application of gene-edited autologous HSPCs is critically limited by the quality of the graft, as HIV also infects HSPCs. By using mobilized HSPCs from healthy donors, the team showed that the $CD34^{+}CD90^{+}$ hematopoietic stem cells (HSCs) express 7-fold lower CD4/CCR5 HIV receptors, higher levels of SAMHD1 anti-viral restriction factor, and possess lower susceptibility to HIV infection than the $CD34^{+}CD90^{-}$ hematopoietic progenitor cells.

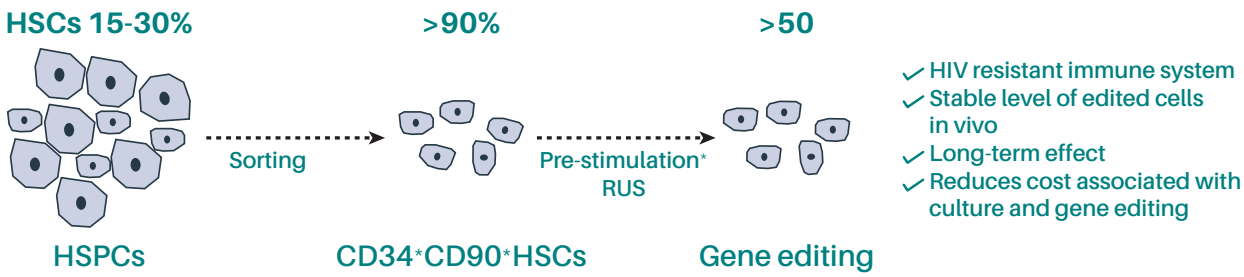


Figure: Schematics of mRNA-based vaccine for SARS-COV-2: *CCR5* gene-edited $CD34^{+}CD90^{+}$ HSCs are the optimal graft for HIV gene therapy (Karuppusamy *et al, Frontiers in Immunology*, 2022)

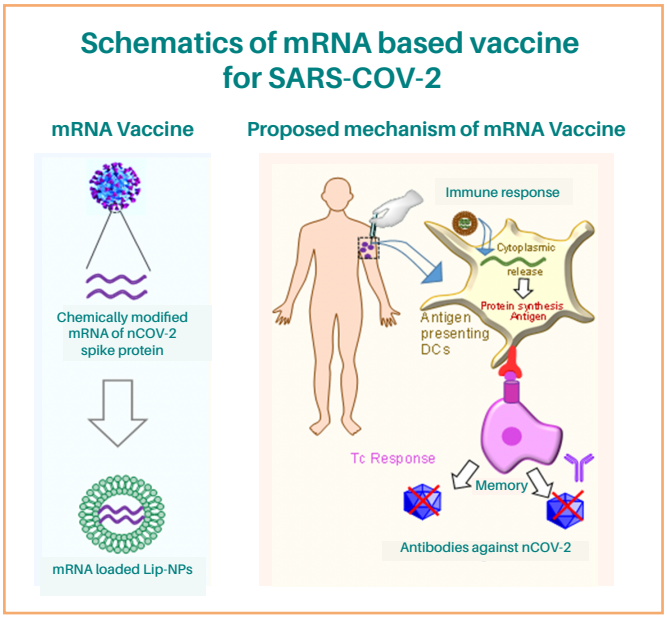
B. Therapeutic genome editing for haemophilia: This project is led by Mohankumar Murugesan. The team worked on developing a technique for haemophilia targeted integration of FVIII/FIX under the endogenous locus in HSPCs utilising the CRISPR/Cas9 technology. Towards this end, they optimized the AAV production and HDR-mediated target integration of GFP in $CD34^{+}$ HSPCs and successfully expressed the transgene under the endogenous locus.

More details of this project can be found at: https://www.cscr.res.in/annual-reports/

4. Other projects:

A. Development of Spike mRNA encapsulated SMART-nanoparticles as a vaccine for SARS-CoV-2 virus: This work is led by Srujan Marepally. They have developed a novel Shikimoylated Mannose Receptor Targeting (SMART) nanoparticle system for delivering mRNAs into dendritic cells for vaccine development, synthesized chemically modified mRNA and validated them functionally. The group also developed tools for COVID-19 research such as HEK-293 cells stably expressing pseudovirion and human ACE-2 receptor for assessing *in vitro* neutralization efficiency of the vaccine. They have successfully demonstrated that vaccinated animals could produce strong immune responses against spike protein and could neutralize SARS-CoV-2 pseudovirus.

Towards developing a novel lipid-mediated gene therapy strategy for haemophilia, galactosylated lipid nanocarriers have been developed by Srujan Marepally. These nanocarriers can specifically deliver nucleic acids including pDNA, siRNA, and mRNA effectively into the liver. Further, safety profiles and therapeutic efficacy are being assessed in haemophilia B mouse model.



More details of this project can be found at: https://www.cscr.res.in/annual-reports/

5. Cellular reprogramming and its applications—disease modelling and haplobanking

The area of cellular reprogramming technology is coordinated by R. V. Shaji at CSCR along with Dolly Daniel. This is now being applied to two areas: disease modelling and haplobanking. Towards the former, reprogramming technology has been applied to develop disease models of various bone marrow failure syndromes – Fanconi anaemia, Diamond Blackfan anaemia and congenital dyserythropoietic anaemia. The models are being used to evaluate disease phenotypes and mechanisms as well as evaluation of gene correction strategies.

A major translational application has been the development of a "*haplobank*"—cells from HLA haplotype homozygous individuals whose mononuclear cells are being converted into iPSC lines for potential use in regenerative medicine. The field and clinical aspects of procuring these peripheral blood samples through our collaborators, the DATRI unrelated donor registry, represented by Nezih Cereb, is being coordinated by Dolly Daniel. So far, 15 GMP cell lines have been produced – one of the largest such collections in the world. This is also being done in collaboration with the international consortium for this effort—Global Alliance for iPSC Therapies (GAiT).

More details of this project can be found at: https://www.cscr.res.in/annual-reports/

6. Control of sickle cell disease & thalassemia major in Odisha program—Creating a model for India

The project aims to reduce the burden of these diseases in the affected populations in Odisha through the combined effort of Ministry of Health, Odisha, NHM Odisha, Christian Medical College, Vellore and Centre for Stem Cell Research (a unit of inStem, Bengaluru), with the support of Department of Biotechnology of the Ministry of Science and Technology, Government of India. The project's primary focus is on addressing Major Haemoglobin Disorders (MHD), a pressing public health concern in the nation. In the state of Odisha, it is estimated that approximately 10% of the population either carry the genetic traits for these disorders or are afflicted by them. Remarkably, this initiative represents the first comprehensive program in India aimed at controlling these significant haemoglobin disorders on such a grand scale. Moreover, pioneering technologies have been implemented for the screening of these haemoglobin disorders and for conducting genetic analyses related to these diseases.

More details of this project can be found at: https://www.cscr.res.in/annual-reports/

7. Core facilities:

A. Good manufacturing practice (GMP) facility at CSCR: The facility is designed to develop and manufacture cell and gene therapy products for clinical applications. It provides the infrastructure for the large-scale expansion of stem cells and genetic modification of cells required to conduct Phase I/II clinical trials in the fields of cell and gene therapy. The GMP facility has two facilities for the manufacture of 1) cell therapy products and 2) genetically modified cells. This facility has the required regulatory central and state authority approvals for manufacturing cell and gene therapy products. More details can be found at https://www.cscr.res.in/cgmp-facility/

B. Imaging and flow cytometry platforms for research: CSCR core facility provides consultation, training, and access to high-end equipment for research applications to all the researchers at CSCR, CMC, and other academic institutions.

We have BD FACS Celesta, BC Cytoflex LX cell analysers, and BD FACS ARIAIII, BD FACS ARIAFusion cell sorters with 3 and 5 lasers respectively at our facility. We have Laser scanning confocal microscope system (Olympus FV1000), Laser scanning multi photon microscope (Olympus FV1000MPE), Leica DMI6000B inverted fluorescence microscope, EVOS FL auto fluorescence imaging system, *in vivo* small animal imaging system (PerkinElmer IVIS Spectrum CT), stereo microscope, and light microscopes in our facility.

We have an offline analysis workstation facility with FlowJo, Kaluza software for flow cytometry data analysis and FV10-ASW software for image analysis.

More details can be found at https://www.cscr.res.in/core-facility/

C. Lab Animal Facility (LAF): The goal of the CSCR- LAF is to promote the humane care and use of laboratory animals by providing information that will enhance animal well-being, the quality of research, and the advancement of scientific knowledge that is relevant to both humans and animals as per the sanction from the Institutional Animal Ethics Committee (IAEC). The LAF is registered with the 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA) for breeding and conducting experiments on small laboratory animals.

The CSCR-LAF maintains several different strains of rodents - mice strains including wild type, transgenic, knockout, and SCID strains and SD rat. More details can be found at http://www.cscr.res.in/laboratory-animal-facility

Other Research Programmes:

For details of research programmes other than those included within this thematic area, please visit the CSCR website: www.cscr.res.in

Publications:

Please see CSCR website for the full list of publications: www.cscr.res.in/research-article

Patents:

1. *A Method for Modification of β-Globin Gene* (Application number 202241030885), dated 30th May 2022. Inventor(s): Mohankumar K. Murugesan, Alok Srivastava, and Kirti Prasad.
2. *A Method for Reactivation of Fetal Hemoglobin and A Composition Thereof* (application no: 202241030465), dated 27th May 2022. Inventor(s): Saravanabhavan Thangavel and Alok Srivastava.
3. *A Method for Downregulation of A Target gene and A Composition Thereof.* (Application No. 202241045242), dated 8th August 2022. Inventor(s): Shaji R. Velayudhan, Alok Srivastava, and Mohankumar K. Murugesan.
4. *A Method for Elevation of Gamma Globin* (Application No.: 202241055876), dated 29th September 2022. Inventor(s): Mohankumar K. Murugesan, Alok Srivastava, Nivedhitha D., and Vignesh R.
5. *Formulations, Lipid compound, methods and thereof* (Application No. PCT/IN2022/050660), dated 22nd July 2022. Inventor(s): Srujan Kumar Marepally and Alok Srivastava.

Awards:

Name of the awardee: **Dr. Mohankumar K. M.**

Name of the award: Ben Barres Spotlight Awards, eLife community (https://elifesciences.org/inside-elife/6794cd8a/ben-barres-spotlight-awards-announcing-the-winners-for-2022)

4.2 Platform for Chemical Biology and Therapeutics (PCBT): Report 2022-2023

Goals:

The Centre for Chemical Biology & Therapeutics (CCBT) was established to explore innovative approaches to modulate intracellular signalling pathways disrupted in disease through a unique, integrated, and multidisciplinary programme. Our first goal was to target domains that recognize phosphorylated proteins – a key class of protein modification vital for signalling to create a unique palette of chemical probes that will not only provide novel insights into disease mechanisms but also help to translate this new knowledge into the discovery of novel approaches for therapy.

Our work provides a framework for chemical biology and translational research across the campus. In this context, the platform will formally offer services as a facility, termed, the Platform for Discovery Biology (PDB) with a dedicated facility manager and facility advisory committee comprising of faculty members from inStem and the BLiSc campus.

Scientific Report:

Since its inception, the CCBT has made headway in expanding the druggable proteome with its unique multidisciplinary format. We have made strong progress towards our first focus, BRCT domains, which represent an important class of domains that recognize pSer/pThr motifs using structurally distinct mechanisms. We have reported (*Cell Chemical Biology*, 2018; *ChemMedChem,* 2019, US2018/0346461 A1) the development of Bractoppin, a first drug-like inhibitor of phosphopeptide recognition by the human BRCA1 tBRCT domain, which selectively inhibits substrate binding in vitro, and in cells, selectively blocks BRCA1-dependent signals triggered by DNA damage. To further develop Bractoppin lead series towards commercialization, several challenges need to be addressed. First, although Bractoppin shows good (~75 nM) potency in vitro, cellular activity is evident at only >1-10 μM. Second, high Plasma protein binding (PPB) is unfavourable. Third, the solubility of Bractoppin (~40 μM) hinders co-crystallization. To progress to other inhibitors of BRCA1, we attempted Fragment-based ligand discovery (FBLD) to identify small organic molecules, typically with MW ~150 Da, that bind the BRCA1 tBRCT domain.

Fragment-based ligand discovery (FBLD) as a strategy to develop a backup series for Bractoppin

We screened 1014 fragments for their binding ability to the BRCA1 t-BRCT domain by NMR spectroscopy using the Saturation Transfer Difference (STD) method. The initial hits were validated by orthogonal thermal shift assay. Using competitive STD-NMR, we further shortlisted active hits potentially binding to the phosphopeptide pocket of the BRCA1 t-BRCT domain (Figure 1). The shortlisted hit molecules were subjected to Heteronuclear Single Quantum Coherence (HSQC) Spectroscopy and the hit binding sites by chemical shift mapping. We shortlisted 15 best fragments for co-crystallization/soaking based on the filtration criteria, as mentioned in Figure 1a. Among the shortlisted hit fragments, fragment 386 showed a better binding signal in the STD NMR. Moreover, its binding was inhibited in the presence of the high-affinity BACH1-phosphopeptide, suggesting that 386 binding pocket overlaps with the phosphopeptide binding pocket of BRCA1 t-BRCT (Figure 1b). 386 binding affinity was measured using isothermal titration calorimetry (ITC), and the study revealed a Kd value of ~100 μM to BRCA1-tBRCT (Figure 1c). Further, an attempt has been made to identify the binding site of 386 using labelled BRCA1-tBRCT protein and NMR experiment. Upon the addition of 386, many peak intensities were significantly reduced compared with the peak intensity of the unbound form (Figure 1d). The co-crystal structure of 386 has been solved to a resolution of 2.5 Å by X-ray crystallography (Figure 1e). Based on the co-

crystal structure of BRCA1-BRCT with fragment 386, structure-based drug design approach was employed to expand the fragment towards the phospho-peptide binding pocket of the BRCA1-BRCT. Newly designed molecules were synthesised and tested for direct binding and in competition with the cognate phosphopeptide. As shown in Figure 1f, in comparison with fragment 386, the new molecules T108 and T109 showed improved affinity in the direct binding and ability to compete with the BACH1-phosphopeptide. The structure-guided optimization of T108 is underway.

To target phosphopeptide recognition for the selective modulation of intracellular signalling via the tBRCT domains of ECT2

Identification of optimized peptide partners for ECT2 using protein engineering approaches

Ect2 is a tandem BRCT-containing protein that functions as a guanine nucleotide exchange factor required for signal transduction pathways involved in the regulation of cytokinesis during mitosis. Although the human phosphorylated Cyk-4 is a well-known binding partner of ECT-2, the molecular details of the interaction and the Cyk-4 sites of phosphorylation that enable ECT2 binding are obscure. Using isothermal titration calorimetry and fluorescence polarization, we investigated the binding affinity of ECT2-BRCT with two human Cyk-4 peptides and four C. *elegans* Cyk-4 peptides phosphorylated on different residues (Table 1). We prioritised three phosphopeptides with Kd values of 2.1, 0.6, and 6.8 μM, respectively, for further crystallization studies. Moreover, the study suggested the potential phosphorylation sites in human Cyk-4 that might increase the binding affinity of Cyk-4 to ECT2. Based on the knowledge gained from C. *elegans* Cyk-4 phosphopeptides, we have designed three human Cyk-4 phosphopeptides. The human peptide 1 was designed in such a way to have two phosphorylation sites that correspond to C. elegans peptide 3; these two phosphosites are highly conserved in both human and C. *elegans* Cyk-4. The measured binding affinity of human peptide 1 showed a Kd value of 4.7 μM, which is ~10 fold higher than the so far reported human phosphopeptide (Kd of 50 μM).

Evaluation of ECT2-BRCT phosphopeptide binding pocket using mutations

Since the crystallisation attempts failed to yield diffraction quality crystals of ECT2 complexed with the C. *elegans* and human Cyk-4 phosphopeptides, we attempted to identify the binding pocket residues through mutational analysis. First, we mutated three highly conserved phosphopeptide binding residues presented in ECT2-BRCT; (i) canonical T153 abd K195 (ii) positive charged cluster residues, R176, K177, and K182 and (iii) the positive charge residues, R138 and H169, present in the interface between BRCT domain 0 and 1 (Figure 2a). The ITC data (presented in the table below) clearly indicated that T153 and K195 have less impact on the binding of C. *elegans* peptide 3 binding. However, the positive charge patch residues R176, K177, and K182 completely abrogated the binding. Similarly, human phosphopeptide binding was significantly affected by R176, K177, and K182 mutants. These data indicated that ECT2-BRCT has a unique phospho recognization motif compared to other known BRCT modules.

Mitosis-dependent Interactors of Ect2

Ect2 has been shown to localize to the central spindle complex on the onset of anaphase of mitosis and found to be essential for the completion of cytokinesis, and the deletion of its tBRCT domain has been shown to affect cytokinesis. Despite the established crucial role of Ect2-tBRCT, the knowledge of its interacting partners (except Cyk4) and the mode of peptide substrate recognition and signal propagation is largely unknown. To understand the myriad functions and the importance of the tBRCT domains in protein–protein interactions, we undertook a proteomics approach to dissect the signalling pathway.

The Ect2-tBRCT interactome in distinct stages of mitosis was identified by mass-spectrometry-based interactome profiling. SAINT analysis for Bayesian false discovery rate (SAINT-BFDR) was used to create a high-confidence interaction list and then the Biological Networks Gene Ontology (BINGO) analysis was performed to fetch biological processes of

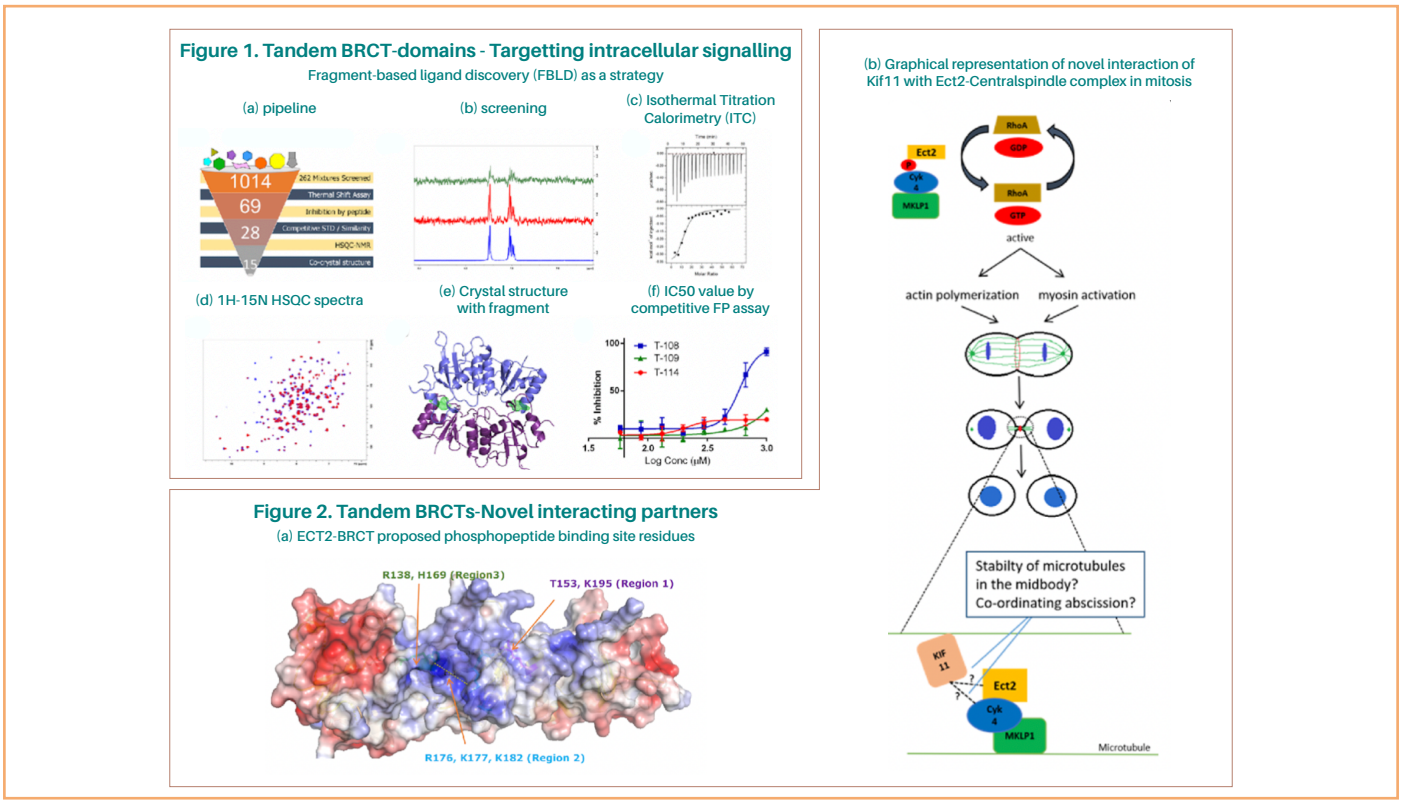
the interactome. Apart from common processes like cellular metabolic, biogenesis, gene expression, and translation, multiple entries for cell cycle-related processes were enriched in the anaphase. The subset of proteins belonging to the central spindle protein interaction network was chosen for validation. From the subset of proteins chosen for validation, a previously unidentified interactor of Ect-2 tBRCT, Kif11, a kinesin motor protein was identified along with components of the central spindle complex and this was shown through immunoprecipitation and immunofluorescence staining (Figure 2b).

Since BRCT domains recognize phosphorylated motifs on interacting partners to mediate cellular signalling processes, and Ect2 also recognizes the phosphorylated form of Cyk4, we wanted to investigate the nature of the interaction of Kif11 with Ect2 and the centralspindlin complex. Co-immunoprecipitation of Kif11 with Ect2 and Cyk4 was performed in the absence and presence of phosphatase enzyme. The interaction of Kif11 with Ect2 appeared to be reduced in the presence of phosphatase, suggesting that Kif11 may interact with the Ect-centralspindlin complex in a phosphorylated manner and that this interaction could be cell cycle-specific due to the occurrence of temporal phosphorylation during mitosis.

Through immunofluorescence microscopy, Kif11 was also shown to localize at the midbody along with Ect2 and the centralspindlin complex but the function of Kif11 during the later stages of mitosis remains elusive. Upon inhibition of Kif11 with Monastrol, a chemical inhibitor of Kif11's motor activity, a delay in cytokinesis was also observed.

Through a mitotic-dependent mass spectrometric study, we identified KIF11 as a novel interactor of Ect2-Centralspindlin complex, which was enriched specifically at the midbody. This could implicate the functional involvement of KIF11 in midbody-microtubule dynamics and/or during abscission.

Supporting Graphic/Image



PCBT achievements in the past year (April 2022-Aug 2023)

Publications

- Asokan M, Joan RF, Babji S, et al. 2023. Interim results from comparison of immune responses elicited by an inactivated and a vectored SARS-CoV-2 vaccine in seronegative and seropositive participants in India. *MedRxIV.* doi: 10.1101/2023.01.03.22284082.
- Wangchuk J, Singh A, Shakthivel S, Sheth D, Sundaramurthy V, Karumbati AS, Miller C, Moore K, Patil SV, Barkate H, Patil SR. 2023. In vitro virucidal assays to evaluate the anti-viral efficacy of nitric oxide nasal spray against different SARS-CoV-2 variants (Submitted to *Nitric Oxide*; doi: 10.2139/ssrn.4532325).
- Subashini M, Puneeth Kumar C, Manvi S, Saranya G, Keerthana S, Kavitha B, Neelagandan K. 2022. Structure of a 14-3-3:FOXO3apS253 phosphopeptide complex reveals 14-3-3 isoform specific binding of FOXO phosphoproteins. *ACS Omega*, 7 (28), 24344–24352. doi: 10.1021/acsomega.2c01700.
- Singh M, Kempanna P, Bharatham K. 2022. Identification of Mtb GlmU Uridyltransferase Domain Inhibitors by Ligand-Based and Structure-Based Drug Design Approaches. *Molecules*, 27, 2805. doi: 10.3390/molecules27092805.
- Jagannath DK, Valiyaparambil A, Viswanath VK, Hurakadli MA, Kamariah N, Jafer AC, Patole C, Pradhan S, Kumar N, Lakshminarasimhan A. 2022. Refolding and characterization of a diabody against Pfs25, a vaccine candidate of *Plasmodium falciparum*. *Analytical Biochemistry*, 655, 114830. doi: 10.1016/j.ab.2022.114830.
- Kavya R, Aouti S, Jos S, Prasad TK, Kumuda KN, Unni S, Padmanabhan B, Kamariah N, Padavattan S, Mythri RB. 2023. High-affinity binding of celastrol to monomeric α-synuclein mitigates in vitro aggregation. *Journal of Biomolecular Structure and Dynamics*. doi: 10.1080/07391102.2023.2175379.
- Rao SS, Parthasarathy K, Sounderrajan V, Neelagandan K, Anbazhagan P, Chandramouli V. 2023. Susceptibility of SARS Coronavirus-2 infection in domestic and wild animals: a systematic review. 3 Biotech, 13(1), 5. doi: 10.1007/s13205-022-03416-8.
- Kumar V, Puneeth Kumar Chunchagatta Lakshman, Kootteri PT, Manjunath K, Sneha Bairy, Akshaya S Vasu, B Ganavi, Subbarao Jasti and Neelagandan Kamariah. Target-based drug discovery: Applications of fluorescence techniques in high throughput and fragment-based screening, Heliyon (under revision, 2023).

Talks

- **Anandi S. Karumbati delivered a talk titled *"Targeting phosphopeptide recognition by tandem BRCT domain family"*** in February 2022 at inStem Annual Review of Research.
- **Neelagandan Kamariah delivered a talk titled *"Structure-guided lead discovery approaches for targeting phosphopeptide recognition to interrupt intracellular signalling"*** at the International Conference on Translational Research on Drug Discovery and Development for Sustainable Healthcare, 2023 at Sathyabama, Chennai.

Conference Presentations

1. roteomics analyses to identify cell cycle dependent mitotic interactors of Epithelial cell transforming 2 (Ect2) tBRCT domain. 2022. Ghosh S, Periasamy J, Nijaguna M, Athar M, Reddy SA, Fathima S, Boggaram S, De S, Sadasivam G, Bharatham K, Karumbati A, Venkitaraman A. Presented at inStem ARR 2022 and IISER TVM 2022: 5th International Chromosome Stability Meeting.
2. Proteomics analyses identify Kinesin-5 (Kif11) as a mitotic interactor of Epithelial cell transforming 2 (Ect2). 2023 Ghosh S, Periasamy J, Nijaguna M, Athar M, Reddy SA, Fathima S, Boggaram S, De S, Sadasivam G, Bharatham K, Karumbati A, Venkitaraman A. Presented at inStem ARR 2023.
3. Structure-guided ligand discovery approaches for targeting phosphopeptide recognition by the BRCA1 tBRCT. 2023. Kootteri PT, Manjunath K, Bairy S, Sathish K, Hurakadli M, Kumar V, Singh M, Bharatham K, Gudla CS, Kamariah N, Venkitaraman A. Presented at the inStem ARR 2023 and 7th Asia Pacific ISSX.
4. Novel Chemical entities targeting SARS-CoV-2 Papain-Like protease (PLpro). 2023. Sheth D, Manjunath K, Kootteri PT, Kumar V, Rohithaswa AC, Sharma AU, Wangchuk J, Bharatham K, Karumbati AS, Kamariah N, Venkitaraman AR. Presented at the inStem ARR 2023 and 7th Asia Pacific ISSX. Won the Best Poster Award.

Conferences organized

1. Dr. Anandi Karumbati was on the Scientific Advisory and Organizing Committee at the Asia Pacific International Society for the Study of Xenobiotics (AP-SSX, January 2023) this year.

Academic/ Commercial Funding

1. Elucidating the Structural and Functional Characterization of Membrane and Envelope Protein from SARS CoV-2 and its Variants in Virus-Like Particle Formation and its Role in the Virus Assembly and Release. Principal Investigator: Dr. Neelagandan Kamariah, Funded by ICMR, (2021–2024).
2. Translational Platforms for Discovery, Repurposing, and Clinical Development for COVID-19 Therapeutics. Principal Investigator: Dr. Anandi Karumbati (along with other BLiSc Investigators)
3. SPARC: Validation of a Novel Target for Oncology

Thus, collectively, PCBT's established and emerging academic links, together with prospective commercial collaborations, validate our measured strategy to extend the scope, impact, and commercialisation of our research programmes.

4.3 Accelerator Program for Discovery in Brain Disorders using Stem Cells

During development, specific cells in the human embryo divide and differentiate to give rise to the adult human brain. These developmental events are influenced by both genetic and environmental factors, and can lead to either normal brain or the development of brain disorder.

Brain disorders are a global health challenge with the vast majority having no effective treatments. Despite obvious differences in their clinical presentation, many of these disorders appear to share molecular, cellular, and circuit mechanisms. Our vision is to accelerate the discovery of these mechanisms and thus facilitate the delivery of effective therapeutics for these disorders.

Among the brain disorders, severe mental illness (SMI) is a major source of disability in young adults with about 2–3% of the population at risk for developing SMI disorders both in India and across the world. These disorders are recognized as one of the major non-communicable diseases (NCD) and a significant contributor to morbidity as articulated by the World Health Organization's New Delhi call for action on combating NCDs in India. Given this huge disease burden, the development of novel ways to diagnose and treat mental illness will have important positive social and economic benefits. To achieve this goal, there is a pressing need to understand the mechanistic basis of these disorders; such discovery could form the basis for the development of novel diagnostic and therapeutic approaches.

The Accelerator Program for Discovery in Brain Disorders using Stem Cells (ADBS) studies five major forms of SMI: schizophrenia, bipolar disorder, obsessive-compulsive disorder, substance dependence, and dementia. These disorders are thought to have a neurodevelopmental origin as well as an inherited basis. However, despite their high heritability, to date, few genetic correlates that account for the high heritability have been identified. To study these disorders, the Department of Psychiatry, thw National Institute for Mental Health and Neurosciences (NIMHANS), and the National Centre for Biological Sciences (NCBS), and inStem have assembled a prospective cohort of patients with a strong family history of SMI. The ADBS is pursuing three distinct but complementary lines of analysis on these families: (i) The families are being clinically characterized in depth to understand changes in structure and function at multiple levels of brain organisation; they are being followed over a period of twenty years at 3-year intervals to define the temporal development of disease through regular and detailed clinical phenotyping. (ii) We have established induced pluripotent stem cell (iPSC) lines and neural stem cell lines from affected individuals in these families and unaffected controls. These lines are being used to generate cellular models to study cellular mechanisms that lead to brain disease.

(iii) Next-generation sequencing and family-based bioinformatics analysis are being used to uncover the genetic basis of SMI.

The multiple types of data generated by the ADBS have been assembled into an integrated database to facilitate the application of sophisticated methods of data analysis to uncover new disease biology. The stem cell lines and other biomaterials are part of a biorepository that will allow the sharing and use of this resource to drive research in the area of SMI. The ADBS has instituted mechanisms to facilitate the sharing of data and resources generated through its activities.

4.4 Leveraging stem cell technology to facilitate discovery for human disease biology in India

Advancements in technology have consistently played a pivotal role in enhancing our understanding of disease mechanisms and fostering innovative therapeutic approaches to improve human health. Stem cell biology, focusing on the study of cells that have the capacity to self-renew and differentiate into multiple cell types, exemplifies how technological progress in pluripotent stem cell (PSC) derivation has uncovered new biological insights and unlocked potential avenues for addressing unmet medical needs through cellular therapies.

The Department of Biotechnology (DBT) has collaborated with a consortium of clinical and basic research institutions to address unmet medical needs by applying stem cell technology to human diseases. The Indo-Japan initiative for Accelerating the application of stem cell technology in human disease (ASHD), based at inStem, focuses on two key clinical areas: (i) mental health disorders through the Accelerator Program for Discovery in Brain Disorders using Stem Cells (ADBS) and (ii) blood disorders through Novel Approaches to Haematological Disorders (NAHD). This program also includes a capacity-building and training component in partnership with the esteemed Centre for iPS Cell Research and Application (CiRA) at Kyoto University, Japan.

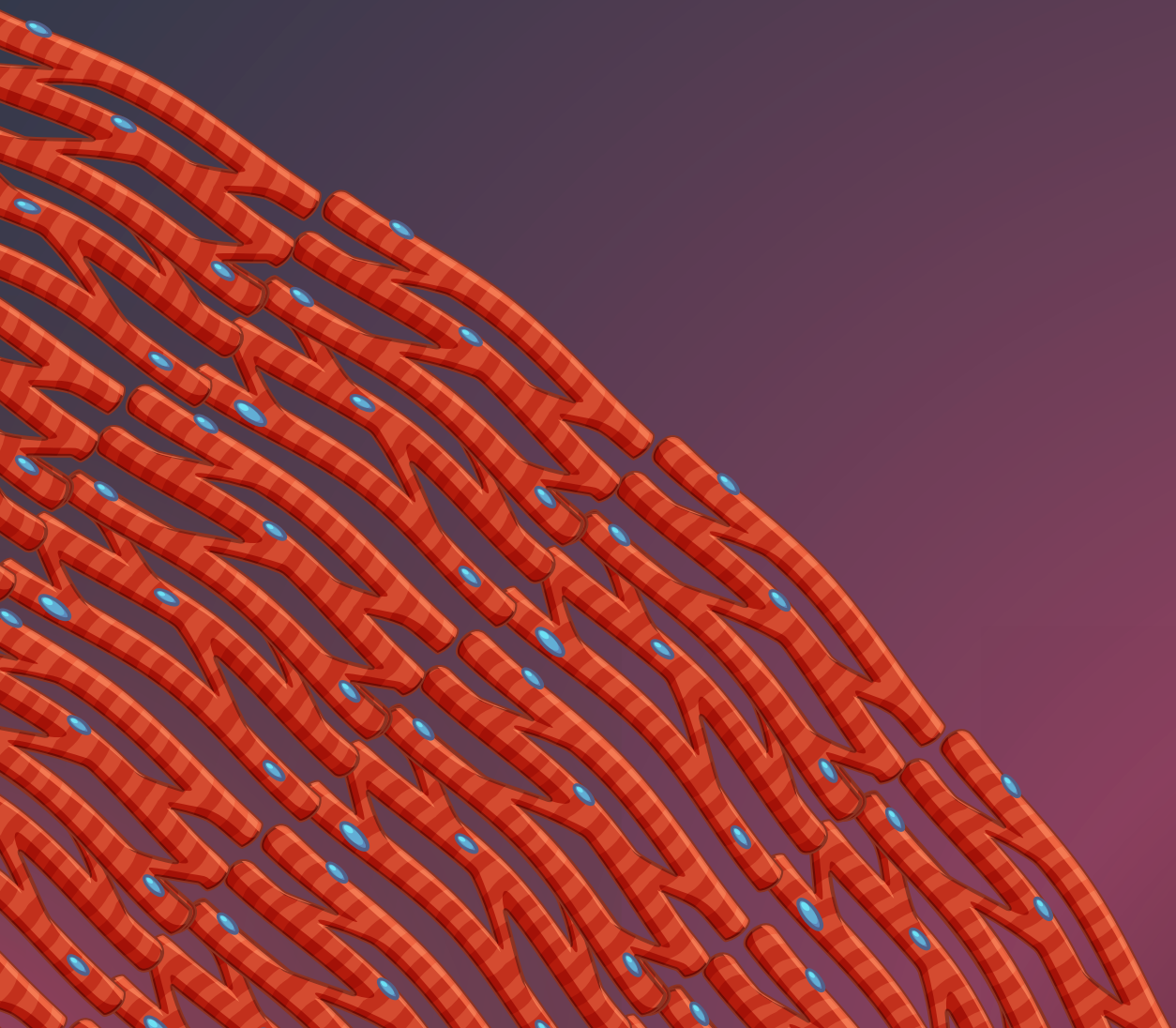
This flagship program from inStem is tailored to augment and harness the existing Indo-Japan initiative, emphasising the utilisation of iPSC technology in conjunction with genome analysis, sequencing, and genome editing techniques to assess biology and explore innovative therapeutic approaches.

Since the project's inception, we have achieved the sequencing of 300 exomes, aligning with our goal of employing next-generation sequencing technology and bioinformatics to facilitate the creation of relevant human sample sets for use in discovery science. Furthermore, we have successfully generated an additional 90 iPSC lines, dedicated to both discovery biology and clinical applications, with quality checks and banking completed for 50 of these lines. Our program places significant emphasis on capacity building, aiming to facilitate the utilisation of stem cell resources developed by researchers in both academic and biotechnology sectors.

In line with this objective, we conducted a training program in iPSC technology in collaboration with the International Stem Cell Banking Initiative (ISCBI). Additionally, during Stem Cell Awareness Week, we organised online lectures by international experts and conducted hands-on training sessions at DBT-inStem. Our next steps include finalising the quality checks and banking of the generated iPSC cell lines. Moreover, we plan to expand our capacity development programs, which were significantly affected by the COVID-19 pandemic. These expansions entail additional hands-on training workshops, improvements in processes and procedures for the stem cell core facility to meet international standards and serve as a national resource centre, as well as establishing a pipeline for the generation, cryopreservation, and distribution of human stem cells and organoids.

05

Research Support



05.
Research Support

5.1 Research Ethics and Integrity Office (RIO)

Research ethics and integrity form the core standards of good research practice to ensure credibility, transparency, reproducibility, accountability, and appropriate credit sharing as well as help in creating and sustaining the public's trust in modern scientific research. Many Indian agencies (e.g., UGC, ICMR) have already created policies for good scientific research practice, but implementation of these policies always remains a challenge. Therefore, DBT-inStem, in collaboration with our neighbour NCBS-TIFR, has set up a dedicated and one-kind Research Ethics and Integrity Office (RIO). The RIO has three functional spheres viz., to formulate relevant policies and interventions, to maintain publication data archive and monitor research outcomes at the institute, and finally conduct training activities for capacity building of various stakeholders on campus. The office has introduced a facility for text (300 document checks completed) and image (1100 image checks completed) integrity checks to the campus community. Standard operating protocol and process for publication data archive has been prepared and made functional for all peer-reviewed publications (12 articles completed).

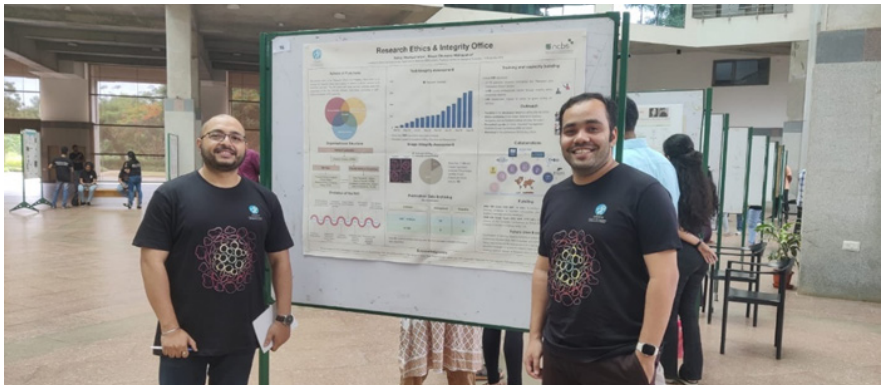
The RIO has also trained 115 doctoral students in Research and Publication ethics as per UGC regulation and conducted monthly research ethics training for 520 campus visitors. Often, the office also helps the institute authority in data collation. RIO has successfully obtained competitive travel to present work at an international conference and a research grant from DBT/Wellcome Trust India Alliance to understand gaps and challenges in academic integrity and regulatory compliance and create a resource database for academic integrity pedagogy. As part of the outreach program, the RIO has also helped other academic institutes in India in academic integrity-related capacity building and training programs.

List of Personnel:

Research Ethics and Integrity Officer, inStem: Dr. Sabuj Bhattacharyya

Research Ethics and Integrity Officer, NCBS-TIFR: Mr. Biswa Bhusana Mahapatra

Project Associate: Ms. Sebanti Tewari



The RIO team presenting at the Annual Research Seminar 2023 at DBT-inStem

5.2 Regulatory Compliance Office (RCO)

Biological research in India is regulated by various guidelines and rules published by Central/State agencies to safeguard ethical, ecological, medical, and environmental interests. These regulations offer structured instructions, principles, and mechanisms to ensure the safe and ethical conduct of research within the country. Compliance with regulatory guidelines is crucial and constitutes an important aspect of modern biological research.

The Regulatory Compliance Office (RCO), a first-of-its-kind office educates and advises campus researchers about regulatory requirements and facilitates the process of obtaining required approvals from Institutional and National statutory committees. The office also assists in ensuring that the campus facilities and research infrastructures are in line with regulatory guidelines and have Environmental, Health, and Safety protocols in place.

Primary functions of the office are:

A. Facilitating Regulatory Approvals:

- Advising the researchers on statutory approval requirements
- Providing feedback on applications
- Acting as a link between researchers and statutory committees (Institutional & Central)

B. Training and capacity-building

- Trainings and workshops on compliance and guidelines
- Course module for Ph.D. students on health research ethics
- Training programmes for regulatory managers and ethics committees

C. Compliant research infrastructure

- Design and function of biocontainment facilities
- Creation of safety and emergency protocols
- Creating processes for biorepositories

During the reporting period, RCO has processed 23 applications for the Institutional Animal Ethics Committee, 5 applications for the Institutional Committee for Stem Cell Research, 20 applications for the Institutional Biosafety Committee, and 22 applications for the Institutional Human Ethics Committee. The RCO is also actively involved in capacity-building and teaching activities. The Regulatory Compliance Officer taught *'Introduction to health research ethics'* as a part of the *'Research and Publication Ethics Course'* developed for the doctoral students at BLiSc. The office also actively collaborates with the Research Ethics and Integrity Office at DBT-inStem to jointly run the research project that aims to understand the gaps and challenges in academic integrity and regulatory compliance in the Indian research ecosystem.

List of Personnel:

Regulatory Compliance Officer, DBT-inStem: Dr. Ketan Thorat

Regulatory Compliance Manager, NCBS-TIFR: Ms. Mouna Nagaraju

5.3 Research Development Office

The Research Development Office (RDO) facilitates research and training on campus via research funding and collaborations. The RDO offers several key services at the boundaries of science, management, resource development, and outreach.

A few key highlights of research funding at DBT-inStem include the award of fellowships in basic biomedical research from the Wellcome Trust India Alliance fellowships, viz. Senior Research Fellowships to Dr. Sunil Laxman, Dr. Minhajuddin Sirajuddin, and Dr. Tina Mukherjee, Intermediate Research Fellowship to Dr. Sudarshan Gadadhar and Early Career Fellowships to Dr. Diya Binoy Joseph and Dr Kruttika Phalnikar (under the mentorship of Dr. Bhavana Muralidharan). The Indian SARS-CoV-2 Genomics Consortium (INSACOG), in which DBT-inStem is a partnering institution, continues with renewed support from DBT.

Private and philanthropic funding supports specific initiatives at DBT-inStem. A significant funding highlight is the award of a grant from the Bill and Melinda Gates Foundation to DBT-inStem to enable generalizable, stem cell-based Target Assessment Packages and Target Enabling Packages for the non-hormonal contraceptive (NHC) initiative. Sun Pharma Advanced Research Company Limited (SPARC) and DBT-inStem have collaborated through the Centre for Chemical Biology & Therapeutics (CCBT) at DBT-inStem for the development of potential drug targets.

The RDO continues to support establishment of national and international collaborations. A consortium led by Dr. Tina Mukherjee was successful in securing the prestigious Research grant from the Human Frontier Science Program (HFSP). This is the first research grant awarded by HFSP with an Indian investigator as the lead. Dr. Arjun Guha, as a member of a consortium of ten Principal Investigators from three countries (USA, Germany, India), was awarded a grant from the Chan Zukerberg initiative to map the Pediatric Inhalational Interface. Dr. Tina Mukherjee and Prof Angela Giangrande from IGBMC, Strasbourg were awarded a fellowship from the University of Strasbourg Institute for Advanced Study (USIAS), Strasbourg, France to address the relationship between innate-immune development and metabolic state with animal growth.

The RDO also launched its new logo and website in August 2023. Notable features of this website are a newly revamped, user-friendly, and expanded funding database as well as a new "Resources" page. The RDO also established a collaboration with ScientifyRESEARCH, a Swedish-based start-up to co-create an open source list of international funding opportunities for Indian researchers with information on funding from several international non-standard funding sources such as NGOs, private foundations, etc. We hope these resources will be useful not only for researchers within our campus but to all researchers in India.

RDO team members:

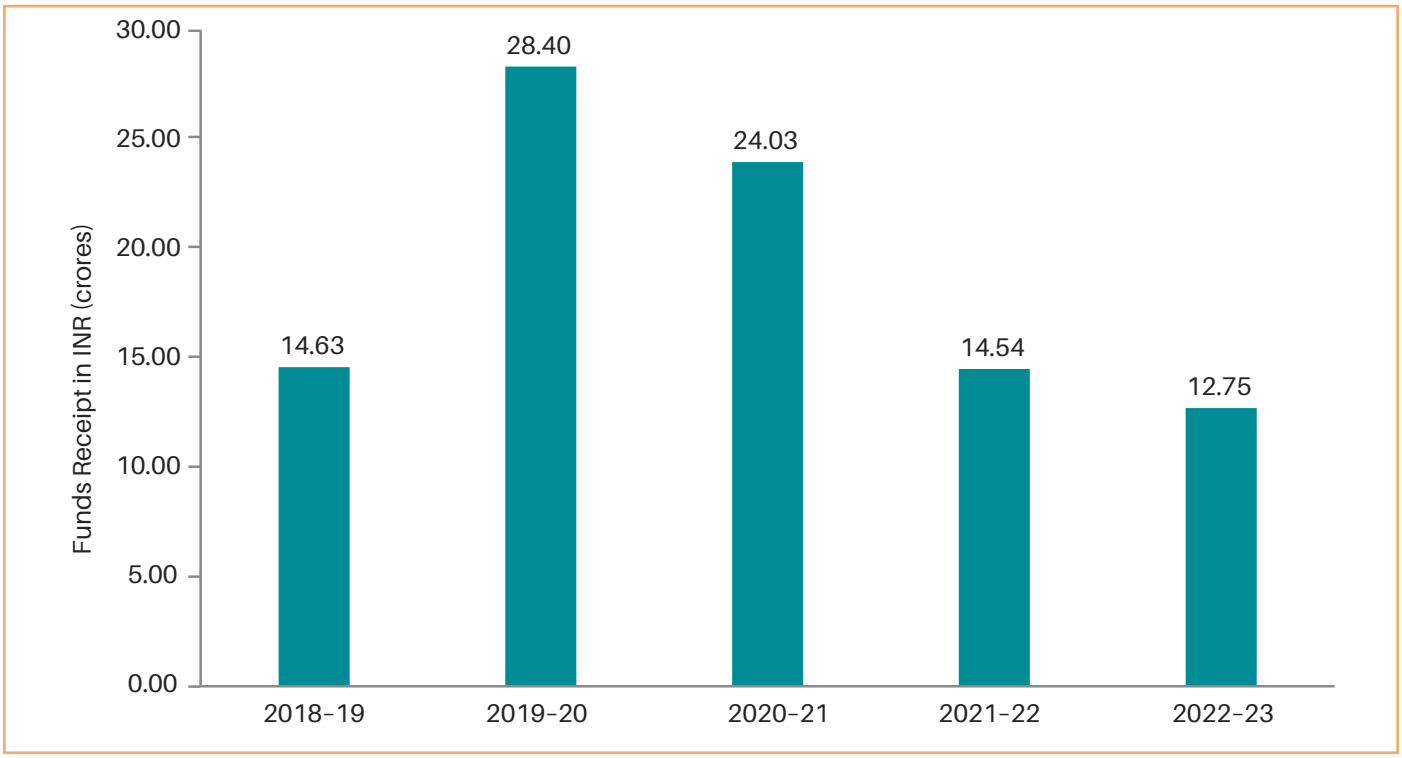
Head, Grants, and Research Collaborations: Dr. Vineetha Raghavan

Grants Advisor: Dr. Malini S. Pillai

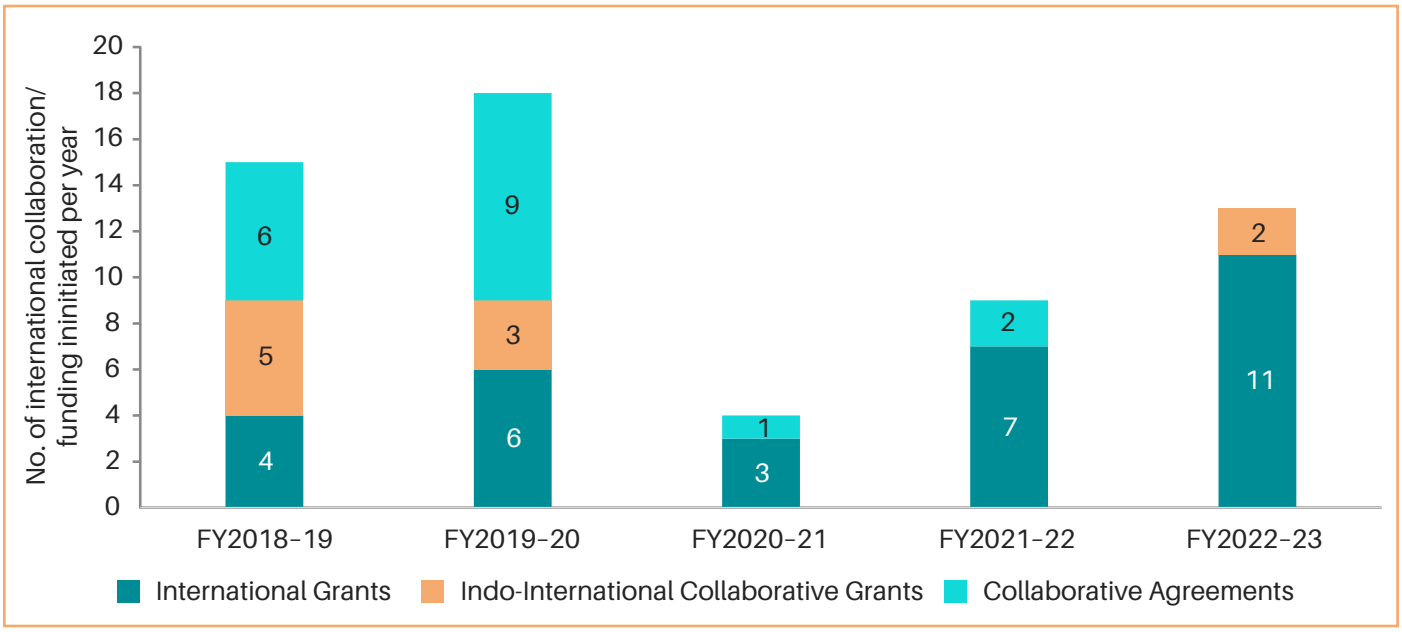
Senior Grants Administrator: Ms. M. C. Aruna

Administrative Assistant: Ms. Supriya R.

Extramural funds at inStem (in INR crores)



International Collaboration/Funding Facilitated by the RDO at BLiSc



5.4 Science Outreach and Communications

Throughout the year, inStem's Comms Office actively focused on Outreach activities at various levels and capacities. Apart from lab visits, popular science lectures were delivered by our faculty and research scholars to make these visits more enriching for the students aiming to study further in various fields of STEM. On special occasions like National Science Day and Rare Disease Day, our faculties interacted with the visiting students to raise awareness and expose them to the wonders of scientific research being done at inStem.

Some of the major outreach events for the year 2022-2023 include the following

1. inStem's Foundation Day

The 14th Foundation Day of DBT-inStem, which falls on 28th August 2022 was celebrated on 27th August 2022. The celebration reflected the breadth of inStem's science and its engagements with the wider research community. The event opened with a talk by Prof. Maneesha Inamdar, Director, inStem, who traced the journey of the institute over the years and the milestones achieved. This was followed by an interaction with Dr. Rajesh Gokhale, Secretary to the Government of India, Department of Biotechnology, who joined the event online. He addressed the inStem community and congratulated the Institute on the occasion of Foundation Day. Following his address, Dr. Gokhale fielded questions raised by the inStem community about the future landscape of Indian science.

The scientific programme for the day began with exciting talks by Early Career Researchers at inStem on its research themes at Bengaluru. The speakers were Dr. Kruttika Phalnikar, a recent awardee of the DBT India Alliance Wellcome Trust Early Career Fellowship, Sunny Kataria, Pratul Jain, Dr. Mohamed Haroon, and Vineeth Vengayil.

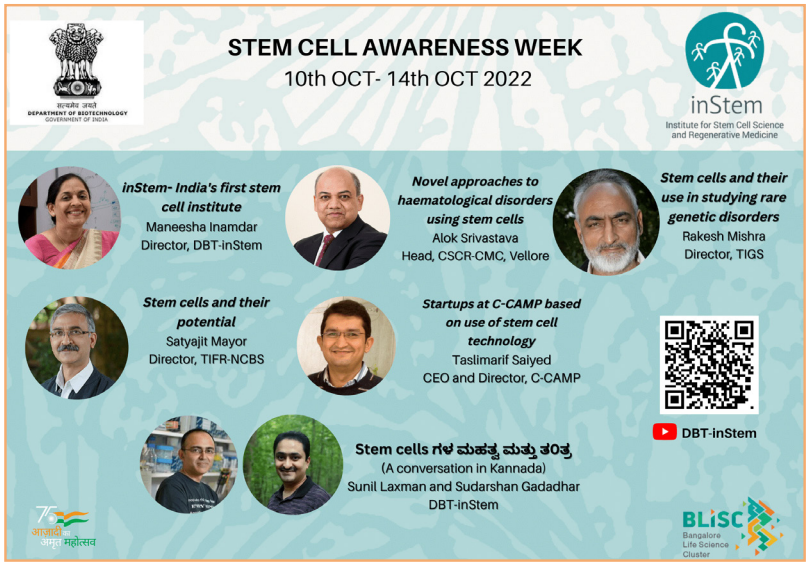
The scientific talks were followed by a poster session, which showcased the excitement and breadth of research at inStem. This was the first live poster session following the lockdowns of the previous years! This session attracted attendees from across the campus who interacted with students and faculty from inStem labs. Following the poster session, the attendees gathered in the auditorium for the Foundation Day lecture by Dr. Alejandro Sanchez Alvarado, member of the inStem Scientific Advisory Board member, and an HHMI Investigator and Executive Director and CEO, Stowers Institute of Medical Research, USA. The talk, titled *"Understanding the sources of regenerative capacity in animals,"* was insightful and thought-provoking as it revisited concepts of plasticity and differentiation and discussed how spatial context and proximity of different cell types influence stem cell function during regeneration. The talk inspired a lively and extended Q&A session. Dr. Arvind Ramanathan, Head Research, inStem, delivered the closing remarks, thanking all members of the inStem and BLiSc community for their commitment and support to make the event possible.

Prof Maneesha Inamdar, Director inStem speaks on Foundation Day, August 2022

Secretary Dr Rajesh S Gokhale interacts with the community at inStem. Foundation Day August 2022

Foundation Day lecture by Prof. Alejandro Sanchez Avarado on inStem Foundation Day, August 2022

2. Stem Cell Awareness Week



As a part of Stem Cell Awareness Week from 10th–14th October 2022, DBT-inStem organized a research talk on 'The History of Stem Cells' by Prof. Peter Andrews, School of Biosciences (formerly the Department of Biomedical Science), the University of Sheffield; Stem Cell Innovator; one of the first Scientists in the UK to work with Human Embryonic Stem Cells (hESCs).

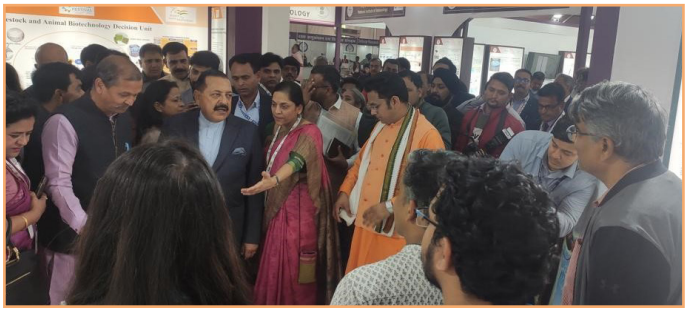
Our first speaker, Prof. Maneesha Inamdar, Director, DBT-inStem spoke on the 'Clinical applications of stem cells and inStem as India's first Stem Cell Institute'. On day 2, Prof. Satyajit Mayor, Director and Scientist at NCBS spoke on 'Stem Cells and their potential'. His lab focuses on understanding the mechanisms of membrane organisation and endocytosis in eukaryotes. On the third day, Dr. Sunil Laxman and Dr. Sudarshan Gadadhar, faculties at inStem, conversed in Kannada about stem cells, their importance, and their unique feature of "self-renewal" and 'need to culture the same'. On day 4, Dr. Taslimarif Saiyed, CEO and Director, C-CAMP spoke on "Start-ups at C-CAMP based on the use of stem cell technology." He highlighted the efforts of C-CAMP in the field of stem cells and introduced Eyestem Research and Pandorum Technologies as two such efforts. On the last day of Stem Cell awareness week, Dr. Rakesh Mishra, Director, TIGS, talked about 'Stem cells and their use in studying rare genetic disorders'. On the same day, Dr. Alok Srivastava, Head, CSCR-CMC, Vellore, spoke about 'Novel Approaches to Haematological Disorders using Stem Cells.'

On 12th and 14th October 2022, inStem conducted a stem cell workshop to train students in good cell culture practice. Students from across the campus participated and got hands-on experience in good cell culture practice. The workshop was opened by Prof. Maneesha Inamdar, Director, inStem, and coordinated by Praveen Wulligundam.

InStem also hosted a series of podcasts with scientists from inStem, National Centre for Biological Sciences (NCBS), the Centre for Cellular and Molecular Platforms (C-CAMP), Tata Institute for Genetics and Society (TIGS), and the Centre for Stem Cell Research (CSCR)-CMC Vellore.

3. India International Science Festival

As an autonomous institute of DBT, inStem participated in IISF 2023 (21st–24th January 2023). The event took place at Maulana Azad National Institute of Technology, Bhopal. DBT-inStem's stall was a part of the DBT pavilion that housed India's major research institutes under DBT. The delegation, led by Prof. Maneesha Inamdar (Director, inStem), included Dr. Dasaradhi Palakodeti (Faculty), Dr. Praveen K. Vemula (Faculty), Nimisha Roy (Comms & Outreach Coordinator), and Ph.D. scholars Ms. Swathi Pavithran, Mr. Ishan Kale, and Mr. Yousaf Edries Hajam. Representatives from CSCR-CMC Vellore presented the stem cell research being done in their institute.



4. International Day of Women and Girls in Science

To commemorate the International Day of Women and Girls in Science on 11th February 2023, DBT-inStem organised and hosted a Panel Discussion on the topic 'Celebrating Women in Different Avenues of Science.' This was a BLiSc event with a diverse set of panellists sharing their experiences and eureka moments.

Other events include the following:

1. Campus visit by student and faculty delegation from Department of Biotechnology, Tamil Nadu Dr. J. Jayalalitha Fisheries University (TNJFU) on 6th February 2023.

2. **Campus visit by a delegation of early career scientists participating in the 2nd Shanghai Cooperation Organisation (SCO)** Young Scientist Conclave on 9th February 2023.

3. **On National Science Day and Rare Disease Day (28th February 2023),** 40 M.Sc. first-year students (Biotechnology) from St. Joseph's College visited us, accompanied by some faculty members. The program included talks by Prof. Arvind Ramanathan (Head-Research, DBT-inStem), Dr. Farah Ishtiaq, and Dr Bhagyashree, and a visit to the insectary and collections facility.

4. **Panel discussion to celebrate International Women's Day:** DBT-inStem organised and hosted a panel discussion on 'Focus on gender equity and accelerating women's equality' on 8th March 2023. The session was moderated by Dr. Tina Mukherjee, faculty at inStem, and had panellists from BLiSc. Overall, the discussion provided valuable insights into the various elements essential for advancing gender equality and was well attended and generated meaningful dialogue and questions on the topic.

5. **INSA Lecture at IISc:** DBT-inStem co-hosted, with the Mathematics Department, IISc, the prestigious Professor Vishnu Vasudeva Narlikar Memorial Lecture (2021) award on 20th March 2023. The award was given to Professor B. V. Rajarama Bhat by Prof. Maneesha Inamdar, Director, inStem and coordinator of the INSA Bengaluru Local Chapter

6. **Campus Visit by students of KV, Hebbal:** About 50 students from Class 12th visited our campus on 21st March 2023. The visit included interactive sessions by our research scholars, Lab visits, and visit to the Collections facility.

Training and Outreach at CSCR:

1. An outreach Program was conducted for the postgraduate students from the Biotechnology department, Thiruvalluvar University, Serkadu, Tamil Nadu on 21st April 2023 at CSCR, Vellore. Basic Scientific talks and practical demonstrations were conducted. Number of Participants: 15
2. Annual Cell and Gene Therapy Symposium: CSCR has been organizing an annual symposium on Cell and Gene Therapy for the last 7 years. This meeting aimed to provide a platform for scientists and physicians working in this field of research to come together and discuss the advances in the field. The 8th Annual Cell and Gene Therapy Symposium was organised from 31st August to 2nd September 2023

Events

1. On 28th February 2023, CSCR hosted a science awareness program to recognize National Science Day for college students to be exposed to the scope of stem cell and gene therapy research in India
2. IVIS® Spectrum CT and Living Imaging system hands-on training was conducted at CSCR by Dr. Shahzada Asad, Senior Product Specialist, Small Animal in vivo Imaging, Perkin Elmer on 6th April 2023
3. Technical presentation on "ImageXpress Confocal HT.ai: A Complete Solution for Automated Imaging and High Content Screening" was given by Dr. Manimaran Paramasivam, Field Application Specialist BR, Spinco Biotech Pvt Ltd, Hyderabad at CSCR on 14th April 2023
4. Hands-on Workshop on Multicolor Advanced Flow Cytometry was conducted on 1st–2nd August 2023 at CSCR, supported by Beckman Coulter Life Sciences. Dr. H. Krishnamurthy from NCBS gave a talk on the basics of flow cytometry as a part of the workshop. Number of participants: 22; Coordinated by Dr. Sandya Rani B, CSCR
5. Biosafety workshop was conducted on 14th August at CSCR. Number of participants: 48; Coordinated by Dr. Gurbind Singh and Dr Sonam Pandey

Note

1. Ms. Trupti Pradhan and Ms. Sheeraza Ahad joined as interns in the Communications Office in July 2022
2. Ms. Malavika Saji joined as an Outreach Assistant in November 2022
3. Dr. Nimisha Roy joined as a Communications and Outreach Coordinator in December 2022

06

Facilities

06.
Facilities

6.1 Stem Cell Facility

Objective: Training and research in culture, differentiation, and genetic modification of human pluripotent stem cells.

The Stem Cell Facility is a central facility of the BLiSc campus, committed to supporting human pluripotent stem cell (hPSC) research on the campus by providing necessary training and infrastructure. The facility also conducts research activities on genetic modification and differentiation of hPSCs.

The Stem Cell Facility has conducted multiple demonstrations and training sessions on Good Cell Culture Practices (GCCP) throughout the year as a part of various stem cell-related events and in-house training activities. During the Stem Cell Awareness week (10^{th}–14^{th} October 2022), the facility demonstrated GCCP methods to twenty participants from various labs of the institute. Subsequently, the facility conducted training sessions in GCCP and an assessment of nineteen people from the institute compromising both existing and new users of the facility.

The facility acted as a crucial platform for conducting the workshops on hPSC research, Part-I and Part-II, conducted in May and August–September 2023, respectively. During the workshops, the facility trained twenty-five selected researchers in GCCP and basic culturing and quality control techniques of hPSCs. The facility also co-organised lecture sessions by world-renowned researchers in stem cell biology and banking including Prof. Glyn Stacey, Prof. Andreas Kurtz, Dr. Tenneille Ludwig, Dr. Charles Hunt, Dr. Laurence Daheron, and Dr. Rachel Steeg.

During the August 2023 workshop, the facility hosted practical sessions on hPSC culture, maintenance, and differentiation under the kind guidance of Dr. Lyn Healy from The Francis Crick Institute, London. Dr. Healy shared her vast experience and knowledge in stem cell culture, maintenance, and differentiation with the participants. Importantly, the facility, in collaboration with Dr. Healy, conducted successful trials of generating gastruloids from hPSCs, which has been very helpful to the ongoing attempts to obtain gastruloids to study early human development at the facility. Currently, this is being taken forward in collaboration with Prof. Inamdar's laboratory.

The facility, with the help of the core faculty, has initiated procedures to build an in-house repository of various hPSC lines developed in India.

The facility is collaborating with Prof. Inamdar's laboratory in the generation of CRISPR-based knock-in reporter lines to study and understand the role of various cellular processes in stem cell maintenance and differentiation. This involves the conventional two-plasmid strategy of CRISPR/Cas9 genome editing. So far, constructs for 12 genes representing various cellular compartments have been generated and their testing and reporter line generation in HEK293 cells is under progress.

Key findings in the past year:

1. Generation of gastruloids, a model for studying early human development using in-house generated hiPSC line.
2. Generation of reporter knock-in constructs for 12 genes related to various organelles, cytoskeletal elements, and cell junction proteins.

Outreach and other activities:

- Regularly conducted training programs in Good Cell Culture Practice (GCCP)
- Stem Cell Awareness Week training programs in October 2023.
- Two workshops on human pluripotent stem cell research (in May and August–September 2023, respectively).

6.2 The Mouse Genome Engineering Facility

Objective: TA national resource to generate, archive, and distribute genetically-altered mouse models globally

The Mouse Genome Engineering Facility (MGEF) at inStem is a state-of-the-art establishment equipped to both create in-house transgenic/knockout animals and serve as a repository for these models on a national scale. This ambitious endeavour caters to 32 different services in the domain of mice model generation and assisted reproductive technologies to academic and non-academic institutions across India.

A total of 236 projects (internal and external), including genome editing, sperm cryo, embryo cryo, and rederivation, have been carried out this year with a substantial amount of revenue generation toward achieving self-sustainability. We conducted two hands-on workshops on 'CRISPR-Cas Genome Editing' and 'Assisted Reproductive Technologies in Mice' and trained 40 participants from various parts of India.

Our capabilities have allowed us to emerge as a national repository for mouse models, cryo-archiving, and distribution of strains to the scientific community nationally and internationally. Since its inception, over 500 strains have been cryo-archived and are easily available for researchers through the dedicated ACoMaS portal (https://www.ncbs.res.in/transgenic).

To learn more about our service portfolio, operational processes, and technological advances, please visit us at https://www.inStem.res.in/research-facilities/acrc.

Facility In-charge: Dr. Mahesh Sahare

Team: Shilpakumari B. A., Reena V., Mahima N., Vaishak Nair, Salil Hangekar, Priya P., Akash Aswini Abhishek Anand, Roopa N., Latha Chukki

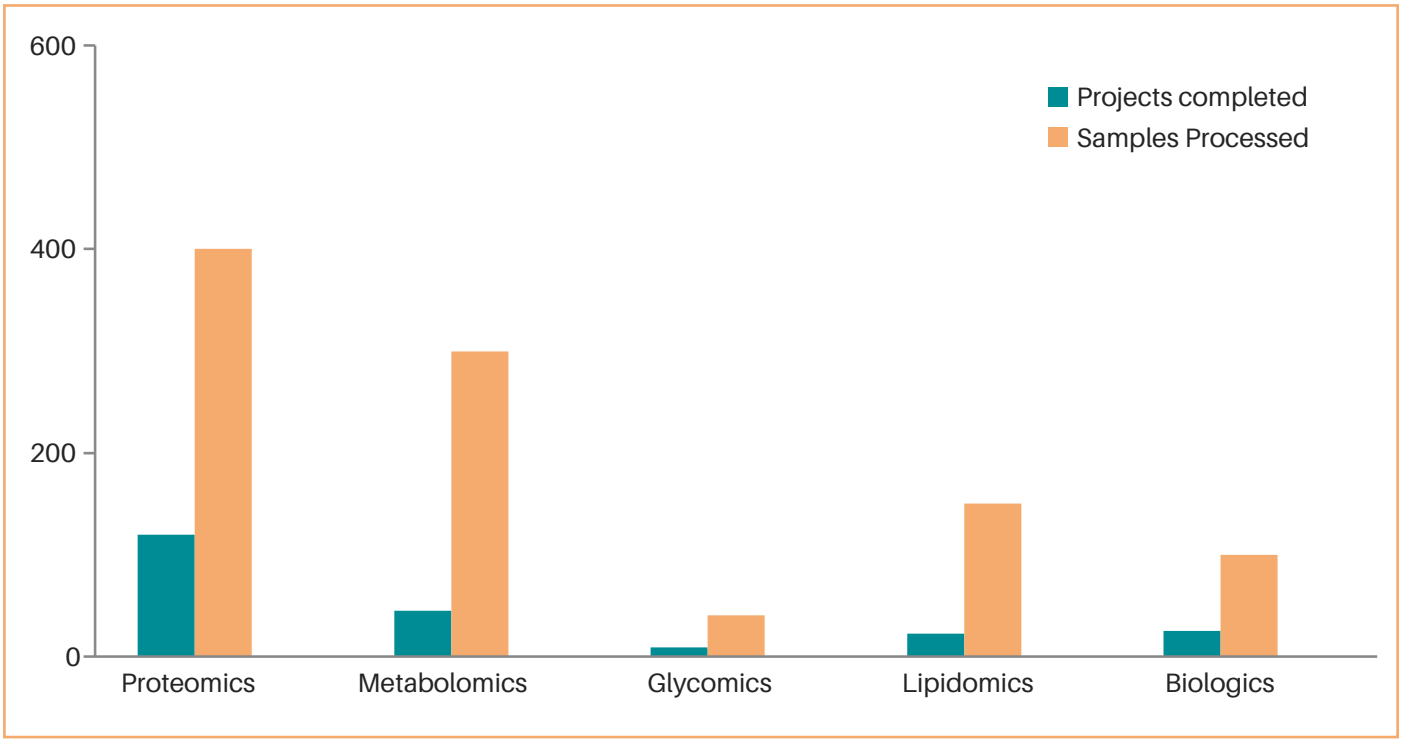
Faculty Advisory Committee: Raj Ladher, Colin Jamora, Dhandapany Prerundurai, Soumyashree Das

6.3 Mass spectrometry facility at BLiSc: Helping to decipher the molecular processes

The mass spectrometry facility at the Bangalore Life Science Cluster (BLiSc) is spread across the campus. The three institutions of the cluster have complementary resources in terms of mass spectrometry instrumentation and capabilities. The mass spectrometry facility at NCBS, inStem, and CCAMP offers cutting-edge technology services and regular training to researchers. The mass spectrometry facility of the cluster has several high-resolution mass spectrometers (Orbitrap and Q-TOF) and multiple Triple Quadrapole-based unit resolution mass spectrometers for qualitative and quantitative analysis of analytes. The newest addition in the facility is the Zeno-TOf 7600 (Sciex) and high sensitivity 6495 Triple Quadrapole system for the quantitation of lipids and metabolites. BLiSc mass spectrometry facility is also equipped with two GC-MS instruments for the identification and quantitation of volatile compounds. The facility has established workflows for the identification of pull-downs, protein digestion products, N-terminal sequencing (mass spectrometry-based), labelled and label-free quantitation of proteins, small molecule quantitation, drug conjugate analysis, and biosimilar characterisation along with some other workflows for vaccine and antibody characterisation.

The mass spectrometry facility is supported by a dedicated staff that provides training to the users and establishes new workflows. At present, Ms. Alifia, Ms. Theja, Ms. Pallavi, Ms. Amrutha, Nirpendra, and Dr. Shadab help run the mass spectrometry facility on the campus.

The mass spectrometry facility has processed approximately 1,000 samples and completed approximately 220 projects in the last year for BLiSc and non-BLiSc users. The facility has been acknowledged in 14 publications.

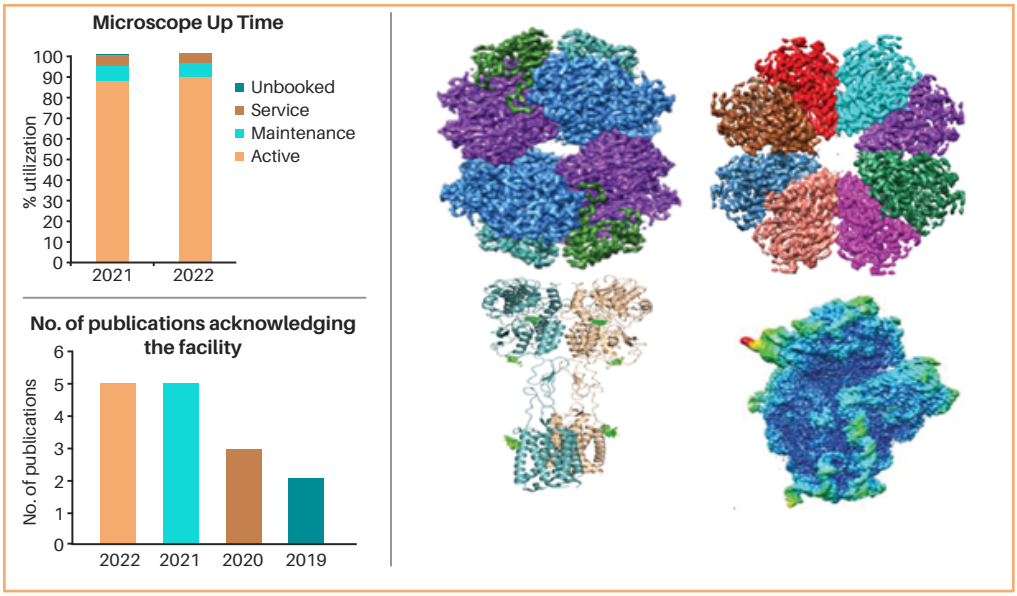


The mass spectrometry facility regularly organises workshops and training programs for non-BLiSc users in its efforts toward capacity building in the field. The facility has organised the following courses on different workflows.

6.4 National Electron Cryo-microscopy Facility

Recent advancements in microscopy technology, high vacuum, direct electron detectors, and computational methods along with enhanced automation have been the prime factors in driving the resolution revolution in CryoEM. This has enabled scientists to obtain near-atomic resolution reconstructions of macromolecular assemblies and provide an understanding of their form and function. The recent pandemic showed that cryoEM has the potential to have a huge impact on biomedical research, including cellular, molecular, and structural biology, and in particular drug discovery and vaccine development. In 2016, the Government of India, through the B-life grant (Department of Biotechnology), invested in the development of a state-of-the-art CryoEM facility located in the BLiSc campus in Bangalore to meet the needs of the Indian scientific community interested in studying macromolecular machines. The National Electron Cryo-Microscopy facility, operational since September 2017, houses a 300 kV Titan Krios G3 (Thermo Fisher Scientific) and provides microscope access as well as scientific and technical support to academic and industrial researchers. The microscope is equipped with a GIF Bioquantum energy filter coupled to a Gatan K2 direct electron detector and features a Falcon III camera in pre-GIF position along with a Volta phase plate.

In 2022, the facility served approximately 27 users, including external and internal users from the BLiSc campus. The facility-in-charge is responsible for data generation, daily operations, and user communications. The facility has been acknowledged in five publications in 2022. Furthermore, the facility was represented by the facility-in-charge at an EMBO workshop in Pune in December 2022, a symposium at NiSER, Bhubaneswar in February 2023, and the Biophysical Society meeting in March 2023. The participation involved conducting practical workshops, engaging in panel discussions, delivering lectures, and giving oral presentations. The facility's prominent achievement is its ability to generate high-resolution structures of complex macromolecules, including challenging targets like membrane proteins and protein complexes. It serves a diverse user community across the country and cultivates collaborative relationships with both academic and industrial partners.



Facility Head:
Sucharita Bose

6.5 Facilities at Centre for Stem Cell Research (CSCR)

A. Good manufacturing practice (GMP) facility at CSCR: The facility is designed to develop and manufacture cell and gene therapy products for clinical applications. It provides infrastructure for the large-scale expansion of stem cells and genetic modification of cells required to conduct Phase I/II clinical trials in the fields of cell and gene therapy. The GMP facility has two manufacturing facilities for 1) cell therapy products and 2) genetically modified cells. This facility has the required regulatory central and state authority approvals for manufacturing cell and gene therapy products. More details can be found at: https://www.cscr.res.in/cgmp-facility/

B. Imaging and flow cytometry platforms for research: CSCR core facility provides consultation, training, and access to high-end equipment for research applications to all the researchers at CSCR, CMC, and other academic institutions.

We have BD FACS Celesta, BC Cytoflex LX cell analysers and BD FACS ARIAIII, BD FACS ARIAFusion cell sorters with 3 and 5 lasers, respectively, at our facility. We have Laser scanning confocal microscope system (Olympus FV1000), Laser scanning multi photon microscope (Olympus FV1000MPE), Leica DMI6000B inverted fluorescence microscope, EVOS FL auto fluorescence imaging system, *in vivo* small animal imaging system (PerkinElmer IVIS Spectrum CT), stereo microscope, and light microscopes in our facility.

We have an offline analysis workstation facility with FlowJo, Kaluza software for flow cytometry data analysis, and FV10-ASW software for image analysis.

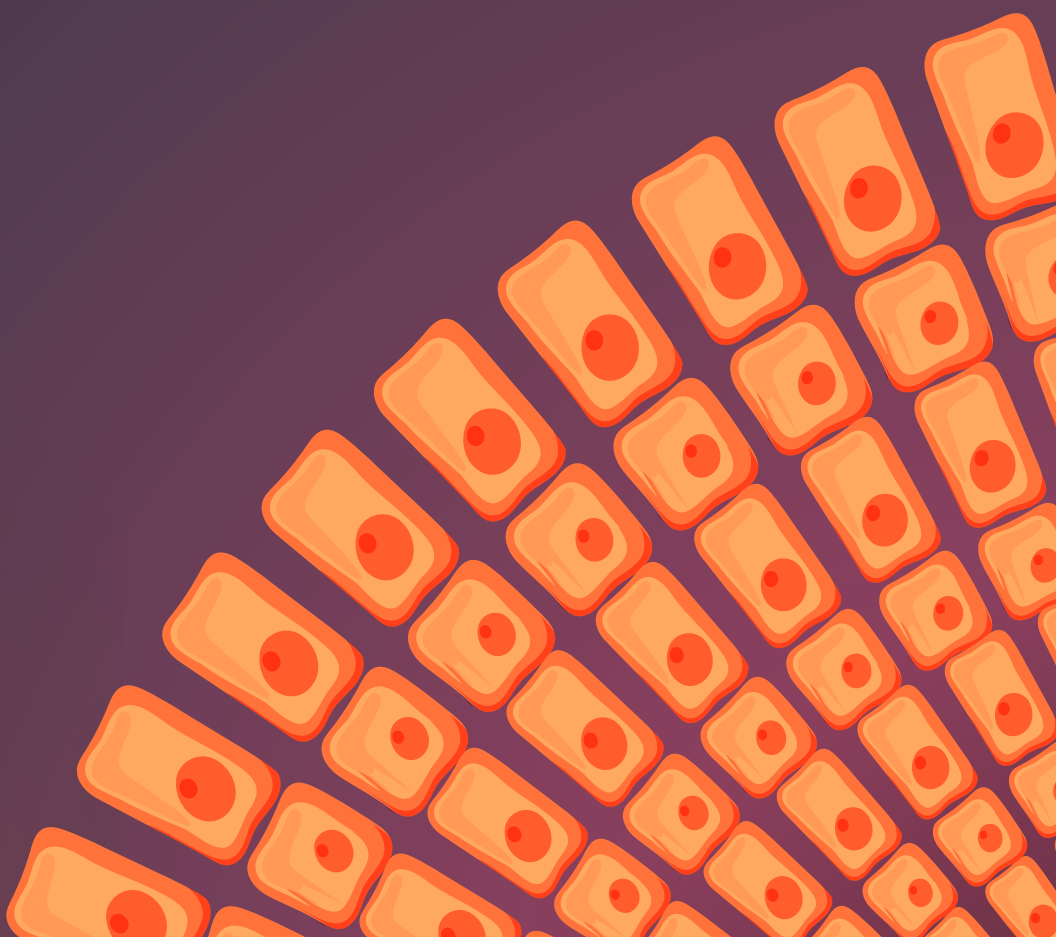
More details can be found at: https://www.cscr.res.in/core-facility/

C. Laboratory Animal Facility (LAF): The goal of the CSCR-LAF is to promote the humane care and use of laboratory animals by providing information that will enhance animal wellbeing, the quality of research, and the advancement of scientific knowledge that is relevant to both humans and animals as per the sanction from the Institutional Animal Ethics Committee (IAEC). The laboratory animal facility is registered with the 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA) for breeding and conducting experiments on small laboratory animals.

The CSCR-LAF maintains several different strains of rodents - mice strains including wild type, transgenic, knock out, and SCID strains and SD rat. More details can be found at: http://www.cscr.res.in/laboratory-animal-facility

07

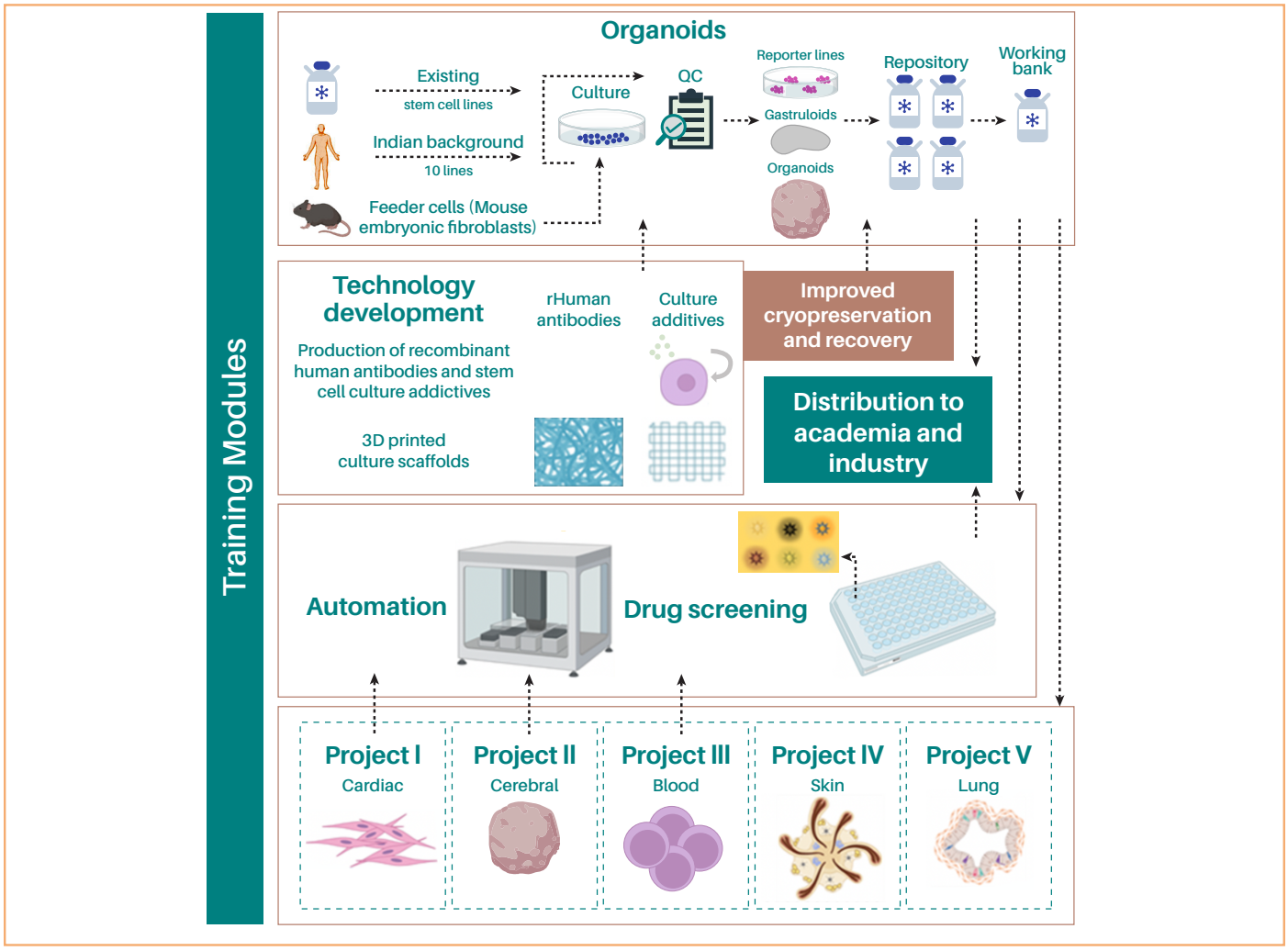
New Initiatives



07.
New Initiatives

7.1 ESCORT: Platform Enabling Stem Cell and Organoid Research and Training

This new initiative is an effort to bring stem cells and organoid biology into drug discovery and regenerative medicine. Globally, the possibilities of halting disease progression and reversing damage, based on stem cell research, are being rapidly explored and have progressed to the clinic. The explosion of human models such as gastruloids, organoids and assembloids that help us understand development and disease trajectories, and using these to find therapeutics, presents an immediate opportunity. This initiative will take bold and inclusive steps to building this knowledge, expertise and capacity in stem cells and regenerative medicine.



Schematic representation summarizing work to be carried out under the ESCORT initiative

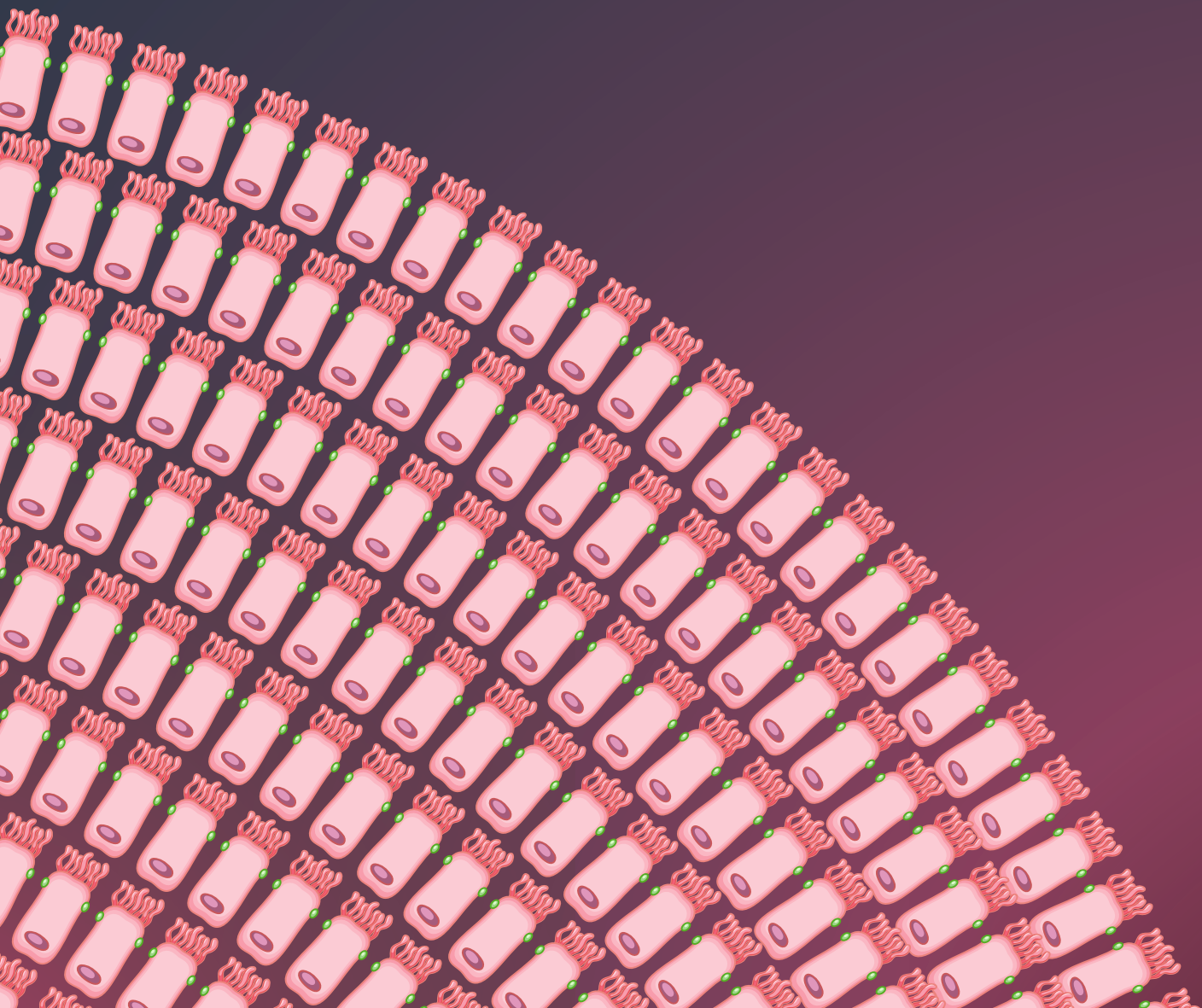
7.2 BMGF project: Stem cells for women's health

Women's health, especially reproductive biology is a greatly understudied area. Understanding mechanisms involved in early development will help transformative discoveries, to develop safer alternatives to existing products. Funded by a grant from the ***Bill and Melinda Gates Foundation***, we will develop tools to enable discovery of candidate drugs and test an array of intervention strategies in vitro. We aim to develop human stem cell and organoid models to help de-risk drug targets and establish new widely applicable paradigms. Stem cell-derived organoids can replicate some aspects of early-stage development and provide robust laboratory models to help de-risk drugs. Simultaneously, developing reagents and substrates to interrogate biologically relevant targets indigenously is also essential. The collective team at inStem aims to fill these gaps and includes stem cell biologists, chemists, and structural biologists from the inStem faculty, and the chemical biology and therapeutics team, along with cryoEM capability from the National Centre for Biological Sciences, bringing substantial experience and expertise to both goals.

This project has the potential to create a robust and sustainable portfolio of stem cell-based applications aimed at facilitating women's reproductive health and choices.

08

Patents and Technologies



08.
Patents and Technologies

List of Patents filed from 1st April 2022-1st June 2023 - DBT-inStem

Title	Inventors	Application Number	Filing Date
A method for reactivation of fetal hemoglobin and a composition thereof	Saravanabhavan Thangavel, Alok Srivastava	202241030885 (India)	27.05.2022
A method for modification of ß-globin gene	Mohankumar K. Murugesan, Alok Srivastava, Kirti Prasad	202241030885 (India)	30.05.2022
Formulations, lipid compound, methods and thereof	Srujan Marepally, Alok Srivastava	PCT/IN2022/050660 (PCT)	22.07.2022
A method for downregulation of a target gene and a composition thereof	Shaji R. Velayudhan, Alok Srivastava, Mohankumar K. Murugesan	202241045242 (India)	08.08.2022
Products and compositions for the treatment of viral infections	Colin Jamora, Tanay Bhatt, Amitabha Majumdar, Naresh Ghatlia	202211048425 (India)	25.08.2022
Method for elevation of gamma globin	Mohankumar K. Murugesan, Alok Srivastava, Nivedhitha D., Vignesh R. A.	202241055876 (India)	29.09.2022

Title	Inventors	Application Number	Filing Date
Compositions and methods for treating a b-thalassemia disease	David I. K. Martin, Mark DeWitt, Mark C. Walters, Wendy J. Magis, Saravanabhavan Thangavel, Dario Boffelli	63/251,229 (USA)	01.10.2022
Therapeutic agents for enhancing epithelial and/or endothelial barrier function	Praveen Kumar Vemula, Nicholas Kenneth Terrett, Sakthimala Jagadeesan, Venkatesh Ravula	63/419,015 (USA)	25.10.2022
Citric acid cycle and lactate transport inhibitors for prevention and/or treatment of skin disorders	Srikala Raghavan, Uttkarsh Ayyangar	PCT/IB2022/061996 (PCT)	10.12.2022
A nucleo-cytoplasmic form of protein lysine methyltransferase-EHMT1N/C, methods and use thereof	Shravanti Rampalli Deshpande, Kriti Kestur Biligiri	202341043660 (India)	01.03.2023
Scaffolds for selective scavenging of storage lesion from biological material and methods thereof	Praveen Kumar Vemula, Manohar Mahato, Subhashini Pandey, Preethem Srinath, Utkarsh Bhutani	PCT/IB2023/052628 (PCT)	17.03.2023

09

Graduate Thesis Awarded

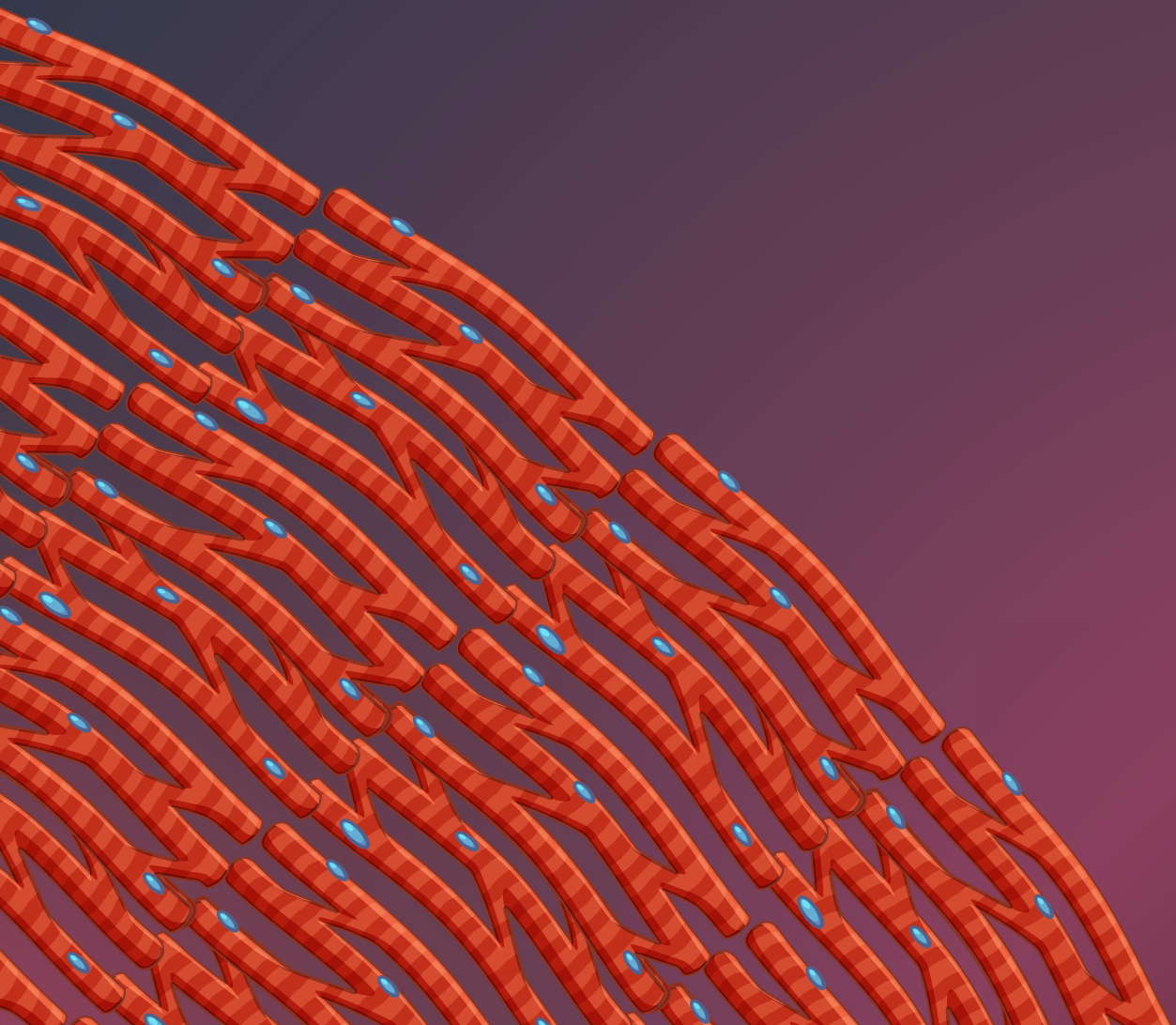
09.
Graduate Thesis Awarded

Name	Supervisor/Guide	Synopsis/Thesis title	Photo
Ashish Dinesh Dhayani	Praveen Kumar Vemula	Biomaterial based strategies for local immunomodulation in vascularized composite allotransplantation (VCA)	
Zirmire Ravindra Kailasrao	Colin Jamora	*Bacopa monnieri* phytochemicals regulate fibroblast cell migration via modulation of focal adhesions	
Oindrila Bhattacharjee	Srikala Raghavan	Elucidating the functions of embryonic macrophages during sterile inflammation and skin development	
Sarayu R.	Ravi S. Muddashetty	APOE4 affects basal and NMDAR-mediated protein synthesis in neurons by perturbing calcium homeostasis	
Vijaya Kumar K.	Sumantra Chattarji	Characterization and interventional strategies in novel transgenic rat models of autism spectrum disorder	
C. S. Anirudh	Akash Gulyani	Multi-layered natural light sensors controlling neural output in flatworms and engineered optical sensors for diagnostics	
Mohamed Mohamed Haroon	Praveen Kumar Vemula	Advanced methods for isolation and characterization of pluripotent stem cells from planaria *Schmidtea mediterranea*	
Subhashini Pandey	Praveen Kumar Vemula	The university of trans-disciplinary health sciences and technology	

Name	Supervisor/Guide	Synopsis/Thesis title	Photo
Michelle Ninochka D'Souza	Dasaradhi Palakodeti	Understanding translation regulation by fragile X messenger ribonucleoprotein (FMRP) through its interaction with the ribosome	
Deblina Sain	Arjun Guha	Role of fragile X mental retardation protein in the lung	
Nivedita Hariharan	Dasaradhi Palakodeti	Exploring ribosome-mediated translation regulation in mammals	
Thorat Ketan Vilas	Praveen Kumar Vemula	Nucleophilic catalytic platforms to alleviate pesticide-induced ache inhibition, neurotoxicity, and mortality	
Ankita Kapoor	Tina Mukherjee	Investigating the non-neuronal function of neurotransmitters in myeloid-like blood progenitor development during *Drosophila* hematopoiesis	
Keerti Ramraj Yadav	S. Ramaswamy	The spectral and structural behavior of Biliverdin IXa bound to Sandercyanin	

10

Administration Report



10.
Administration Report

The institute has completed fourteen years in its pursuit of excellence in stem cell research and allied areas. The Centre for Stem Cell Research (CSCR) is a translational unit of inStem located at Christian Medical College Campus, Bagayam, Vellore. The accounts of CSCR are integrated into the accounts of the institute.

The table below indicates the status of grants received and personnel on roll up to 31st March 2023

Status of Grants

57.38	16.23	59
Core grants received (INR in Crore)	EMG grants received (INR in Crore)	Number of active grants (Nos)

Personnel on roll

393
Staff (incl. Contractual & Outsourced Employees; Nos)

Important administrative events that occurred during 2022-2023 are as follows:

- Hindi Pakhwada was observed in September 2022
- Vigilance Awareness Week was celebrated in the month of October-November 2022
- Planned activities pertaining to Swachh Bharat Abhiyan and special campaign were observed during 2022-2023
- 1,089 orders for 1,165 indents valued at Rs. 20,15,96,684/- were issued
- Status of vacancy positions (number) of various posts (as of 31st March 2023)

Cadre	Approved	Filled	Vacant	Advertised
Scientific	42	22	20	13
Admin	22	18	04	05
Technical	27	16	11	06
Total	**91**	**56**	**35**	**24**

- As part of Swachh Bharat Abhiyan, purchase records pertaining to the year 2016-2017 were weeded out in October 2022
- 20 RTI queries and 5 RTI appeals have been answered through the RTI online portal; 21 RTI queries were answered after being transferred from DBT
- 4 grievances were resolved during this period

The following important meetings were conducted during 2022-23 in the normal course of its activities:

Sl. No.	Meeting	Date
1	30th Finance Committee	26.09.2022
2	31st Finance Committee	07.06.2023
3	32nd Governing Body	04.10.2022
4	33rd Governing Body	24.08.2023
5	14th AGM inStem Society	01.12.2022

The following audits were conducted during 2022-23:

Sl. No.	Type of Audit	Date
1	Statutory audit FY 2022-23	June-October 2023
2	Internal Audit from IAW, DST for the period 2019-20 and 2020-21	September 2022

The following employees joined inStem during 2022-23:

Sl. No.	Name	Designation
	Scientific Staff	
1	Prof. Maneesha S. Inamdar	Director
2	Prof. Apurva Sarin	Distinguished Scientist
	Technical Staff	
1	Pankaj	Engineer (Instrumentation)
2	Hemanth Kumar Killewala	Technical Assistant (Electrical)
3	Ramkumar R.	Technical Assistant (Electrical)
4	Ankit Kumar	Technical Assistant (Instrumentation)
	Administrative Staff	
1	Maniarasan B.	Junior Management Assistant
2	Sunitha R.	Section Officer (Administration)

Mr. Rajesh R. was promoted to Pay Level 12 under CSIR-MANAS Scheme in October 2022 and assigned the additional responsibilities of Sr. Engineer (In Charge).

The following employees were confirmed to their substantive positions after completion of a probation period of two years during the year 2022-23:

1. Mr. Ramanathan K., Senior Administrative Officer
2. Mr. Amit Kumar, Administrative Officer (Hospitality & Services)
3. Mr. Anup Kumar, Administrative Officer (Establishment)
4. Mr. Gnanasampathan, Clerk (Hospitality and Services)
5. Mr. Vinod Kulkarni, Clerk (Academic Office)
6. Mr. Raghuram G., Clerk (Accounts)
7. Mr. Umesha T., Technical Officer-II (Civil)
8. Mr. Thiyagarajan M., Senior Technical Officer (HVAC)
9. Mr. Naresh Kumar Yadav, Technical Officer-I (Instrumentation)
10. Mr. Rakshith Komalan H. K., Senior Technical Officer (Civil)

Budget and Finance 2022-23

Sources of Funds:

The financial resources of the Institute are the Core Plan Grant-in-Aid provided by the Department of Biotechnology, Government of India, against annual budgetary projections made by the Institute. Other resources are in the form of research grants provided by various national and international agencies and from services rendered by DBT-inStem. The components of the core grants are essentially for meeting expenditures on salaries, operating expenses, equipment, infrastructure, furnishing etc.

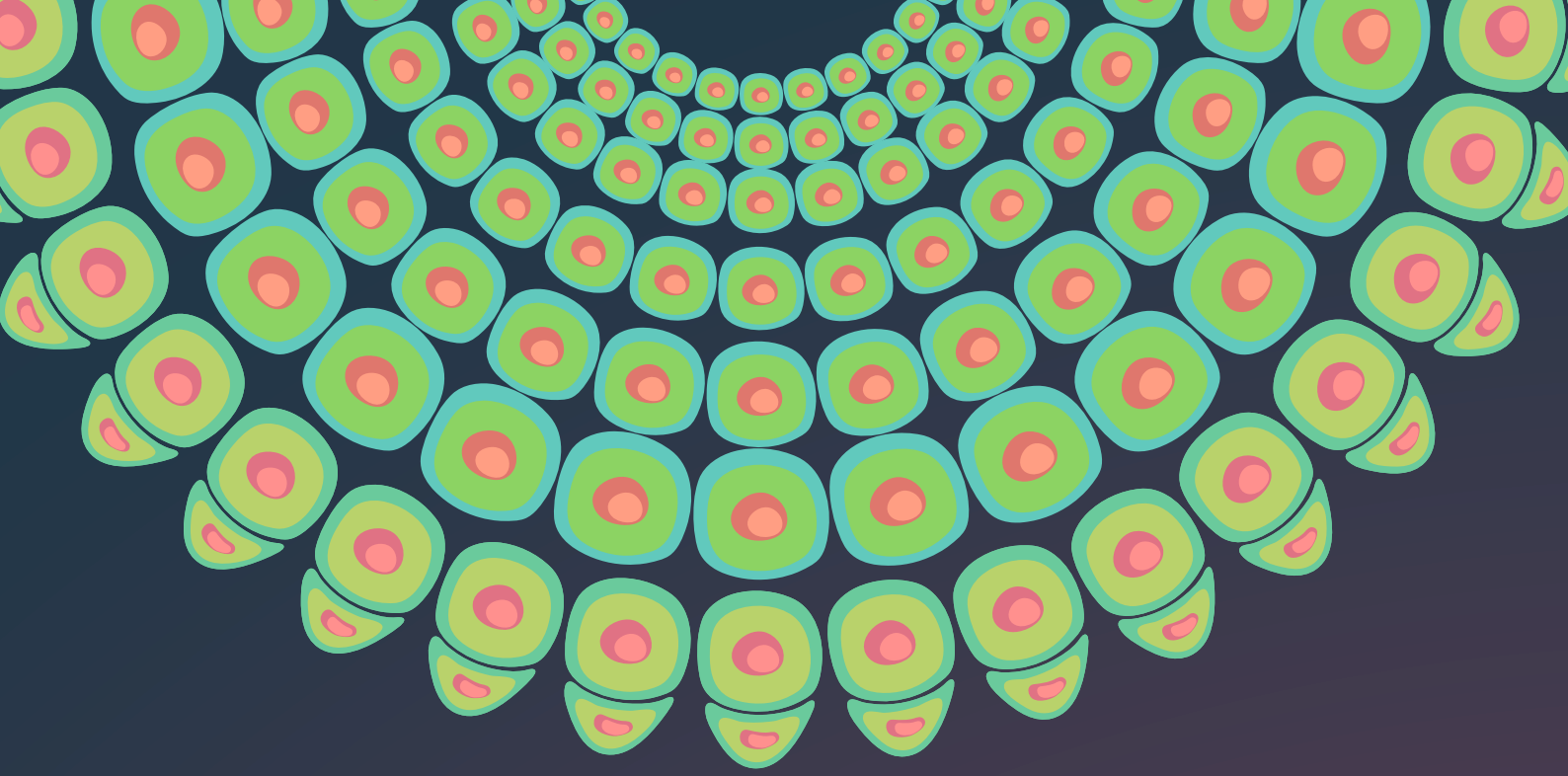
Receipts during the year 2022-23:

Sl. No.	Particulars	Amount in Lakhs	Percentage (%)
1	Grant-in-Aid	5,738.00	75.66
2	Extra Mural Projects	1,622.92	21.40
3	inStem Services	108.72	1.43
4	Miscellaneous Receipts	114.80	1.51
	Total	**7,584.44**	**100.00**

Application of Funds during 2022-23 (excluding Extra Mural Grants):

Sl. No.	Particulars	Amount in Lakhs	Percentage (%)
	Recurring		
1	Salaries and wages	1,418.17	23.91
2	Operating expenses	2,625.75	44.28
	Total	**4,043.92**	**68.19**
	Non-Recurring		
1	Equipment, infrastructure, and furnishing	1,886.18	31.81
	Total	**1,886.18**	**31.81**
	Grand Total	**5,930.10**	**100.00**

Prakash A. K.
Registrar, DBT-inStem



11

New Appointments

11.
New Appointments

11.1 Reena Singh

Dr. Reena Singh joined DBT-inStem as Assistant Investigator/Scientist E in June 2023. She is heading the CardioMet Translational Lab, focussed on unravelling the underlying mechanism of stem cells'/progenitors' specification, differentiation, and maturation during organ development. Her lab utilises long-standing expertise in human pluripotent stem cell-derived 3-D organoids and mouse developmental genetics to identify factors playing critical roles in cardiovascular development, congenital, and chronic cardiometabolic diseases, identify novel therapeutic targets, and develop proof-of-concept stem cell replacement therapies for cardiometabolic disease management and cure.

Dr. Singh received her Ph.D. in *"Molecular Medicine"* from Hannover Medical School, Germany, and did her Postdoctoral training at Victor Chang Cardiac Research Institute, Sydney, Australia. Before joining DBT-inStem, she was working as a Research-focused academic at The University of Sydney, School of Medical Sciences, Department of Physiology, where she established *"Stem Cells for Type 1 Diabetes"* research, attracting substantial Philanthropic Funds and competitive federal funds such as Diabetes Australia Research Program Grant as Chief Investigator. As a lecturer, she also engaged in teaching and training junior staff and students at The University of Sydney and The University of New South Wales, Australia. Her research contributions include publications in *Circulation Research, Nature Cell Biology, Cell, eLife,* and *Stem Cells Translational Medicine*.

11.2 Srujan Kumar Marepally

After completing his Ph.D. in liposomal nucleic acid delivery in 2011 from the Indian Institute of Chemical Technology (CSIR-IICT-Hyderabad, India), he pursued post-doctoral research at Florida A&M University (USA), focusing on the development of topical/transdermal drug/nucleic acid delivery systems for treating chronic skin inflammations. In 2013, he relocated to the Institute for Stem Cell Sciences and Regenerative Medicine (inStem, Bengaluru, India) as a DST-Fast Track Young scientist.

In 2015, he established his independent research lab at the Centre for Stem Cell Research (CSCR, CMC-Vellore, India). His research interests encompass the development of lipid nanoparticle-enabled nucleic acid therapeutics for monogenic disorders and mRNA-based vaccines. He has a record of publishing over 45 research articles in internationally peer-reviewed journals and has successfully secured funding from Indian central funding agencies, including DST, SERB, DBT, and BIRAC.

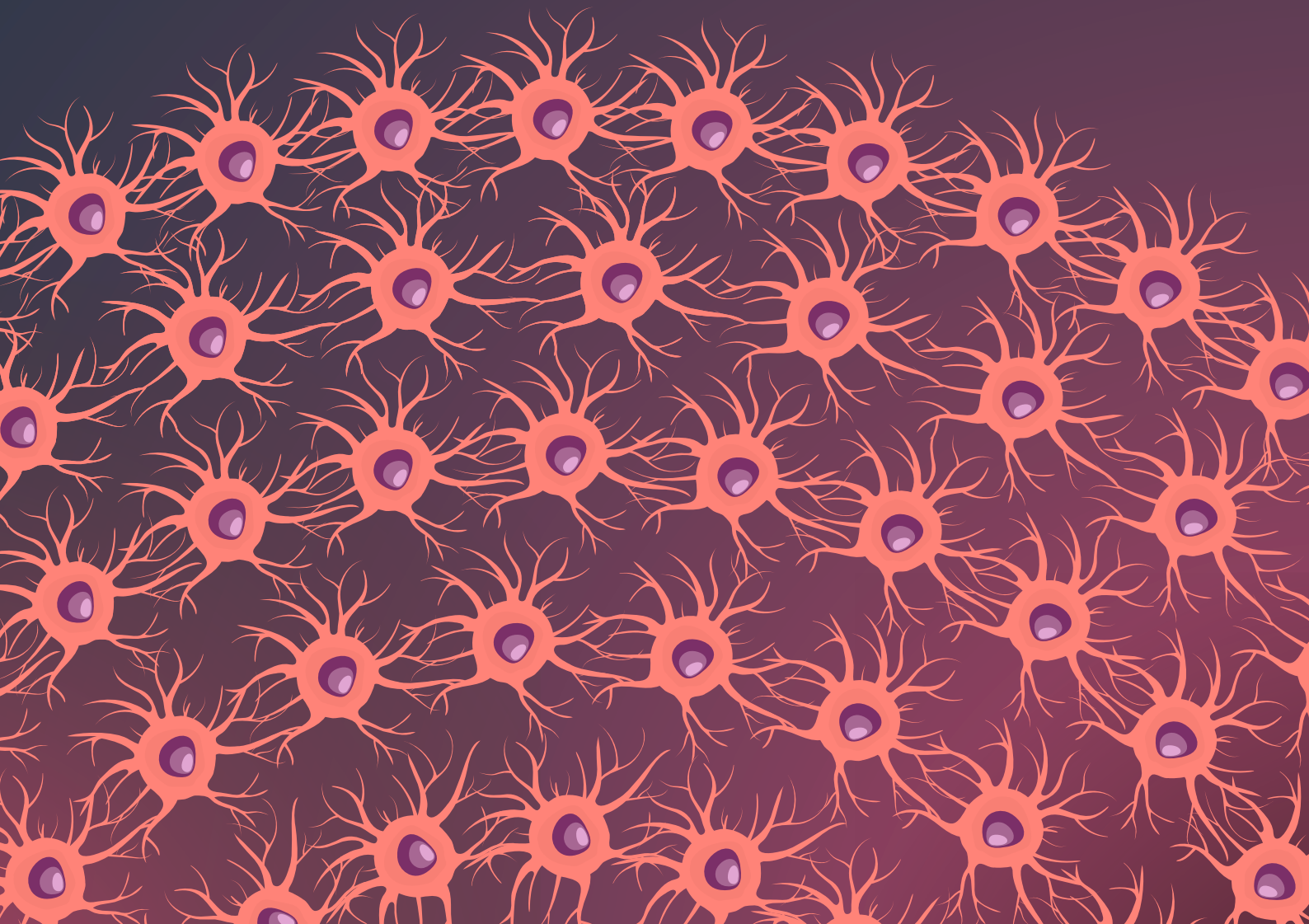
11.3 Srinivasarao Repudi

Srinivasarao Repudi joined as a faculty (Assistant Investigator/Scientist E) at DBT-inStem in August 2023 under the theme of *"Modelling Neurodevelopment and Disease"*. He obtained his Master's degree in biotechnology (2005–2007) from Pondicherry Central University, Pondicherry. In 2007, he joined CSIR-Indian Institute of Chemical Biology, Kolkata under the supervision of Dr. Malini Sen for his Ph.D, where he focused on the role of WISP3 (Wnt Induced Secreted Protein-3) in cartilage maintenance. In 2015, he moved to Prof. Rami Aqeilan's laboratory at Hebrew University of Jerusalem, Israel for doing postdoctoral studies on understanding the function of WWOX (WW domain containing oxidoreductase) in brain development and disease using mouse models and human brain organoids. His contributions paved the way to treat children with WOREE (WWOX Related Epileptic Encephalopathy) by AAV-mediated gene therapy, which is currently recommended for clinical trials. From August 2022–July 2023, he worked as a research fellow in Prof. John Hammer's laboratory at the National Institutes of Health, Bethesda, USA, where he gained experience in oligodendrocyte cell biology and myelination.

His research primarily focuses on understanding the interplay between neurons and oligodendrocytes in brain development and neurodevelopmental disorders, with particular emphasis on myelination impairment, abnormal neuronal hyperexcitability, and impaired motor coordination. His lab uses human brain organoids (derived from CRISPR-edited ESCs or patient-derived iPSCs) and conditional mouse models to dissect the cellular (cell autonomous and non-cell autonomous) and molecular events underlying brain physiology and disease progression and find alternative treatment strategies through regenerative medicine approaches.

12

Leadership Committee



12.
inStem Leadership Committees 2022-23

12.1 Director

- **Dr. Thangaraj K.**, Director (Addl. Charge), inStem (from 1st March to 18th August 2022)
- **Prof. Maneesha S. Inamdar**, Director, inStem (from 19th August 2022)

12.2 Society

- **Dr. Jitendra Singh,** Union Minister for Science and Technology, New Delhi - President
- **Dr. C. N. Ashwath Narayan,** Minister-in-charge of the Department handling Biotechnology in Karnataka
- **Dr. Rajesh S. Gokhale,** Secretary to the Government of India, Department of Biotechnology, Ministry of Science & Technology
- **Prof. Ravichandran,** Secretary DST, New Delhi
- **Dr. Shekhar C. Mande,** Secretary CSIR, New Delhi
- **Dr. E. V. Ramana Reddy,** Principal Secretary-in-charge of the Department handling Biotechnology in Karnataka
- **Mr. Chaitanya Murti,** Joint Secretary (Admin), DBT, New Delhi
- **Shri Vishvajit Sahay,** Additional Secretary & Financial Advisor, DBT, New Delhi
- **Prof. Sharath Chandra,** Hon. Director, Centre for Human Genetics, Bengaluru
- **Dr. Kiran Mazumdar-Shaw,** Chairperson & Managing Director, Biocon India Ltd., Bengaluru
- **Prof. Goverdhan Mehta,** Former Director, IISc & CSIR Bhatnagar Fellow, Hyderabad
- **Prof. P. Balaram,** Former Director, IISc, Bengaluru
- **Dr. Jyotsna Dhawan,** Chief Scientist, CCMB, Hyderabad
- **Prof. Satyajit Mayor,** Centre Director, NCBS, Bengaluru (till 21st February 2023)
- **Prof. L. S. Shashidhara,** Centre Director, NCBS-TIFR, Bengaluru (22nd February 2023)
- **Dr. Thangaraj K.,** Director (Addl. Charge), inStem (from 1st March to 18th Aug 2022) – Member Secretary
- **Prof. Maneesha S. Inamdar,** Director, inStem (from 19th August 2022) – Member Secretary

12.3 Governing Body

- **Dr. Rajesh S. Gokhale,** Secretary to the Government of India, DBT, New Delhi-Chairperson
- **Dr. Thangaraj K.,** Director (Addl. Charge), inStem (from 1st March to 18th August 2022)

- **Prof. Maneesha S. Inamdar,** Director, inStem (from 19th August 2022)
- **Mr. Vishvajit Sahay,** Additional Secretary & Financial Advisor, DBT, New Delhi
- **Dr. Alka Sharma,** Senior Adviser/Scientist H, DBT, New Delhi
- **Mr. Chaitanya Murti,** Joint Secretary (Admin), DBT, New Delhi
- **Dr. Sangita M. Kasture,** Scientist 'F', DBT, New Delhi
- **Dr. Arvind Ramanathan,** Head-Research, inStem, Bengaluru
- **Dr. Dasaradhi Palakodeti,** Scientist-F, inStem, Bengaluru
- **Prof. Satyajit Mayor,** Centre Director, NCBS-TIFR, Bengaluru (till 21st February 2023)
- **Prof. L. S. Shashidhara,** Centre Director, NCBS-TIFR, Bengaluru (from 22nd February 2023)
- **Dr. J. V. Peter, Director,** CMC, Vellore
- **Prof. Jayaram N. Chengalur,** Director TIFR, Mumbai – Member
- **Dr. Vidita A. Vaidya,** Professor, TIFR, Mumbai
- **Dr. Gagandeep Kang,** Department of Gastroenterology, CMC, Vellore
- **Dr. Soniya Nityanand,** Director, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow
- **Dr. Dinakar M. Salunke,** Director, ICGEB, New Delhi
- **Mr. Ramanathan K.,** Head-Admin & Finance, inStem, Bengaluru (Non-member Secretary)

12.4 Scientific Advisory Board

- **Prof. B. Ravindran,** Professor Emeritus and Former Director, Institute of Life Science (DBT-ILS), Bhubaneswar – Chairperson
- **Prof. Alejandro Sánchez Alvarado,** Howard Hughes Medical Institute, Stowers Institute for Medical Research, USA
- **Prof. Gagandeep Kang,** Department of Gastroenterology, CMC, Vellore and Former Executive Director, DBT-THSTI, Faridabad.
- **Dr. Satyajit Rath,** Indian Institute of Science Education and Research (IISER), Pune
- **Dr. Dinakar Salunke,** International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi
- **Prof. Helen Skaer,** Emeritus Professor of Developmental Biology, University of Cambridge, UK
- **Prof. Mriganka Sur,** Newton Professor, Simons Centre for the Social Brain, MIT, Harvard, USA
- **Dr. K. Thangaraj,** Centre for DNA Fingerprinting and Diagnostics (DBT-CDFD), Hyderabad
- **Prof. Vidita Vaidya,** Department of Biological Science, TIFR, Mumbai
- **Prof. Umesh Varshney,** J. N. Tata Chair Professor, Dean, Faculty of Science, IISc, Bangalore

12.5 Finance Committee

- **Mr. Vishvajit Sahay,** Additional Secretary & Financial Advisor – Chairperson
- **Dr. Thangaraj K.,** Director (Addl. Charge), inStem (from 1st Mar to 18th Aug 2022)
- **Prof. Maneesha S. Inamdar,** Director, inStem, Bengaluru (From 19th Aug 2022)
- **Dr. Sangita M. Kasture,** Scientist 'F', Nodal Officer, DBT, New Delhi
- **Dr. M. Krishna Murthy,** Joint Registrar (Finance), IISc, Bengaluru
- **Mr. R. Shivakumar,** Sr. Head, IAW, Department of Space, Bengaluru
- **Prof. Alok Srivastava,** Head-CSCR, CMC, Vellore
- **Dr. Thangaraj K.,** Director, CDFD, Hyderabad
- **Mr. Ramanathan K.,** Head-Admin & Finance, inStem, Bengaluru

13

Memoriam

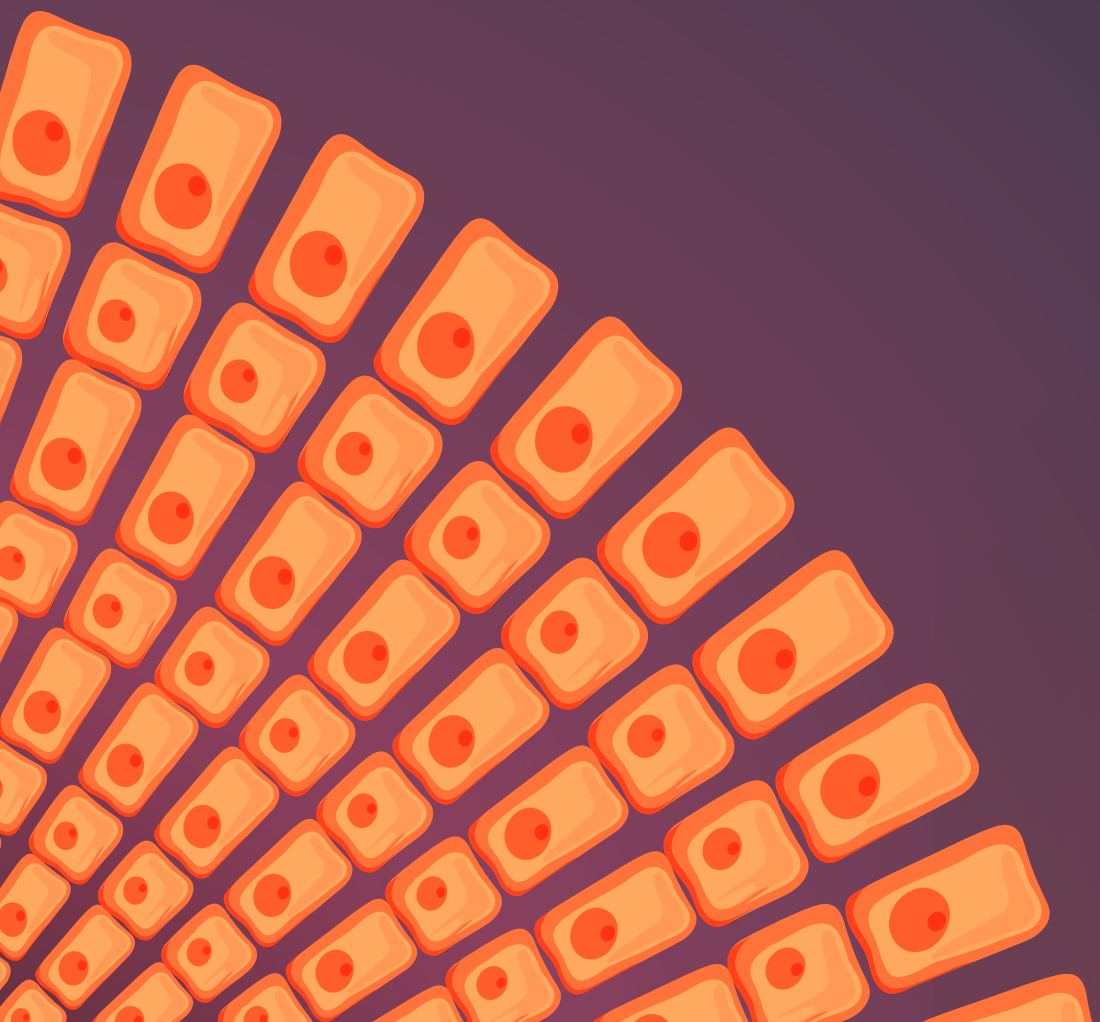
13.
Memoriam

Mr. Knight Paul Pandian

Coordinator and Convener at BLiSc, guided the campus by his knowledge and experience for more than a decade, till his untimely passing away in January 2023

14

Financial Report



Annexure – 2

T. Ramachandran & Co.,
Chartered Accountants

INDEPENDENT AUDITOR'S REPORT

To,
The Members
Governing Council of
M/s. Institute for Stem Cell Science and Regenerative Medicine
Bangalore-560065

Report on the Audit of the standalone Financial Statements

Opinion

We have audited the financial statements of "Institute for stem Cell Science and Regenerative Medicine", which comprises the Balance Sheet as at 31st March 2023, the Income and Expenditure Account and Receipts and Payments account for the year then ended, and notes to the financial statements, including a summary of significant accounting policies.

In our opinion and to the best of our information and according to the explanations given to us, the accompanying financial statements give a true and fair view of the financial position of the Institute as at 31st March 2023, of its financial performance and Receipts and Payments for the year ended in accordance with the Accounting Standards issued by the Institute of Chartered Accountants of India (ICAI).

Basis for Opinion

We conducted our audit in accordance with the Standards on Auditing (SAs) issued by ICAI. Our responsibilities under those Standards are further described in the Auditor's Responsibilities for the Audit of the Financial Statements section of our report. We are independent of the entity in accordance with the Code of Ethics issued by ICAI and we have fulfilled our other ethical responsibilities in accordance with the Code of Ethics. We believe that the audit evidence we have obtained is sufficient and appropriate to provide a basis for our opinion.

M/s T. Ramachandran & Co. BANGALORE Chartered Accountants

Other Matters

We have not audited the financial statements of CSCR at Vellore, whose financial statements reflect total assets of Rs. 14.41 crores, total revenue of Rs.6.08 crores and excess of expenditure over income of Rs. 1.68 crores for the year ended as on 31-03-2023. These financial statements have been audited by other auditor whose reports have been furnished to us by the Management.

Responsibilities of Management and Those Charged with Governance for the Financial Statements

Management is responsible for the preparation of the financial statements in accordance with generally accepted accounting principles in India. This responsibility includes the design, implementation and maintenance of internal control relevant to the preparation and presentation of the financial statements that give a true and fair view and are free from material misstatement, whether due to fraud or error.

In preparing the financial statements, management is responsible for assessing the entity's ability to continue as a going concern, disclosing, as applicable, matters related to going concern and using the going concern basis of accounting unless management either intends to liquidate the entity or to cease operations, or has no realistic alternative but to do so.

Those charged with governance are responsible for overseeing the entity's financial reporting process.

Auditor's Responsibilities for the Audit of the Financial Statements

Our objectives are to obtain reasonable assurance about whether the financial statements as a whole are free from material misstatement, whether due to fraud or error, and to issue an auditor's report that includes our opinion. Reasonable assurance is a high level of assurance, but is not a guarantee that an audit conducted in accordance with SAs will always detect a material misstatement when it exists. Misstatements can arise from fraud or error and are considered material if, individually or in the aggregate, they could reasonably be expected to influence the economic decisions of users taken on the basis of these financial statements.

As part of an audit in accordance with SAs, we exercise professional judgment maintain professional skepticism throughout the audit. We also:

• Identify and assess the risks of material misstatement of the financial statements, whether due to fraud or error, design and perform audit procedures responsive to those risks, and obtain audit evidence that is sufficient and appropriate to provide a basis for our opinion. The risk of not detecting a material misstatement resulting from fraud is higher than for

M/s T. Ramachandran & Co. BANGALORE Chartered Accountants

one resulting from error, as fraud may involve collusion forgery, intentional omissions, misrepresentations, or the override of internal control.

• Obtain an understanding of internal control relevant to the audit in order to design audit procedures that are appropriate in the circumstances, but not for the purpose of expressing an opinion on the effectiveness of the entity's internal control.

• Evaluate the appropriateness of accounting policies used and the reasonableness of accounting estimates and related disclosures made by management.

• Conclude on the appropriateness of management's use of the going concern basis of accounting and, based on the audit evidence obtained, whether a material uncertainty exists related to events or conditions that may cast significant doubt on the entity's ability to continue as a going concern. If we conclude that a material uncertainty exists, we are required to draw attention in our auditor's report to the related disclosures in the financial statements or, if such disclosures are inadequate, to modify our opinion. Our conclusions are based on the audit evidence obtained up to the date of our auditor's report. However, future events or conditions may cause the entity to cease to continue as a going concern.

We communicate with those charged with governance regarding, among other matters, the planned scope and timing of the audit and significant audit findings, including any significant deficiencies in internal control that we identify during our audit.

We also provide those charged with governance with a statement that we have complied with relevant ethical requirements regarding independence, and to communicate with them all relationships and other matters that may reasonably be thought to bear on our independence, and where applicable, related safeguards.

For T. Ramachandran & Co.
Chartered Accountants
Firm Registration Number: 009009S

M/s T. Ramachandran & Co. BANGALORE Chartered Accountants

T. Ramachandran
Partner
Membership No.: 207600

Date: 04.10.2023
Place: Bangalore
UDIN: 23207600BGTZSF6569

File No.InStem/G/110/22-23/

inStem

UTILISATION CERTIFICATE

(Towards establishment of Institute for Stem Cell Science and Regenerative Medicine for the financial year: 2022-23 (01-04-2022 to 31-03-2023)

1. Title of the Project/Scheme : Institute for Stem Cell Science and Regenerative Medicine

2. Name of the Organization: : Institute for Stem Cell Science and Regenerative Medicine

3. Department of Biotechnology Sanction Order No and date of sanctioning the project: : No. BT/PR7972/MED/14/1208/2006 dated 25.08.2008

4. Amount brought forward from the previous financial year 2021-22 quoting DBT letter No. & date in which the authority to carry forward the said amount was given: : Rs. 1,29,16,945/-

5. Amount received from DBT during the financial year 2022-23 (Please give No. & date of sanction orders showing the amount paid):

1	No. BT/PR7972/MED/14/1208/2006	10.05.2022	Rs. 8,25,00,000/-
2	No. BT/PR7972/MED/14/1208/2006	10.05.2022	Rs 15,00,00,000/-
3	No. BT/PR7972/MED/14/1208/2006	10.05.2022	Rs. 4,40,00,000/-
4.	No. BT/PR7972/MED/14/1208/2006	01.09.2022	Rs. 6,01,00,000/-
5.	No. AID-19015/1/2020-MED-DBT	16.12.2022	Rs. 7,50,00,000/-
6.	No. AID-19015/1/2020-MED-DBT	16.12.2022	Rs. 4,40,00,000/-
7.	No. AID-19015/1/2020-MED-DBT	13.03.2023	Rs. 2,24,00,000/-
8.	No. AID-19015/1/2020-MED-DBT	15.03.2023	Rs. 3,12,00,000/-
9.	No. AID-19015/1/2020-MED-DBT	24.03.2023	Rs. 6,46,00,000/-
			Rs.57,38,00,000/-

6. Other receipts/interest earned, if any on the DBT grants: : Rs.5,81,934/-

7. Total amount that was available for expenditure incurred during the financial year (Sl.No. 4, 5, and 6): : Rs.58,72,98,879/-

8. Actual expenditure incurred during the financial year (Statement of expenditure is enclosed) : Rs.60,58,82,710/-

9. Unspent balance refunded, if any (Please give details) : Rs.15,35,484/- refunded vide Bharatkosh (T. No. 1909220004172)

Amount lapsed and returned back from TSA : (a) **GIA Capital:** Rs. 1,12,63,366/- lapsed and returned back from TSA on 31.03.2023 due to RBI glitch.
(b) **GIA Manpower** Rs. 6,46,00,000/- lapsed and returned back from TSA on 31.03.2023 due to RBI glitch.
(c) **GIA General** Rs. 67,104/- lapsed and returned back from TSA on 31.03.2023 due to RBI glitch.

9A. Interest refunded, if any (Please give details) : Rs. 3,11,844/- refunded vide Bharatkosh (T.No. 1204220008897)
Rs. 5,11,565/- refunded vide Bharatkosh (T No. 1909220003399)
Rs. 5,81,934/- refunded vide Bharatkosh (T No. 2104230005883)
Rs. 4,55,192/- refunded vide Bharatkosh (T No. 0410230010218)

RAMACHANDRAN & CO. BANGALORE Chartered Accountants

10. Negative Balance amount at the end of the financial year: (as on 31.03.2023) : Rs.9,74,55,128/-

Note:
(1) As on 31.03.2023, balance is TSA pertaining to inStem GIA General amounting to Rs.67,104/-, GIA Capital amounting to Rs. 1,12,63,366/-, GIA Manpower amounting to Rs. 6,46,00,000/- lapsed and returned back to GoI.
(2) Refund of interest of Rs.14,05,343/- was processed in 2022-23, BharatKosh details for all refunds are provided at SL No.9A.

11. Negative Balance at the end of Financial Year 2022-23 vide letter no. & date : Rs. 9,74,55,128/-

CERTIFICATE

Certified that the amount of **Rs.60,58,82,710/-** mentioned against col.8 has been utilized on the project/scheme for the purpose for which it was sanctioned and that the deficit balance as on 31.03.2023 is **Rs. 9,74,55,128/-.**

Certified that I have satisfied myself that the conditions on which the grants in aid was sanctioned have been duly fulfilled /are being fulfilled and that I have exercised the following checks to see that money was actually utilized for the purpose for which it was sanctioned.

1. Verification of audited books of accounts

2. Checking of vouchers and bank balances

(T Ramachandran)
Partner (M No. 207600)

M/s. T. RAMACHANDRAN & CO. BANGALORE

(A.K. Prakash)
Registrar
ए.के. प्रकाश / A K Prakash
कुलसचिव / Registrar
मूल कोशिका विज्ञान एवं पुनर्योजी औषधि संस्थान (डीबीटी-इन्स्टेम)
Institute for Stem Cell Science and Regenerative Medicine (DBT - inStem)
जैव प्रौद्योगिकी विभाग, विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार के अधीन स्वायत्त संस्थान

(Prof. Maneesha S Inamdar)
Director
प्रो. मनीषा एस इनामदार / Prof. Maneesha S Inamdar
निदेशक / Director
मूल कोशिका विज्ञान एवं पुनर्योजी औषधि संस्थान (डीबीटी-इन्स्टेम)
Institute for Stem Cell Science and Regenerative Medicine (DBT-inStem)

(Madhu Chandan Roy)
Admin Officer (F& A)
मधु चंदन रॉय / Madhu Chandan Roy
प्रशासनिक अधिकारी (वित्त एवं लेखा)
Administrative Officer (Finance & Accounts)
स्टेम कोशिका विज्ञान और पुनर्योजी औषधि संस्थान
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जैव प्रौद्योगिकी विभाग, भारत सरकार के अधीन स्वायत्त संस्थान
(An under Department of Biotechnology, Govt. of India)
जीकेवीके पोस्ट, बेल्लारी रोड / GKVK Post, Bellary Road
बेंगलूरु - ५६० ०६५ / Bengaluru - 560 065

(Ramanathan K)
Senior Administrative Officer
रामनाथन के / Ramanathan K
वरिष्ठ प्रशासनिक अधिकारी / Senior Administrative Officer
स्टेम कोशिका विज्ञान और पुनर्योजी औषधि संस्थान
Institute for Stem Cell Science and Regenerative Medicine
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बेंगलूरु - ५६० ०६५ / Bengaluru-560 065

inStem
Institute for Stem Cell Science and Regenerative Medicine

INSTITUTE FOR STEM CELL SCIENCE & REGENERATIVE MEDICINE, BANGALORE

STATEMENT OF EXPENDITURE FOR THE PERIOD FROM 01.04.2022 TO 31.03.2023

(Amount in Rs.)

Sl.No.	Particulars	Unspent balance as on 01.04.2022 as per Audited SOE & UC	Grants received from DBT during the period 01.04.2022 to 31.03.2023	Other receipts/interest earned on the DBT Grants	Total	Expenditure incurred (excluding commitments) from 01.4.2022 to 31.3.2023	Interest or Unspent amount Refunded/Amount Lapsed in TSA	Balance as on 31.03.2023
1	2	3	4	5	6=3+4+5	7	8	9=6-7-8
	INSTEM :							
A	GIA - Capital							
(i)	Equipments & Accessories	-8,96,173	8,14,00,000	-	8,05,03,827	9,44,65,544	1,03,38,599	-2,43,00,316
	Total (A)	**-8,96,173**	**8,14,00,000**	-	**8,05,03,827**	**9,44,65,544**	**1,03,38,599**	**-2,43,00,316**
B	GIA - Salary							-
(ii)	Manpower	-4,57,720	11,62,00,000	-	11,57,42,280	11,81,88,580	4,86,95,413	-5,11,41,713
	Total (B)	**-4,57,720**	**11,62,00,000**	-	**11,57,42,280**	**11,81,88,580**	4,86,95,413	**-5,11,41,713**
C	GIA - General							
(iii)	Recurring Expenses	15,35,484	21,64,31,633	0	21,79,67,117	21,59,09,337	16,02,588	4,55,192
	Total (C)	**15,35,484**	**21,64,31,633**	-	**21,79,67,117**	**21,59,09,337**	**16,02,588**	**4,55,192**
D	Other receipts	-	-		-			-
E	Interest Earned	5,11,565	-	-	5,11,565	-		5,11,565
F	Interest Refunded	-	-		-	-	5,11,565	-5,11,565
	GRAND TOTAL (A+B+C+D+E+F) - INSTEM	**6,93,156**	**41,40,31,633**	-	**41,47,24,789**	**42,85,63,461**	**6,11,48,165**	**-7,49,86,837**
	CSCR Vellore :							
G	GIA - Capital	-	8,36,00,000		8,36,00,000	8,26,76,737	9,24,767	-1,504
H	GIA - Salary	-3,15,955	3,64,00,000		3,60,84,045	3,61,95,521	1,59,04,587	-1,60,16,063
I	GIA - General	1,22,27,900	3,97,68,367		5,19,96,267	5,84,46,991		-64,50,724
J	Interest Earned	3,11,844		5,81,934	8,93,778	-		8,93,778
K	Interest Refunded			-	-	-	8,93,778	-8,93,778
	GRAND TOTAL (G+H+I+J+K) - CSCR	**1,22,23,789**	**15,97,68,367**	**5,81,934**	**17,25,74,090**	**17,73,19,249**	**1,77,23,132**	**-2,24,68,291**
	GRAND TOTAL - INSTEM + CSCR	**1,29,16,945**	**57,38,00,000**	**5,81,934**	**58,72,98,879**	**60,58,82,710**	**7,88,71,297**	**-9,74,55,128**

Note: (1) As on 31.03.2023, balance in TSA (inStem & CSCR) towards GIA Mapower, General & Capital amounting to Rs.6,46,00,000, Rs.67104 & Rs.1,12,63,366 lapsed and returned back to GoI respectively.
(2) Refund of opening interest of Rs.5,11,565/- was processed in 2022-23 BharatKosh details for all refunds are provided in UC.
(3) Refund of opening interest of Rs.3,11,844/- was processed in 2022-23 & current year interst of Rs. 5,81,934/- was processed in 2023-24, BharatKosh details for all refunds are provided in UC.
(4) Refund of opening balance of Recurring Expenses of inStem amounting Rs.15,35,484 was made during Sep-22, BharatKosh details for all refunds are provided in UC.
(5) Refund of closing balance of Recurring Expenses of inStem amounting Rs.4,55,192/- was made on 04.10.2023 after finalization of accounts, BharatKosh details for all refunds are provided in UC.

(T Ramachandran)
Partner (M.No.207600)
Place: Bangalore
Date: 04.10.2023

M/s. T. RAMACHANDRAN & CO. BANGALORE Chartered Accountants

(Madhu Chandan Roy)
Admin Officer (F&A)

मधु चंदन रॉय / Madhu Chandan Roy
प्रशासनिक अधिकारी (वित्त एवं लेखा)
Administrative Officer (Finance & Accounts)
मूल कोशिका विज्ञान और पुनर्योजी औषधि संस्थान
Institute for Stem Cell Science and Regenerative Medicine (inStem)
जैव प्रौद्योगिकी विभाग, भारत सरकार के अधीन स्वायत्त संस्थान
(An autonomous institute under Department of Biotechnology, Govt. of India)
जीकेवीके पोस्ट, बेल्लारी रोड / GKVK Post, Bellary Road
बेंगलूरु-560 065 / Bengaluru - 560 065

Ramanathan K
Sr.Admin Officer

(AK Prakash)
Registrar

ए.के. प्रकाश / A K Prakash
कुलसचिव / Registrar
मूल कोशिका विज्ञान एवं पुनर्योजी औषधि संस्थान (डीबीटी-इनस्टेम)
Institute for Stem Cell Science and Regenerative Medicine (DBT - inStem)
जैव प्रौद्योगिकी विभाग, विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार के अधीन स्वायत्त संस्थान

(Prof. Maneesha Inamdar)
Director

प्रो. मनीषा एस इनामदार / Prof. Maneesha S Inamdar
निदेशक / Director
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Institute for Stem Cell Science and Regenerative Medicine (DBT-inStem)

INSTITUTE FOR STEM CELL SCIENCE & REGENERATIVE MEDICINE, BANGALORE
(Registered under the Karnataka Societies' Registration Act)
GKVK, BELLARY ROAD, BANGALORE - 560 065
RECEIPTS AND PAYMENTS STATEMENT FOR THE YEAR ENDED MARCH 31, 2023

(Amount -Rs)

RECEIPTS	CURRENT YEAR	PREVIOUS YEAR	PAYMENTS	CURRENT YEAR	PREVIOUS YEAR
I. Opening Balances			I. Expenses		
a) Cash in hand	24	1,224	a) Establishment Expenses	14,18,16,876	12,22,74,961
b) Bank Balances			b) Administrative Expenses	26,25,75,884	23,48,85,104
i) in current accounts	59,00,638	6,34,81,240		**40,43,92,760**	**35,71,60,065**
ii) in deposit accounts	16,30,11,390	30,60,03,848	**II. Payments made against projects**	**16,34,06,279**	**20,84,83,315**
iii) in savings accounts	18,26,24,829	5,21,06,464	**III. Investments made**		
	35,15,36,881	**42,15,92,776**	a) Out of Earmarked/End. Funds	-	-
II. Grants Received			b) Out of own funds	-	-
a) From Govt. of India	49,78,69,530	39,64,00,000	**IV. Increase in Current Assets**	**-**	**-**
b) From State Govt.	-	-	V. Capital Expenditure		
	49,78,69,530	**39,64,00,000**	a) Purchase of fixed assets-Projects	74,35,905	1,12,22,521
III. Project Receipts-Projects	**16,22,92,882**	**18,19,53,848**	b) Exp. On Building	83,91,678	2,84,87,598
			c) Exp on Equipments & Furnitures	17,27,90,721	5,66,62,841
IV. Decrease in Current Assets	**56,35,483**	**2,32,87,604**		**18,86,18,304**	**9,63,72,960**
V. Interest Received			VI. Refund of surplus money/Loans	-	-
a) On Bank deposits	71,48,057	1,10,99,832	a) To the Govt. of India	5,79,43,052	1,63,49,698
b) on Loans, Advances etc.		-	a) To the Govt. of India-EMG	6,04,83,929	3,94,49,957
	71,48,057	**1,10,99,832**		**11,84,26,981**	**5,57,99,655**
VI. Other Income (Specify)	**2,05,06,927**	**81,06,378**	VII. Finance Charges (Interest)	-	-
VII. Amount Borrowed	-	-	VIII. Decrease in Current Liabilities	**1,00,58,506**	**-2,69,12,437**
VIII. Any other receipts	-	-	IX. Closing Balances:		
IX. Increase in Current Liabilitites	-	-	a) Cash in hand	24	24
			b) Bank Balances		
			i) in current accounts	90,21,512	59,00,638
			ii) in deposit accounts	3,05,97,889	16,30,11,390
			iii) in savings accounts	12,04,67,505	18,26,24,829
				16,00,86,930	**35,15,36,880**
TOTAL	**1,04,49,89,760**	**1,04,24,40,438**	**TOTAL**	**1,04,49,89,760**	**1,04,24,40,438**

Vide our report of even date
For T Ramachandran & Co
Chartered Accountants

(T Ramachandran)
Partner (M.No.207600)

Place: Bangalore
Date : 04.10.2023

M/s.T.RAMACHANDRAN & CO. BANGALORE Chartered Accountants

(Madhu Chandan Roy)
Admin Officer (F&A)

(Ramanathan K)
Sr.Admin Officer

(AK Prakash)
Registrar

ए.के. प्रकाश / A K Prakash
कुलसचिव / Registrar
मूल कोशिका विज्ञान एवं पुनर्योजी औषधि संस्थान (डीबीटी-इनस्टेम)
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(Prof. Maneesha Inamdar)
Director

प्रो. मनीषा एस इनामदार / Prof. Maneesha S Inamdar
निदेशक / Director
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INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE

(Registered under the Karnataka Societies' Registration Act.)

GKVK, BELLARY ROAD, BANGALORE - 560 065

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED MARCH 31, 2023

(Amount- Rs.)

Particulars	Schedule	Current Year	Previous Year
INCOME			
Income from Projects - to the extent of expenditure included	3	16,34,06,279	20,84,83,315
Income from Sales and Services	12	1,08,72,378	27,44,267
Grants/Subsidies	13	34,41,32,896	31,27,00,000
Fees/Subscriptions	14	-	-
Income from Investments	15	-	-
Income from Royalty, Publications etc.	16	-	-
Interest earned	17	18,45,775	34,37,598
Other Income	18	96,34,549	58,77,112
Increase/(decrease)in stock of Finished goods and works-in-progress	19	-	-
TOTAL (A)		**52,98,91,877**	**53,32,42,292**
EXPENDITURE			
Establishment Expenses	20	14,18,16,876	12,22,74,961
Other Administrative Expenses	21	26,25,75,884	23,55,42,315
Expenditure on Grants/Subsidies etc.	3	16,34,06,279	20,84,83,315
Interest	23	8,23,409	1,20,83,693
Depreciation (Net Total at the year -end -corresponding to Sch.8)		30,93,00,483	32,26,85,016
TOTAL (B)		**87,79,22,932**	**90,10,69,300**
Balance being excess of Expenditure over Income (A-B)		**-34,80,31,055**	**-36,78,27,007**
Less- Transfer to Capital Reserve - equivalent to depreciation charges	2(1)	30,93,00,483	32,26,85,016
Less- Transfer to/from General Reserve - Recurring Grant Account	1(B)	-3,87,30,571	-4,51,41,991
Balance being surplus/deficit carried to Corpus/Capital Fund		-	-

Vide our report of even date
For T Ramachandran & Co
Chartered Accountants

(T Ramachandran)
Partner (M.No.207600)

Place: Bangalore
Date : 04.10.2023

M/s. T. RAMACHANDRAN & CO. BANGALORE Chartered Accountants

(Madhu Chandan Roy)
Admin Officer (F&A)

मधु चंदन रॉय / Madhu Chandan Roy
प्रशासनिक अधिकारी (वित्त एवं लेखा)
Administrative Officer (Finance & Accounts)
स्टेम कोशिका विज्ञान और पुनर्योजी औषधि संस्थान
Institute for Stem Cell Science and Regenerative Medicine (inStem)
जैव प्रौद्योगिकी विभाग, भारत सरकार के अधीन स्वायत्त संस्थान
An Autonomous Institute under Department of Biotechnology, Govt. of India
GKVK Post, Bellary Road / GKVK Post, Bellary Road
बेंगलूरु / Bengaluru

(Ramanathan K)
Sr.Admin Officer

(AK Prakash)
Registrar

ए.के. प्रकाश / A K Prakash
कुलसचिव / Registrar
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(Prof. Maneesha Inamdar)
Director

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INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE

(Registered under the Karnataka Societies' Registration Act.)

GKVK, BELLARY ROAD, BANGALORE - 560 065

BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

Particulars	Schedule	Current Year	Previous Year
CORPUS/CAPITAL FUND AND LIABILITIES			
CORPUS/CAPITAL FUND	1	2,07,58,788	14,40,54,767
RESERVES AND SURPLUS	2	2,36,59,84,315	2,48,66,66,494
EARMARKED/ ENDOWMENT FUNDS	3	14,82,33,552	21,19,64,501
SECURED LOANS AND BORROWINGS	4	-	-
UNSECURED LOANS AND BORROWINGS	5	-	-
DEFERRED CREDIT LIABILITIES	6	-	-
CURRENT LIABILITIES AND PROVISIONS	7	7,43,14,825	8,43,73,331
TOTAL		**2,60,92,91,480**	**2,92,70,59,094**
ASSETS			
FIXED ASSETS	8	2,41,31,08,230	2,53,37,90,410
INVESTMENTS - FROM EARMARKED /ENDOWMENT FUNDS	9	-	-
INVESTMENTS - OTHERS	10	600	600
CURRENT ASSETS, LOANS, ADVANCES ETC.	11	19,61,82,650	39,32,68,083
TOTAL		**2,60,92,91,480**	**2,92,70,59,094**
SIGNIFICANT ACCOUNTING POLICIES	24		
CONTINGENT LIABILITIES AND NOTES ON ACCOUNTS	25		

Vide our report of even date
For T Ramachandran & Co
Chartered Accountants

(T Ramachandran)
Partner (M.No.207600)

Place: Bangalore
Date:

M/s. T. RAMACHANDRAN & CO. BANGALORE Chartered Accountants

(Madhu Chandan Roy)
Admin Officer (F&A)

मधु चंदन रॉय / Madhu Chandan Roy
प्रशासनिक अधिकारी (वित्त एवं लेखा)
Administrative Officer (Finance & Accounts)

(Ramanathan K)
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INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
(Registered under the Karnataka Societies' Registration Act.)
GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE-1 - CORPUS/CAPITAL FUND:	Current Year	Previous Year
(A) NON-RECURRING GRANT		
Balance at the beginning of the year	12,88,04,727	13,02,55,166
Add: Contributions during the year	15,37,36,634	8,37,00,000
Less: Expenditure incurred during the year	18,11,82,399	8,51,50,439
Adjustments, if any	-4,47,30,580	-
BALANCE AS AT THE YEAR END (A)	**5,66,28,382**	**12,88,04,727**
(B)RECURRING GRANT		
Balance as at the beginning of the year	1,52,50,040	6,46,58,036
Grants returned to DBT	-1,23,89,063	-42,66,005
Adjustment pertaining to previous years	-	-
Transferred from Income & Expenditure	-3,87,30,571	-4,51,41,991
BALANCE AS AT THE YEAR END (B)	**-3,58,69,594**	**1,52,50,040**
TOTAL (A) + (B)	**2,07,58,788**	**14,40,54,767**

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GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE -2 - RESERVES AND SURPLUS:	Current Year	Previous Year
1: CAPITAL RESERVE		
As per last account	2,48,66,66,494	2,71,29,78,550
Less: Adjustments of previous years	-	-
Addition during the year (See Note -1 below)	18,86,18,304	9,63,72,960
Less: Deduction during the year(See Note -2 below)	30,93,00,483	32,26,85,016
TOTAL	2,36,59,84,315	**2,48,66,66,494**
2: REVALUATION RESERVE:	-	-
3: SPECIAL RESERVES:	-	-
4: GENERAL RESERVE:	-	-
Total Reserves & Surplus	**2,36,59,84,315**	**2,48,66,66,494**

Note 1 : This represents Total additions made to the Fixed Assets during the year, consisting of Rs.17,71,42,282 (FY 21-22 Rs. 8,51,50,439/-) acquired out of Core Funds and Rs.74,35,905/- (FY 21-22 Rs.1,12,22,521/-) acquired out of Project Funds.
Note 2 : This represents the Depreciation on Fixed Assets for the year, consisting of Rs.22,39,83,122/- (FY 21-22 Rs.22,50,19,805/-) on Fixed Assets acquired out of Core Funds & Rs.8,53,17,361/- (FY 21-22 Rs.9,76,65,211/-) on Fixed Assets acquired out of Project Funds.

M/s. T. RAMACHANDRAN & CO. BANGALORE Chartered Accountants

INSTITUTE FOR STEM CELL SCIENCE & REGENERATIVE MEDICINE, BANGALORE
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GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULE FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

SCHEDULE -3-EARMARKED / ENDOWMENT FUNDS

(Amount -Rs.)

Sl No.	Project Title (Name of PI if applicable)	Budget code	Funding Agency	Opening Balance	Received during the year	Refund during the year	Interest received	Interest Refunded	Expenditure		Total Expenditure	Balance as on 31-03-2023
									Capital	Revenue		
A	*From Government*											
1	DBT JRF (8138-Oindrilla Banerjee)	8138	DBT	-89,200	1,30,200		-			25,138	25,138	15,862
2	Mahendra Rao (8146)	8146	-	15,82,387	2,67,772		-				-	18,50,159
3	DBTRA Fellowship (8149-Bhakti J Vyas)	8149	DBT	1,02,000	68,600		-			68,600	68,600	1,02,000
4	DBTRA Fellowship (8152-Sarayu R)	8152	DBT	-	1,28,800		-			1,28,800	1,28,800	-
5	DBTRA Fellowship (8153-Mohd M)	8153	DBT	1,29,515	1,29,546		-			1,55,033	1,55,033	1,04,028
6	ICMR Fellowship(8155-Radhika Rao)	8155	ICMR	27,208			-				-	27,208
7	ICMR Fellowship (8158-Edries Y H)	8158	ICMR	2,07,265			-				-	2,07,265
8	ICMR Fellowship (8159-Isha Rana)	8159	ICMR	-22,400			-				-	-22,400
9	CSIR Fellowship (8162-Abhinava Yadav)	8162	CSIR	15,068			-				-	15,068
10	CSIR Fellowship (8164-Lakshmi Kripa)	8164	CSIR	20,000			-				-	20,000
11	Lady Tata Fellowship (8165-Subhasini Pandey)	8165	Lady Tata	-	3,69,450	1,06,950	-			2,62,500	2,62,500	-
12	CSIR Fellowship (8166-Imtiyaz Gulami)	8166	CSIR	34,998			-				-	34,998
13	DBT RA Fellowship (8167-Anupam Dutta)	8167	DBT	13,048			-				-	13,048
14	DBT RA Fellowship (8168-Anusree Mahanta)	8168	DBT	66,567			-				-	66,567
15	DST Inspire Fellowship (8172-Vineeth V)	8172	DST	-	5,56,025		-		-	5,56,025	5,56,025	-
16	DBT JRF Fellowship (8173-Harshadri)	8173	DBT	9,166			-				-	9,166
17	DBT JRF Fellowship (8178-Uttkarsh I)	8178	DBT	30,000	5,77,990		-			5,47,990	5,47,990	60,000
18	DBT JRF Fellowship (8179-Sonal Joshi)	8179	DBT	20,000			-				-	20,000
19	DBT JRF Fellowship (8180-Vishwaja Javeri)	8180	DBT	1,68,760			-				-	1,68,760
20	DST Inspire fellowship (8181- Manisha Goyal)	8181	DST	2,44,280	4,61,280	7,05,560	-				-	-
21	DBT SRF Fellowship (8182-Pratul Jain)	8182	DBT	1,15,320	59,520		-			1,32,320	1,32,320	42,520
22	DST Inspire fellowship (8184- Michelle Dsouza!)	8184	DST	4,89,150			-			4,74,150	4,74,150	15,000
23	DST Inspire fellowship (8185-Sreesa Sreedhran)	8185	DST	-32,240	5,40,800	20,000	-			4,88,560	4,88,560	-
24	DBT RA Fellowship(8187-Aditi Jain)	8187	DBT	25,000	3,89,560	1,83,963	-			2,30,597	2,30,597	-
25	DBT RA Fellowship(8188-Gaurav Singh)	8188	DBT	75,000	8,16,320		-			7,28,484	7,28,484	1,62,836
26	ICMR Fellowship (8189-Pratul Jain)	8189	ICMR	-71,300	5,53,400		-			4,60,375	4,60,375	21,725
27	ICMR Fellowship (8190- Vinay J Rao)	8190	ICMR	1,401	5,53,400		-			5,33,400	5,33,400	21,401
28	DBT JRF Fellowship (8191-Radhika Agrawal)	8191	RCB-DBT	69,730	4,91,280		-			5,29,720	5,29,720	31,290
29	DBT JRF Fellowship (8192-Ravi Kiran)	8192	RCB-DBT	17,984	4,91,280		-			4,61,455	4,61,455	47,809
30	SERB Purdue University Overseas Visiting Doctoral Fellowship	8193	SERB	4,50,000	17,28,000		-			16,92,000	16,92,000	4,86,000
31	CSIR Contingency received to Sreenath R. (8127)	8127	CSIR	1,176	-		-				-	1,176
32	Sumana Ghosh (8186)	8186	CSIR	39,758	-		-				-	39,758
33	Aswhin Venkateshvaran	8194	DBT	-	4,49,019		-			4,21,600	4,21,600	27,419
34	Sneha Muralidharan	8195	DBT		7,49,360		-			6,99,360	6,99,360	50,000
35	Diksha Gogia	8196	DBT	-	2,70,204		-			2,04,600	2,04,600	65,604
36	Saraswati Sanjay Chavda	8197	DBT	-	4,92,440	77,150	-			4,15,290	4,15,290	-
37	*Sahana Vasudevan*	8198	***DBT***	-	1,24,893		-			*116560*	1,16,560	8,333
38	Debasna Pritimanjari Panigrahi	8199	DBT	-	1,24,893		-			1,16,560	1,16,560	8,333
39	Susobhan Mahanty	8200	DBT	-	1,24,893		-			1,16,560	1,16,560	8,333
40	Other Miscelleaneous Grant			82,979							-	82,979
41	Novel Cell Surface Markers for endodermal stem and progenitor cells in health and disease (8214) & CSIR	8214	DBT	3,70,118			-				-	3,70,118
42	Therapeutic approaches to augmentation of Adult cardiac stem cells (8217- Prof. Jyotsna Dhawan)	8217	DBT	7,54,889			-				-	7,54,889
43	DBT Twinning Programme for the North East - Molecular Mechanism of target recognition and clevage by the CRISPR-CAS bacterial immune system (8220-Prof. Ramaswamy)	8220	DBT	3,88,853			-				-	3,88,853
44	Centre for Brain Development and Repair - CBDR (8221- Prof. Sumantra Chattarji)	8221	DBT	71,81,210		5910000	-	12,71,210			-	-
45	Muscle SC self renewal: A stressful matter (8225- CEFIPRA Grant - Prof. Jyostns Dhawan)	8225	CEFIPRA	1,87,054			-				-	1,87,054
46	Molecular mechanisms that regulate cyto skeletal modelling in cardiac hypertrophy by developing an in-vitro human cariomyocyte culture microfluidic system (8229 - Prof. Jyotsna Dhawan)	8229	DST	4,51,756			-				-	4,51,756
47	Imaging synaptic plasticity an control: Novel protein carbon nanotube fluorscent sensors for regulation of protein translation-(8239 Dr. Akash Gulvani)	8239	DBT	5,36,842			-				-	5,36,842
48	Structure-Function studies on nucleotide sugar transporters Indo Argentina program-(8249-Prof.Ramaswamy)	8249	DST	-43,335			-				-	-43,335

M/s. T. RAMACHANDRAN & CO. BANGALORE Chartered Accountants

Sl No.	Project Title (Name of PI if applicable)	Budget code	Funding Agency	Opening Balance	received during the year	Refund during the year	Interest received	Interest Refunded	Expenditure		Total Expenditure	Balance as on 31-03-2023
49	Programme support on metabolic control of cell fate-(8250-Prof. Apurva sarin)	8250	DBT	6,38,859		6,38,859	-				-	-
50	Accelerating the application of stemcell technology in human disease (ASHD)-8251 (Prof. Apurva, Dr. Raghu Padinjat)	8251	DBT	1,81,30,274	-	12,87,020	3,31,265	11,04,415		1,57,10,631	1,57,10,631	3,59,473
51	Mining the Genome and Metagenome of Marine Microbiome for PKS-NRPS Biosynthetic Gene Cluster and Bioactive Small Molecules : A Cordinated R & D Initiative in marine Genomics (8257-Dr. Praveen Kumar Vemula)	8257	DBT	1,74,294			-				-	1,74,294
52	Ramalinga Swamy Grant (8258-Tina Mukharjee)	8258	DBT	2,63,267			-	12,958			-	2,50,309
53	NeuroStem : Stem cell Models for Discovery of RNA-Mediated regulation in Neurodeneration(8260-Dr.Ravi Muddshetty)	8260	DBT	-3,96,121			-				-	-3,96,121
54	Genetic Program Controlling Mesoderm Differentiation(8272-Ramkumar Sambasivan)	8272	DBT	1,84,669			-				-	1,84,669
55	Accelerating the application of stemcell technology in human disease (ASHD)-8273 (Prof. Apurva, Dr. Raghu Padinjat)	8273	DBT	3,18,925		3,18,925	-				-	-
56	Implementation of Phase-II for Centre for Chemical Biology and Therapeutics (CCBT)(8274-Prof. Ashok Venkitaraman)	8274	DBT	1,13,192	1,64,37,375		2,46,077	8,64,723		1,49,74,443	1,49,74,443	9,57,478
57	Financial approval of the Swarna Jayanti Fellowship to Dr.Dasaradhi Palakodeti(8276-Dasaradhi Palakodeti)	8276	DST	-22,253			-	14,625			-	-36,878
58	Role of Mechanical Signaling in Maintaining Stem Cell Quiescence in Mouse Skin(8277-Srikala Raghavan)	8277	SERB	-8,07,594			-				-	-8,07,594
59	Indo French Centre for Promotion of Advances Research-IFCPAR (8279-Dr Minhal S)	8279	CEFIPRA	4,18,863			-			7,18,182	7,18,182	-2,99,319
60	Scientists without boundaries at the Bangalore life science cluster (8283)	8283	TTK	64,33,643			1,81,719			19,64,962	19,64,962	46,50,400
61	Prophylactic catalytic dermal cream to prevent pesticide exposure during farming practices (8290 Sandeep C)	8,290	SERB	4,199		4,199	-				-	-
62	Structure-Function Studies on Nucleotide Sugar Transporters (8291-S Ramaswamy)	8291	SERB	-6,69,094			-				-	-6,69,094
63	Sialic Acid Scavening,Catabolic & Sialyation Pathways : Putative Targets for New Antimicrobial Agents (8294 S Ramaswamy)	8294	DBT	1,06,276		1,06,276	-				-	-
64	Tata Education & Development Trust (8292)	8292	TIGS	-10,40,054	43,10,283		-			86,64,984	86,64,984	-53,94,755
65	Identification of regulators of myeloid-cell homeostasis predisposing animals to metabolic disorders and insulin resistance (8451 Tina Mukharjee)	8,451	DBT	4,538		4538	-			-	-	-0
66	N PDF Fellowship to Dr. Venkatramanan G. Rao(8454)	8454	SERB	-5,20,166			-				-	-5,20,166
67	Elucidating the function of the euchromatic histone lysine methyltransferase 1(EHMT 1) in obesity and diabetes (8455 Mahua Chakraborty)	8455	DST	-8,99,103			-				-	-8,99,103
68	8456 Tina Mukharjee	8456	CEFIPRA	-84,206			-			4,65,540	4,65,540	-5,49,746
69	Bugwork Ltd 8457 Ramaswamy	8457	BUGWORK	1,00,307			-				-	1,00,307
70	Regulationof damage-induced cellular plasticity in the lung(8458 Arjun Guha)	8458	SERB	5,098			-				-	5,098
71	Characterization of novel variant human embryonic stem cells with features of neoplastic progression 8460 Shravanti Rampalli	8460	SERB	12,387			-				-	12,387
72	Dissecting the Role of chromatin remodeling factor TIP60 in regulation of regeneration in Planaria (8461 Bharti Jaswal)	8,461	DST	-97,582			-	11,216			-	-1,08,798
73	Molecular, structural and functional mapping of eye regeneration using novel light sensing assays (8463 Akash Gulyani)	8463	SERB	-2,27,284			-				-	-2,27,284
74	Pro Adjuvant polymer based Dissovable Microneedlies for Transdermal Sustained Delivery of vaccines(58465 Suman Pahal)	8465	DST	2,923			-	2,923			-	-
75	Fabrication of Catalytic nano-Fibre based facemask and clothing to prevent pesticide-induced neuronal dysfunction and mortality(8468-Prof. Praveen Kumar Vemula)	8468	DBT	3,99,839	13,65,705	7,30,669	5,310	18,076		9,95,230	9,95,230	26,879
76	DST post Doctoral fellowship in Nano Science & Technology TO Dr. Utkarsh Bhutani(8470-Dr. Naveen Kumar)	8470	DST/JNCASR	35,201		12,881	-			22,320	22,320	-0
77	Profiling of bromodomain specific interacting partners using unnatural amino acid mutagenesis (Ramalingaswami Fellowship)-(8473-Dr. Sonia Sen)	8,473	DBT	-2,08,072	800000		24,338	70,411		-	-	5,45,855
78	National post doctoral fellowship to Dr. Siddhartha Datta(8476-Dr.Siddhartha Datta/Sumantra Chatterjee)	8476	SERB	9,65,850		9,65,850	-				-	-0
79	Analysis of deficient fear learning and memory in a novel rat model of fragile X syndrome FXS(8477-Dr.Pradeep kumar Mishra)	8477	DST	1,26,197		24,959	-	18,372	24,999		82,866	-0
80	An ex-vivo method metabolic capacity to determine protein as an early diagnostic marker(8478-Dr.Aditi Bhattcharya)	8478	BIRAC	5,29,178		5,29,178	-				-	-
81	Inducible gene drive based approach to control infectious insect vectors(8480-Dr. Baskar Bakthavachalu)	8480	BIRAC	-3,45,323			-				-	-3,45,323

M/S.T. RAMACHANDRAN & CO. BANGALORE Chartered Accountants

Sl No.	Project Title (Name of PI if applicable)	Budget code	Funding Agency	Opening Balance	Received during the year	Refund during the year	Interest received	Interest Refunded	Expenditure		Total Expenditure	Balance as on 31-03-2023
82	Leveraging stem cell technology to facilitate discovery for human disease biology in India(8479-Prof.Apurva Sarin/ Prof.Raghu Padinjat)	8479	DBT	1,75,59,463	-	51,21,992	4,04,427	9,18,387		1,14,95,696	1,14,95,696	4,27,815
83	Regulation of metabolic homeostasis by Trna modifications(8483-Dr.Sunil Laxman)	8483	SERB	-1,44,690	14,00,000	3,412	2,587	4,320		7,11,355	7,11,355	5,38,810
84	Delineating the Immune-Epithelial Crosstalk in Embryonic Skin(8484-Dr.Srikala Raghavan/Dr.Dasaradhi Palakodeti)	8484	DBT	35,225	29,54,080	11,96,804	-	24,364		17,12,884	17,12,884	55,253
85	Architecture of axonemal doublet microtubule inner junction(8485-Dr.Minhaj Sirajuddin)	8485	SERB	2,81,863	20,00,000		6,441			18,94,702	18,94,702	3,93,602
86	Understanding selective drug mechanisms using hypertrophic patient-specific induced pluripotent stem cell (Ipsc)-derived cardiomyocytes (8487- Dr. Dhandapany)	8487	SERB	6,37,319	15,00,000		15,703			20,44,782	20,44,782	1,08,240
87	Purchase of materials for RTPCR test of COVID-19 Pandemic (8491)	8491	Karnataka State Government Funds	27,175	-		-			27,175	27,175	-
88	High-affinity binders against Covid 19 spike protein using display libraries (8492)	8492	DBT	31,03,500			-	1,63,555		37,37,237	37,37,237	-7,97,292
89	COVID-19 Bioresource (8493)	8493	DBT	26,14,829	27,43,131	4,20,011	36,562	2,15,969	1,836	39,90,608	39,92,444	7,66,098
90	Chromatin regulation of human cortical development by LSD1 and its role in intellectual disability (8494)	8494	SERB	53,253	10,50,000		1,771			10,40,815	10,40,815	64,209
91	Single Cell metabolic maps of senescent cells and senescence driven cancer cells in aging (8495)	8495	SERB	-1,12,319			-			5,77,382	5,77,382	-6,89,701
92	Inspire Faculty Fellowship award to Dr.Vasanth Thamodaran (8496)	8496	DST	32,12,275		31,28,263	-	84,012			-	-0
93	Epigenetic Regulation of the Wound Healing Program (8498)	8498	DBT	-21,69,701	77,52,040	33,34,510	-	12,517		22,19,317	22,19,317	15,995
94	National Post Doctoral fellowship awarded to Dr. Shalini Sanyal (8500)	8500	SERB	-1,09,010	10,68,400		-			9,34,813	9,34,813	24,577
95	Characterize the role of Angiogenin and Trna derived small RNAs in early embryogenesis (8502)	8502	DBT	35,92,630	30,31,005	24,06,585	70,783	2,19,271	9,00,000	30,54,118	39,54,118	1,14,444
96	Genomic Surveillance for SARS-Cov-2In India: Indian SARS-CoV-2 Genomics Consortium (INSACOG) (8503)	8503	DBT	1,46,21,889	5,48,000	46,40,661	3,03,186	4,46,717		1,00,82,511	1,00,82,511	3,03,186
97	Deciphering the molecular mechnaisms of dysregulation caused by mutations in LSD1 leading to Intellectual Disability (IYBA-2022)	8605	DBT	78,857	15,70,213		61,053	62,498	9,95,000	6,14,258	16,09,258	38,367
98	Pre-Clinical evaluation of promising drugs for a rare syndromic cardiomyopathy in children	8606	BIRAC	14,19,569	5,92,200		26,172			23,54,063	23,54,063	-3,16,122
99	Translational platforms for discovery, repurposing and clinical development for Covid-19 therapeutics	8607 & 8608	DBT	2,43,57,197	-	1,58,00,220	5,62,746	4,46,261	29,63,111	51,47,605	81,10,716	5,62,746
100	Immune Cell lipid homeostasis by the Notch pathway functions as a central regulator of animal growth control both in homeostasis and conditions of dietary Stress	8609	SERB	11,30,954			27,100			8,07,549	8,07,549	3,50,505
101	NPDF to Dr. Abrar Rizvi	8,610	SERB	8,98,461			18,194			9,59,655	9,59,655	-43,000
102	Novel micropatterned cardiac organoid model for deciphering the pa	8611	DBT	25,69,528	26,14,091	26,81,699	52,827	47,078		24,20,215	24,20,215	87,454
103	Faculty Travel Award to Dr. Tina Mukherjee	8613	SERB	-	3,45,215		1			3,45,115	3,45,115	101
104	Faculty Travel Award to Dr. Tina Mukherjee for collaboration visit of Prof. Ramas	8615	SERB	-	2,15,754		1,162			1,57,673	1,57,673	59,243
105	Elucidating the Structural and Functional characterization of Membrane and Envelope Protein from SARS CO V-2 and its variants in Virus like Particle formation and its role in the virus assembly and release	8616	ICMR	-	15,06,249		2,490			12,61,607	12,61,607	2,47,132
106	The role of Galectin-3/LGALS3 in mediating epithelial defences against urinary tract infection by constitutive activation of the NF-κB pathway.	8617	SERB	-	15,20,000		5,192			10,24,721	10,24,721	5,00,471
107	Enabling Covid-19 vaccine and therapeutics development using animal models	8618	BIRAC	-	3,37,06,000		3,53,313				-	3,40,59,313
108	Developing CRISPR-Cas9 based genome engineering technology for homology independent genetic manipulation at endogenous genomic loci in a tissue specific manner	8619	DBT	-	20,22,000	15,12,205	-			5,09,795	5,09,795	-
109	NPDF to Ms. Sreeja Kumari Dhanya	8624	SERB		11,18,400	11,18,400	-				-	-
110	Development of machine for rapid fabrication of the biocompatible rnicroneedles, followed by clinical testing u	8628	SERB	-	11,54,296		10,609			1,42,185	1,42,185	10,22,720
111	NPDF Fellowship of Shabhir Ahmad	8629	SERB	-	11,18,400		8,530			3,04,600	3,04,600	8,22,330
112	Genomic Surveillance for SARS-Cov-2In India: Indian SARS-CoV-2 Genomics Consortium (INSACOG) - Phase II	8635	DBT	-	4,68,720		663			4,05,480	4,05,480	63,903
113	Total : (A)			11,09,59,398	10,55,60,482	5,29,91,739	27,60,222	60,33,878	48,84,946	11,80,29,752	11,80,02,898	4,22,51,787
B												
114	From other than Government			-								-
115	Workshop on X-Ray Crystallography (8223-Dr. Vinod Nayak)	8223	AVANTOR	1,14,591			-				-	1,14,591
116	Regulation of epithelial stem cell homing in cutaneous wound healing (8226- Dr. Subhasri Ghosh)	8226	WT/DBT IA	-1,59,784			-				-	-1,59,784
117	Research Project awarded by L'Oreal (8232- Dr. Colin Jamora)	8232	L'OREAL	15,86,744			48,236			1,58,528	1,58,528	14,76,452

Sl No.	Project Title (Name of PI if applicable)	Budget code	Funding Agency	Opening Balance	Received during the year	Refund during the year	Interest received	Interest Refunded	Expenditure		Total Expenditure	Balance as on 31-03-2023
118	Agreement with Unilever(8245-Dr. Colin Jamora)	8245	Unilever Industries	26,90,354	2,00,000		74,669			11,47,635	11,47,635	18,17,388
119	Innovative multi-model approach to identify novel candidate genes and	8278	WT/DBT IA	31,46,289	81,08,242		78,160	11,82,748	6,48,957	82,58,946	89,07,903	12,42,040
120	Metabolic Regulation of Fungal Morphogenesis(8286-Dr Sriram V)	8286	WT/DBT IA	31,217			-				-	31,217
121	Structural and functional insights into bacterial sialic acid transport (829	8293	WT/DBT IA	22,82,047	8,74,029		53,007	77,602		25,62,188	25,62,188	5,69,293
122	Support world class research in Neurobiology(8467-Prof. Apurva Sarin)	8467	Prathika Trust	2,39,98,735			7,06,435			46,02,399	46,02,399	2,01,02,771
123	Donation from Kiran Mazumdar Shah(8474-Prof. Apurva Sarin)	8474	Kiran Mazumdar Shah	2,17,81,468			6,75,573			8,94,897	8,94,897	2,15,62,144
124	Regulation of cerebral cortical development by chromatin modifiers in H	8475	WT/DBT IA	21,46,654	42,19,435		60,373	1,46,677	2,08,702	45,44,442	47,53,144	15,26,641
125	Joint Agreement between Instem and Eyestem(8482-Dr.Arjun Guha)	8482	Eyestem-Ccamp	2,26,809			-				-	2,26,809
126	Gates Foundation Grant-8242	8242	GF	4,66,895			-				-	4,66,895
127	Fraxa-8247	8247	FRAXA	1,92,928			-			19,950	19,950	1,72,978
128	Results of EMBO Young Investigator Programme Selections-2016(8275-Minhajuddin Sirajuddin)	8275	EMBO	27,79,462	1,54,460		79,302			7,71,302	7,71,302	22,41,922
129	Simons Autism Research Project (8282-Sumnatra Chatterji)	8282	EDINBURGH	78,484			-				-	78,484
130	Develop an Insect (Mosquito) repellent formulation based on the natural insecticide nootkatone -8288 Praveen Vernula)	8288	MANUS	4,61,327			13,662			80,640	80,640	3,94,349
131	Directors Descreationory Fund (8296)	8296	FLEXI	1,49,74,571	4,36,480		-			59,65,690	59,65,690	1,19,31,380
132	Terumo Inc (8466) Mahendra Rao	8466	Terumo	23,71,119			-				-	23,71,119
133	Collaborative Agreement between Phoremost & Instem(8471-Dr.Kavitha Bharatham/Dr.Anandi Karumbat)	8471	Phoremost/ Funded	6,82,833			-			6,82,833	6,82,833	0
134	Collaborative Agreement between Artus & Instem(8472-Dr.Praveen Kumar Vemula)	8472	Artus/ Funded	35,48,801			75,038			33,78,373	33,78,373	2,45,467
135	CSIR & Project Cost Contingency			5,72,013			-				-	5,72,013
136	Covid funds (8042)	8042	PNBHL	33,019			-			33,019	33,019	-0
137	(NCBS-Covid Salary-8400)	8400		-59,962	60,000		-			38	38	-
138	Early Career Fellowship to Dr. Anusree Mahanta (8486)	8486	WT/DBT IA	18,45,683	13,18,588		44,501	51,285		25,58,941	25,58,941	5,98,546
139	TIGS Covid Testing Funds (8488)	8488	TIGS	11,64,707			-			18,006	18,006	11,46,701
140	Indigenisation of Diagnostics (INDX) (8497)	8497	C-CAMP	18,82,521			25,858		16,93,300	2,15,079	19,08,379	0
141	Investigation of the Susceptibility UPK3A+Mouse Airways Cells to AJAGI1+AJAGI2 (8499)	8499	Genentech	5,05,851			10,768			4,90,266	4,90,266	26,354
142	EMBO Global Investigator Network (8501)	8501	EMBO	7,35,077	10,00,118		24,756			8,43,654	8,43,654	9,16,296
143	Milestone-I identify potent compounds against SARS-Cov-2 virus in 8-point DR for compounds	8600	BUGWORK	94			-			94	94	-
144	SCO-088 compound with human p210c-ABL wild type co-crystallization activity requirements	8601	Sun Pharma Advanced Research Company Limited	8,82,353	-		-			8,82,353	8,82,353	0
145	Covid RT-PCR for Saliva Samples	8602	CMC Vellore/NCBS/CCAMP	87,70,156	1,06,57,391		2,89,092			79,01,642	79,01,642	1,18,14,997
146	Establish assay and research capabilities (As per agreement)	8604	Mitosciences Research PVT LTD	3,93,704	8,00,000		13,732			6,71,105	6,71,105	5,36,331
147	Anti SARS Activity Service Pipeline	8612	C-CAMP	8,78,343	6,77,500		22,581			11,58,269	11,58,269	4,20,155
148	EMBO Meeting for Organoid meeting 06.02.23-09.02.2023	8614	EMBO	-	36,92,206		-			14,12,819	14,12,819	22,79,387
149	NUS	8621	NUS	-	41,14,744		42,005			1,07,471	1,07,471	40,49,278
150	USIAS	8622	USIAS	-	16,49,384		11,424			5,59,579	5,59,579	11,01,229
151	Understanding and manipulating how resource allocations drive collective cell functions	8623	Welcome Trust	-	1,37,68,159		1,40,459			3,68,369	3,68,369	1,35,40,249
152	Role of tubulin posttranslational modifications in regulating primary cilia functions in wound healing and tissue homeostasis	8625	Welcome Trust	-	42,98,979		45,063				-	43,44,042
153	Regulation of immune defenses and homeostasis at the urethral barrier	8626	Welcome Trust	-	7,02,685		7,366				-	7,10,051
	Sub Total : (C)			10,10,05,104	5,67,32,400	-	25,42,060	14,58,312	25,50,959	5,02,88,527	5,28,39,486	10,59,81,766
	D. CSCR - CMC- VELLORE			-							-	-
	Interest received on Grants			-							-	-
	Grand Total: (A+B+C+D)			21,19,64,501	16,22,92,882	5,29,91,739	53,02,282	74,92,190	74,35,905	16,34,06,279	17,08,42,184	14,82,33,552

M/s. T. RAMACHANDRAN & CO. BANGALORE Chartered Accountants

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
(Registered under the Karnataka Societies' Registration Act.)
GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE -4 - SECURED LOANS AND BORROWINGS:	Current Year	Previous Year
1. Central Government	-	-
2. State Government (Specify)	-	-
3. Financial Institutions	-	-
(a) Term Loans	-	-
(b) Interest accrued and due	-	-
4. Banks	-	-
(a) Term Loans	-	-
- Interest accrued and due	-	-
(b) Other Loans (Specify	-	-
- Interest accrued and due	-	-
5. Other Institutions and Agencies	-	-
6. Debentures and Bonds	-	-
7. Others (Specify)	-	-
TOTAL	-	-

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
(Registered under the Karnataka Societies' Registration Act.)
GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE -5 - UNSECURED LOANS AND BORROWINGS:	Current Year	Previous Year
1. Central Government	-	-
2. State Government (Specify)	-	-
3. Financial Institutions	-	-
(a) Term Loans	-	-
(b) Interest accrued and due	-	-
4. Banks	-	-
(a) Term Loans	-	-
- Interest accrued and due	-	-
(b) Other Loans (Specify	-	-
- Interest accrued and due		-
5. Other Institutions and Agencies	-	-
6. Debentures and Bonds	-	-
TOTAL	-	-
Note: Amounts due within one year		

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
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GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE-6 - DEFERRED CREDIT LIABILITIES	Current Year	Previous Year
a) Acceptances secured by hypothecation of capital equipment and other assets.	-	-
b) Others	-	-
TOTAL	-	-
Note: Amounts due within one year	-	

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
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GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE -7 CURRENT LIABILITIES AND PROVISIONS	Current year	Previous Year
A. CURRENT LIABILITIES	-	-
1. Acceptances	-	-
2. Sundry Creditors	-	-
(a) For Goods	2,90,07,633	4,45,74,378
(b) Others	41,49,321	27,79,130
3. Advance Received	-	-
4. Interest accrued but not due on:	-	-
(a) Secured Loans/Borrowings	-	-
(b) Unsecured Loans /borrowings	-	-
5. Statutory Liabilities :	-	-
(a) Overdue	-	-
(b) Others	18,97,236	29,75,442
6. Other Current Liabilities	3,26,19,236	2,85,93,978
TOTAL (A)	**6,76,73,426**	**7,89,22,928**
B. PROVISIONS	-	-
1. For Taxation	-	-
2. Gratuity	0	21,34,181
3. Superannuation/Pension	-	-
4. Accumulated Leave Encashment	0	32,14,471
5. Trade Warranties/Claims	-	-
6. Others*	66,41,399	1,01,751
TOTAL (B)	**66,41,399**	**54,50,403**
Grand TOTAL (A+B)	**7,43,14,825**	**8,43,73,331**

*The Bruhat Bengaluru Mahanagara Palike (BBMP) has indicated an amount of Rs. 65.47 Lakhs as payable towards Property Tax (Service Charges) for the period from 2019-20 to 2023-24 in respect of inStem and the same has been provisioned in the Books.

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(Registered under the Karnataka Societies' Registration Act)
GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULE FORMING PART OF BALANCE SHEET FOR THE PERIOD ENDED MARCH 31, 2023

SCHEDULE - 8

(Amount -Rs.)

DESCRIPTION	GROSS BLOCK				Rate	DEPRECIATION				NET BLOCK	
	As on 1-4-2022	Additions	Deductions	Up to 31-03-2023		As on 1-4-2022	Additions	Deductions	Up to 31-03-2023	As on 31-03-2023	As on 31-03-2022
(A) Own Funds											
Land Development Works	17,01,110	-	-	17,01,110	0%	7,97,071	-	-	7,97,071	9,04,039	9,04,039
Land (Nominal Value)	1	-		1	0%	-	-	-	-	1	1
Other Misc. facilities	25,26,642	-	-	25,26,642	10%	14,39,006	1,08,764	-	15,47,770	9,78,872	10,87,636
Buildings (Residential)*	19,30,49,174	-		19,30,49,174	5%	6,44,54,239	64,29,747	-	7,08,83,986	12,21,65,188	12,85,94,935
Buildings(Non-Residential)	11,96,25,797	-	-	11,96,25,797	10%	6,74,97,730	52,12,807	-	7,27,10,537	4,69,15,260	5,21,28,067
Laboratory Equipment	61,12,04,986	10,95,16,369	-	72,07,21,355	15%	34,36,89,468	4,98,26,444	-	39,35,15,912	32,72,05,443	26,75,15,518
Laboratory Equipment(Work in Progress)	3,53,38,680	-	3,53,38,680	-	15%	-	-		-	-	3,53,38,680
Computer Equipment	68,52,726	99,94,950	-	1,68,47,676	40%	60,38,198	21,89,503	-	82,27,701	86,19,975	8,14,528
Office Equipment	65,26,451	19,27,846	-	84,54,297	15%	44,11,954	4,04,235	-	48,16,189	36,38,108	21,14,497
Furniture & Fixture	1,86,02,999	40,13,499	-	2,26,16,498	10%	71,58,146	12,02,226	-	83,60,372	1,42,56,126	1,14,44,853
Capital / Building	2,03,91,33,350	83,91,678	-	2,04,75,25,028	10%	69,47,82,600	13,44,93,156	-	82,92,75,756	1,21,82,49,272	1,34,43,50,750
Sub Total (A)	**3,03,45,61,916**	**13,38,44,342**	**3,53,38,680**	**3,13,30,67,578**		**1,19,02,68,412**	**19,98,66,881**	-	**1,39,01,35,293**	**1,74,29,32,285**	**1,84,42,93,504**
(B) Project Funds											
Furniture & Fixture	3,15,984	-	-	3,15,984	10%	2,18,505	9,748	-	2,28,253	87,731	97,479
Laboratory Equipment	1,05,60,12,547	68,25,137	-	1,06,28,37,684	15%	58,97,10,131	7,13,94,107	-	66,11,04,238	40,17,33,446	46,63,02,416
Capital / Building	18,91,80,635	6,10,768	-	18,97,91,403	10%	5,12,67,952	1,38,77,862	-	6,51,45,814	12,46,45,589	13,79,12,683
Sub Total (B)	**1,24,55,09,166**	**74,35,905**	-	**1,25,29,45,071**		**64,11,96,588**	**8,52,81,717**	-	**72,64,78,305**	**52,64,66,766**	**60,43,12,578**
(C) CSCR -Vellore											
Buildings	2,46,00,000	-	-	2,46,00,000	10%	40,55,853	20,54,415	-	61,10,268	1,84,89,732	2,05,44,147
Laboratory Equipment	24,90,02,142	8,26,76,737	-	33,16,78,879	15%	18,46,01,604	2,20,61,366	-	20,66,62,970	12,50,15,909	6,44,00,538
Computer Equipment	3,57,46,731	-	-	3,57,46,731	40%	3,57,46,306	170	-	3,57,46,476	255	425
Furniture & Fixture	7,875	-	-	7,875	10%	4,977	290	-	5,267	2,608	2,898
Sub Total (C)	**30,93,56,748**	**8,26,76,737**	-	**39,20,33,485**		**22,44,08,740**	**2,41,16,241**	-	**24,85,24,981**	**14,35,08,504**	**8,49,48,008**
(D) Wadhwani Foundation											
Laboratory Equipment	6,84,372	-	-	6,84,372	15%	4,48,834	35,331	-	4,84,165	2,00,207	2,35,538
Computer Equipment	8,48,633	-	-	8,48,633	40%	8,47,851	313	-	8,48,164	469	782
Sub Total (D)	**15,33,005**	-	-	**15,33,005**		**12,96,685**	**35,644**	-	**13,32,329**	**2,00,676**	**2,36,320**
Grand Total (A+B+C+D)	**4,59,09,60,835**	**22,39,56,984**	**3,53,38,680**	**4,77,95,79,139**		**2,05,71,70,425**	**30,93,00,483**	-	**2,36,64,70,908**	**2,41,31,08,231**	**2,53,37,90,410**

*The residential building (50 Nos. Flats) at CB Site Yelahanka is constructed jointly by NCBS and inStem and the land on which it is constructed belong to NCBS. The cost is shared between both the Institutes and there is an MoU signed between both the Institutes to this effect.

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
(Registered under the Karnataka Societies' Registration Act.)
GKVK, BELLARY ROAD, BANGALORE - 560 065
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(Amount- Rs.)

SCHEDULE -9 - INVESTMENTS FROM EARMARKED /ENDOWMENT FUNDS	Current Year	Previous Year
1. In Government Securities	-	-
2. Other approved securities	-	-
3. Shares	-	-
4. Debentures and Bonds	-	-
5. Subsidiaries and Joint Ventures	-	-
6. Others (to be specified	-	-
TOTAL	-	-

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SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE -10 - INVESTMENT OTHERS	Current Year	Previous Year
1. In Government Securities	-	-
2. Other approved securities	-	-
3. Shares	-	-
4. Debentures and Bonds	-	-
5. Subsidiaries and Joint Ventures - Shares of C-CAMP- (Company registered under Section 8 Company Act)	600	600
6. Others (to be specified	-	-
TOTAL	600	600

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE

(Registered under the Karnataka Societies' Registration Act.)

GKVK, BELLARY ROAD, BANGALORE - 560 065

SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE -11 - CURRENT ASSETS, LOANS, ADVANCES ETC.	Current year	Previous Year
A. CURRENT ASSETS:	-	-
1. Inventories:	-	-
a) Stores and Spares	-	-
b) Loose Tools	-	-
c) Stock-in-trade	-	-
Finished Goods	-	-
Work -in-progress	-	-
Raw Materials	-	-
2. Sundry Debtors:	-	-
a) Debts outstanding for above six months	2,07,835	-
b) Others	2,56,806	54,649
3. Cash balances in hand (including cheques/drafts)	24	24
4. Bank Balances:	-	-
a) With Scheduled Banks:	-	-
- On current Accounts	90,21,512	59,00,638
- On Deposits Accounts(includes margin money	3,05,97,889	16,30,11,390
- On Savings Accounts	12,04,67,505	18,26,24,829
b) With Non-Scheduled Banks:	-	-
- On current Accounts	-	-
- On Deposits Accounts(includes margin money	-	-
- On Savings Accounts	-	-
5. Post Office Savings Accounts	-	-
TOTAL (A)	**16,05,51,571**	**35,15,91,529**

B. LOANS, ADVANCES AND OTHER ASSETS		
1. Loans:		
a) Staff	-	-
b) Other Entities engaged in activities /	-	-
Objectives similar to that of the Entity	-	-
c) Others (specify)	-	-
2. Advances and other amounts recoverable in	-	-
cash or in kind or for value to be received:	-	-
a) On Capital Account	-	-
b) Prepayments	-	0
c) Others	2,93,13,939	3,66,81,862
3. Income Accrued:	-	-
a)On investments from earmarked/endow. Funds	-	-
b) On investments - others	1,07,530	12,65,409
c) On Loans & Advances	-	-
d) Others	-	-
(includes income due unrealized Rs.....)	-	-
	-	-
4. Claims Receivable:	62,09,610	37,29,283
TOTAL (B)	**3,56,31,079**	**4,16,76,554**
GRAND TOTAL (A+B)	**19,61,82,650**	**39,32,68,083**

M/s. T. RAMACHANDRAN & CO. BANGALORE Chartered Accountants

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SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE -12 : INCOME FROM SALES AND SERVICES	Current Year	Previous Year
1) Income from Sales	-	-
a) Sale of Finished Goods	-	-
b) Sale of Raw Material	-	-
c) Sale of Scraps	-	-
2) Income from Services:	-	-
a) Labour and Processing Charges	-	-
b) Professional /Consultancy Services	-	-
c) Agency Commission and Brokerage	-	-
d) Maintenance Services (Equipment/ Property)	-	-
e) Others (Facility User charges)	1,08,72,378	27,44,267
TOTAL	**1,08,72,378**	**27,44,267**

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
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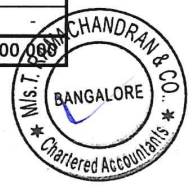
GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE -13: GRANTS/SUBSIDIES (Irrevocable Grants and Subsidies received)	Current Year	Previous Year
1) Central Government	34,41,32,896	31,27,00,000
2) State Government(s)	-	-
3) Government Agencies	-	-
4) Institutions/Welfare Bodies	-	-
5) International Organizations	-	-
6) Others (specify)-PNB	-	-
TOTAL	**34,41,32,896**	**31,27,00,000**



INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE

(Registered under the Karnataka Societies' Registration Act.)

GKVK, BELLARY ROAD, BANGALORE - 560 065

SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE-14: FEES/SUBSCRIPTIONS	Current Year	Previous Year
1. Entrance Fees	-	-
2. Annual Fees/Subscriptions	-	-
3. Seminar/Programme Fees	-	-
4. Consultancy Fees	-	-
5. Others (Specify)	-	-
TOTAL	-	-

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE

(Registered under the Karnataka Societies' Registration Act.)

GKVK, BELLARY ROAD, BANGALORE - 560 065

SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE-15: INCOME FROM INVESTMENTS	Current Year	Previous Year
A.Investment from Earmarked Fund	-	-
1) Interest	-	-
a) On Govt. Securities	-	-
b) Other Bonds/Debentures	-	-
2) Dividends	-	-
a) On Shares		-
b) On Mutual Fund Securities	-	-
3) Rents	-	-
4) Others (Specify)	-	-
TOTAL (A)	-	-
B.Investment -Others		
1) Interest	-	-
a) On Govt. Securities	-	-
b) Other Bonds/Debentures	-	-
2) Dividends	-	-
a) On Shares	-	-
b) On Mutual Fund Securities	-	-
3) Rents	-	-
4) Others (Specify)	-	-
TOTAL (B)	-	-
Total of income from investment (A+B)	-	

M/s. T. RAMACHANDRAN & CO. BANGALORE * Chartered Accountants *

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GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE - 16: INCOME FROM ROYALTY, PUBLICATIONS ETC.	Current Year	Previous Year
1) Income from Royalty	-	-
2) Income from Publications	-	-
3) Others (Specify)	-	-
TOTAL	-	-

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
(Registered under the Karnataka Societies' Registration Act.)
GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE - 17 : INTEREST EARNED	Current Year	Previous Year
1) On Term Deposits:		
a) With Scheduled Banks	16,06,400	3,11,844
b) With Non-Scheduled Banks	-	-
c) Interest of CSCR Vellore	-	-
d) Others	-	-
2) On Savings Accounts:		
a) With Scheduled Banks	-	27,45,473
b) With Non-Scheduled Banks	-	-
c) With Institutions	-	-
d) Others	-	-
3) On Loans:		
a) Employees /Staff	-	-
b) Others	-	-
4) Interest on Debtors and Other Deposits	2,39,375	3,80,281
TOTAL	**18,45,775**	**34,37,598**

M/S. T. RAMACHANDRAN & CO. BANGALORE * Chartered Accountants *

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE

(Registered under the Karnataka Societies' Registration Act.)

GKVK, BELLARY ROAD, BANGALORE - 560 065

SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE - 18: OTHER INCOME	Current Year	Previous Year
1) Profit on Sale /disposal of Assets:	-	-
a) Owned assets	-	-
b) Assets acquired out of grants, or received free of cost	-	-
2) Export Incentives realized	-	-
3) Fees for Miscellaneous Services	41,00,317	51,46,048
4) Miscellaneous Income *	55,34,232	7,31,064
TOTAL	**96,34,549**	**58,77,112**

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE

(Registered under the Karnataka Societies' Registration Act.)

GKVK, BELLARY ROAD, BANGALORE - 560 065

SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE - 19: INCREASE/DECREASE IN STOCK OF FINISHED GOODS & W.I.P	Current Year	Previous Year
a) Closing stock	-	-
- Finished Goods	-	-
- Work-in-progress	-	-
TOTAL (A)	-	-
b) Less: Opening Stock - Finished Goods	-	-
- Work-in-progress	-	-
Total (B)	-	-
NET INCREASE/(DECREASE) (A+B)	-	-

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
(Registered under the Karnataka Societies' Registration Act.)
GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF BALANCE SHEET AS AT MARCH 31, 2023

(Amount- Rs.)

SCHEDULE - 20: ESTABLISHMENT EXPENSES	Current Year	Previous Year
a) Salaries and Wages	10,75,02,291	9,57,49,994
b) Prior Period Salaries	-	-
c) Contribution to Provident Fund-NPS*	94,13,566	89,76,955
d) Contribution to other Fund (specify) - LS &Pension Contributions	89,27,600	56,98,386
e) Staff Welfare /expenses	14,31,817	15,69,883
f) Expenses on Employees' Retirement and Terminal Benefits	-	-
g) Others (specify)	-	-
h) Fellowships (JRF/SRF)	1,45,41,602	1,02,79,743
TOTAL	**14,18,16,876**	**12,22,74,961**

M/s. T. RAMACHANDRAN & CO. BANGALORE Chartered Accountants

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
(Registered under the Karnataka Societies' Registration Act.)
GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF INCOME & EXPENDITURE ACCOUNT FOR THE YEAR ENDED MARCH 31, 2023

(Amount - Rs.)

SCHEDULE - 21: OTHER ADMINISTRATIVE EXPENSES ETC.	Current Year	Previous Year
a) Purchases - Laboratory & Computer Consumables	7,93,13,399	5,09,00,458
b) other Laboratory expenses	38,94,600	40,37,962
c) Membership Fees	11,85,938	-
d) Electricity and power	5,52,46,106	6,12,45,627
e) Water charges	30,41,007	27,70,350
f) Contract for Services-CSIR	68,38,739	76,92,755
g) Repairs & Maintenance	5,01,12,862	4,92,97,209
h) Training	-	-
i) Rent, Rates, Taxes and fees	95,47,219	46,02,119
j) Vehicles running and maintenance	6,48,000	5,94,000
k) Potage, Telephone and Communication charges	18,70,174	21,63,259
l) Printing and Stationery	12,00,707	7,46,388
m) Travelling & Conveyance Expenses	58,02,967	38,82,255
n) Expenses on Seminars/Workshops	8,82,082	-
o) Subscription Expenses	4,47,550	8,54,301
p) Expenses on Fees - Consultancy Fee/Honorarium	28,48,029	33,74,484
q) Auditors Remuneration	1,34,650	1,51,775
r) Hospitality Expenses	1,94,050	1,30,142
s) Security Charges	1,28,81,863	1,38,67,354
t) Bank Charges	46,099	30,236
u) Other Contingent Expenditure	23,70,158	18,34,156
v) Advertisement & Publicity	24,10,252	27,78,100
w) Sports facility management	-	-
x) Campus maintenance	1,32,69,889	1,50,39,448
y) Canteen Expenses	68,96,062	83,26,473
z) Professional fees	1,20,767	81,092
z) Other office Expenses	13,72,715	11,42,371
TOTAL	**26,25,75,884**	**23,55,42,315**

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
(Registered under the Karnataka Societies' Registration Act.)
GKVK, BELLARY ROAD, BANGALORE - 560 065
SCHEDULES FORMING PART OF INCOME & EXPENDITURE ACCOUNT FOR THE YEAR ENDED MARCH 31, 2023

(Amount - Rs.)

SCHEDULE - 22: EXPENDITURE ON GRANTS, SUBSDIES ETC.	Current Year	Previous Year
a) Grants given to Institutions/Organizations	-	-
b) Subsidies given to Institutions/Organizations	-	-
c) Expenditure incurred on Grants (As per Schedule -3)	16,34,06,279	20,84,83,315
TOTAL	**16,34,06,279**	**20,84,83,315**

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE BANGALORE
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SCHEDULES FORMING PART OF INCOME & EXPENDITURE ACCOUNT FOR THE YEAR ENDED MARCH 31, 2023

(Amount - Rs.)

SCHEDULE - 23: INTEREST	Current Year	Previous Year
a) On fixed loans	-	-
b) On other Loans (including Bank Charges)	-	-
c) Others (specify)	8,23,409	1,20,83,693
TOTAL	**8,23,409**	**1,20,83,693**

M/s. T. RAMAN & CO. * Chartered Accountants * BANGALORE

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE, BANGALORE

(Registered under the Karnataka Societies' Registration Act)
GKVK, BELLARY ROAD, BANGALORE - 560 065

SCHEDULE FORMING PART OF ANNUAL ACCOUNTS FOR THE PERIOD ENDED MARCH, 31, 2023

SCHEDULE 24 - SIGNIFICANT ACCOUNTING POLICIES

1. ACCOUNTING CONVENTION
The Financial statements are prepared on the basis of historical cost convention.

2. INVESTMENTS
Investment are carried at cost. The decline in their value, which is other than temporary is provided for.

3. PROPERTY, PLANT & EQUIPMENT (PPE)

3.1 PPE are capitalized at cost of acquisition inclusive of inward freight, duties and taxes and incidental and direct expenses related to acquisition and it is carried in the balance sheet net of accumulated depreciation.

3.2 Cost of PPE acquired out of project funds are also taken as Assets by debiting the appropriate head of Fixed Assets of the Institute and by crediting corresponding amount to Capital Reserve. Every year Capital reserve is reversed to the extent of depreciation, calculated under the WDV method at the rates of depreciation prescribed under the Income Tax Rules, 1962. In the event of the asset being returned to the agency sanctioning the grant, the written down value will be adjusted by reversing the entries.

4. DEPRECIATION

4.1 Depreciation is provided on written down value method as per rates specified in the Income-tax Act, 1961.

4.2 Depreciation of assets commences when the assets are ready for their intended use which is generally on commissioning/installation. The total amount of depreciation on assets acquired out of Core and EMG funds for the year is transferred from Capital Reserve to Income and Expenditure Account.

5. GOVERNMENT GRANTS / SUBSIDIES

5.1 Grants received from the Government are of two types: (a) Non-Recurring Grants - which are for the purpose of acquiring Capital Assets. The amount of grants received is initially credited to Corpus / Capital fund account and expenditure incurred for acquisition of capital assets is debited thereto. The balance in this account represents the unspent amount of non-recurring grant. The amount equivalent to capital assets added during the year is added to capital reserve account. (b) Recurring Grants - which are for the purpose of recurring expenditure and are taken directly to Income & Expenditure Account. Unspent balance/excess of expenditure over income is shown in Reserves & Surplus Account distinctly under General Reserve. The combined balance in this account is the total unspent balance of grants.

5.2 Government grants / subsidy are accounted on receipt basis.

6. EARMARKED/ENDOWMENT FUNDS

6.1 Project Funding by both Government and non-government agencies to whom a statement of account of the expenditure incurred together with a utilization Certificate of the amount released has to be furnished are accounted under this heading. Fellowships/Scholarships sanctioned by UGC/CSIR and other agencies are also accounted under this head in order to watch the balance available/recoverable on each such award. Based on the conditions and limits stipulated in the sanction order, expenditure is incurred.

6.2 Such Earmarked/Endowment Funds towards specific projects, to the extent unspent is carried in the Balance Sheet as a liability under the head "Earmarked/Endowment Funds". Project-wise details of funds received and corresponding expenditure during the year is furnished along with opening and closing unspent balances of specific funds under Schedule 3. Upon Completion of the project in its entirety, the same is removed from the list in Schedule 3. When tangible Fixed Assets are acquired out of the projects funds, the appropriate head of Fixed Assets is debited with corresponding credit to Capital Reserve. Every year Capital reserve is reversed to the extent of depreciation, calculated under the WDV method at the rates of depreciation prescribed under the Income Tax Rules, 1962.

7. FOREIGN CURRENCY TRANSACTIONS

7.1 Transactions denominated in foreign currency are accounted at the exchange rate prevailing at the date of the transaction.

7.2 Current assets, foreign currency loans and current liabilities are converted at the exchange rate prevailing as at the year end. The resultant gain / loss is adjusted to cost of fixed assets, if the foreign currency liability relates to fixed assets, and in other cases is considered to revenue.

8. LEASE

Lease rentals are expensed with reference to lease terms.

9. RETIREMENT BENEFITS

9.1 The provision for leave encashment is provided based on the actuarial valuation. The Institute has a plan with Life Insurance Corporation of India who provides the actuarial valuation.

9.2 The provision for gratuity is provided based on the actuarial valuation. The Institute has a group gratuity plan with Life Insurance Corporation of India who provides the actuarial valuation.

10. REVENUE RECOGNITION

10.1 INCOME FROM FACILITY SERVICES: Income is derived from services in relation to usage of facilities available at the institute by external users and are billed at specified rate on accrual basis along with applicable GST.

10.2 INTEREST FROM BANKS: Interest earned is accounted on accrual basis as per bank statement.

10.3 MISC INCOME: Charges for internal usage of facilities at the institute for research purposes is accounted (net) under this head. As this is purely for internal purposes no GST is applicable.

R. RAMACHANDRAN & CO. BANGALORE Chartered Accountants

INSTITUTE FOR STEM CELL SCIENCE AND REGENERATIVE MEDICINE, BANGALORE

(Registered under the Karnataka Societies' Registration Act)
GKVK, BELLARY ROAD, BANGALORE - 560 065

SCHEDULE FORMING PART OF ANNUAL ACCOUNTS FOR THE PERIOD ENDED MARCH, 31, 2023

SCHEDULE 25 - CONTINGENT LIABILITIES AND NOTES ON ACCOUNTS

1. CONTINGENT LIABILITIES

1.1 In respect of:
- Bank guarantees given by / on behalf of the Entity - Rs. NIL /- (Previous year Rs. NIL/-)
- Letters of Credit opened by Bank on behalf of the Entity - Rs. NIL/- (Previous year Rs. NIL/-)
- Bills discounted with banks Rs. NIL/- (Previous year Rs. NIL/-)

1.2 Disputed demands in respect of:
- Income-tax Rs.NIL/- (Previous year Rs. NIL/-)
- Sales-tax Rs. NIL/- (Previous year Rs. NIL/-)
- Municipal Taxes Rs. NIL/-(Previous year Rs. NIL/-)

1.3 In respect of claims from parties for non-execution of orders, but contested by the Entity - Rs. NIL/- (Previous year Rs. NIL/-)

1.4 NCBS–TIFR was the executing agency for the construction of inStem Building. Accordingly, NCBS - TIFR has issued work orders for Construction of Buildings for Laboratories & Associated facilities and one such work order was issued on M/s. URC Constructions in pre-GST regime.

Against the said Work Order M/s. URC Construction have claimed amount towards GST at the rate of 18% which has been disputed by NCBS- TIFR based on circular No. SE/TAS/GST/04 from CPWD dated 22.09.2017. Currently the subject matter is under arbitration (A. C. No. 401 / 2022) and the decision of the arbitrator is yet to be pronounced. As per Executing Agency (NCBS - TIFR), the total claim of M/s. URC Construction is about Rs.3.48 Crores towards unpaid GST.

As on 31.03.2023, an advance amount of Rs.1.52 Crore is with NCBS.

2. CAPITAL COMMITMENTS

Estimated value of contracts remaining to be executed on capital account and not provided for Rs. NIL/- (Previous year Rs.NIL/-)

3. LEASE OBLIGATIONS

M/S T RAMACHANDRAN & CO. BANGALORE Chartered Accountants

Future obligations for rentals under finance lease agreements for plant and machinery amount to Rs.NIL/- (Previous year Rs. NIL/-).

4. CURRENT ASSETS, LOANS AND ADVANCES

In the opinion of the Management, the current assets, loans and advances have a value on realization in the ordinary course of business, equal at least to the aggregate amount shown in the Balance Sheet.

5. TAXATION

The Society is registered under section 12A of the Income Tax Act, 1961 under the category Charitable Trust. The Society is filing the income tax return by claiming exemption under section 11 of the Income Tax Act, 1961.

6. FOREIGN CURRENCY TRANSACTIONS (Amount in Rs.)

6.1 Value of Imports Calculated on C.I.F. Basis:

Particulars	Current Year	Previous Year
Purchase of Finished Goods	NIL/-	NIL/-
Raw Material & Components (including in transit)	NIL/-	NIL/-
Capital Goods	7,35,67,362/-	3,75,45,529/-
Stores, Spares & Consumables	1,49,71,715/-	1,65,17,507/-

6.2 Expenditure in foreign currency:

Particulars	Current Year	Previous Year
Travel	NIL/-	NIL/-
Interest payment	NIL/-	NIL/-
Collaboration Expense	NIL/-	NIL/-
Remuneration	14,08,841/-	14,94,647/-
Publication charges & Training	25,57,084/-	11,56,484/-

6.3 Earnings:

Particulars	Current Year	Previous Year
Value of Exports	NIL	NIL

7. Remuneration to auditors

Particulars	**Amount(Rs.)**
As Statutory Auditors*	134,650/-
For Taxation Matters	NIL/-
For Certification	NIL/-

* Audit fee for inStem and CSCR is Rs.94,400/- and Rs.40,250/- respectively.

8. The Institute, National Centre for Biological Sciences (NCBS), Tata Institute Genetics and Society and C-Camp are located in a common campus. As per the MOU entered into between the four(4) entities, common expenditure incurred by any institutes is shared by all other three entities. The Institute accounts these expenditures on the basis of the Debit Note raised by respective institutes.

9. Expenses are allocated between Core and EMG based on the scientific progress achieved in laboratories utilising common inputs.

10. Share Certificates of C-Camp are held in the name of persons employed in the BLiSC campus as representatives of the institute.

11. The Institute's Building and Infrastructure are located on Lease Hold Land. The lease deed is between The University of Agricultural Sciences (UAS) and Department of Bio-Technology, Ministry of Science and Technology (DBT) have entered into a Lease Deed on 04-11-2009 whereby the UAS has granted 20 acres of land on 49 years of lease to DBT for establishment of the Institute.

12. Corresponding figures for the previous year have been regrouped / rearranged, wherever necessary.

13. Schedules 1 to 25 are annexed to and from an integral part of the Balance Sheet as at March 31, 2023 and the Income and Expenditure Account for the year ended on that date.

इन्स्टेम
inStem

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