inStem
CSCR
CENTRE FOR STEM CELL RESEARCH
(a unit of inStem, Bengaluru)
Christian Medical College Campus
Bagayam, Vellore - 632002

ANNUAL REPORT

2024

NOT TO BE MINISTERED UNTO BUT TO MINISTER
CHRISTIAN MEDICAL COLLEGE
VELLORE
INDIA

CSCR

Center for Stem Cell Research

(a unit of inStem Bengaluru)
at Christian Medical College Vellore, India

Content

Annual Report

2024

1st March, 2025

FOREWORD

Dear Friends,

The Centre for Stem Cell Research (CSCR) at Christian Medical College Vellore was established with a vision to promulgate innovations in the area of cell biology, gene therapy, and stem cell research, eventually with the fostering hand of Institute for Stem Cell Science and Regenerative Medicine (inStem) in Bengaluru, along with the Christian Medical College, Vellore, India.

The initial years and foundational contributions of Prof Alok Srivastava and his colleagues and the department of Haematology at CMC, Vellore were epochal in ensuring a sound foundation.

This compendium, which enlists the work of many scientists who have burnt the midnight oil and put in many decades of thought with their dedicated teams to conjure transformational change, has massive value that is both currently relevant and eventually translational in making a difference towards patient care.

Looking forward into the immediate future, CSCR has made great beginnings in cell and gene therapy, particularly in the field of Haematology.

This beckons us to diversify and move forward in multiple directions, making forays into the territories of cancer, drug development, metabolic disorders, bone issues, neurological diseases, and many more areas in medical science.

In the years to come, the CSCR, together with inStem and CMC, Vellore would like to welcome basic scientists, translational biologists, clinicians and other innovators from across the country and also the world to amalgamate their ideas and skills and ensure that its contributions to science in India are both unique and progressive.

I would like to thank all the scientists and our RDO who have contributed massively towards the preparation of this document.

We would like to thank Dr. Maneesha Inamdar, the Director of inStem, Bengaluru, Prof Vikram Mathews, the Director at CMC Vellore and Dr Solomon Satish, the Principal at CMC Vellore for their enduring support.

Bonne Lecture!
Yours Sincerely,

Prof. Nihal Thomas, MD, MNAMS, DNB(Endo), FRACP(Endo) FRCP(Edin), FRCP(Glas),FRCP(London) FACP, FAMS,PhD(Copenhagen)
Head, Centre for Stem Cell Research (a unit of inStem, Bengaluru)
Senior Professor, Department of Endocrinology, Diabetes and Metabolism, Principle Coordinator, ICMR Collaborating Centre of Excellence in Diabetes
Christian Medical College, Vellore, India
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Centre for Stem Cell Research (CSCR)
(a unit of inStem, Bengaluru)
Christian Medical College Campus, Bagayam,

The Centre for Stem Cell Research (CSCR) was sanctioned in December 2005 as a public-private partnership between the Christian Medical College (CMC) Vellore and the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India. After the project phase in July 2011, CSCR (www.cscr.res.in) was integrated with the Institute for Stem Cell Science and Regenerative Medicine (inStem), Bengaluru (www.instem.res.in) as its translation unit through a tripartite partnership located at and managed by CMC, Vellore.

Mandate

The mandate of CSCR is to apply stem cell science and gene manipulation technologies to regenerative medicine to create innovative therapeutic options for the management of human diseases with unmet needs. CSCR has evolved thematic research to address selected areas of therapeutic needs. It also contributes to the development of trained personnel for this field through doctoral programs as well as other training opportunities. Its facilities and expertise are available for all scientists and institutions working in this field in the country.

Foundation:

2005 – 2010: A project with a difference

Though initiated as a project through the MoU between DBT, and CMC, Vellore, CSCR was managed differently and had a Governing Body, chaired by the Secretary DBT and also had a Finance Committee. There also was a DBT-designated Scientific Advisory Committee that reviewed the work done at CSCR every year. In addition, there were two committees appointed by CMC, Vellore to help with the initial scientific and administrative management of CSCR.

2011 onwards: CSCR – the translational unit of the inStem, Bengaluru

As mentioned above, CSCR, as the translational unit of inStem Bengaluru, is located at the Bagayam campus of CMC, Vellore with its focus in the last decade on regenerative medicine through cell and gene therapies. It continues to have a separate Scientific Advisory Committee which meets annually to review its scientific directions and progress.

The past decade – Growth and consolidation…..

It is now nearly 20 years since CSCR was sanctioned in 2005. As was already anticipated when crafting this project, success would depend on finding the right

people to make a good team. The first decade was extremely challenging from that perspective but over the last decade, a good scientific team has been put together who have created a very collaborative and mutually supportive environment that has helped establish cutting-edge technologies in cell and gene therapy research.

As the reports from individual labs will show, overall three thematic areas evolved: i. Musculoskeletal Regeneration; ii. Cellular Reprogramming and its Applications; iii. Gene Therapy of Haematological Diseases. Exceptional achievements have been recorded in all of them. The gene therapy program particularly has generated novel targets with a range of technologies from viral vectors to the full spectrum of gene editing methods to generate intellectual property-protected potential gene therapy products.

The past 1-2 years – Touching excellence…..

The past two years have been specially rewarding and successful for CSCR with the successful initiation of a first-in-human clinical trial of gene therapy of haemophilia A using a novel lentiviral vector-mediated transduced autologous haematopoietic stem cell-based expression of a novel FVIII transgene. This clinical trial which was initiated in June 2022 completed recruitment and therapy of the last participant by June 2024. The results have been excellent so far with all participants showing clinically important responses with measurable FVIII expressions. This achievement was announced by the Hon Union Minister of Science and Technology on the National Science Day in February 2024. (https://pib.gov.in/PressReleaseIframePage.aspx?PRID=2009823).

CSCR is specially privileged to have two scientists being recognized in the same year by the prestigious DBT-Wellcome Trust Team Science grants. It is also very gratifying to see our students getting post-doc positions in the best labs in the world.

It is obvious from the above that in the last decade, CSCR has established itself as a leading centre for cell and gene therapy in India and is also being recognized for its work internationally. There is increasing participation by leading international experts in its annual cell and gene therapy symposium. CSCR has achieved its given mandate of bringing cutting-edge translational regenerative medicine science to India in a way that can address several unmet health care needs as well as give a thrust and advantage to the biotechnology industry in the country.

Alok Srivastava
Former Head, CSCR

Annual Report

2024

VISION

To lead in stem cell science and regenerative medicine through translational research

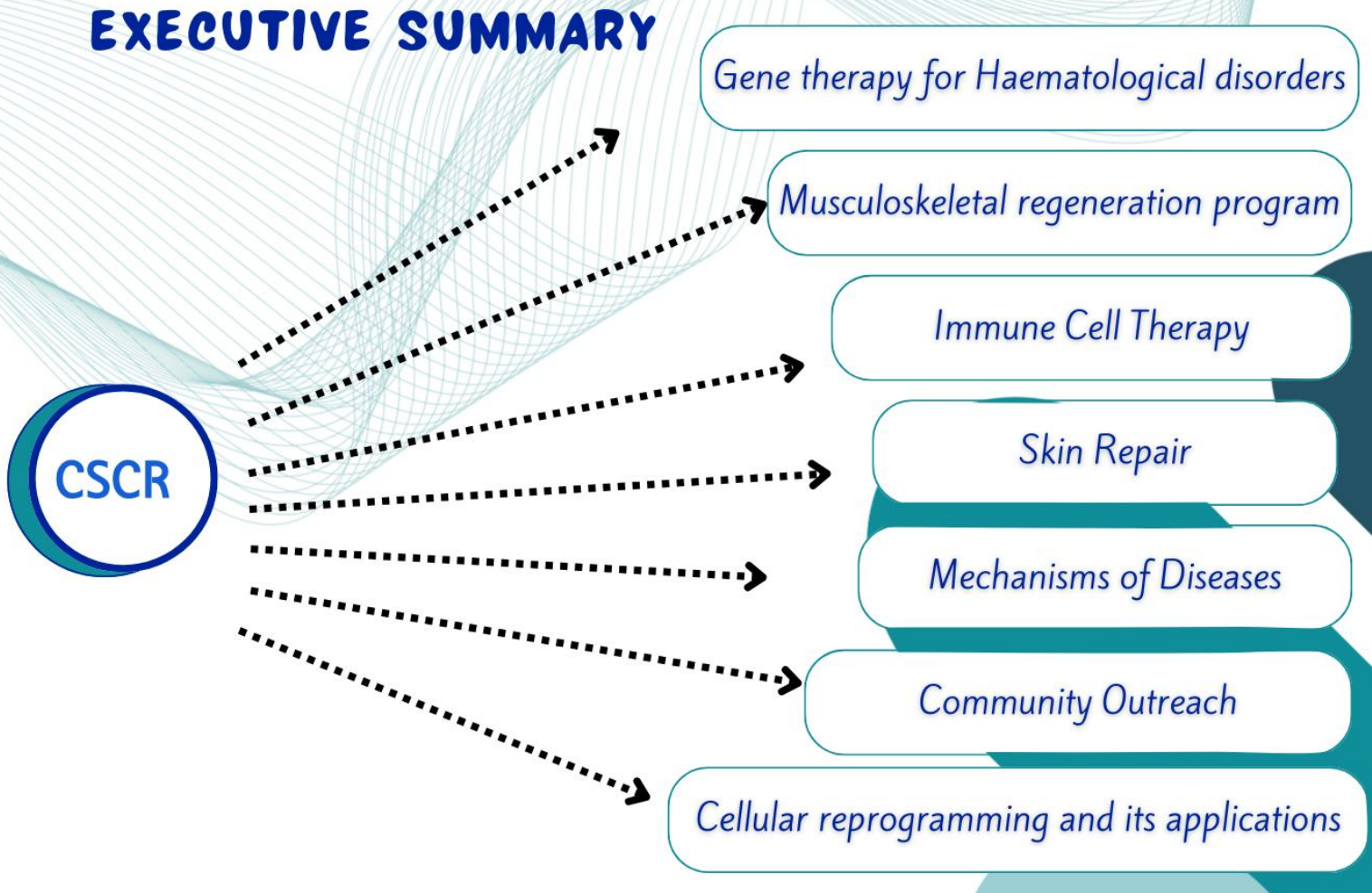
MISSION

Develop cell and gene therapy for unmet medical needs in India and make them accessible to all patients

FOCUS Area

Cell and gene therapy for haematological and musculoskeletal disorders

EXECUTIVE SUMMARY



A. THEMATIC RESEARCH PROGRAM

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.

1. Musculoskeletal Regeneration Program

This program has two components. The first one was coordinated by Vrisha Madhuri. After her superannuation in January 2024, this program is now completed. The major focus was on clinical and preclinical translation related to physis, articular cartilage, bone, and muscle regeneration. Towards this, we have two major areas of focus. The first is a cell-based therapy for bone, cartilage, and muscle regeneration. In collaboration with the Karolinska Institute, Sweden, we completed a Phase I/II clinical trial for the treatment of osteogenesis imperfecta using fetal liver-derived mesenchymal stem cells. The trial yielded significant improvements in bone quality and a reduction in fracture rates. Following this success, we developed technology for the indigenous production of fetal liver MSC at CSCR. In collaboration with TIGS, Bengaluru, we also developed technology for the production of iPSC-derived iMSCs. Additionally, we completed another Phase I/II clinical trial using culture-expanded muscle-derived stem cells to treat urinary sphincter incontinence, resulting in significant improvement in quality of life.

The second is the cell-free therapy for cartilage and bone regeneration using biomolecules. In collaboration with multidisciplinary groups from SCTIMST, Trivandrum, Kerala, and CSCR, we identified suitable biomaterials with kinetics for the sustained release of therapeutic biomolecules. The newer initiative includes the use of extracellular vesicles for the treatment of osteoporosis in genetic defect animal and cellular models. We are currently testing a point-of-care device in a large animal model for treating physeal bar and articular cartilage repair. This device aims to be a single-step procedure, eliminating the need for cell expansion in GMP conditions.

The second program is coordinated by Elizabeth Vinod and includes Solomon Sathishkumar, Alfred Job Daniel, Abel Livingston, and Viju Daniel Varghese in the group. Their current research focuses on evaluating the regenerative potential of chondroprogenitors for chondral defects, comparing them to commonly used cells such as BM-MSCs and chondrocytes. They are also investigating the use of soluble factors derived from these progenitors to cultivate injectable therapeutic molecules, using animal osteoarthritic models. Additionally, in collaboration with VIT, the lab is conducting studies using progenitors within nano-composite bioscaffolds as alternative extracellular matrix options.

2. Gene Therapy Program

This program was coordinated by Alok Srivastava. The other investigators in this group include RV Shaji, Mohankumar Murugesan, Saravanabhavan Thangavel, Srujan Marepally, and Gurbind Singh. This involves two major components – The first is directed towards haemophilia where two technologies were being pursued. First, the AAV vector-based gene therapy for haemophilia B in collaboration with Emory University, Atlanta, USA, and the University of Florida, Gainesville, USA.

However, the so far insurmountable technical challenges with GMP AAV production in India have forced its closure.
The second component is the use of a novel lentiviral vector-mediated autologous haematopoietic stem cell-based gene therapy for haemophilia A. This has been done in collaboration with Emory University, USA.

A very special achievement has been the completion of phase 1 of this first-in-human clinical trial of gene therapy for haemophilia A by June 2024 after having been initiated in June 2022. A total of six participants have been treated with no major unexpected safety concerns. It is highly gratifying that there has also been a clinically significant therapeutic response in all of them. These findings have been reported in several international meetings earlier this year. It was also mentioned by the Union Minister for Science and Technology in his address to the country on National Science Day in February 2024.

The second part of the gene therapy program involves gene therapy for the major haemoglobin disorders. Here again, two different technological approaches have been taken. First, a lentiviral vector-based gene addition of beta or gamma globin gene and a gene-modulation technologies using shRNA against the BCL11A gene as well gene editing approaches using CRISPR-Cas9 and base editing technologies to modulate HbF levels. For all of these, proof-of-concept studies have been completed in animal models.

3. Cellular Reprogramming and Its Applications - Disease Modelling and Haplo-banking

The area of cellular reprogramming technology is coordinated by R. V. Shaji at CSCR along with Dolly Daniel from the Department of Immunohematology and Transfusion Medicine, CMC. This is now being applied to two areas: disease modelling and haplobanking.
Important disease models for otherwise difficult-to-study bone marrow failure syndromes such as Fanconi anemia, Diamond Blackfan anemia, and congenital dyserythropoietic anemias have been created and used for understanding disease biology towards uncovering the basis of disease phenotypes and evaluation of gene correction strategies.

The translational application of iPSCs towards establishing "haplobank" – cells from HLA haplotype homozygous individuals has been achieved. So far, 10 GMP grade cell lines have been produced covering many of the most common HLA haplotypes in Indian donor registries. Final characterization work and reporting them to repositories remains to be completed. This has been done in collaboration with the international consortium for this effort – Global Alliance for iPSC Therapies (GAiT).

A. NOVEL APPROACHES TO HEMATOLOGICAL DISEASES (NAHD) PROGRAM

This program was initiated in 2016, through the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India as a major effort for 'Accelerating the application of Stem cell technology in Human Disease' or ASHD program and was located at the following institutions – The Christian Medical

College (CMC) with the Centre for Stem Cell Research (CSCR), a unit of inStem, at Vellore which focuses on applications in haematological diseases and the other component at the National Centre for Biological Sciences (NCBS), Institute for Stem Cell Science and Regenerative Medicine (inStem), and the National Institute for Mental Health and Neurosciences (NIMHANS) at Bengaluru focuses on neurological disorders. It was completed in April 2023.

The program at CSCR / CMC - Novel Approaches to Haematological Disorders (NAHD) aims to enhance current methods/technologies including gene therapy for hereditary blood disorders such as haemophilia, thalassemia and sickle cell disease, all of which are causes of significant morbidity and mortality in India. This part was completed in April 2023. The details are presented in the reports of individual scientists or other faculty involved. In addition, there is a community outreach component within NAHD related to developing a model for the control of the major haemoglobin disorders in India. This is being implemented in Odisha in collaboration with the Government of Odisha through the National Health Mission program. This part was completed in April 2024. This program successfully developed a comprehensive model for disease control and burden reduction. This model has now been handed over to the Government of Odisha for further implementation and expansion across the state.

The Major Components of this Program are:

Clinical Trials for Gene Therapy of Haemophilia

»» Hemophilia B (see the report by Alok Srivastava)
»» Hemophilia A (see the report by Alok Srivastava)
»» AAV Antibody Assays

Gene Therapy for Thalassemia and Sickle Cell Disease

»» Lentiviral Approach (see report of R V Shaji)
»» Gene Editing (see reports of Saravanabhavan / Mohankumar)

Applications of Induced Pluripotent Stem Cell (iPSC)Technology

»» Disease Modelling for Erythroid Disorders (see the report of R V Shaji)
»» Haplobanking (see report of Dolly Daniel)

Control of Sickle Cell Disease & Thalassemia Major in Odisha Program

»» Behaviour Change & Communication [BCC] (See report of Shantidani Minz)
»» Training of personnel (See report of Kuryan George and Jiji Elizabeth Mathew)
»» Laboratory Services [Screening & Diagnosis] (See report of S. C. Nair and R. V. Shaji)
»» Data Management (See report of Venkat Raghava)

Further details of the projects, which are within the thematic research programs that are ongoing in CSCR, are shown in the reports of the individual scientists.

B. RESEARCH PROJECTS

Given the overall mandate of translation of basic research at CSCR, the focus continues supporting and harnessing available interest and expertise at the Christian Medical College, Vellore, to address unmet clinical needs.
It is hoped that one or more of these will attract enough people and acquire the necessary depth of work to evolve into a thematic program aimed at solving an unmet therapeutic need in the country. Currently, these include the following:

1. **Project title: Immune Cell Therapy**
   Name of the Investigator: Thiyagaraj Mayuranathan

   Project title: Generation of Off-the-Shelf Allogenic Anti-CD19 CAR-T Cells for B-Cell Malignancies
   Thiyagaraj Mayuranathan is working on the generation of allogenic CD19 CAR-T cells from healthy donors by two different modalities. First, they plan to eliminate T-cell receptor alpha constant (TRAC) and Beta-2 microglobulin (B2M) genes using base editors to avoid graft-versus-host disease (GvHD) and rejection. Second, they plan to generate virus-primed CD19 CAR-T cells to recognize and eliminate CD19+ tumor cells.

2. **Cell Therapy for Ocular Disorder**
   Project title: Cell Therapy for Ocular Disorder
   Name of the Investigator: Jeyanth Rose
   The Ocular stem cell Lab is focused on the therapeutic use of Mesenchymal Stem Cells (MSCs) and MSC-conditioned medium in treating ocular surface disorders and in mitigating the mast cell response in severe allergies related to the conjunctiva. Our other interests include translational work with corneal scarring, dry eye, and infections of the ocular surface.

3. **Mechanisms of Disease**
   Name of the Investigator: Eunice Sindhuvi
   Project title 1: Biology of Iron in RBC Regeneration: Upcoming Players of Regenerative Medicine

   Eunice Sindhuvi is working on the characterization of human erythroblasts at distinct stages and its implications on iron regulatory mechanisms during normal erythropoiesis in vitro. They hypothesize that by studying the behaviour of HSCs in an increased erythropoietic state of pregnancy will lead to a better understanding of RBC regeneration. They are also elucidating the role of Mesenchymal Stem Cells, Immune and Telomere Biology in the regeneration and differentiation of Haematopoietic Stem Cells.

   Project title 2: Elucidating the Role of Mesenchymal Stem Cells, Immune and Telomere Biology in Regeneration and Differentiation of Haematopoietic Stem Cells
   The indefinite self-renewal and potential to differentiate into other types of cells represent stem cells as frontiers of regenerative medicine. Hematopoietic stem cells (HSCs) are arguably the well-characterized tissue-specific stem cells and are in routine use clinically. Stem cell transplantation in the backdrop of regenerative medicine banks on the unique potential of stem cells to regenerate the entire hematopoietic system. Recent studies suggest that mesenchymal stem cells (MSCs) are critical for forming a niche that maintains and directs HSCs self-renewal and differentiation.

4. Skin Regeneration

Name of the Investigator: Susan Jehangir Homi

Project title: GMP Grade Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) with an *Aloe vera* Polycaprolactone Composite Scaffold in the Topical Treatment of Partial-thickness Burns in a Rat Burn Model: Validation and Toxicity Testing.

Susan Jehangir is working on validating and testing the toxicity of GMP grade Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) with an *Aloe vera* Polycaprolactone (A-PCL) composite scaffold for the topical treatment of deep second degree burns in a rat burn model. The findings will make an important contribution to the field of regenerative medicine, particularly in terms of improving the quality and effectiveness of skin restoration procedures. Future research will concentrate on scaling up production processes and investigating the use of this technology in various therapeutic settings that need successful tissue regeneration.

C. LIST OF PATENTS FILED:

2023-2024

1. A METHOD FOR MODIFICATION OF ß-GLOBIN GENE. Mohankumar K. Murugesan, Alok Srivastava, Kirti Prasad. (Indian Patent Application No. 202241030885)' May 2023 (Waiting for approval for filing RFE)
2. A METHOD FOR REACTIVATION OF FETAL HEMOGLOBIN AND A COMPOSITION THEREOF. Saravanabhavan Thangavel, Alok Srivastava; (Indian Patent Application No. 202241030465), May 2023. (Waiting for approval for filing RFE)
3. STRATEGIES FOR PRECISION EDITING OF HOMOLOGUS REGIONS. Inventor(s): Mohankumar K. Murugesan, Alok Srivastava, Anila George, Nithin Sam Ravi, Kriti Prasad (Application No: 202341082277), 4 Dec, 2023.

2022-2023

1. COMPOSITIONS AND METHODS FOR TREATING A ß-THALASSEMIA DISEASE. Inventor(s): David I.K. Martin, Mark DeWitt, Mark C. Walters, Wendy J. Magis, Saravanabhavan Thangavel and Dario Boffelli, (U.S. Pat. App. No. 63/251,229), October, 2022.
2. A METHOD FOR ELEVATION OF GAMMA GLOBIN, Mohankumar K. Murugesan, Alok Srivastava, Nivedhitha D, Vignesh R; (Patent Application No.: 202241055876), September 2022.
3. A METHOD FOR DOWNREGULATION OF A TARGET GENE AND A COMPOSITION THEREOF.
4. Inventor(s): Shaji R Velayudhan, Alok Srivastava, Mohankumar K. Murugesan; (Application No. 202241045242); Aug 2022
5. FORMULATIONS, LIPID COMPOUND, METHODS AND THEREOF. Inventor(s): Srujan Marepally, Alok Srivastava, (PCT Application No.: PCT/IN2022/050660); July-2022

6. A METHOD FOR MODIFICATION OF ß-GLOBIN E. Inventor(s): Mohankumar K. Murugesan, Alok Srivastava, Kirti Prasad; (Application No. 202241030885), May 2022
7. A METHOD FOR REACTIVATION OF FETAL HEMOGLOBIN AND A COMPOSITION THEREOF. Inventor(s): Saravanabhavan Thangavel, Alok Srivastava; (Application no: 202241030465), May 2022.

D. NO. OF PUBLICATIONS (2023-2024): 34

E. MAJOR EXTRAMURAL GRANTS APPROVED (2023-2024)

A. Department of Biotechnology, Ministry of Science & Technology, New Delhi

1. **Project Title:** Novel Applications of Technology for Haematological Disorders – Translational Research to Clinical Trials (NAT-HD)
   Coordinator: Alok Srivastava; **Joint Coordinator**: R V Shaji
   Duration: 2024 – 2027

Sub-Projects details:

(i) **Name of Principal Investigator:** R. V. Shaji
Project A1: Generation of novel lentiviral gene therapy vectors for haemoglobinopathies

(ii) **Name of Principal Investigator:** Saravanabhavan Thangavel
Project A2: The safety and scale-up studies with gene-edited Haematopoietic Stem and Progenitor cells for the treatment of β-haemoglobinopathies

(iii) **Name of Principal Investigator:** Mohankumar K. Murugesan
Project A3: Targeting the gamma-globin regulatory elements using base editors for clinical application in patients with β-haemoglobinopathies

(iv) **Name of Principal Investigator:** Alok Srivastava
Project C2: Lentiviral vector transduced hematopoietic stem cells (HSC) gene therapy product for Phase 1 clinical trial to treat patients suffering from hemophilia A.

2. **Project Title:** Point of Care Cartilage Repair Device (CARD) for chondrocyte autologous therapy (CAT).
   Name of Principal Investigator: Susan Homi Jehangir/ Dr. Vrisha Madhuri
   Project Duration: 2022 - 2025

B. Science and Engineering Research Board (SERB), DST, New Delhi

1. **Project Title:** Comparative profiling of the immunogenic and immunomodulatory properties between bone marrow-derived mesenchymal stem cells, migratory chondroprogenitors, fibronectin assay-derived chondroprogenitors, and chondrocytes, from non-diseased and osteoarthritic human articular cartilage.

   Name of Principal Investigator: Elizabeth Vinod
   Project Duration: 2023 - 2026

2. **Project Title:** Precise correction of HbE and major β-thalassemia mutation using base editors.
   Name of Principal Investigator: Mohan Kumar Murugesan
   Project Duration: 2023 -2026.

C. Indian Council of Medical Research (ICMR), New Delhi

1. **Project Title:** Therapeutic rescue of neutrophil maturation arrest by base editing of ELANE in severe congenital neutropenia
   Name of Principal Investigator: Mohan Kumar Murugesan
   Project Duration: 2023 - 2026

D. DBT/Wellcome Trust India Alliance

1. **Project Title:** Functional assessment of genetic variants associated with fetal hemoglobin levels using base editor-mediated saturated mutagenesis for the treatment of beta-hemoglobinopathies.
   Name of Principal Investigator: Mohankumar Murugesan
   Project Duration: 2023 - 2028

2. **Project Title:** In vivo gene editing of hematopoietic stem and progenitor cells for HIV gene Therapy.
   Name of Principal Investigator: Saravanabhavan Thangavel
   Project Duration: 2023 – 2028

E. DBT Ramalingaswami Re-entry Fellowship

Project Title: Generation of efficient Chimeric Antigen Receptor (CAR) T-cells to treat B cell acute lymphoblastic leukemia.
Name of Principal Investigator: Thiyagaraj Mayuranathan
Project Duration: 2024 – 2026

1. MUSCULOSKELETAL REGENERATION PROGRAM

VRISHA MADHURI
Former Professor, Department of Pediatric Orthopedics, CMC
Former Adjunct Scientist, CSCR, Vellore

Dr. Vrisha's Team Members

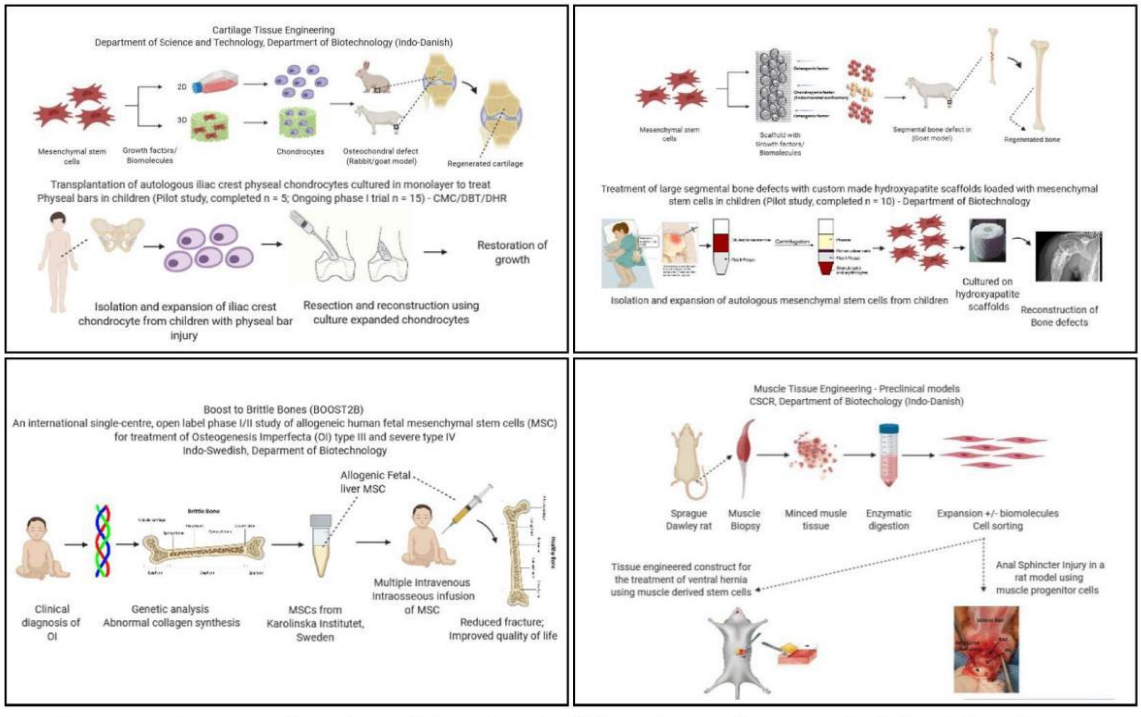
The major focus of our research group is on clinical and preclinical translation related to physis, articular cartilage, bone, and muscle regeneration. In the last year, the focus of work has been on two clinical trials and two large animal studies. In the field of bone regeneration, a phase I/II clinical trial in collaboration with Karolinska Institutet, Sweden for the treatment of osteogenesis imperfecta has completed 2 years of follow-up with 3 patients receiving cell therapy and 14 serving as concurrent controls. The results establish the safety of the intraosseous injection procedure and cell therapy. The efficacy in terms of growth, fracture rate, bone density, and quality of life is also established.

We are exploring the possibility of establishing novel cell-free therapy for cartilage and bone tissue regeneration in large animals. We have generated functionalized scaffolds with biomolecules to act as a cell-free therapeutic product that can augment the regeneration of cartilage without leading to hypertrophy. We have an ongoing large animal trial for biomolecules and cells on scaffolds for cartilage regeneration in a critical-size defect. Experiments are completed and we are following up for results at 6 months. The in vitro work for bone tissue engineering using novel scaffolds and the establishment of critical size defects is completed.

A novel point-of-care device for cartilage tissue repair is under development under a new DBT project (ATGC) with large animal validation of the device. This device can bypass the need for a GMP facility to expand the cells and reduce the two-step surgical procedure to a one-time procedure, where the harvest and implantation both take place at the same time. The device for harvest of cells and protocols for digestion within one hour have been completed and in vitro cultures on the chosen scaffolds and in-vivo work will follow.

Under the ICMR funding, we have optimized and standardized cGMP protocols for the expansion of muscle-derived stem cells (MDSCs) for the treatment of urinary incontinence, a phase I/II clinical trial. Two patients have undergone transplantation of MDSCs into damaged urinary sphincter with clinical improvement in Stamey grade at three months follow up.

Working on the treatment strategy for Osteogenesis Imperfecta (OI) We have created cellular disease models of OI using iPSC technology from patient-derived cells with variants in the COL1A1, COL1A2 SERPINF1, and WNT1 genes using gene editing technology. These cellular models will be used for screening small molecules that can rescue the disease phenotype. Further, we are exploring the use of iPSC derived from fetal MSC and differentiated to iMSC as an alternate source of stem cells for treating osteoporotic disorders.



Regenerative strategies using cell-based and cell-free therapy for musculoskeletal disorders

PUBLICATIONS

1. Selina A, Kandagaddala M, Kumar V, Abraham SSC, Danda S, Madhuri V. SERPINF1 gene variants causing late-onset progressive deforming osteogenesis imperfecta– A study of 18 patients from India. Bone Rep. 2023 Jun 1;18:101690.
2. Jaybhaye A, Lg S, Dash N, Verghese V, Chacko A, Madhuri V, Palocaren T, Gahukamble A, James D, Prakash J, Rose W. Clinical Spectrum and Microbial Etiology of Bone and Joint Infections in Children: A Retrospective Analysis from South India. Am J Trop Med Hyg. 2023 Apr 10;108(5):936-941. doi: 10.4269/ajtmh.22-0327.
3. Selina A, James D, Madhuri V. A Novel Biallelic Splice Site Variant in the SPARC Gene Causing Severe Osteogenesis Imperfecta. Indian J Pediatr. 2023 Mar 30. doi: 10.1007/s12098-023-04541-9.

INVITED TALKS

1. Dr. Vrisha M. presented 'Stem cell technology in bone repair and regeneration' at the PK Duraiswamy Oration at the North Zone Orthopaedic Association meeting on 13th May 2023.
2. Dr. Vrisha M. delivered the POSICON oration on 'Research in the making of the Paediatric Orthopaedics' on February 11th, 2023.
3. Dr. Vrisha M. gave a guest lecture on 'Stem cell therapy in Orthopaedics Present and the future' at the Kerala Orthopaedic Association in February 2023.
4. Mr. Ashis Kumar presented "Boost to brittle bone: An evaluation of safety and efficacy of multiple intravenous and intraosseous injections of fetal liver-derived MSCs in children with Osteogenesis Imperfecta (An exploratory open-label phase I/ II clinical trial)" in DBT-inStem Annual Review of Research on 23-25, March 2023.

ELIZABETH VINOD
Physician (Associate Professor), Department of Physiology, CMC, Vellore
Adjunct Scientist, CSCR, Vellore

Dr. Elizabeth's Team Members

II. Articular cartilage Derived Chondroprogenitors for Cartilage Repair and Regeneration

Cartilage repair necessitates adjunct therapies due to its avascular and aneural nature. While cell-based approaches using mesenchymal stem cells and chondrocytes have been explored, fibro-hyaline cartilage formation remains a limitation. Articular cartilage-derived chondroprogenitors offer promise in overcoming this, as they exhibit higher chondrogenic potential and lower hypertrophic phenotype.

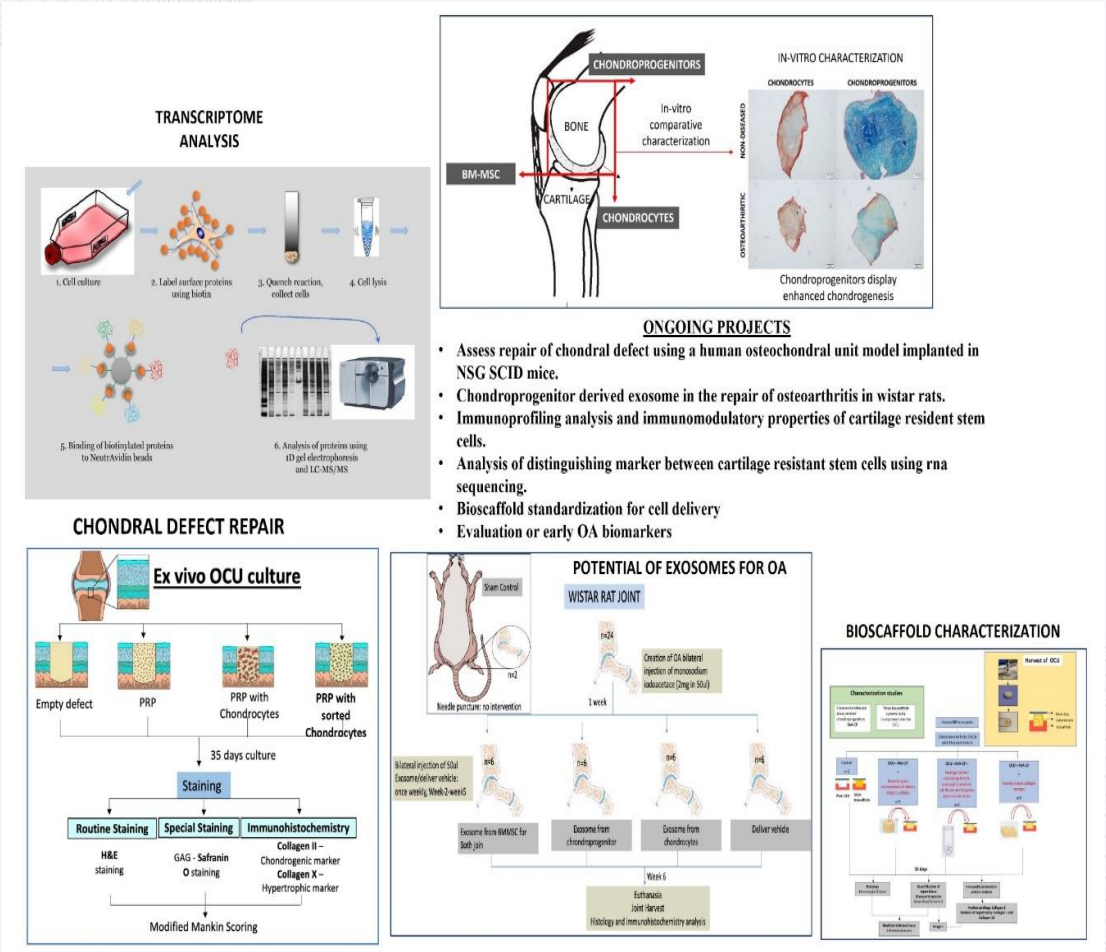
In a proof-of-principle study, we report progenitors are more likely than chondrocytes and bone marrow-derived MSCs to exhibit chondrogenesis and are less likely to undergo hypertrophy. These characteristics are essential for the generation of hyaline-like repair. Our work uses both in vitro and in vivo settings to characterize cartilage-derived progenitors and evaluate their possible implications for cartilage regeneration. Currently, our work includes:

- Isolation of progenitors using migratory assays, fibronectin adhesion assays, sorting based on the combinatorial expression of surface markers, and evaluating their repair potential for chondral defects using human osteochondral units using ex-vivo and in vivo NSG-SCID mice experiments.
- Assessing the potential of exosomes derived from these cells in alleviating chemically induced osteoarthritis using in vivo experiments
- Comparative analysis for obtaining distinguishing markers between cartilage resident cells using RNA sequencing and transcriptome analysis and assessing

how physiological stimuli (i.e., appropriate mechanical load) alter the qualitative and quantitative composition of the surfaceome during chondrogenesis

- Immunogenic and immunomodulatory profiling
- Chondroinducive properties of bioscaffolds such as PRP and nano composite scaffolds and and assessing the ideal parenteral solution for the delivery of chondroprogenitors in terms of their maintained viability and differentiation potential.

We hypothesize that a better understanding of these progenitors, in comparison to other cell types, will enable us to create a detailed biological profile and develop better approaches toward the treatment of cartilage pathologies.



Articular Cartilage Derived Chondroprogenitors for Cartilage and Regeneration

PUBLICATIONS

1. Jeya Lisha J, Ganesh Parasuraman, Soosai Manickam Amirtham, Deepak Vinod Francis, Abel Livingston, Anjali Goyal, Boopalan Ramasamy, Solomon Sathishkumar, Elizabeth Vinod. Comparative Assessment of Chondral Defect Repair Using Migratory Chondroprogenitors Suspended in either Gelled or Freeze-dried Platelet-Rich Plasma: An In Vitro and Ex Vivo Human Osteochondral Unit Model Study. The Knee. April 2024.

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4. Deepak Vinod Francis, Aishwarya Jayakumaran Rajeswari, John Bino Stephen, Ganesh Parasuraman, Jeya Lisha J Abel Livingston, Sandya Rani, Alfred Job Daniel, Solomon Sathishkumar, Elizabeth Vinod. An ultrastructural report of human articular cartilage resident cells in correlation with their phenotypic characteristics. Journal of Histotechnology. November 2023.
5. Soosai Manickam Amirtham, Ganesh Parasuraman, Jeya Lisha J, Deepak Vinod Francis, Abel Livingston, Grace Rebekha, Solomon Sathishkumar and Elizabeth Vinod. Decellularized Fetal Collagen Exhibits Chondroinductive Potential for Bone Marrow-Derived Mesenchymal Stem Cells by Enhancing Glycosaminoglycan Production. European Journal of Anatomy. July 2023.
6. Elizabeth Vinod, Jeya Lisha J, Ganesh Parasuraman, Abel Livingston, Alfred Job Daniel, Solomon Sathishkumar. Evaluation of Ghrelin as a distinguishing marker for human articular cartilage-derived chondrocytes and chondroprogenitors. Journal of Clinical Orthopaedics and Trauma. June 2023.
7. Ganesh Parasuraman, Elizabeth Vinod, Abel Livingston, Solomon Sathishkumar and Deepak Vinod Francis. Comparative assessment of the morphology and antigenicity of human osteochondral units using formalin and coagulant fixative. European Journal of Anatomy. May 2023.

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2.1 LENTIVIRAL VIRAL VECTORS BASED GENE THERAPY

ALOK SRIVASTAVA
Former Senior Professor, Department of Haematology, CMC
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I. Lentiviral Vector-Based Gene Therapy for Hemophilia A

As reported in 2023, the lentiviral vector-based gene therapy for haemophilia A, a first-in-human application, has been in a phase I clinical trial since June 2022. The pre-clinical data that allowed an IND to be filed was already published in 2018 and has been described before in the previous annual reports. (Doering et al Human Gene Therapy 2018; 29:1183)

The main points to recall would be the following:

1. An investigational new drug (IND) application was filed in 2018 in India. It took >3 years for it to be approved in 2021 with multiple rounds of review in multiple committees – some of whom had to form new subcommittees with adequate 'expertise' to even review the proposal. The chronology of events is shown below:

Clinical Trial of Gene Therapy for Haemophilia A – Chronology of Communications

Dates of Communication with CDSCO / RCGM	Application/Response /Presentation
17-07-2018	Application in Form 44 for conducting a phase-1 clinical trial titled "Gene therapy for hemophilia A with a high expression factor VIII transgene in autologous hematopoietic stem cells (CD68-ET3-LV-CD34+)"
22-11-2018	First Response to CDSCO, Reference: Your letter dated 12.09.2018 ,File No. Stm-Cl/13/CSCR/18-BD
03-07-2020	Second Response to CDSCO letter dated 10.05.2019
08-04-2019	Presentation to CBBTDEC
05-2019	CBBTDEC presentation Comments sent to CDSCO
06-2019	**Completed the following:** 1.Research agreement with Emory University / Expression Therapeutics, USA for use of this vector in India with full 'freedom to operate' – if found to be successful in this Phase 1 trial. 2.US-FDA approval obtained.
22-01-2020	Presentation to RCGM Subcommittee
22-06-2020	RCGM approval for not repeating the preclinical toxicity studies on India and Recommended to conduct transduction for three patients
03-07-2020	Third Response to CDSCO letter dated 10.05.2019
17-03-2021	Responses with **3 patient data report and Protocol amendment / Form-44 sent to CDSCO**
06-05-2021	CT-10 application submitted online
10-06-2021	CT-10 application physical copy with response to CDSCO
29-07-2021	Permission, from CDSCO, to conduct clinical trial, in Form CT-06
13-08-2021	Permission, from CDSCO, to manufacture drug product, in form CT-11
28-12-2021	Manufacturing licence obtained from SLA (Form-29)

2. It has been a learning experience for everyone involved in this process in the country as the first such proposal and that too a first-in-human study. However, it is necessary that going forward the review processes be streamlined to ensure much more rapid decisions avoiding delays of this nature that can slow down the whole program.

3. The clinical trial has progressed well since its initiation with a total of 5 participants having been treated so far. The overall schema for the clinical trial is shown in the figure 1 below:

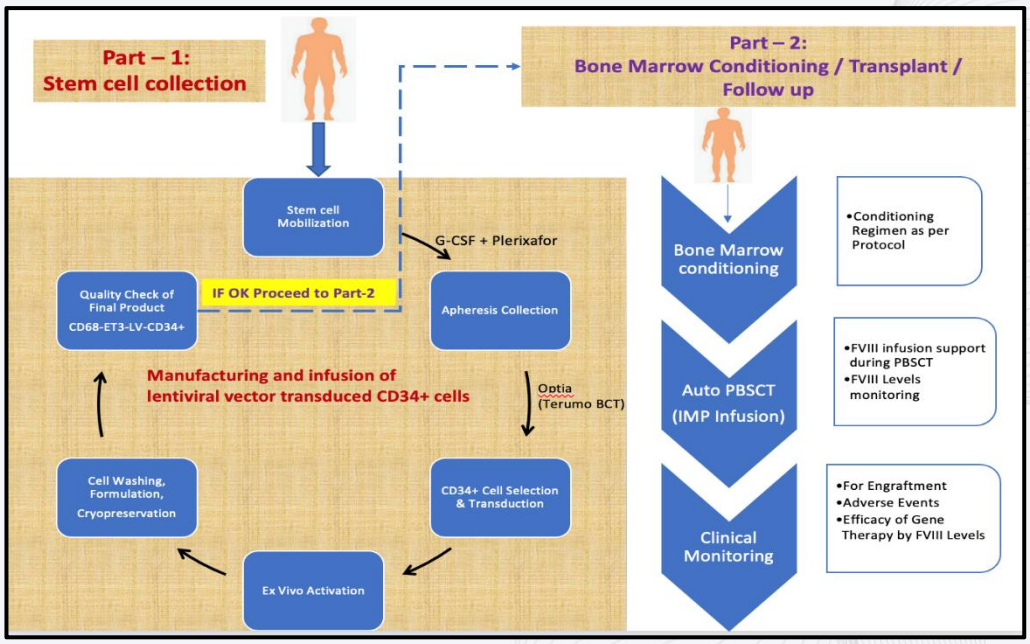

Figure 1: Outline of clinical trial protocol for gene therapy haemophilia A using a lentiviral vector transduced autologous haematopoietic stem cell with a novel FVIII transgene

The results have been quite remarkable so far:

i. No major safety issues or concern have occurred or have been identified with any patient in this clinical trial so far.
ii. Its feasibility has been well established with local manufacturing of the final drug product being consistently achieved for all 5 participants so far.
iii. What has been most rewarding for the trial participants and the investigators is the clinically significant response from the 1st participant onwards. This is also a first in some ways as there is no example of the first participant of new technology for gene therapy showing a consistent therapeutic response, as per all information available.
iv. An important aspect to be emphasized is the very elaborate consenting process followed for this study with community-based awareness of the haemophilia patients being initiated several years ago through meetings with patient advocacy societies in different cities in southern India and then further engagement with this group after approval of the study, followed by multiple rounds of discussion by the investigating team at CMC Vellore and formal video recorded consenting process. However, it did not end there. We also have established an independent external committee to evaluate the understanding of the participant and his family of the information provided and certify that it is adequate. This goes beyond the regulatory requirements in India and most places in the world. Finally, apart from the institutional DSMB, we also established

an international Scientific Advisory Committee (SAC) of experts in this field to guide us on any unusual circumstances that may arise during this clinical trial.

v. These data have been submitted to the regulator in India and shared with the DBT earlier this year. They have also been presented at three international meetings over the last 2 months. The data shown in the abstract presented at the last such meeting in April 2024 is shown below table 1A & B.

vi. The plan going forward is to treat one more patient whose product was manufactured but could not be infused as he developed low titer inhibitors before infusion of the IND product while on regular prophylaxis with clotting factor concentrates. He has now become inhibitor-negative after receiving nearly 15 months of immune tolerance induction therapy. This matter has been reviewed by the international SAC for this clinical trial who were convinced of the investigating team's recommendation to proceed with the inclusion of this participant in this clinical trial. The request was forwarded to the institutional IRB as a protocol exception for their opinion. Approval of the IRB has recently been received and the plan is to proceed with the inclusion of this final participant in the clinical trial under intimation to the regulator which has also already been done.

Table 1A. Gene Therapy of Haemophilia A with LV-CD68-ET3 transduced autologous HSC – Outcomes.

	P1	P2	P3*	P4*
Age (years)	33	31	34	22
Weight (kg)	49	51	60	48
CD34+ PBSC (x10^6/kg	7.5	7.0	8.7	9.1
CD34+ HSCT (x10^6/kg)	5.6	5.3	5.9	6.2
Viability (%)	95.4	94.7	95.7	95.8
VCN – CFU Day 14 Post-Trans	1.02	0.57	1.49	0.62
VCN – PB Day +30	0.07	0.12	2.5	1.45
Engraftment Day – Neutrophil	+12	+10	+10	+12
Engraftment Day – Platelet	+15	+15	+12	+15
Severe neutropenia (days)	11	7	7	10
Severe thrombocytopenia (days)	1	2	2	4

** Received product with modified trnsduction protocol*

Table 1B. Gene Therapy of Haemophilia A with LV-CD68-ET3 transduced autologous HSC – Outcomes.

	P1	P2	P3*	P4*
Last FVIII CFC infusion day	+15	+20	+11	+17
FVIII:C (%) – Day +60	7.9	4	28	14.2
FVIII:C (%) – Highest till last f/u	8.7**	4**	45.3**	21.3**
Inhibitor assay	Neg	Neg	Neg	Neg
Annual Bleed Rate – Pre-GT	30	20	36	120
Annual Bleed Rate – Post-GT	0	0	0	0
Follow-up (months)	22	14	8	6

**Received product with modifies transduction protocol*
***One Stage assay (OSA) results; Chromogenic substrate assay (CAS) results correlate with OSA values (within limits of lab CV)*

As these data are still unpublished, they should not be distributed or disseminated.

vii. The next step is to publish this data and the manuscript is under preparation.

In the meantime, it was good to find this clinical trial gets top mention in the announcement by the Hon'ble Minister of Science and Technology in the National Science Day announcements this year on 28th February 2024 along with the Secretary, DBT. (https://pib.gov.in/PressReleaseIframePage.aspx?PRID=2009823)

This clinical trial is a culmination of >10 years of international collaboration with the faculty of Emory University, led by Dr. Trent Spencer, in a unique arrangement where we have collaborated in the pre-clinical work and obtained an agreement to have the freedom to develop this technology as an indigenous product in India. It should be noted that while there are two other AAV vector-based products for gene therapy of haemophilia A, certain limitations have led to progressively dropping FVIII levels after the first 6-12 months in those participants. Compared to that, the FVIII levels have been steady so far with the longest follow-up reaching 2 years in this clinical trial.

While remaining cautiously optimistic about the long-term outcome of this clinical trial, very diligent follow-up must be maintained for these participants for the next several years monitoring both efficacy but also safety from the oncological transformation perspective given that the lentiviral vector has been used for gene therapy for haemophilia for the first time. A similar clinical trial for patients with haemophilia A with inhibitors is also underway at the University of Wisconsin, USA but only one patient has been recruited so far in that clinical trial. While no safety

issues were encountered, there is limited information available on the overall clinical outcome.

II. AAV Vector-Based Gene Therapy for Hemophilia B

As has been reported earlier, this program, which should have gone into clinical trial several years ago with all the pre-clinical work having been done and published, (Hum Gene Ther. 2020 Oct;31(19-20):1114-1123. doi: 10.1089/hum.2020.099.), has had to be halted due to challenges in GMP grade vector production at costs that would make sense for clinical use in India. To that end, with all proof-of-concept studies completed, efforts are on to find suitable industry partners working with our main collaborator at the University of Florida, Dr. Arun Srivastava. However, the lack of personnel to take even small-scale GMP-level manufacturing forward at the institutional level in CSCR does not make it feasible to pursue this further at CSCR, at this time. The expertise and materials developed for this purpose can be shared with any entity that may get involved in the future to further develop this technology.

Anti-AAV Antibody Assays

In this context, it should be mentioned that the technologies developed to measure anti-AAV antibody levels through assays established in-house by the Clinical Virology department in CMC, Vellore is a unique opportunity to help the efforts of any centre / entity that wishes to use AAV3, 5, 8 based products for gene therapy in India to screen for these pre-existing antibodies to select suitable participants for clinical trials or patients for treatment. Again, this technology can also be shared with others for wider application as needed.

R.V SHAJI
Professor, Department of Haematology, CMC
Adjunct Scientist , CSCR

Dr. Shaji's Team Members

III. Pre-clinical Lentiviral Gene Therapy Vectors for Haematological Diseases

One of the primary focuses of our laboratory is the development of lentiviral vectors for gene therapy targeting hemoglobinopathies. We utilize gene addition vectors that express both beta and gamma globin genes. We have created five distinct gene addition vectors and tested them in an ex vivo erythropoiesis system. We identified a promising vector, CMCEU-1, which exhibited high levels of human globin gene expression, including adult hemoglobin (HbA). In animal experiments using the CMCEU-1 vector within a mouse sickle cell model, we observed significant expression of human adult hemoglobin, leading to the correction of the sickle cell disease phenotype in the mouse model. We are currently testing these vectors in CD34+ cells obtained from patients with homozygous beta-thalassemia, with plans to transplant the transduced cells into immunocompromised mice. We have established methods to enhance viral titer and explored the use of small molecules to improve transduction efficiency in CD34+ cells.

In another approach, we generated lentiviral vectors designed for lineage-specific expression of an shRNA aimed at downregulating B-cell lymphoma/leukemia 11A (BCL11A) specifically in human erythroid cells. BCL11A is a transcription factor that represses gamma-globin gene (HBG) expression in adults and represents a key target for therapies aimed at increasing HbF levels. To achieve erythroid-specific BCL11A knockdown, we developed lentiviral vectors tailored to the erythroid lineage. Experiments conducted in HUDEP-2 cell lines and erythroid cells derived from CD34+ HSPCs have validated the efficacy of BCL11A knockdown. Furthermore, mouse

transplantation experiments employing CD34+ HSPCs from healthy donors demonstrated successful engraftment and multilineage reconstitution without any lineage bias. These lentiviral vectors exhibit robust, erythroid-specific expression of shRNAs, making them well-suited for the therapeutic reactivation of HbF in hemoglobinopathies. We have also engineered several novel lentiviral vectors with modified transcription regulatory sequences to enhance the transduction efficiencies of the BCL11A shRNA vector, thereby further improving the downregulation of BCL11A and the upregulation of HbF in adult erythroid cells.

Building on our experience in generating lentiviral vectors for gene therapy applications, we have expanded our efforts to develop vectors for other hematological diseases, including Diamond-Blackfan anemia and Fanconi anemia. These vectors have been thoroughly tested in patients' fibroblasts and induced pluripotent stem cells (iPSCs), with future experiments set to be conducted using CD34+ cells from patients.

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2. Jebanesan DZP, Illangeswaran RSS, Rajamani BM, Vidhyadharan RT, Das S, Bijukumar NK, Balakrishnan B, Mathews V, Velayudhan SR, Balasubramanian P. Inhibition of NRF2 signaling overcomes acquired resistance to arsenic trioxide in FLT3-mutated Acute Myeloid Leukemia. Ann Hematol. 2024 Jun;103(6):1919-1929. doi: 10.1007/s00277-024-05742-8.
3. Benjamin ESB, Vinod E, Illangeswaran RSS, Rajamani BM, Vidhyadharan RT, Bagchi A, Maity A, Mohan A, Parasuraman G, Amirtham SM, Abraham A, Velayudhan SR, Balasubramanian P. Immortalised chronic myeloid leukemia (CML) derived mesenchymal stromal cells (MSCs) line retains the immunomodulatory and chemoprotective properties of CML patient-derived MSCs. Cell Signal. 2024 Apr;116:111067. doi: 10.1016/j.cellsig.2024.111067. Epub 2024 Jan 27. PMID: 38281615.
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expression sensitizes chronic myeloid leukemia cells to imatinib in vitroand reduces disease burden in vivo. Front Pharmacol. 2023 May 31;14:1187066. doi: 10.3389/fphar.2023.1187066.

6. Illangeswaran RSS, Jebanesan DZP, Sivakumar KK, Vidhyadharan RT, Rajamani BM, Janet NB, David E, Velayudhan SR, Mathews V, Balasubramanian P. Corrigendum to "Chemotherapeutic drugs elicit stemness and metabolic alteration to mediate acquired drug-resistant phenotype in acute myeloid leukemia cell lines" [Leukemia Res. 128, 107054]. Leuk Res. 2023 Jul;130:107329. doi: 10.1016/j.leukres.2023.107329. Epub 2023 May 29.
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INVITED TALKS

1. 2nd Rare Genetic Diseases Research Summit-2023 (REDRESS 2023), on the 23rd and 24th of November, 2023 at TIGS, Bengaluru.
2. Manipal Genetics Update VII on Cellular and Animal Models for Rare Genetic Diseases' from January 18-20, 2024.
3. Molecular Pathology Association of India Annual Meeting on 17 to 18 February 2024 at AIIMS, New Delhi.

GRANTS

S. No.	PROJECT TITLE	ROLE	FUNDING AGENCY	Duration
1	Development of novel gene therapy vectors	PI	DBT	2024-2027
2	Mission Program project in Pediatric Rare Disorders.	PI	DBT	2023-2028
3	iPSC-based model for studying AML chemoresistance.	PI	ICMR	2024-2029
4.	Development of an iPSC-based model for chronic myeloid leukemia for drug screening.	PI	DST	2023-2026

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2.2 GENOME-EDITING BASED GENE THERAPY

SARAVANABHAVAN THANGAVEL
Scientist- E, CSCR

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Gene-Edited Hematopoietic Stem and Progenitor Cells (HSPCs) for GeneTherapy Applications

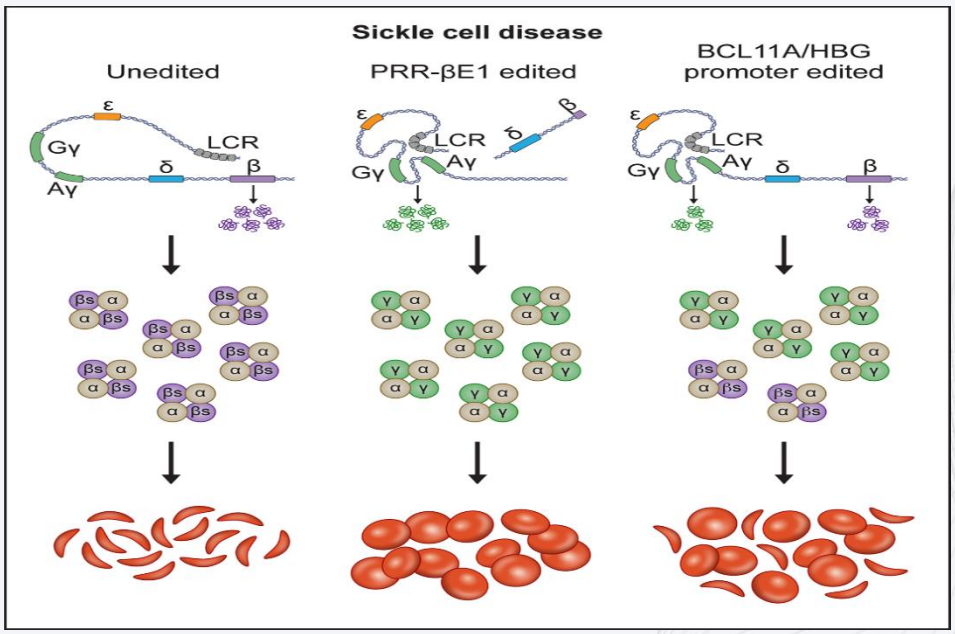
SUMMARY

The gene-edited autologous hematopoietic stem and progenitor cells (HSPCs) have shown great potential to cure several inherited and acquired hematological disorders. By focusing on gene editing of autologous HSPCs, we aim to develop curative therapies for the most common monogenic-inherited disorder, beta-hemoglobinopathies, as well as the infectious disease HIV.

KEY FINDINGS IN THE PAST YEAR

β-hemoglobinopathies gene therapy: Reactivation of fetal hemoglobin (HbF) is a commonly adopted strategy to ameliorate β-hemoglobinopathies. However, the continued production of defective adult hemoglobin (HbA) limits HbF tetramer production affecting the therapeutic benefits. We evaluated deletional hereditary persistence of fetal hemoglobin (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 hematopoiesis in immunocompromised mice producing HbF-positive cells in vivo. Importantly, the editing induced therapeutically significant levels of HbF to

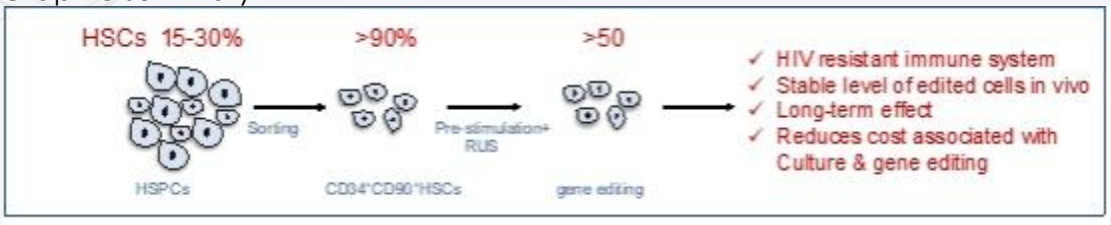
reverse the phenotypes of both sickle cell disease and β-thalassemia major. These findings imply that PRR-βE1 gene editing of patient HSPCs could lead to improved therapeutic outcomes for β-hemoglobinopathy gene therapy. Recently, we have developed a strategy to gene edit PRR-βE1without the ex vivo culture of HSPCs and currently working on overcoming the limitations associated with it.



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

HIV Gene Therapy: CCR5 gene edited autologous HSPCs can be a potential alternative to hematopoietic 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 the HSPCs. Last year, by using mobilized HSPCs from healthy donors, we 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. To demonstrate that the CD34+CD90+ HSC population is an ideal graft for HIV gene therapy, we sort purified CD34+CD90+ HSCs, and gene edited the CCR5 with 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.

Graphic summary



Karuppusamy K et al., Frontiers in Immunology 2022. The Graphical abstract shows the approach for HIV gene editing therapy. Furthermore, we have developed a protocol to edit the target locus with Cas9 mRNA instead of RNP and have established an off-target analysis system.

PUBLICATIONS

1. Venkatesan V, Christopher AC, Rhiel M, et al & Thangavel S (2023) Editing the core region in HPFH deletions alters fetal and adult globin expression for the treatment of β-hemoglobinopathies. Mol Ther Nucleic Acids. https://doi.org/10.1016/j.omtn.2023.04.024
2. Current approaches and potential challenges in the delivery of gene editing cargos into hematopoietic stem and progenitor cells. R Murugesan, KV Karuppusamy, S Marepally, Thangavel S (2023). Frontiers in Genome Editing. https://doi.org/10.3389/fgeed.2023.1148693
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GRANTS (2022/2023)

1. Preclinical Gene editing studies for the treatment of HIV infection (2022 – 2025). Funding agency: DBT, India
2. The safety and scale-up studies with gene-edited Hematopoietic Stem and Progenitor cells for the treatment of β-hemoglobinopathies (2023-2026). Funding agency: DBT, India
3. In vivo gene editing of hematopoietic stem and progenitor cells for HIV gene therapy (2023-2028). Funding agency: DBT Wellcome Alliance

INVITED TALKS

1. Stem cells- Theory and demo class to the students of Thiruvalluvar University. March-April-2023.
2. Genome edited stem cells for therapeutic applications" conducted by Sri Balaji Vidyapeeth, Puducherry (ADHR sponsored training programme). 10th Feb 2023.

MOHANKUMAR MURUGESAN
Scientist-E, CSCR

Dr. Murugesan's Team Members

Therapeutic Genome Editing for Hematological Disorders

SUMMARY

The primary goal of my research is to develop preclinical genome editing strategies for treating monogenic hematological disorders. We utilize advanced genome engineering platforms based on the CRISPR/Cas9 system, such as base editors and prime editors, to correct or create specific mutations in hematopoietic stem cells. For beta hemoglobinopathies, our focus is on precisely correcting disease-specific mutations and creating beneficial mutations to reactivate developmentally silenced fetal hemoglobin. To minimize undesired effects in other cell lineages, we employ a lineage-specific approach by disrupting major transcriptional factors or regulatory elements specifically in erythroid cells to elevate fetal hemoglobin levels. In the case of hemophilia, our strategy aims to achieve lineage-specific expression of FVIII in hematopoietic stem cells using CRISPR/Cas9-mediated homology-dependent repair.

In addition, we also developed the nuclease-deficient base editor to be completely devoid of large deletions that occur while editing at the highly homologous region.

Genome-Editing-Based Gene Therapy – Overall Research Activity:

Recent advancements in base editing for the reactivation of fetal hemoglobin (HbF) have shown promise, particularly in targeting the gamma-globin promoter. However, base editing at the gamma-globin locus has been associated with genotoxic events, such as a 4.9 kb large deletion in the intervening region due to simultaneous nicking in the HBG1 and HBG2 genes. Although the deletion frequency is lower compared to traditional Cas9 editing, it poses a challenge to the therapeutic potential of this approach. To address this issue, we evaluated if replacing the nickase Cas9 (nCas9) in ABE8e with a catalytically inactive deadCas9 (dCas9) could overcome large deletions while maintaining editing efficiency. Using three therapeutically relevant guide RNAs (gRNAs) targeting the gamma-globin promoter, our study compared the editing outcomes and deletion frequencies of dCas9, nCas9, dCas9-ABE8e, and nCas9-ABE8e. The findings indicated that while nicking induced large deletions, the frequency of these deletions was reduced with efficient base editing. Notably, the use of dCas9-ABE8e resulted in no appreciable deletions, suggesting it as a safer approach for maintaining genome integrity in therapeutic genome editing at the gamma-globin locus. Additionally, dCas9-ABE8e demonstrated efficient editing in primary human CD34+ hematopoietic stem and progenitor cells (HSPCs), achieving therapeutically significant outcomes. This work highlights dCas9-ABE8e as a promising tool for safer and more effective therapeutic genome editing to reactivate HbF in the treatment of hemoglobinopathies (1).

In our lab, base editors (BEs) were used to correct mutations responsible for β-thalassemia/HbE in HUDEP-2 cells. We screened various BE variants for their ability to correct a spectrum of β-thalassemia mutations integrated into the genome. These corrections were introduced at the endogenous HBB gene locus using BEs. We developed cellular models of β-thalassemia/HbE to assess the efficiency of these corrections and the subsequent restoration of functional β-globin. Our findings indicated that most bystander edits near the target sites did not interfere with hemoglobin expression and are not predicted to be pathogenic. Additionally, we validated the effectiveness of BEs in correcting the pathogenic HbE variant in patient cells with severe β0/βE-thalassemia. This work establishes a novel platform for screening and selecting optimal BE tools for therapeutic genome editing, demonstrating precise, efficient, and scarless correction of pathogenic point mutations across multiple regions of the HBB gene, including the promoter, intron, and exons (2).

Also, we explored the potential of targeting BCL11A-XL to treat β-hemoglobinopathies by increasing fetal hemoglobin (HbF) production. BCL11A-XL normally represses the fetal globin genes (HBG1/2) using its zinc-finger domains (ZnF4, ZnF5, and ZnF6). By using CRISPR-Cas9 and base editing to disrupt these domains, we aimed to reduce BCL11A-XL's repressive action and increase HbF levels. Our experiments showed that creating indels in the ZnF domains with CRISPR-Cas9 prevented BCL11A-XL from binding to the HBG1/2 promoters, leading to higher HbF levels but it disrupted the normal RBC development. Notably, base editing mediated disruption of ZnF4 significantly increased HbF without affecting many other genes involved in erythroid maturation and resulted in near-normal erythroid maturation. However, we still

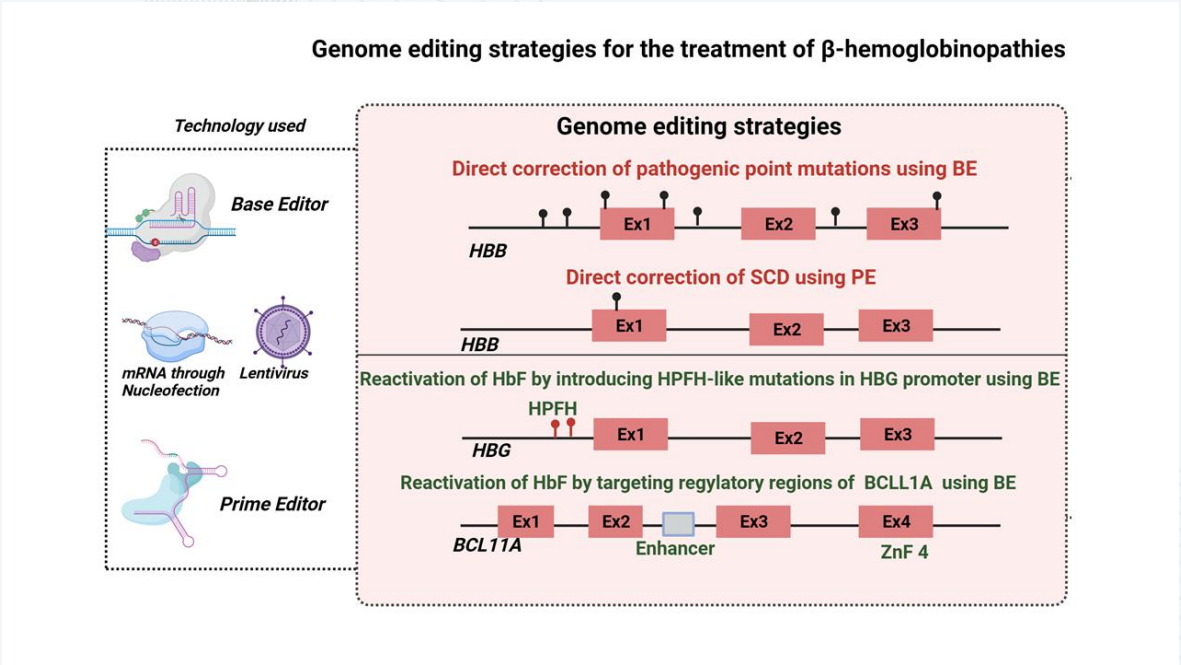
observed a reduction in engraftment and B cell development in vivo. These findings highlight the specific roles of the ZnF domains in BCL11A-XL and suggest a potential for introducing targeted mutations to selectively increase HbF while preserving other blood functions (3)

In addition to the current research activity, we have identified other novel targets for sickle cell disease and beta-thalassemia for enhancing HbF levels. Additionally, we have developed a strategy for integrating FVIII/FIX in HSPCs for hemophilia treatment, demonstrating successful transgene expression under the endogenous locus.

FUTURE 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 the validation, the HSPCs obtained from healthy donors will be edited at a clinical scale in pre-GMP conditions to confirm the reproducibility during scaling up.

Graphic summary:



PUBLICATIONS

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2. Kirti Prasad, Nivedhitha Devaraju, Anila George, Nithin Sam Ravi, Joshua Paul P, Gokulnath Mahalingam, Vignesh Rajendiran, Lokesh Panigrahi, Vigneshwaran Venkatesan, Kartik Lakhotiya, Yogapriya Periyasami, Aswin Anand Pai, Yukio Nakamura, Ryo Kurita, Poonkuzhali Balasubramanian, Saravanabhavan Thangavel, Shaji R Velayudhan, Srujan Marepally, Alok Srivastava, Mohankumar KM.(2023)Precise

correction of a spectrum of β-thalassaemic mutations in the coding and non-coding regions by base editors Molecular Therapy - Nucleic Acids. (Online)

3. Vignesh Rajendiran, Nivedhitha Devaraju, Nithin Sam Ravi, Lokesh Panigrahi, Joshua Paul, Chandrasekar Gopalakrishnan, Stacia Wyman, Keerthiga Ariudainambi, Gokulnath Mahalingam, Yogapriya Periyasami, Kirti Prasad, Anila George, Dhiyaneshwaran Sukumaran, Sandhiya Gopinathan, Aswin Anand Pai, Yukio Nakamura, Poonkuzhali Balasubramanian, Rajasekaran Ramalingam, Saravanabhavan Thangavel, Shaji R Velayudhan, Jacon E Corn, Merlin Crossley, Srujan Marepally, Alok Srivastava, Mohankumar KM. (2024) Base editing of key residues in the BCL11A-XL-specific zinc finger domains de-represses fetal globin expression. Molecular Therapy. 10.1016/j.ymthe.2024.01.023.

INVITED TALKS

1. Indira Gandhi Medical College and Research Institute, Puducherry- Lecture on "Genome Engineering for Hemoglobinopathies"- Strategies of Stem Cell Therapy (2023).
2. Sun Pharma Science Foundation's National Annual Conference- Presentation under "Genome-editing to treat hematological diseases" (2023).
3. Centre for Stem Cell Research- Presentation in 8th Annual Cell and Gene Therapy Symposium (2023)
4. Centre for Stem Cell Research- Presentation in Stem Cell Awareness Day (2023)
5. An event conducted by eLife journal for the Ben Bern's award – Presentation on Precise genome editing for beta-hemoglobinopathies to the post-graduate life science students (2023)
6. Frontiers in Genome Engineering- Presentation in "Precise genome editing at highly homologous regions without inducing large deletions" (2023)
7. National Centre for Biological Sciences (NCBS)-Presentation in the Advanced genome editing strategies for the treatment of haematological disorders" (2024).

INVESTIGATORS

SARAVANABHAVAN THANGAVEL, Ph.D.
Scientist-E, CSCR, Vellore

MOHANKUMAR MURUGESAN, Ph.D.
Scientist-E, CSCR, Vellore

2.3 NON-VIRAL VECTOR BASED NUCLEIC ACID TRANSFER

SRUJAN KUMAR MAREPALLY
Scientist- E, CSCR

Dr. Marepally's Team Members

Our research is interdisciplinary with an interface of chemistry and biology, emphasizing lipid-mediated nucleic acid therapeutics. The primary goal is to translate the concept of nucleic acid therapy into a clinically viable application. To this end, We have been developing cationic lipids to deliver nucleic acids including pDNA, and mRNA. Our disease models include nucleic acid therapeutics for hemophilia and RNA-based therapeutics and vaccines for infectious diseases.

Base-Modified Factor VIII mRNADelivery with Galactosylated LipidNanoplexes as a Protein Replacement Therapy for Haemophilia A

The bleeding disorder, Hemophilia A, needs a systemic functional Factor VIII protein infusion at prophylactic schedules. Recently, chemically modified mRNAs have emerged as promising protein replacement therapies to reduce repeated infusions and improve the safety profiles. However, base

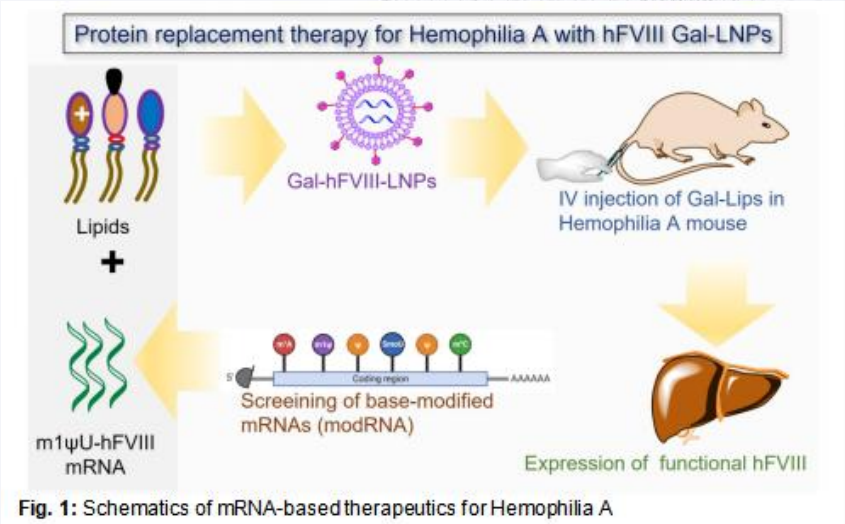

Fig. 1: Schematics of mRNA-based therapeutics for Hemophilia A

modifications are confined mainly to uridine, and the influence of base modifications on mRNA translation kinetics to specific cell types remains unexplored. In this study, towards developing mRNA therapeutics for Haemophilia, we synthesized chemically modified mRNAs with commercially available base modifications of Adenine, Guanine, Uridine, and Cytidine, evaluated in vitro transcription yield and translation kinetics in hepatic cell lines using reporter eGFP mRNA. Our findings demonstrated that mRNA with N1-methyl pseudouridine (m1Ψ) improved 5-12-fold higher translation efficiency in both hepatocytes and endothelial cell lines. As a proof of concept, towards developing mRNA therapy for Hemophilia A, where FVIII is deficient, we developed an m1Ψ modified functional m1Ψ-hFVIII mRNA with our liver-targeting lipid nanoplexs (Gal-LNP) system. We screened LNP delivery efficiencies in both hepatic and non-hepatic cell lines. Our study reveals that Gal-LNPs could deliver mRNAs 50% superior into liver and endothelial cells compared to non-targeting LNPs and the commercial control Lipofectamine messenger Max (LF MessengerMax), whereas the difference between Gal-LNPs, LNPs, and LF MessengerMax was not evident. Finally, we evaluated the therapeutic efficacy in the FVIII deficient Hemophilia A mouse model. We demonstrated that functional hFVIII mRNA encapsulating lipid nanoparticles Gal-m1Ψ-hFVIII-LNPs prevented bleeding after 15 days of administration, without affecting the safety profiles. In the present proof-of-concept study, an optimal lipid nanoparticle-enabled mRNA-based protein replacement therapy has been developed for a liver disorder, Hemophilia A (Fig. 1). Optimized mRNA synthesis for superior expression kinetics and hepatic cells and its delivery with liver-targeted nanoparticles may emerge as protein replacement therapies for monogenic liver disorders.

PUBLICATIONS

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3. Porkizhi A, Durga K, Mahalingam G, Ashish K Goel, Uday Z, Alok S, Srujan M, Lipid-nanoparticle-enabled nucleic acid therapeutics for liver disorders. Acta Pharmaceutica Sinica B. 2024 (In press)
4. Mahalingam G, Marepally S*. In a quest for bivalent mRNA vaccine for respiratory viruses: An effective strategy to overcome antigenic competition. Molecular Therapy. 2024 32(4):873-874.
5. Gokulnath M, Hari Krishnareddy R, Porkizhi A, Karthik V. K, Yogapriya P, Aruna M, Kanimozhi S, Salma M, Vigneshwar R, Mahesh M, George V, Mohankumar M, Saravanabhavan T, Alok S, Srujan M. SMART-lipid nanoparticles enabled mRNA vaccine elicits cross-reactive humoral responses against the omicron sub-variants. Molecular Therapy, 2024 32(5):1284-1297.

6. Vignesh R#, Nivedhitha D#, Nithin SR, Lokesh P, Joshua P, Chandrasekar G, Keerthiga A, Gokulnath M, Yogapriya M, Stacia W, Kirti P, Anila G, Dhiyaneshwaran S, Sandhiya G, Aswin AP, Ryo K, Yukio N, Poonkuzhali B, Rajasekaran R, Saravanabhavan T, Shaji R V, Merlin C, Srujan M, Alok S, KM Mohankumar* Targeted disruption of BCL11A-XL specific zinc finger motif for de-repression of fetal globin expression. Molecular Therapy, 2024, 32(3):663-677.

INVITED TALKS

1. Delivered an invited lecture on "Development of lipid-based nucleic acid therapeutics" organized by PopVax on 31st October 2023.

INVESTIGATOR

SRUJAN MAREPALLY, Ph.D.
Scientist-E, CSCR, Vellore

3. CELLULAR REPROGRAMMING AND ITS APPLICATIONS

R.V SHAJI
Professor, Department of Haematology, CMC
Adjunct Scientist, CSCR

iPSC-Based Disease Modeling of Hematological Diseases

We utilized induced pluripotent stem cells (iPSCs) to model four hematological diseases: Fanconi anemia (FA), Diamond-Blackfan anemia (DBA), congenital dyserythropoietic anemia (CDA), and chronic myeloid leukemia (CML). The project's objective is to leverage these iPSCs and their differentiated hematopoietic progenitors to investigate disease mechanisms and conduct drug screening. After genotyping the patients through exome sequencing, we reprogrammed their fibroblasts to generate iPSCs. We successfully developed iPSCs from patients with DBA and FA. Our findings indicate that both RPS19 (associated with DBA) and FA pathway genes (associated with FA) play pivotal roles in the reprogramming process and iPSC maintenance. To overcome the reprogramming limitations of FA patient fibroblasts, we employed doxycycline-inducible lentiviral vectors to complement defective genes with normal wild-type cDNAs before reprogramming. This strategy enabled the successful reprogramming of fibroblasts with defects in FANCA, FANCL, FANCF, FANCI, and FANCC genes. The derived iPSCs exhibited pluripotency marker expression and the potential for in-vitro trilineage differentiation. When differentiation was performed without complementation (by removing doxycycline from the medium), the hematopoietic progenitors (HPCs) displayed the phenotype of bone marrow failure. We have identified novel mechanisms involved in the pathogenesis of DBA and FA using these iPSCs. Currently, we are conducting CRISPR-gRNA screening to identify genes that can ameliorate the FA phenotypes of FA-iPSCs.

We successfully generated iPSCs from patients with chronic myeloid leukemia (CML). We found that inhibiting the tyrosine kinase activity of the Bcr-Abl fusion protein is necessary for the successful generation of CML-iPSCs. Various tyrosine kinase inhibitors were tested for their ability to generate stable iPSCs from CML patients' blood cells. Our current experiments are focused on generating iPSCs from both CML patients who responded to the drugs and those who did not respond.

Additionally, we employed gene editing strategies to introduce mutations in disease-associated genes into iPSCs. CRISPR-Cas9-based non-homologous end joining (NHEJ) and base editing were used to introduce mutations in DBA and CDA-associated genes in a normal iPSC line. We have generated new iPSC lines with doxycycline-inducible expression of ABE8e (for adenine base editing) and Cas9 (for creating indels). These cell lines serve as valuable tools for rapidly generating mutations for disease modelling. A novel base-editing strategy facilitated efficient mutation generation in iPSCs.

Importantly, we established a strategy to create heterozygous mutations in ribosomal protein genes of normal iPSCs for disease modelling. This gene editing approach was essential for generating isogenic iPSCs, a critical element of disease modelling. Hematopoietic progenitors and erythroid cells derived from DBA and CDA iPSCs, generated by gene editing, exhibited disease phenotypes during differentiation. We are currently conducting CRISPR-gRNA screening to identify factors that can restore the normal phenotype in iPSC-derived erythroid cells.

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1. Nandy K, Babu D, Rani S, Joshi G, Ijee S, George A, Palani D, Premkumar C, Rajesh P, Vijayanand S, David E, Murugesan M, Velayudhan SR. Efficient gene editing in induced pluripotent stem cells enabled by an inducible adenine base editor with tunable expression. Sci Rep. 2023 Dec 11;13(1):21953. doi: 10.1038/s41598-023-42174-2.
2. Rani S, Thamodaran V, Nandy K, Fouzia NA, Maddali M, Rajesh P, Vijayanand S, David E, Velayudhan SR. Establishment and characterization of CSCRi006-A: an induced pluripotent stem cell line generated from a patient with Diamond-Blackfan Anemia (DBA) carrying ribosomal protein S19 (RPS19) mutation. Hum Cell. 2023 Nov;36(6):2204-2213. doi: 10.1007/s13577-023-00946-y.
3. 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 Res. 2023 Sep;71:103159. doi: 10.1016/j.scr.2023.103159.
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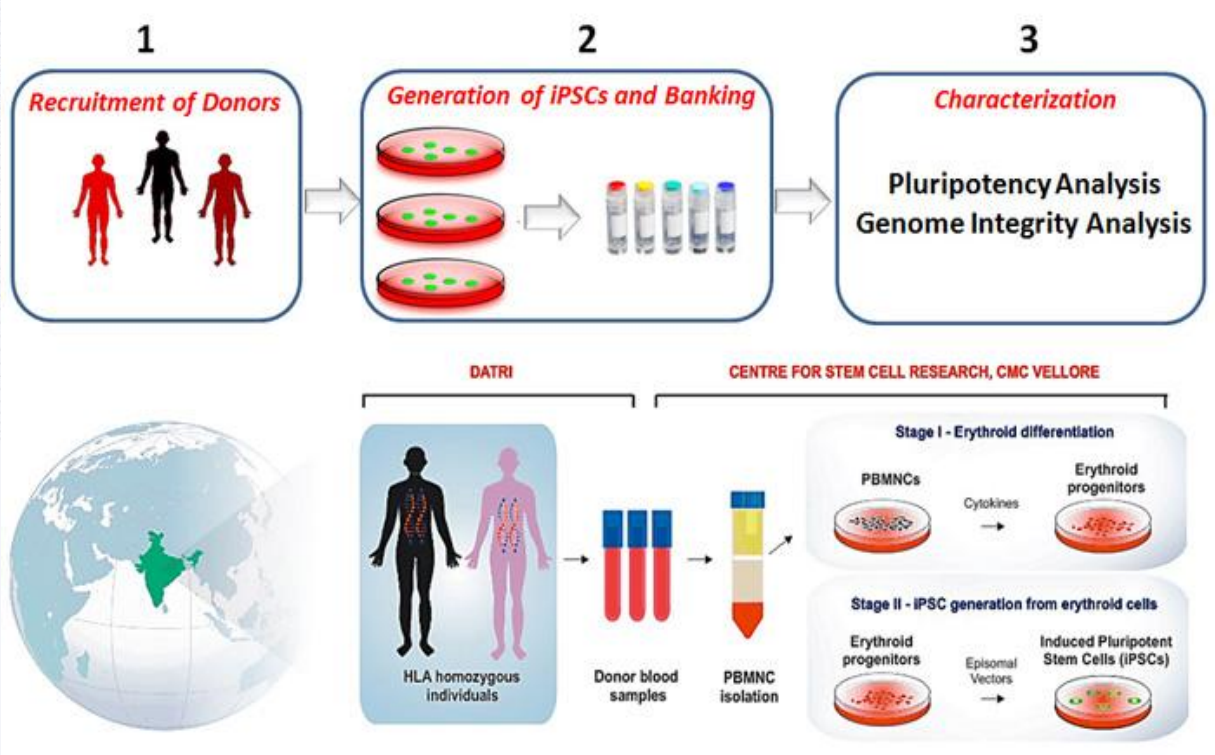
INVESTIGATOR

R.V SHAJI, Ph.D.
Professor, Department of Haematology, CMC, Vellore
Adjunct Scientist, CSCR, Vellore

II. Applications of Induced Pluripotent Stem Cell (iPSC) Technology: Haplobanking



Schematic Representation of Haplobanking from Donors with Homozygous HLA

The conversion of somatic cells into induced pluripotent stem cells (iPSCs) and their cell-type specific differentiation have revolutionized the field of regenerative medicine and have raised the prospects of personalized medicine to cure various diseases. The Generation of induced pluripotent stem cell (iPSC) lines from specific donors who are likely to be immune compatible with a large part of the population, enables to development iPSC banks which can theoretically provide matching cells for recipients worldwide. The haplobanking project involves identifying HLA homozygous donors from the Indian population and generating iPSC lines from their cultured cells through good manufacturing practice (GMP). The Centre for Stem Cell Research collaborates with the blood stem cell registry, DATRI, a non-governmental organization located in Chennai for the collection of blood samples from suitable donors across India. These are subsequently HLA-typed and screened for infectious diseases at the Christian Medical College, Vellore.

A robust method has been established at the Centre for Stem Cell Research to generate iPSC lines from cultured erythroid progenitor cells derived from peripheral blood mononuclear cells (PBMNCs).

For future clinical applications using iPSCs, there is a global initiative to generate iPSCs from individuals who have homozygous HLA haplotypes. Our centre has joined the Global Alliance of iPSC Therapies (GAiT) for haplobanking of iPSCs from normal donors of Indian origin. As a first step, a bank of PBMNC from 235 donors with homozygous haplotypes from various regions of the country was generated.

Following this, we established a highly efficient feeder-free and xeno-free protocol to generate GMP-grade iPSCs. So far, iPSCs from 10 donors have been generated which cover the top 10 HLA haplotypes. The isolated clones were expanded from passage 2 to passage 10 to make the seed stock. Expanded cells were analyzed for pluripotency marker expression and in-vitro differentiation to three germ layers. In the future, we will perform a comprehensive characterization of the iPSC lines from all the donors.

TEAM MEMBERS

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Adjunct Scientist, CSCR, Vellore

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Adjunct Scientist, CSCR, Vellore

GURBIND SINGH, Ph.D.
Scientist-D, CSCR, Vellore

ALOK SRIVASTAVA, M.D., FRACP, FRCPA, FRCP
Former Senior Professor, Department of Haematology, CMC, Vellore
Former Adjunct Scientist / Former Head, CSCR

4. IMMUNE CELL THERAPY

THIYAGARAJ MAYURANATHAN
DBT-Ramalingaswami Re-entry Fellow, CSCR

Dr. Mayuranathan's Team Members

Strategy-I: Generation of Off-the-Shelf Allogenic Anti-CD19 CAR-T Cells for B-Cell Malignancies

In recent years, autologous chimeric antigen receptor (CAR) T-cell therapy has revolutionized the treatment landscape for haematological malignancies. Following the remarkable clinical outcomes, the Food and Drug Administration (FDA) approved six CAR-T cell products for B-cell leukemia, non-Hodgkin lymphoma, and multiple myeloma. However, the safety, efficacy, and accessibility of this therapy have been hampered by exhaustion and poor persistence of infused CAR-T cells in vivo, cytokine-related toxicities, antigen escape by tumor cells, and bottlenecks in the production of autologous products. Alternatively, using allogeneic CAR-T cells from healthy donors has several potential benefits over autologous approaches, such as the immediate availability of cryopreserved batches for patient treatment, the ability to modify cells over time, redosing or combination of CAR-T cells targeting different targets and reduced cost using an industrialized process. However, allogeneic CAR-T cells may cause fatal graft-versus-host disease (GvHD) and may be rapidly rejected by the host immune system.

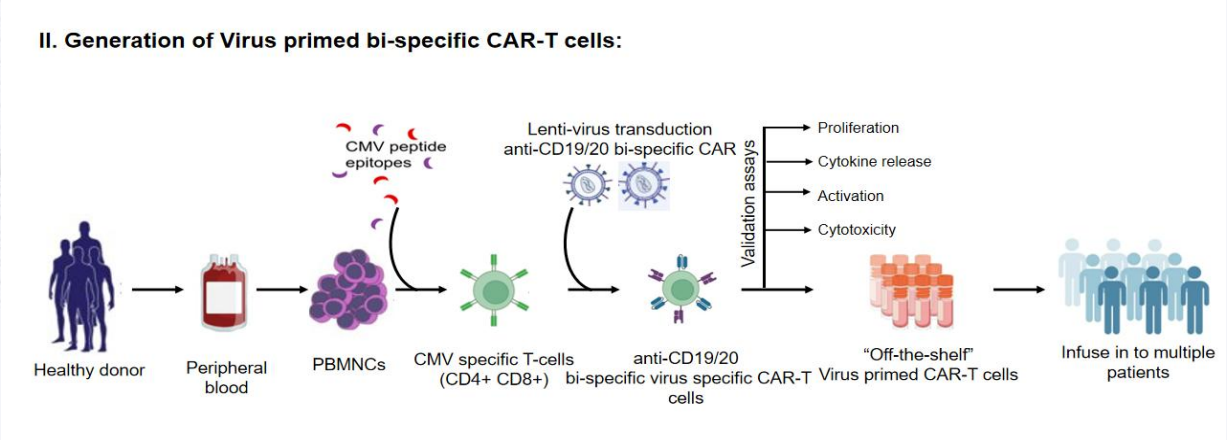
Therefore, we aim to generate off-the-shelf allogenic anti-CD19 CAR-T cells by two different strategies (described below) to avoid the host immune rejection and GvHD. In the first approach, we plan to generate the CD19 CAR-T cells from healthy donors followed by gene editing to eliminate T-cell receptor alpha constant (TRAC) and Beta-2

microglobulin (b2M) genes to avoid graft-versus-host-disease (GVHD). In the second approach, we plan to develop combination immunotherapy, in which healthy donors T-cells will be primed with cytomegalovirus (CMV) followed by lentiviral transduction of anti-CD19 CAR to eliminate CD19+ tumor cells and the risk of inducing GVHD in the allogeneic hematopoietic cell transplant (HCT) setting, by removing alloreactive T-cells. The anti-CD19 CAR-T cells developed by both modalities will be tested for their tumor-killing potency in vitro and in vivo.

T-cells (CD3+ cells) will be purified from the peripheral blood of healthy volunteers by magnetic-activated cell sorting (MACS) separation. Selected T-cells will be transduced with lentivirus carrying CD19 CAR followed by electroporation of C-base editor (CBE) mRNA and sgRNAs to introduce the stop codon in T-cell receptor alpha constant (TRAC) and Beta-2 microglobulin (b2M) genes. CD19 CAR-T with TRAC and b2M knockdown cells will be further stimulated and expanded in vitro to assess the potency of generated allogenic CAR-T cells by measuring the proliferation (standard cell count), activation (measuring CD25 and CD69 by flow cytometry), cytokine profile (measuring IL-2, TNFa and INFg by ELISA) and cytotoxicity activity by co-culturing with CD19+ malignant cells (NALM-6 and RAJI cell lines).

Strategy-II: Generation of Virus Primed Bi-Specific (CD19/20) CAR-T Cells:

Peripheral blood mononuclear cells (PBMNCs) will be purified from the blood of healthy volunteers with certain serostatus. Then, PBMNCs will be cultured with CMV-specific peptides to generate virus-specific cells. Further, CMV-primed T-cells (CD4/8) will be selected and purified by MACS and flow cytometry based on CD4/8, IFN and CD154 expression. Selected virus-specific T-cells will be transduced with lentivirus carrying CD19/20 bispecific CAR. Virus-primed CD19/20 bispecific CAR-T cells will be further stimulated and expanded in vitro to assess the potency of generated Virus primed allogenic bispecific CAR-T cells by measuring the proliferation (standard cell count), activation (measuring CD25 and CD69 by flow cytometry), cytokine profile (measuring IL-2, TNFa and INFg by ELISA) and cytotoxicity activity by co-culturing with CD19+ malignant cells (NALM-6 and RAJI cell lines).



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TEAM MEMBERS

INVESTIGATOR

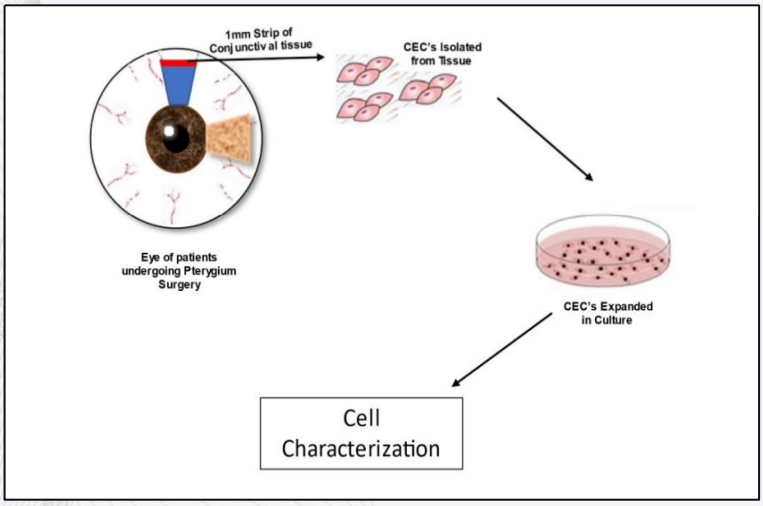
THIYAGARAJ MAYURANATHAN, Ph.D.
DBT-Ramalingaswami Re-entry Fellow, CSCR, Vellore

CO-INVESTIGATOR

MOHANKUMAR MURUGESAN, Ph.D.
Scientist-E, CSCR, Vellore

5. CELL THERAPY FOR OCULAR DISORDERS

JEYANTH ROSE
Associate Surgeon, Department of Ophthalmology, CMC
Adjunct Scientist, CSCR



Schematic Representation of Isolation and Culture of Conjunctiva Epithelial Cells (CEC's)

SUMMARY

The ocular stem cell lab has made significant strides this year in advancing our understanding of the therapeutic use of Mesenchymal Stem Cells (MSCs) in treating ocular disorders. Our focus has been in the areas of corneal scarring, dry eye and allergic conjunctivitis.

A major highlight of our research this year has been the investigation into the use of mesenchymal stem cells (MSCs) to mitigate mast cell activation in allergic conjunctivitis. Our ongoing studies are examining whether MSCs can modulate immune responses and reduce mast cell activation. Early in vitro results have demonstrated that MSCs stimulated with TNFa release anti-inflammatory cytokines and can influence mast cell behavior, reducing the release of pro-inflammatory mediators such as histamine and cytokines. This finding opens new avenues for treating allergic conjunctivitis with cell-based therapies that offer longer-lasting relief compared to conventional antihistamines and corticosteroids.

We are also exploring lipid nanoparticle-enabled siRNA delivery as a method to target and silence viral genes responsible for HSV-1 replication. This method holds the potential for not only treating acute HSV-1 infections but also preventing recurrences, offering a significant advancement over existing antiviral treatments and preventing corneal blindness.

PUBLICATION

- J Rose, Aarwin Joshua, Alo Sen, Bharathi Mohan Raj. Joshua Paul, Sanita Korah, Hannah Mary T Thomas, Tulasi Geevar, Sukesh Chandran Nair, "Impressions of an impression" – A standardized, cost-effective, non-invasive test to assess the cellular profile of the Tarsal conjunctiva of the human eye (under review).
- J Rose, Aarwin Joshua, Joshua Paul, Alo Sen, Sanita Korah, A standardized approach to isolate, Culture and Compare Human Conjunctival Epithelial cells from two different sources. South Asian Journal of Experimental Biology (accepted)

INVESTIGATOR

JEYANTH ROSE, M.S.
Associate Surgeon, Department of Ophthalmology, CMC, Vellore
Adjunct Scientist, CSCR, Vellore

6. MECHANISMS OF DISEASES

EUNICE SINDHUVI
Professor, Department of Haematology, CMC
Adjunct Scientist, CSCR

I. *Elucidating the Role of Mesenchymal Stem Cells, Immune and Telomere Biology in Regeneration and Differentiation of Haematopoietic Stem Cells*

The indefinite self-renewal and potential to differentiate into other types of cells represent stem cells as frontiers of regenerative medicine. Hematopoietic stem cells (HSCs) are well-characterized tissue-specific stem cells used clinically. The destruction of HSCs by various causes, such as chemicals, cytotoxic drugs, radiation, immune disorders, and genetic mutations, leads to bone marrow failure. One such scenario is observed in aplastic anemia (AA), where immunologic mechanisms are believed to be the prime cause, along with other causative factors, namely telomere biology and constitutional gene defects, leading to the loss of HSCs. Aplastic anemia results in peripheral pancytopenia and marrow hypoplasia, and its incidence is believed to be 2-3 times higher in Asia. Hematopoietic stem cell transplantation (HSCT) or immunosuppressive therapy, a combination of anti-thymocyte globulin (ATG) and cyclosporine+/-Eltrombopag are the mainstays of treatment of AA.

FINDINGS

- Hematopoietic stem cells reside in specialized microenvironments in the bone marrow and provide signals that support fundamental HSC properties. Mesenchymal stromal cells (MSCs) possess the capacity to differentiate into specific cell types, abundant production of soluble growth factors and cytokines, and their immunomodulating properties. Studies showed a decreased proliferation potential in AA-MSCs and their differentiation capacities, for instance, an increased tendency to differentiate towards adipocytes and a decreased propensity towards the osteogenic lineage. AA-MSCs showed a reduced ability for immunomodulation and hematopoietic support compared to healthy controls. Incongruously, few recent data have shown that the proliferative, functional, and immunomodulatory properties of MSCs are comparable to normal controls. This discrepancy could be due to the heterogeneity of the study populations.
- The proliferative potential of HSCs reduces with age and leads to the shortening of telomeres. Telomeres are DNA-protein structures that protect the ends of linear chromosomes from degradation. Telomere erosion results in critically short telomeres, leading to apoptosis, and genomic instability. Telomerase enzyme prevents replication-dependent loss of telomere and cellular senescence. Studies demonstrated that the telomere length (TL) of leukocytes in patients with AA is shorter than in age-matched healthy controls. In addition, lower TL is associated with frequent relapses, clonal evolution to MDS, and poor survival in patients treated with ATG. Donor and patient TL also influence outcomes in patients undergoing unrelated HSCT for AA. There is limited data in studying the telomere length in patients with AA in the Indian

population. Therefore, our study investigated the role of mesenchymal stromal cells, and molecular and immune mechanisms that support HSC regeneration in aplastic anemia.

II. *Biology of Iron in RBC Regeneration: Upcoming Players of Regenerative Medicine*

Regeneration of red blood cells (RBCs) through an *in vitro* system serves as a model addressing blood transfusion shortages and enhancing therapeutic options for hematological diseases. The pivotal regulation of iron metabolism, particularly in hemoglobin synthesis, is essential for erythroid regeneration. A physiological increase in erythroid production is observed in pregnancy during the second trimester. We studied the role of iron and erythroid regulators in pregnant women at different gestational time points and delivery in pregnant women with iron deficiency anemia (IDA). Additionally, we established a pregnant mouse model exposed to various iron diets to observe the roles of erythroid, iron, and various regulators in different organs during non-pregnancy, mid-gestation, and late gestation. Furthermore, we explored the impact of iron deficiency on erythroid differentiation in human hematopoietic stem cells (HSCs) and HSCs of patients with polycythemia vera. Iron and erythroid regulators were also examined in conditions of pathologically increased erythropoiesis, such as beta-thalassemia and polycythemia vera.

FINDINGS:

- Our findings illustrated a gradual increase in erythropoietin and a decline in ferritin levels from the initial to the third trimester, indicating the utilization of mobilized iron to support increased erythropoiesis for placental and fetal development. Statistical analysis revealed a decline in erythropoiesis during the initial two months of pregnancy, followed by a rise from the second trimester. This aligns with the redirection of increased iron from plasma to the bone marrow to support erythropoiesis. In the third trimester, erythroid activity stabilizes, with a significant portion of iron devoted to fetal iron endowment. During delivery, placental iron transporters like ferroportin and GDF15 respond to maternal iron deficiency by increasing their expression, facilitating adequate iron passage to the fetus.
- In the context of pathological erythropoiesis in polycythemia vera (PV), erythropoietic activity governs the amount of iron transferred to the bone marrow by modulating intracellular iron in erythroid cells and systemic iron regulation. In thalassemia, iron requirements in the bone marrow are influenced by ineffective erythropoiesis, impacting hepcidin regulation.
- Both animal and *in vitro* studies revealed that systemic and intracellular iron deficiency modulates increased erythropoiesis and erythroid differentiation, respectively. Observations from animal models indicated that placental prolactin and estrogen may contribute to the regulation of increased erythropoiesis. Maternal regulatory hormone hepcidin expression was found to be diminished in cases of iron deficiency and elevated in iron overload mice compared to controls. This corresponded with a reduction in liver iron stores, transferrin receptor, and bmp6 expression at mid-gestation, key regulators of hepcidin production. Our study also demonstrated diminished bmp6 expression at mid-gestation, suggesting that erythroferrone (erfe) secreted by erythroid

cells might inhibit bmp6 activity. During mid-gestation, elevated expression in erythroid cells was noted, potentially aiding in suppressing the inhibition of BMP6 signaling.

Our study suggests that systemic and intracellular iron transport for medullary erythropoiesis depends on the rate of erythroid activity.

INVESTIGATOR

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7. SKIN REGENERATION

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GMP Grade Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) with an *Aloe-vera* Polycaprolactone Composite Scaffold in the Topical Treatment of Partial-thickness Burns in a Rat Burn Model: Validation and Toxicity Testing

PROJECT OVERVIEW

Dr. Jehangir's Team Members

This project aims to validate and test the toxicity of GMP-grade Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) with an Aloe-vera Polycaprolactone (A-PCL) composite scaffold for the topical treatment of deep second-degree burns in a rat burn model. Building on previous research at CSCR, which demonstrated enhanced skin repair and reduced fibrosis using in-house fabricated A-PCL with WJ-MSCs, this study seeks to confirm these promising results with GMP-grade materials [1]. The project has secured funding from the CSCR seed grant and will commence following the necessary approvals from institutional and animal ethics committees.

Objectives and Methodology

The primary objectives of this study include isolating and characterizing GMP-grade mesenchymal stem cells from human umbilical cord Wharton's jelly, preparing and testing the GMP grade A-PCL scaffold, and assessing both the in vitro cytotoxicity and the in vivo safety and efficacy of these components in a rat model. Human umbilical cord samples will be collected with patient consent, and WJ-MSCs will be isolated and cultured using established protocols. The cells will be characterized through surface marker expression analysis, differentiation assays, karyotyping, and HLA typing to ensure their quality and suitability for clinical use. Additionally, the sterility, mycoplasma presence, and endotoxin levels of the cell cultures will be meticulously tested to comply with GMP standards.

EXPECTED OUTCOMES AND FUTURE DIRECTIONS

This study is anticipated to validate the therapeutic potential of GMP grade WJ-MSCs in combination with an A-PCL scaffold for burn treatment, providing robust preclinical data on their efficacy and safety. Successful completion of this project could pave the way for subsequent clinical trials and the development of advanced wound care therapies. The findings will contribute significantly to the field of regenerative medicine, particularly in enhancing the quality and outcomes of skin repair treatments. Future work will focus on scaling up production processes and exploring the application of this technology in other clinical scenarios requiring effective tissue regeneration.

PUBLICATIONS

- Jehangir, S., Ramesh, S., Thomas, M. et al. Wharton's Jelly Mesenchymal Stem Cells on a Novel Aloe Vera-Polycaprolactone (A-PCL) Composite Scaffold in Burns. Regen. Eng. Transl. Med. (2022).

INVITED TALKS

- Preclinical assessment of the efficacy of Wharton'sJelly Mesenchymal Stem Cells on a Scaffold of *Aloe vera* Polycaprolactone in Wound Healing using a Rat Burn Model. 43rd Annual Scientific Meeting, Australian and New Zealand Burns Association (ANZBA), Hobart, 15th– 18th October 2019.

INVESTIGATOR

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8. COMMUNITY OUTREACH PROGRAMME

Program for Control of Thalassemia and Sickle Cell Disease in Odisha- Creating a Model for India

Beta thalassemia major and sickle cell disease are major hemoglobin disorders that cause significant morbidity and mortality in India. These are well-defined monogenic disorders with clear Mendelian autosomal recessive inheritance and prevention is possible and effective. However, preventive strategies have not been comprehensively planned and implemented in India. To address this, a program aimed at creating a model for the control of major hemoglobin disorders in India was proposed.

This program was initiated after signing an MoU with Govt. of Odisha in Dec 2017. A collaboration was established with the Department of Health and Family Welfare and the National Health Mission of the Govt. of Odisha to develop a model for the control of sickle cell disease and thalassemia in Odisha and the population-based program for the control of major hemoglobin disorders was initiated in Odisha in 2019. A detailed plan was prepared for the project based on a situation analysis conducted by the Indian Institute of Public Health, Bhubaneswar (IIPHB).

This program employs a comprehensive approach along with several novel technologies that are used in screening and genetic diagnosis of major haemoglobin disorders, all aimed at prevention and control. The program is based on providing information on which people can opt for carrier testing and prenatal diagnosis and then use that knowledge to reduce the burden of these diseases in the community.

This program is being implemented in collaboration with the Government of Odisha. It was initiated in the first phase in six districts (Balasore, Bargarh, Cuttack, Jharsuguda, Koraput, Sambalpur) and progressed to 9 second-phase districts (Raygada, Nabrangpur, Puri, Bhadrak, Angul, Kandhamal, Mayurbhanj, Malkangiri, Sundargarh).

The priorities of the project are

1. To increase awareness of MHD, its genetic transmission, and options for prevention, and also to increase access to testing, counselling, and prenatal diagnosis.
    - *Through BCC (Behavioral Change & Communication) outreach to the community*
    - *Through screening & diagnosis of high-risk populations including pre-natal diagnosis by utilizing state-of-the-art technologies in a convenient and cost-effective manner*
2. To Provide training to health care workers for improved diagnosis and treatment of MHD.
    - *By training the health care professionals and field workers in the state health service*

1. Behaviour Changes and Communication (BCC)

The project seeks to address existing misconceptions about these diseases through BCC activities. A combination of population-level and individual-level approaches are being used to create awareness and behavior change. The deliverables include the development of a media plan, development, and production of BCC materials, piloting of media and material, and identification and training of district implementing agencies. In the past year, the project had made a presence in the whole state with particular activities in 15 districts through BCC activities.

As per the project mandate, various BCC activities were implemented across the state and in designated districts in a phased manner. To enhance community awareness, purpose-driven materials such as flipbooks and targeted outdoor creatives including hoardings, tin plates, and leaflets were collaboratively designed by the team. Additionally, to promote testing and awareness among ethnic groups, folk-based shows were organized in remote areas of each district.

Region	**BCC Activities Completed**	
First & second phase districts (15 districts)	Wall paintings	In 15 districts (3676 wall paintings)
	Folk shows	In 15 districts (580 shows)
	Leaflets	In 15 districts (3.89 lakh Nos)
	Flex banners	In 15 districts (1452 Nos)
	Posters	In 15 districts (5000 Nos)
	Wall painting	In 15 districts (2239 Nos)
	Flipbook for ANM	In 15 districts (3966 Nos)
	Hoardings (CHC)	In 4 districts (61 Nos)
	Tin Plates	In 15 districts (3000 Nos)
	Sun boards	In 15 districts (3820 Nos)
	Paper Stickers	In 15 districts (29,000 Nos)
	Vinyl Stickers	In 15 districts (10,000 Nos)
	Multi-color posters	In 15 districts (10,000 Nos)
	Multi-color folders	In 15 districts (10,000 Nos)
	Hoardings (Highway)	In 6 districts (36 Nos)
Whole state	• Radio airing (Radio Jingles, Radio spots) – Aired on 17 channels on 3 occasions. • Awareness message through newspaper advertisement – 6 papers on 3 occasions (Dussera, Rathyathra-2) • Bus Branding – 100 buses across the state • Calendars – A total of 7000 calendars with messages distributed in 30 districts in 2 successive years (to NHM and health officials) • Social Media – Regular updates on Facebook, Instagram, and Twitter	

Major achievements were:

- *Community-level sensitization for the disease happened through the distribution and display of BCC creatives and items.*
- *Demand generation for testing amongst communities across the planned districts*
- *Awareness generation among service providers and the community about the importance of the program*
- *Sensitization for testing and prevention among the beneficiaries*

2. Laboratory Service [Screening & Diagnosis]

The priority groups and the point of screening are listed below:

Priority group	Point of screening
Antenatal women	Periphery – RI centres/PHC/CHC
Spouses of AN women (positives)	Periphery – RI centres/PHC/CHC
High school & College students (2^{nd} & 3^{rd} year onwards)	Schools & Colleges
Walk-ins	CHCs

The Lab strategies were formulated by a series of meetings with professional bodies, and diagnostics companies by CMC and NHM. The cell counter lab sites across different districts are assessed and finalized in 15 districts by a series of meetings with District Administrative Health officials and program managerial staff at district levels. As per assessment by district NHM wings, Odisha, the 62 CHCs in 15 districts were selected for setting up testing labs for the launch of the program.

Screening has been initiated among pregnant women in this program. The samples from pregnant women are collected at Routine Immunization sites and the initial processing of samples occurs at the cell counter site. A two-step approach was envisaged for the program. The first step is a screening test and the samples found positive on screening will be sent for confirmatory tests. However, the strategy didn't materialize due to the inconsistency of correlation between the CBC test and the HPLC in the project, increased workload of LTs in CHC, and delays in sample transport by vaccine delivery persons. Later, the project started sending all samples to the central HPLC lab for confirmation.

A. *The lab set up with cell counter machine was installed at 62 CHC/SDH in 15 districts (Koraput, Sambalpur, Bargarh, Jharsuguda, Cuttack, Balasore, Rayagada, Angul, Nabarangpur, Puri, Bhadrak, Kandhamal, Mayurbhanj, Malkangiri & Sundargarh) and antenatal screening started in 143 blocks from 15 districts (Koraput, Sambalpur, Bargarh, Jharsuguda, Cuttack, Balasore, Rayagada, Angul, Nabarangpur, Puri, Bhadrak, Kandhamal, Mayurbhanj, Malkangiri & Sundargarh).*

The cell counter site LTs were provided with laptops installed with an interface for the data management system and the LTs were given hands-on training on sample

processing by Central HPLC Lab LTs. LTs were trained in data entry also. The support of Govt LT is being compensated by monthly incentives by CMC. Lab logistics and registers are supplied by CMC to all lab sites. Logistics are distributed till the ANM level. Sample transport system established with help of vaccine delivery person supported by NHM and courier part paid by CMC.

B. HPLC Haemoglobin Testing

HPLC Lab with five HPLC instruments from 2 manufacturers at **SCB Medical College, Cuttack** to test all samples from the districts.

- Total number of samples collected (till Apr 2024): 2,26,576

C. Genetic Testing: Sentinel Surveillance and PND Centre at Medical College

RT PCR machine was installed at *SCB Medical College Cuttack and VSS Medical College Burla* for establishing the genetic diagnosis lab. Sentinel surveillance by cord blood sampling of newborn babies had been initiated in *SCB Medical College Cuttack, VSS Medical College Burla & SLN Medical College, Koraput*. The Professors, scientists, and LTs were provided with hands-on training.

The Genetic lab received samples from the sentinel surveillance centers to monitor the trend of births with sickle cell disease and thalassemia major as baseline data. Chorionic villous sampling (CVS) started at SCB Cuttack, VSS Burla, and AIIMS Bhubaneswar and planning to start at SLN MCH, Koraput. Genetic testing of Chorionic Villous Samples is being carried out by the Genetic Lab at SCB Cuttack with cross-checking at CMC Vellore. **Nurse counsellor** staff were appointed for the Sentinel surveillance and PND counselling and testing.

- Total number of sentinel samples collected (till Apr 2024): 16,072
- Total number of CVS samples collected (till Apr 2024):104

3. Training of Administrative Officers/ Programme Managers/ Medical Officers /LT/ ANM & ASHA

The training was given to healthcare workers for both project implementation and to strengthen diagnosis and counselling. Training requirements of all Medical Officers, Administrative and Programme Officers, ANMs, and ASHAs for 15 districts were prepared and submitted to NHM. Block-wise training plan with a budget has been prepared for these districts. Training of field functionaries (ANM & ASHA) in sample collection and data management systems was completed in 15 districts.

a. Medical Officers, Administrative and Programme Officers:

Training on Clinical Management of MHDs: 310 doctors (block-level Medical Officers) across the state were trained in clinical management.

Training for MOs and Admin Officers: The Orientation of Medical Officers and administrative officers of all 15 districts is completed. Training of Block data managers on data management system completed in ToT.

Chorionic Villous Sampling Training: Hands-on training on CVS provided and certified to 11 O&G doctors nominated by all the seven Govt. Medical Colleges in Odisha and AIIMS BBSR at SCB, MCH Cuttack by CMC Vellore.

b. Workshop and Training of Trainers (ToTs):

A workshop is being conducted for all higher-level health officials in a district to familiarise and sensitize the project as part of the launch of the project in each district. Training of Trainers was completed in 15 districts and the trainers are being used in - block-level training.

c. Training for LT/ ANM & ASHA

Training manual for the field program implementation at the State and District level, Odia Handouts for the training of ANMs and ASHAs were prepared and training completed in 15 districts.

Lab technicians were trained by running the screening test on a cell counter, data entry, and reporting procedures.

The details of training conducted in 15 districts are given below:

S. No.	District	Accomplished Training & Number of Participants
1	Koraput	• Workshop (administrative staff from District and Block)-48 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-40 • Block level ANM training HW (M), HW(F), LHV, and other Field staff. -443 • ASHA orientation-2584
2	Sambalpur	• Workshop (administrative staff from District and Block)-21 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-24 • Block level ANM training HW (M), HW(F), LHV, and other Field staff. -225 • ASHA orientation-1018
3	Jharsuguda	• Workshop (administrative staff from District and Block)-22 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-15 • Block level ANM training HW (M), HW(F), LHV, and other Field staff. -111 • ASHA orientation-610
4	Bargarh	• Workshop (administrative staff from District and Block)-30 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-35 • Block level ANM training HW (M), HW(F), LHV, and other Field staff. -355 • ASHA orientation-1432
5	Balasore	• Workshop (administrative staff from District and Block)-32 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-30 • Block level ANM training HW (M), HW(F), LHV, and other Field staff. -379 • ASHA orientation-2284
6	Cuttack	• Workshop (administrative staff from District and Block) 51 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-44 • Block level ANM training HW (M), HW(F), LHV and other Field Staff - 701 • ASHA orientation-197
7	Bhadrak	• Workshop (administrative staff from District and Block)-21 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-28 • Block level ANM training -363

8	Nabarangpur	• Workshop (administrative staff from District and Block)-28 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-39 • Block level ANM training -587
9	Raygada	• Workshop (administrative staff from District and Block)-35 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-41 • Block level ANM training -391
10	Angul	• Workshop (administrative staff from District and Block)-25 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-32 • Block level ANM training -367
11	Puri	• Workshop (administrative staff from District and Block)-31 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-44 • Block level ANM training -494
12	Kandhamal	• Workshop (administrative staff from District and Block)-37 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-50 • Block level ANM training -284
13	Mayurbhanj	• Workshop (administrative staff from District and Block)-57 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-81 • Block level ANM training -976
14	Malkangiri	• Workshop (administrative staff from District and Block)-20 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-21 • Block level ANM training -243
15	Sundaragarh	• Workshop (administrative staff from District and Block)-37 • TOT (BDM, LT, Counsellor, LHV/Staff Nurse)-59 • Block level ANM training -848

4. Data Management

A comprehensive data management system in a field screening programme has been implemented. Each person who is tested is assigned a unique identification number (UID) which will link the Laboratory Information System and the Medical Information System through a bar code-enabled system and the results are entered into a web-based information system. Samples collected from all districts are being captured in the project data management interphase.

A contract had been made with an IT company to develop a web application for screening & diagnosis, an android application for collecting data of screened individuals, and a separate web application for sentinel surveillance. The android application will support the ANMs to register the beneficiaries, collect the samples, and electronically ship the samples to respective cell counters. The web application supports the CHC/cell counter LTs to receive the samples, process the samples, and ship the samples to the central HPLC lab. It even allows walk-in registration at the CHC level. The web application also enables the pathologist to make the diagnosis online and the generated reports can be downloaded by each block data manager for their block.

The master data is required to create credentials for every ANM and LT for sample collection and processing and data entry. The master data is collected in a systematic and standardised manner to ensure the accuracy and completeness of the data, the master data collected is used for programme planning, monitoring, and evaluation, as well as identifying service gaps and improving the service quality. This includes basic information about the ANM and LT, where the samples will be collected, as well as other relevant details.

Android app and web-based application to facilitate data management for each category of staff involved in the project were developed. A web application had also been developed for NHM officials to monitor the performance of the project in their

corresponding blocks and districts. Android Application was developed and currently, version 3.0 was developed and deployed to the Play Store. Training for the field staff to use the app and enter the data is completed in all 15 districts.

5. Monitoring & Evaluation

The progress of this program is being monitored weekly by teams from both CSCR / CMC Vellore and NHM, Govt. of Odisha. The external evaluation has been contracted to IIPH Gandhinagar. The contract was signed with the Indian Institute of Public Health Gandhinagar (IIPHG) and the baseline evaluation was completed and the report has been submitted.

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9. RESEARCH DEVELOPMENT OFFICE (RDO)

The Research Development Office (RDO) at the Centre for Stem Cell Research (CSCR) was created to provide executive and administrative support to the research and development activities of the Centre. RDO assists the CSCR faculty and students in various activities related to their research.

Image Credit: Sonam Pandey, RDO, CSCR

These include funding source information dissemination, assistance with proposal development, and management of research projects. RDO coordinates funding information and provides services related to research administration. It also helps faculty and students identify sources of funding for research and scholarly activities. RDO also assists in formulating research policies, implementing research administration strategies, and developing initiatives to enhance research and funding performance. The Office undertakes research information management and provides administrative support for the development of research. Besides, the Office coordinates the management of research projects and initiatives supported by CSCR and also the internal and external research assessment exercises, research capacity-building programs as well as national/international research collaborations.

The Office coordinates meetings of research groups and communicates with funding agencies/collaborators to keep track of the progress of research work at the Centre. RDO helps in organizing various scientific meetings that take place at CSCR. Preparation and submission of project reports to the funding agencies and collation of the annual report of the Centre is also coordinated by this office.

RDO liaises with the researchers and administration of the Centre to develop new initiatives and to set policies aimed at planning for the improved quality of research and to ensure their proper and timely implementation. It also monitors the implementation of research plans to ensure timeliness and results in relation to planned objectives and expected outcomes. To a certain extent, it also helps the researchers in CSCR in the timely utilization of funds.

In the past few years, this office has focused its work towards these goals. Several collaborations were established and MoUs signed for the research work that is ongoing at CSCR. For the gene therapy program, research agreements were signed between CSCR, the University of Florida, and Emory University, USA. Collaborations are being established with the Government of Odisha and the Indian Institute of Public Health Bhubaneswar for the development of a population-based control program for thalassemia and sickle cell disease in Odisha (under the NAHD program). Several research grant applications were submitted in the past year for both intramural/extramural funding. The process of submission and post & pre grants management at the Centre was also streamlined. These efforts are coordinated by this office.

SONAM PANDEY Ph.D
Scientist, Research Development Office

10. CORE FACILITIES AND INSTRUMENTATION

CSCR Core Facilities:

Core Facilities Team Members

The Core Facilities at CSCR host state-of--the-art instrumentation to aid researchers both within and outside CSCR. The Core Facilities provide expertise in sample processing and analysis and also help in experiment design. All facilities are accessible to not only scientists working full-time at CSCR but also to all other scientists in CMC, Vellore who require these technologies/platforms for their work.

A. Molecular Biology Facility:

The Molecular Core Facility involved in providing high-end molecular biology services for the users (in-house and off-campus). The facility currently has a 3500 8-capillary DNA sequencer from Applied Biosystems, and an Applied Biosystems QuantStudio 12K Flex Real-time PCR for high throughput analysis and Beckman ultracentrifuge, Beckman High-speed centrifuge, Sepctramax multimode reader, ExPERT GTX flow electroporation system.

Genetic Analyzer 3500

I. Genetic Analyzer 3500:

The Applied Biosystems 3500 Series Genetic Analyzer is designed to maintain the unsurpassed application versatility that life science researchers expect from Applied Biosystems instruments. The 3500 platform can run a wide variety of Sanger sequencing and fragment analysis applications, including de novo sequencing and resequencing (mutational profiling), microsatellite analysis, MLPA,

AFLP, LOH, and SNP validation or screening. The total number of sequencing samples processed during June 2022- May 2023 for CSCR and other departments of CMC is shown in Figure: 1(CSCR) and Figure: 2(CMC).

Figure 1: Total number of Sequencing samples processed from May 2023 to April 2024 for CSCR

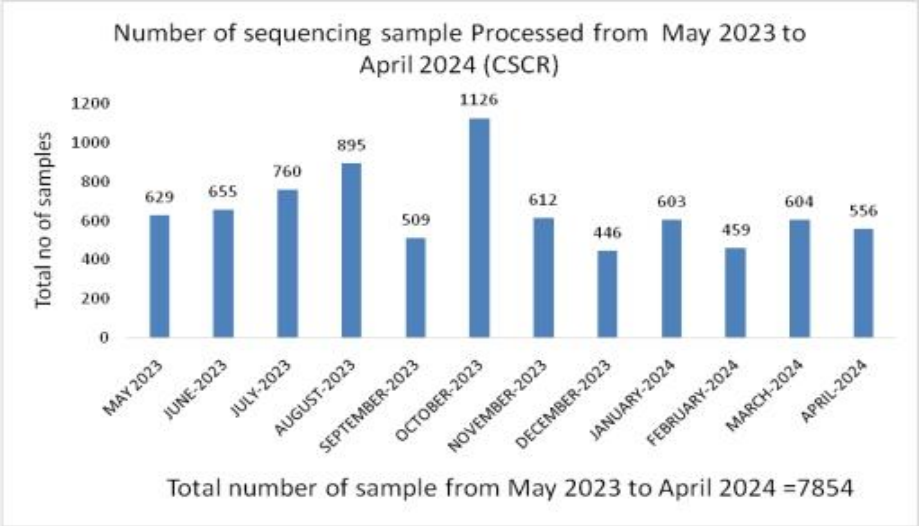
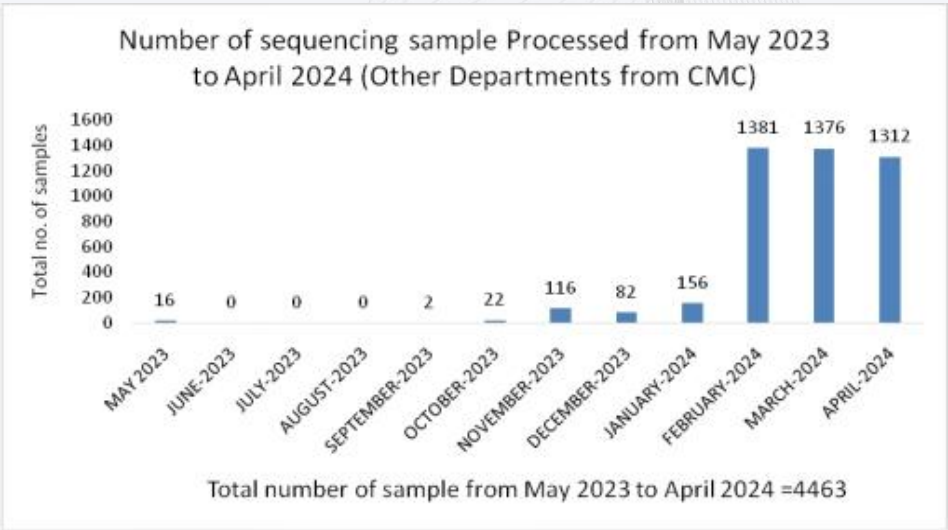


Figure 2: Total number of sequencing samples processed from May 2023 to April 2024 for other departments from CMC



II. Quant Studio12 K & 6 K Flex Real-Time PCR System:

QuantStudio 12K Flex system is designed for maximum throughput, outstanding flexibility with 5 inter-changeable blocks, scalability, and user-friendliness. This system is widely used in gene expression analysis, SNP genotyping, copy number analysis, digital PCR technology, Micro RNA, and other noncoding RNA analysis.

The Quant Studio 6 Flex Real-Time PCR System is ideal for laboratories with multiple applications and end users on a limited budget.

Quant Studio12 Flex Real-Time

III. Jess - Chemiluminescent & Fluorescent Western Blotting Protein Simple:

Jess from Protein Simple, a Bio-Techne brand, automates the protein separation & immune detection of traditional western blotting. Multiplex with chemiluminescence & fluorescence channels.

Chemiluminescent & Fluorescent Western

B. Tissue Culture Facility:

The Tissue Culture (TC) Facility is the most widely used core facility. The TC facility is a full-service cell culture shared resource. The TC facility located on the ground floor and first floor houses the basic equipment required for cell culture experiments. Users from within the center and adjunct scientists from CMC are provided with access to the facility and all the equipment. The facility is supplied with HEPA-filtered air to maintain a sterile environment within the lab. The vinyl flooring helps in the easy cleaning of the facility. The users are also provided with lint-free lab coats for use within the facility.

AutoMACS NEO Separator

The autoMACS NEO Separator is a benchtop magnetic cell separator that allows gentle isolation of cells with various separation strategies. The autoMACS NEO Separator features automated sample labelling, sample loading onto the column, and elution of the unlabelled negative cell fraction as well as the labelled positive cell fraction. Sensor-controlled fluidic level detection and automated buffer dispensing allow for fully automated cell isolation, which is constantly monitored. The touchscreen and software allow for intuitive planning and handling of cell separations. The MACS MiniSampler S holds the MACS Chill Racks and MACS Reagent Racks. The MACS Reagent Rack 8 allows for automated labelling with eight reagents. Up to six samples are placed in MACS Chill Racks of different sizes that keep the samples chilled at 4-8˚C.

The instrument can be used either for the automated separation of manually labelled cells or for completely automated labelling and separation of cells. In the end, the labelled and unlabelled cells are eluted into individual tubes. Isolated PBMCs, dissociated tissue, blood products as well as suspensions containing bacteria or yeast can be applied as starting material.

Cryostat: Leica CM1900

C. Histopathology Facility:

I. Cryostat:

Leica CM1900

The Leica CM1900 is a rapid sectioning cryostat for advanced routine diagnostics in histology and clinical histopathology. This Leica cryostat offers extremely rapid specimen freezing and frequent changes in specimen temperature, meeting even the highest demands for smooth operation and enhanced safety.

EG 1150H

II. Embedding system:

EG 1150H

It is a paraffin embedding station with microprocessor control. It is designed for embedding histological tissue specimens in molten paraffin for use in pathological laboratories.

TP1020

III. Tissue Processor:

TP1020:

The Leica TP1020 is an automatic tissue processor designed for laboratory applications. It is used for the fixation, dehydration, and infiltration of histological tissue samples with fixatives, alcohol, solvents, and paraffin wax.

Microtome

IV. Microtome:

The RM2245 is a semi-motorized rotary microtome, designed for routine in histopathology. Manual sectioning is enhanced by a high-precision motorized specimen feed, which results in efficient operation with maximum section quality and reproducibility.

V. Cytospin:

Cytospin is a low-speed centrifuge used to separate and deposit a monolayer of cells on glass-slides while maintaining cellular integrity. This is widely used in histology, cytology, and immunochemistry.

Cytospin

TEAM MEMBERS

Technical Officer

A. RAJESH, M.Sc., M.Phil.

Technical Staff

T. ABDUL MUTHALLIB, B.Sc.
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GURBIND SINGH, Ph.D.
Scientist-D, CSCR, Vellore

Flow Cytometry Facility

The CSCR Flow Cytometry and Cell Sorting Laboratory provides a broad array of instrumentation, support, education, and consultation to the research community. A wide variety of cell sorting modes are supported, from one-way to four-way tube cell sorting, plate sorting, slide sorting using high speed to low speed, different sizes of nozzles with 18 colors, and 20 parameters. Additionally, a wide variety of cell analysis services (up to 19 colors, 21 parameters) are offered. Currently, the facility offers two cell sorters (BD FACSAria FUSION and BD FACS Aria III) two analyzers (BD FACS Celesta and BC Cytoflex LX), and two computers dedicated to the offline analysis of the flow cytometry data using FlowJo and Kaluza software.

I. BD FACS Aria Fusion

BD FACSAria Fusion High-Speed Cell Sorter (5 Laser) Comprises of: Fixed Optical Bench with Pre-Aligned Fixed Solid State Lasers: 488nm Blue laser, 640nm Red Laser, 405nm Violet laser, 355nm UV laser, 561nm Yellow Green laser. 20 Parameters: Forward Scatter, Side Scatter & 18 colors/Fluorescence Detectors. Two-way and four-way sorting into microtubes, 12 x 75-mm, 15 mL tubes, and automated cell deposition unit (ACDU) for sorting into slides, 6, 24, 48, 96, and 384-well plates. Sample injection chamber for various sample input tubes, including microtubes, 12 x 75-mm, and 15 ml tubes. Nozzle Size 70, 85, 100, and 130-micron sizes. Temperature control option for sort collection devices. Sample input agitation for mixing of samples and sample temperature control accessories. Analysis rate of 70,000 events/second and sorting rate of 70000 events/second with purity of more than 98% for 4-way sorting. Fluorescence sensitivity <87 MESF for FITC and <29 MESF for PE.

II. BD FACS Aria III:

The BD FACSAria III flow cytometer is a high-speed fixed-alignment benchtop.
Cell sorter. With its fixed-optics design and digital electronics, the BD FACSAria III flow cytometer measures up to 11 colors simultaneously and supports a wide range of applications in immunology, genomics, cancer, and stem cell research. A patented flow cell with a gel-coupled cuvette and patented octagon and trigon detection system allows the system to achieve unrivaled sensitivity and resolution.

BD FACSAria III cell sorter with a five laser (Near UV-375nm, Violet-405nm, Blue-488nm, Yellow-Green-561nm, Red-633nm) and 11

color setup has a throughput of 70,000 events per second and can do one-way, two-way, three-way, 4-way sorting, and single-cell sorting.

III. BD FACS Celesta:

BD FACS celesta is a multi-laser flow cytometer with 3 lasers (blue-488nm, violet-405nm, and yellow-green-561 nm) and 12 12-colour setup for delivering high sensitivity and performance. In the BD FACSCelesta, the optical and electronics systems—lasers, filters, detectors, optical paths, and signal processing technologies—have been engineered to get the most out of BD Horizon Brilliant™ dyes.

IV. BC CytoFLEX-LX:

The High-performance BC CytoFLEX-LX Flow cytometer analyser with Five High Power Lasers 488nm, 638nm 405nm, 561nm and 355nm, 19 Colors & 21 Parameters (R3 B3-V5-YG5–UV3 System) is used for qualitative and quantitative measurement of biological and physical properties of cells and other particles. The system offers the ability to configure the violet laser detector (VSSC) to collect side scatter to better resolve nanoparticles from noise. It has a superior acquisition rate of 30,000 events per second and a superior signal-processing digital system with 7 dynamic range.

Number of Principal Investigators using Core Flow Cytometry Facility for the Research (May 2023-April 2024):

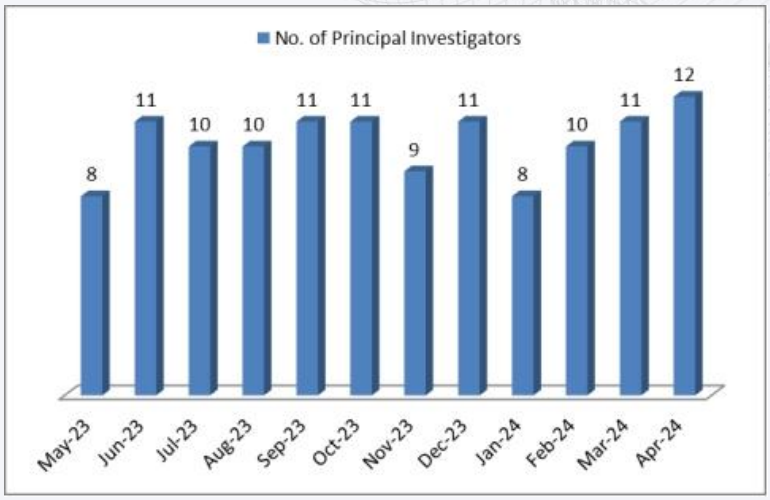

Figure 1: The above figure shows the number of Principal Investigators from CSCR as well as other departments of CMC and other academic institutions using our flow cytometry facility from May 2023 to April 2024.

Internal and External users for Flow Cytometry Facility:

S. No	Institute/ Department	Number of PIs
1	CSCR, Vellore	13
2	Dept. of Haematology, CMC, Vellore	2
3	Dept. of Paediatric Ortho, CMC, Vellore	1
4	Dept. of Physiology, CMC, Vellore	4
5	Dept. of Anatomy, CMC, Vellore	1
6	Dept. of Biochemistry, CMC, Vellore	3
7	VIT, Vellore	2

The above table shows the number of principal investigators from CSCR, different departments of CMC, and other academic institutions using the CSCR core Flow Cytometry facility.

CSCR - Centre for Stem Cell Research (A unit of InStem), CMC Campus, Vellore
CMC - Christian Medical College, Vellore
VIT - Vellore Institute of Technology, Vellore

TEAM MEMBERS

Technical Staff

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N. JOSEPH JOEL, B.Sc., M.L.T.
Graduate Technician Trainee, CSCR, Vellore

Scientist In-Charge

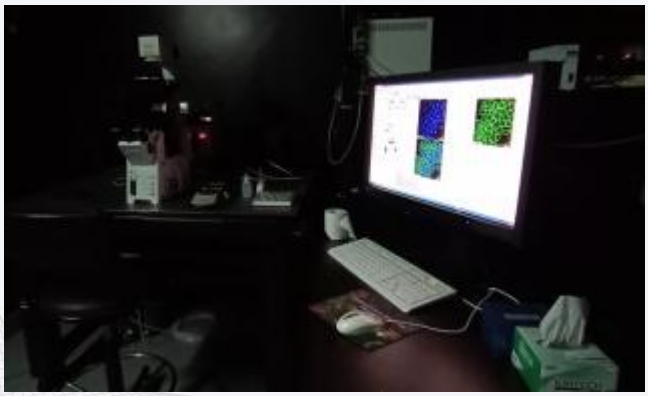
B. SANDYA RANI, Ph.D.
Scientist-D, CSCR, Vellore

Imaging Facility

The CSCR Microscopy Core facility offers training and access to a variety of light and fluorescence microscopes; the core can also do imaging for users who are not trained and offer fluorescence and confocal imaging.

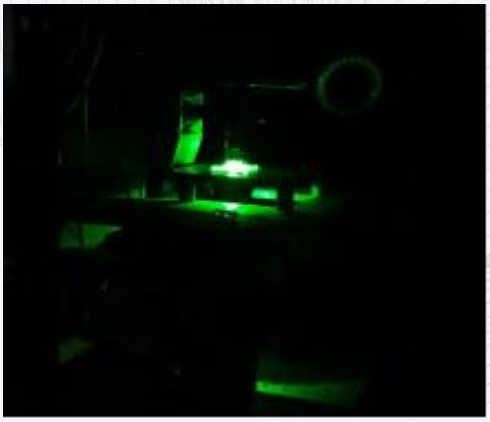
The CSCR Microscopy Core is a full-service facility serving the research community. We aim to provide personalized assistance on all aspects of imaging, from tips on sample preparation to training on our microscopes to processing and analysis of image data. Our facility currently houses one Multiphoton Laser Scanning Microscope (OLYMPUS FV1000 MPE), confocal (OLYMPUS FV1000), and three fluorescence (EVOS FLAuto, LEICA DMI6000B, LEICA DMi8), and four widefield light microscopes, and one computer dedicated to image processing and analysis.

I. Laser Scanning Confocal Microscope System (Olympus FV1000)



The Olympus FV1000 confocal system comprises a motorized microscope with z focus drift compensation facility for bright field, differential interference contrast and fluorescence imaging with a motorized XY scanning stage, and a CO2 incubation facility for live cell imaging. It is equipped with the following lasers - 405nm, Multi-Argon (458nm, 488nm, and 515nm), 559nm, and 635nm. Apart from regular confocal imaging, this microscope can be used to perform Multi-Area Time Lapse, FRET, FRAP, FLIM, and diffusion experiments.

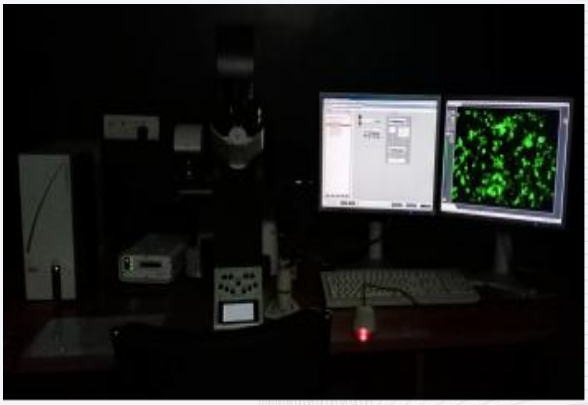
II. Laser Scanning Multi Photon Microscope (Olympus FV1000MPE)



The FV1000MPE is an upright multiphoton laser-scanning microscope coupled with a Mai Tai HP-Deep See–OL laser with automated broadband wavelength tuning from 690 to 1040nm for deep tissue imaging.

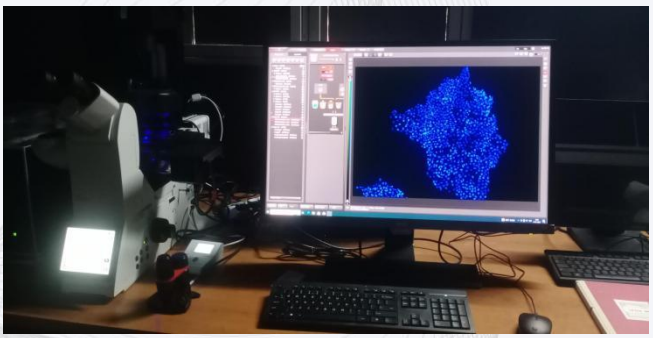
The systems support multicolor fluorescent studies for imaging of living, whole mount, or thickly sliced specimens. Dynamic biological processes can be imaged hundreds of micrometers within living cells and tissues. Provides support for applications where phototoxicity/photobleaching is a concern such as time course studies of living cells and tissues. Low magnification lens and long working distance stage allow imaging of large samples, embryos, and animals.

III. Leica DMI6000B Inverted Fluorescence Microscope



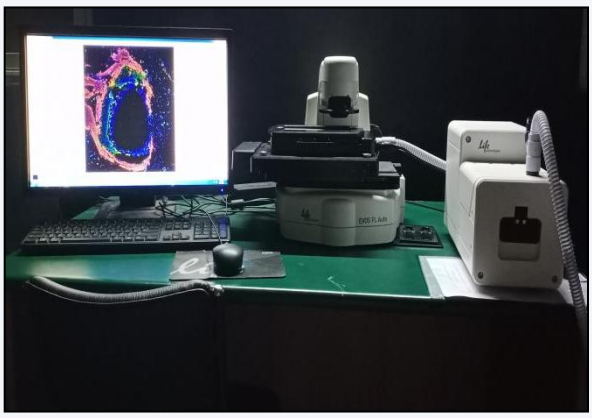
The Leica DMI6000B is an inverted fluorescence microscope comprising 6 interchangeable filters for detecting various fluorochromes. It has two independent cameras – DFC295 for high-resolution bright-field imaging and DFC360 FX for high-frame-rate fluorescence imaging. It is also equipped with a fluorescence intensity manager and programmable function keys for easy access to functions.

IV. Leica DMi8 Inverted Fluorescence Microscope



The Leica DMi8 is a premium-class modular research microscope with all options of intelligent automation. DMi8 is a motorized inverted fluorescence microscope with high-resolution dual-mode camera and software. The features include a motorized magnification changer, motorized objective turret, motorized Z-focus, electronic focus repositioning, electronic parfocality, and automatic lowering before the objective change. Touch screen for intuitive control of all functions, contrasting techniques including status display. The objective includes 10X, 20X, 40X, 63X and 405, GFP and Y3 filter cubes. Installed with high-resolution K3C color camera and K5 monochrome camera for superior quality imaging. LAS X premium software for users with a wide variety of multi-dimensional imaging requirements with advanced functionality including image stitching, environmental control, and mobile connection.

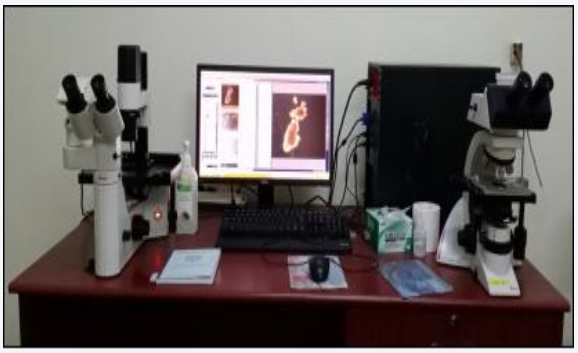
V. EVOS FL Auto Imaging System



The EVOS® FL Auto Imaging System is a fully automated, digital, inverted multi-channel fluorescence and transmitted light imaging system with outstanding workflow efficiency. Designed to meet demanding requirements over a broad

range of applications, the EVOS® FL Auto system supports high-resolution mosaic tiling, multi-position well scanning, cell counting with thresholding, and time-lapse studies. The intuitive interface, proprietary light cubes, dual cameras, precision automated stage, and parfocal optical system enable us to produce publication-quality images in seconds. The EVOS® FL Auto system can be programmed to run acquisition routines, 8-point time-lapse experiments, and tile-stitch scans in nearly any vessel through the sensitive touch-screen display.

VI. Light Microscopes



Olympus BX43F upright microscope, **Leica DMIL** (upright), and **Leica DMI1000** (inverted) microscopes are available for users to perform routine light microscopy imaging. DMIL and DMI1000 microscopes are provided with an interchangeable Leica DFC290 camera for high-resolution bright-field imaging. The **Leica DMI1000** is also installed in the tissue culture facilities of individual labs and the Core tissue culture area.

ZEISS Primovert is an inverted transmitted-light microscope of compact design with a small footprint. Bright-field and phase contrast images can be taken. It is primarily used to examine cell and tissue cultures as well as sediments in culture flasks, petri dishes, and microtiter plates.

VII. Training Sessions

The Imaging Core Facility conducts training sessions regularly for both first-time and experienced users. The training sessions comprise specifically designed modules which include theory and practical sessions. The final authorization is given to the user upon completing the required modules. The hands-on training sessions are tailored to the specific application requirements of each user so that they get the maximum benefit out of these systems. Apart from in-house training, the imaging core organizes sessions by application specialists from Leica, Zeiss, and Olympus.

Number of Principal Investigators using Core Imaging facility for the research (May 2023-April 2024):

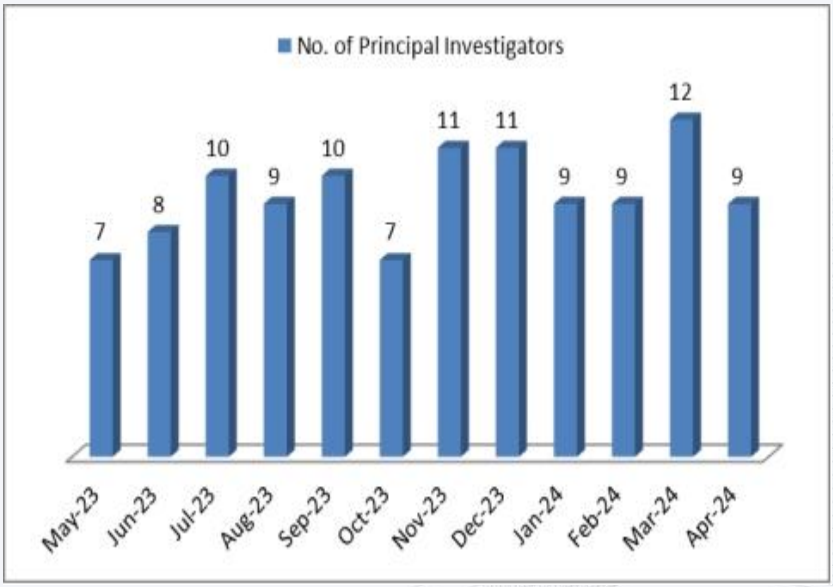


Figure 2: The above figure shows the number of Principal Investigators who utilize the imaging facility at CSCR from May 2023 to April 2024.

Internal and External Users for Imaging Facility:

S. No	Institute/ Department	Number of PIs
1	CSCR, Vellore	8
2	Dept. of Haematology, CMC, Vellore	2
3	Dept. of Paediatric Ortho, CMC, Vellore	1
4	Dept. of Physiology, CMC, Vellore	3
5	Dept. of Anatomy, CMC, Vellore	1
6	Dept. of Biochemistry, CMC, Vellore	2
7	Wellcome Trust Research Laboratory, CMC, Vellore	1
8	Dept. of Child Health, CMC, Vellore	1
9	VIT, Vellore	2

The above table shows the number of principal investigators from CSCR, different departments of CMC, and other academic institutions using the CSCR core Imaging facility.

CSCR - Centre for Stem Cell Research (A unit of InStem), CMC Campus, Vellore
VIT- Vellore Institute of Technology, Vellore

Scientist In-Charge

B. SANDYA RANI, Ph.D.
Scientist-D, CSCR, Vellore

GMP Facility

GMP Facility Team Members

1. About the Facility

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.

GMP facility has two sections 1) cell therapy product manufacturing facility and 2) genetically modified cells manufacturing facility. The cell therapy product manufacturing facility has a total area of 1200 square feet. It is divided into four independent ISO Class 7 manufacturing suites and a common staging room. Each suite is equipped with a Class II biological safety cabinet, CO_2 incubators, a high-speed centrifuge, and an inverted phase-contrast microscope with a camera. The facility also has a raw material and product storage room with a controlled rate freezer, liquid nitrogen containers for storage of cellular products in cryovials, and cryopreservation bags in the vapour phase.

The facility for genetic modification of cells has a total area of ~2800 square feet. Clean rooms are divided into two independent suites. Each suit has ISO class 7 and class 6 areas for isolation, genetic modification, and final filling of the product. Each suite is equipped with the necessary equipment required for cell enrichment, culture, genetic modification, and cryopreservation of the cells.

This facility has been granted a license from the Central Drugs Standard Control Organization (CDSCO) and the Office of Director of Drug Control, State licensing authority (SLA), Chennai Tamil Nadu in Form CT11 and Form 29 for manufacturing of genetically modified hematopoietic stem cells to conduct the following phase 1 clinical trials "Gene therapy product for hemophilia A with high expression FVIII transgene in autologous hematopoietic stem cells (CD68-ET3-LV-CD34+) for patients suffering with severe hemophilia A and to manufacture "Culture expanded satellite cells/myoblast for the treatment of urinary incontinence in female patients with urethral sphincter insufficiency".

2. Services

The following are the services provided by the GMP facility:

- Clean-room suites for manufacturing of clinical grade products under GMP conditions for clinical applications.
- Process development for genetic modification of cells by using viral and non-viral methods.
- Derivation and expansion of various cell types such as mesenchymal stem cells, induced pluripotent stem cells, T cells, chondrocytes, and skeletal muscle-derived stem cells.
- Genetic modification of cells by using viral and nonviral methods.
- Cryopreservation and storage of cell and gene therapy products.

Quality control

- Endotoxin testing using the endo-safe PTS system.
- Mycoplasma testing for various types of samples.
- Molecular characterization and immunophenotyping of various cell types.
- Viability and Colony forming unit assay.
- DNA isolation and vector copy number determination.
- PCR and QPCR-based assay.

Regulatory approval

- Provides support in the regulatory approval process - Evaluate and interpret regulations for cell and gene-based therapy from relevant agencies to apply for clinical trial and manufacturing licenses from relevant regulatory authorities.

3. Facility Maintenance

- Manufacturing suites are cleaned regularly (including ceiling, wall, and floor).
- Change over cleaning between each manufacturing batch.
- Environmental monitoring program for both viable & non-viable contaminates.
- Daily checks for door pressure, temperature, and humidity.
- Liquid nitrogen level monitoring and scheduled filling of the storage tanks.

4. Current Projects

The GMP facility is involved with the following projects:

a. Manufacturing of Genetically Modified Hematopoietic Stem Cells for Phase 1 Clinical Trial for the Treatment of Patients Suffering from Severe Hemophilia A

During the previous year, we manufactured genetically modified hematopoietic stem cells from 3 severe hemophilia A patients by using a clinical-grade lentiviral vector with the FVIII gene. For this, patients underwent mobilization of CD34 cells and apheresis. CD 34 cells were enriched by using cliniMACS and cultured in an HSPC culture medium for pre-stimulation. These HSPCs were transduced with lentiviral vectors in appropriate culture conditions. The final product was cryopreserved and tested for various quality control parameters. To manufacture and perform the quality control it takes 21 days per sample. During this procedure, we use the facility for viral-mediated transduction. We also optimized the transduction conditions by using transduction enhancers and established a robust single transduction protocol for manufacturing the hemophilia A gene therapy product.

b. Culture-expanded Satellite Cells/Myoblast for the Treatment of Urinary Incontinence in Female Patients with Urethral Sphincter Insufficiency.

During the previous year, we manufactured 2 batches of muscle-derived stem cells from urinary incontinence patients. For this, abdominal skeletal muscle tissue was collected from the patients and digested using collagenase type II enzyme. Dissociate cells were separated from the clumps and replated in a non-coated culture flask. After the attachment of cells, muscle-derived stem cells in the supernatant were collected, centrifuged, and plated on a myo-cult coated culture flask.

They were expanded for 3 passages and assessed by using various quality control parameters for clinical use. For manufacturing and quality control of each product takes 21-25 days.

c. Derivation and Expansion of iPSC Lines using Nonviral Reprogramming Methods

During the previous year, clones from passage 2 were thawed and expanded to passage 10 to generate seed stock from 6 homozygous HLA donors. After expansion, iPSC clones were found to have OCT-4, Nanog, TRA 1-60, SSEA4, etc. marker expression and normal karyotype. For reprogramming, peripheral blood samples were used from homozygous HLA haplotyped donors for reprogramming process. Mononuclear cells were isolated and erythroid progenitor cells were expanded for electroporation with GMP-grade reprogramming plasmids in xenofree conditions. For expansion of cells from each donor it takes 40-50 days.

5. Training and Access

The GMP facility staff are regularly trained to carry out product and process development activities and manufacture various clinical-grade cellular and gene therapy products. Access to the facility is limited only to GMP facility-trained staff. The services are available for investigators from Christian Medical College, Vellore, and other non-profit organizations.

TEAM MEMBERS

Technical Officer

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PRAVEENA M , B.Sc., PGMLT
Assistant Graduate Technician, CSCR, Vellore

Scientist In-Charge

GURBIND SINGH, Ph.D.
Scientist-D, CSCR, Vellore

Laboratory Animal Facility

Laboratory Animal Facility Team Members

Specific Pathogen Free (SPF) laboratory animal facility (LAF) is located in the basement of the CSCR building with a total floor space area of 5000 sq. ft. The CSCR-LAF maintains several different strains of mice - including wild, transgenic, knock-out, and SCID strains and Sprague Dawley rats. Most of these strains are bred under strictly inbred conditions. Animals are housed in individually ventilated caging (IVC) systems for breeding, holding, and experimentation. The environmental parameters are maintained as per the CPCSEA guidelines. A qualified veterinarian supervises day to day functioning of the facility and provides all necessary veterinary care to ensure that healthy animals are available for research. The LAF is also providing hands-on training for the research scholars of CSCR on animal handling and bio-methodologies and ensuring the ethical treatment of animals.

Specialized Pieces of Equipment

The CSCR-LAF is equipped with in vivo small animal imaging system (IVIS CT spectrum), animal blood counter, zoom stereo microscopes, multiphoton microscope, and blood irradiator with Co-60 as a source, in addition to a couple of Isoflurane anaesthetic vaporizers, induction and heating chambers.

Quality control (QC)

A quality control program for environmental microbiological monitoring, genetic monitoring, and health monitoring of animals by culture analysis, ELISA and PCR tests are regularly followed. The reports of QC tests are maintained in the LAF office.

Individually Ventilated Cages (IVC)

IVIS CT spectrum

Ongoing projects (approved by IAEC):

S.NO	Project title	Duration	Animal stains
1	Lipid nanocarrier-guided chemically modified factor IX messenger RNA therapy for Hemophilia B	3 years	Balbc/j
2	In vivo efficacy and safety studies of CSCR-ST04, the gene-edited autologous hematopoietic stem cells for gene therapy of beta hemoglobinopathies	3 years	NBSGW
3	Precise correction of Hbe and major b-thalassemia mutation using base editors	2 years	NBSGW
4	Strategies to optimize the Treosulfan/Thiotepa/Fludarabine regimen and post-transplantation in haematopoietic stem cell	1 year	Balbc/j
5	To evaluate the effect of GSK 4716 ligand in human chronic myeloid leukemia xenograft mouse model	3 years	NSG
6	Generation of novel Lentiviral gene therapy vectors for haemoglobinopathies	2 years	NBSGW
7	Liver targeting lipid nanocarrier guided, No End DNA gene therapy for hemophilia A & B	3 years	Balbc/j, HEMO A & HEMO B
8	Target disruption of the binding domain in the BCLIIA for therapeutic reactivation of fetal hemoglobin	2 years	NBSGW
9	Modulating retinoid X-receptor-A (RXRA) and its coregulators to overcome chemoresistance in myeloid leukemia	2 years	NSG
10	Can anti-helminthics improve chemosensitivity in acute myeloid leukemia by mediating nuclear hormone receptor expression	2 years	NBSGW

TEAM MEMBERS

Veterinary Officer

R.VIGNESHWAR, M.V.Sc.

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R. PAVITHRA J, B.Sc., P.G.D.M.L.T
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S. ASHOK KUMAR, D.M.L.T
Sel. Gr. III Technician, CSCR, Vellore

Faculty Support

SRUJAN MAREPALLY, Ph.D.
Scientist-E, CSCR, Vellore

11. ANNUAL CELL & GENE THERAPY SYMPOSIUM

8th ANNUAL SYMPOSIUM ON CELL AND GENE THERAPY (a Hybrid event)

31st August, 1st and 2nd September, 2023

The Centre for Stem Cell Research (CSCR), a unit of inStem, Bengaluru, managed by Christian Medical College (CMC), Vellore organized the 8th Annual Symposium on Cell and Gene Therapy on 31st August, 1st and 2nd September, 2023. This symposium brought together scientists, physicians, and all others interested in and responsible for developing this field in the country. Dr. Maneesha Inamdar, Director, inStem, Bangalore addressed the participants through a virtual platform.

The program this year focused on Gene Editing, Applications of iPSC Technology, Gene Therapy, Manufacturing and Regulatory aspects in Cell and Gene Therapy, Technology Advances in cell and gene therapy, Safety Aspects of Gene Therapy, and Cell Therapy in Cartilage Repair. About 351 participants from across the country and 31 speakers from around the world took part in the symposium.

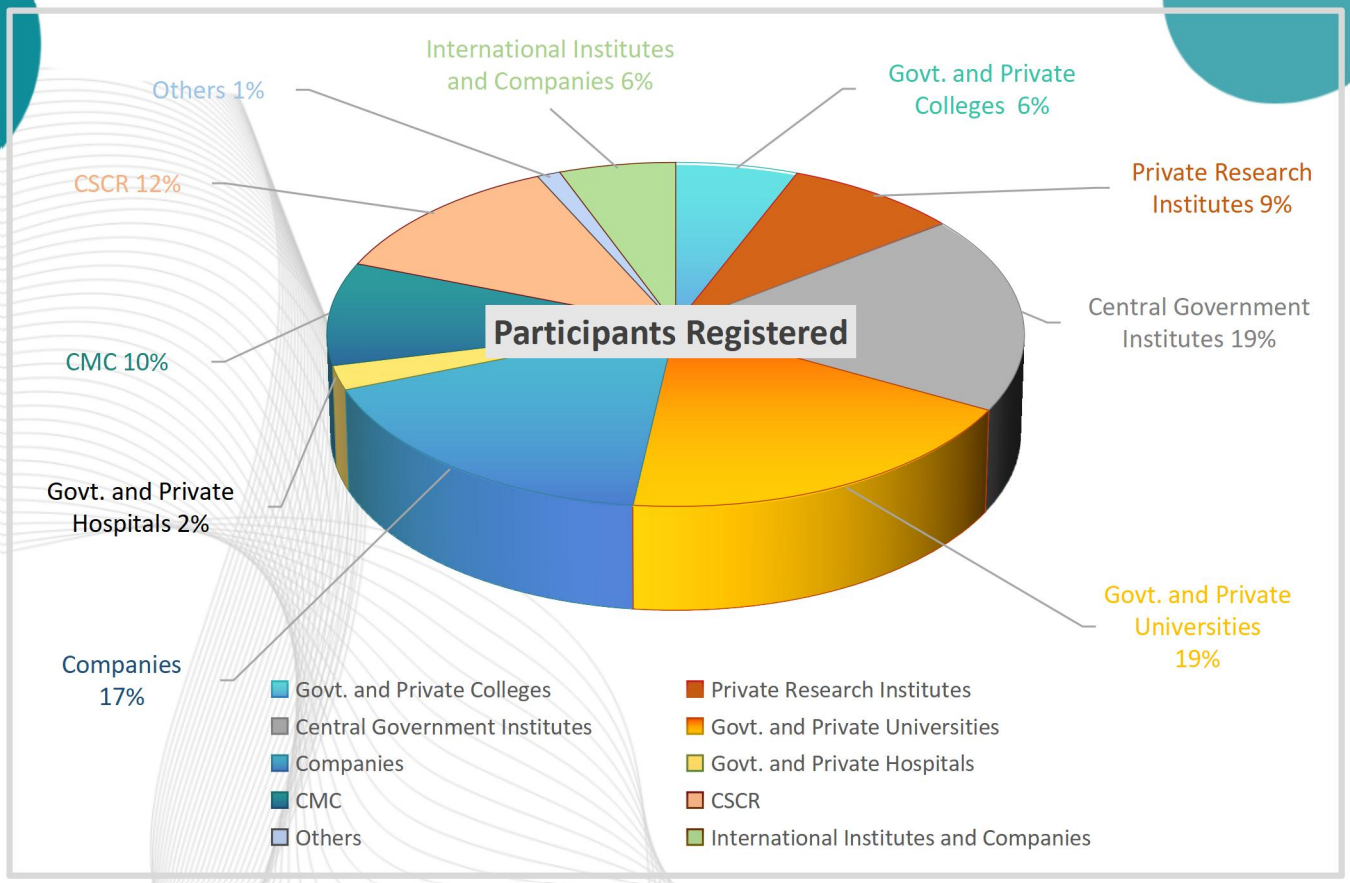
The first day of the symposium was devoted to Cell Therapy in Cartilage Repair, Safety Aspects of Gene Therapy. Participants from the industry also gave a presentation on advanced technologies developed for the processing and manufacturing of cell therapy products. On the first day, GMP manufacturing and regulatory aspects in cell and gene therapy were also discussed. There were presentations on Lentiviral Vector Manufacturing for use in Phase I/II Clinical Trials and manufacturing of CAR T cells for Point-of-Care treatment of refractory hematological malignancies.

The keynote address of the symposium was delivered by Prof. Martin H. Steinberg from Boston University Chobanian & Avedisian School of Medicine, Boston, USA. He delivered the keynote address titled "Gene Therapy and Fetal Hemoglobin: Progress Toward a Cure for Sickle Cell Disease."

The second day of the symposium featured a variety of discussions on immune cell therapy, applications of iPSC technology as well as technology advances. The keynote address of the symposium was delivered by Dr. Roger Hajjar from Gene and Cell Therapy Institute, Mass General Brigham, Cambridge, USA. He delivered the keynote address titled "Clinical Cardiac Gene Therapy Comes of Age".

The third day of the symposium featured a variety of discussions on gene editing, non-viral nucleic acid transfer, and industry updates on cell and gene therapy. All participants praised the symposium as a one-of-a-kind event in the country.

CSCR plans to hold this meeting on an annual basis to help promote this area of research in India through cross-discipline dialogue and collaborations on a diverse range of inter-connected issues relevant to the field.


Participants Registered
Others 1%
International Institutes and Companies 6%
Govt. and Private Colleges 6%
CSCR 12%
Private Research Institutes 9%
CMC 10%
Central Government Institutes 19%
Govt. and Private Hospitals 2%
Govt. and Private Universities 19%
Companies 17%
Govt. and Private Colleges
Private Research Institutes
Central Government Institutes
Govt. and Private Universities
Companies
Govt. and Private Hospitals
CMC
CSCR
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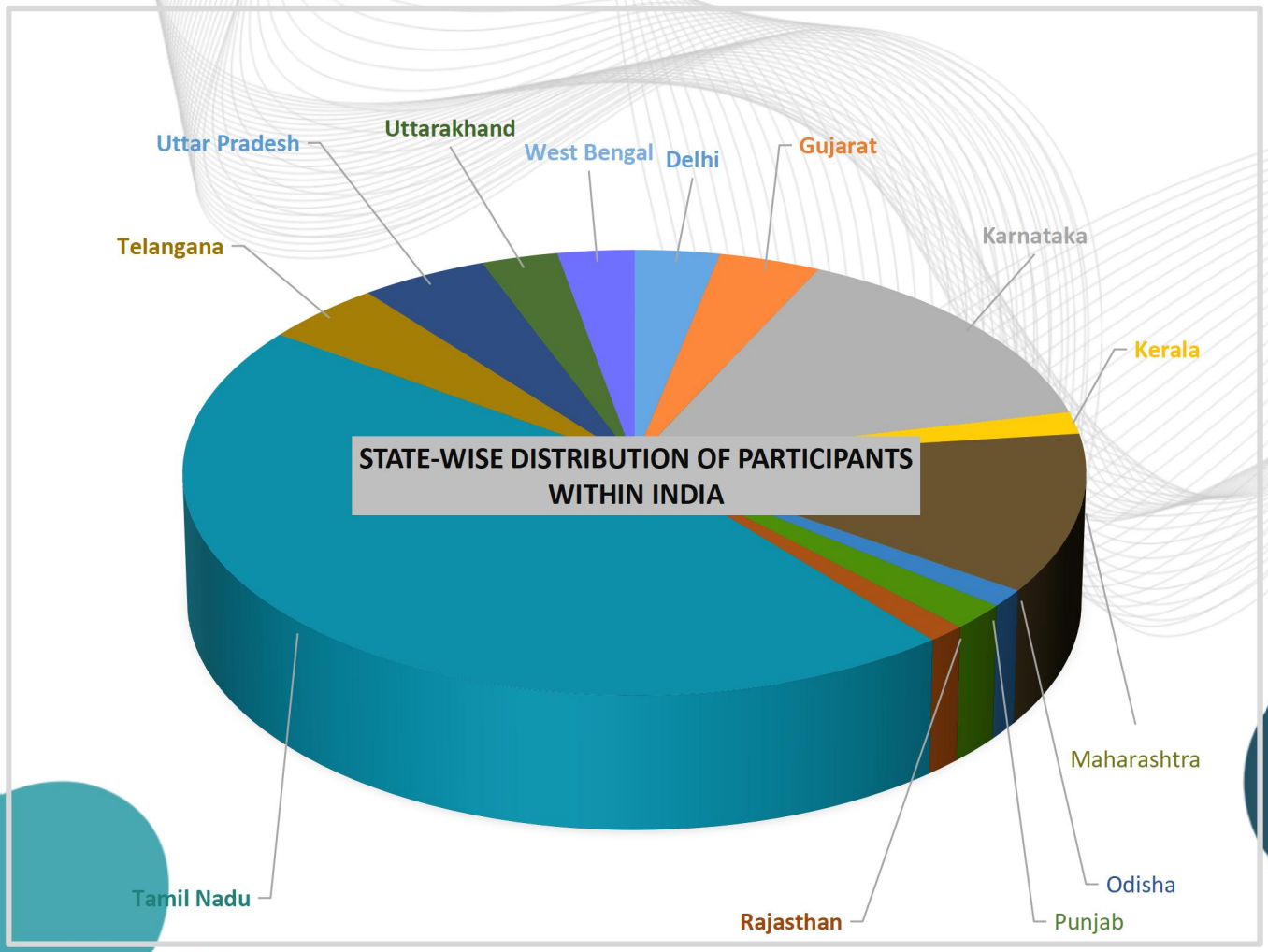
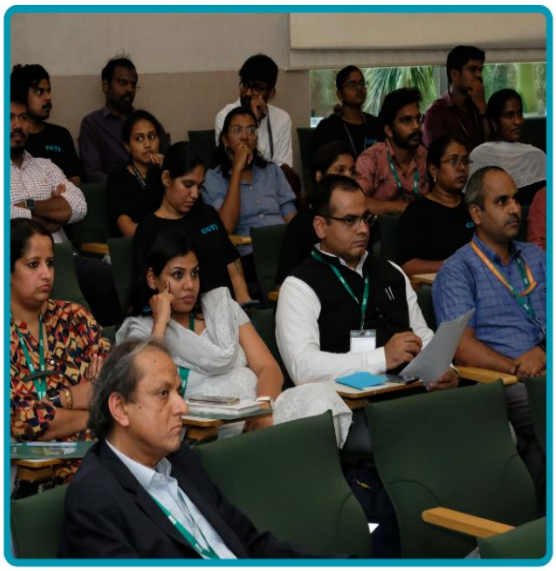
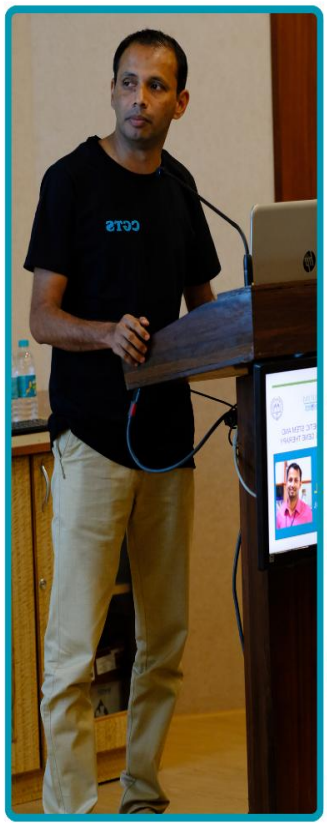

STATE-WISE DISTRIBUTION OF PARTICIPANTS WITHIN INDIA
Uttar Pradesh
Uttarakhand
West Bengal
Delhi
Gujarat
Telangana
Karnataka
Kerala
Maharashtra
Odisha
Punjab
Rajasthan
Tamil Nadu

Glimpses of 8th Annual Cell & Gene Therapy Symposium - 2023

Inaugural Session



Glimpses of 8th Annual Cell & Gene Therapy Symposium - 2023



Scientific Session

TALKS AND Q&A

12. EDUCATION & TRAINING

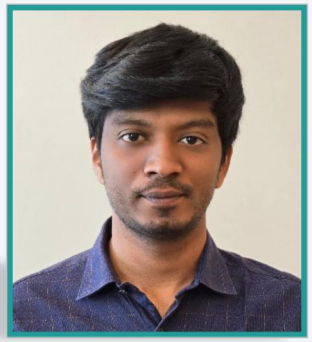
Education & Training

I. PhD Program

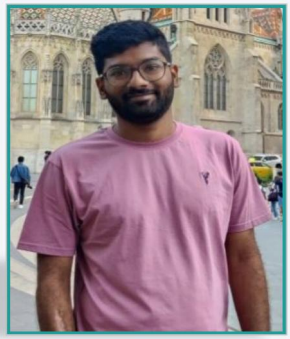
CSCR has an active PhD programme and the students can register for PhD under Sree Chitra Thirunal Institute of Medical Sciences and Technology (SCTIMST), Thiruvananthapuram, Thiruvalluvar University, Vellore and Manipal University, Manipal.

S.No. Details of Ph.D. Thesis Awardees

1. **Name: Nithin Sam R**
   Registered University: SCTIMST University, Kerala
   Thesis title: Genome editing strategies for the treatment of hereditary haematological disorder
   Thesis Guide: Dr. Mohankumar K.M.
   Date of Ph.D award: 31st October 2023



2. **Name: Vignesh R**
   Registered University: SCTIMST University, Kerala
   Thesis title: Pre-Clinical Study for Therapeutic Genome Editing in Beta-Haemoglobinopathies
   Thesis Guide: Dr. Mohankumar K.M.
   Date of Ph.D award: 14th March 2024



II. Other training programs

Short-term student projects (Bi-annual)

S. No	Name of the Students	Duration	Qualification	Name of the University	Principal Investigator
1.	Ms. Ramyadevi	Jul – Dec 2023	M.Tech - Biotech	VIT University, Vellore	Mohankumar KM
2.	Ms. Devika Gopi	Jul – Dec 2023	B.Tech – Biotech & Biochemical Engineering	APJ Abdul Kalam Technological College, Kerala	Mohankumar KM
3.	Mr. Jhagan R	Jul – Dec 2023	BS-MS in Biological Sciences (Integ.)	Indian Institute of Science Education & Research Tirupati.	Saravanabhavan T
4.	Mr. Udit Millenn R	Jul – Dec 2023	M.Tech - Biotech	VIT University, Vellore	Saravanabhavan T
5.	Ms. Ramya A	Jul – Dec 2023	M.Sc - Biotech	DKM College, Vellore	Saravanabhavan T

6.	Ms. Jayashree K	Jul – Dec 2023	M.Sc - Biotech	DKM College, Vellore	Srujan K Marepally
7.	Mr. Yuvachandranviva V	Jul – Dec 2023	M.Tech - Biotech	VIT University, Vellore	Srujan K Marepally
8.	Mr. Yogesh D	Jul – Dec 2023	M.Sc – Biotech (Integ.)	VIT University, Vellore	Srujan K Marepally
9.	Ms. Jayashree K	Jan – June 2024	M.Sc - Biotech	DKM College, Vellore	Srujan K Marepally
10.	Mr. Yuvachandranviva V	Jan – June 2024	M.Tech - Biotech	VIT University, Vellore	Srujan K Marepally
11.	Mr. Yogesh D	Jan – June 2024	M.Sc – Biotech (Integ.)	VIT University, Vellore	Srujan K Marepally
12.	Ms. Divyaa Sree S	Jan – June 2024	M.Sc – Biotech (Integ.)	VIT University, Vellore	Thiyagaraj M
13.	Ms. Mariya Sneha Rani J	Jan – June 2024	M.Sc – Biotech	Kalasalingam Academy of Research and Education Madurai.	Elizabeth Vinod
14.	Ms. Merin Mary Zachariah	Jan – June 2024	B.Tech - Biotech	Karunya Institute of Technology and Sciences, Coimbatore	Elizabeth Vinod
15.	Mr. Kevin Johnston A	Jan – June 2024	MSc Zoology	MSc Zoology Christ (Deemed to be) University, Bengaluru.	Saravanabhavan T
16.	Mr. Jeevan Kumar	Jan – June 2024	M.Sc – Biotech	VIT University, Vellore	Saravanabhavan T
17.	Ms. Swathi K	Jan – June 2024	MSc Zoology	MSc Zoology Christ (Deemed to be) University, Bengaluru.	Saravanabhavan T
18.	Ms. Jayasri R	Jan – June 2024	B.Tech Industrial Biotech	Anna University, Guindy, Chennai	Mohankumar KM

13. SCIENCE OUTREACH AND COMMUNICATION- ANNUAL ACTIVITIES

Science Outreach and Communication-Annual Activities

- The Stem Cell Awareness Program organized on 13th October 2023. Total participants: 95 (5 colleges).
- CSCR Foundation Day celebration on 1st Dec 2023. Total participants: 117
- Technical presentation (Virtual) on "Applications of NanoFCM" by Ms. Anindita Guha, Product and Application Specialist, SG Instruments, Delhi on 26th Dec 2023 - Coordinated by Dr. Sandya Rani. B. Total participants: 5
- Hands-on Instrument Demonstration - Moxi GO II QC Cell analyzer by Dr. Hemanth Agarwal, Application Scientist, LabGig, Bangalore at CSCR on 29th Dec 2023 - Coordinated by Dr. Sandya Rani. B. Total participants: 17
- On February 28, 2024, On the occasion of National Science Day, CSCR organized a one-day program on 28th Feb 2024 for 175 school students and 10 teachers from 6 schools in Vellore, followed by Scientific talks, a physical tour, and science experiments.
- Benchtop Confocal Microscope BC-43 ANDOR Demo and hands-on training conducted at CSCR on 6th and 7th March 2024 jointly by Toshniwal Brothers (SR) Pvt. Ltd. and CSCR. Coordinated by Dr. Sandya Rani. B, Scientist-D. Total participants: 15.

CSCR Day Celebration

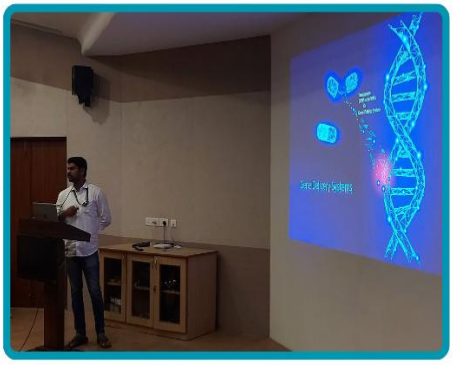
1st December, 2023

Scientific Talks

CSCR Day Celebration

1st December, 2023

Demonstration Activities



Stem Cell Awareness Day

13-10-2023

"Science is a continuous journey; there is always something new and exciting to discover."

Sir C V Raman

14. PERSONNEL AT CSCR

PERSONNEL AT CSCR

Scientists /Adjunct Scientists

Dr. Nihal Thomas	Professor and Head
Dr Alok Srivastava	Former Professor and Head
Dr. Mohankumar Murugesan	Scientist-E
Dr. Saravanabhavan Thangavel	Scientist-E
Dr. Srujan Kumar Marepally	Scientist- E
Dr. Gurbind Singh	Fellow-E (Scientist-D)
Dr. Sandhya Rani	Fellow-E (Scientist-D)
Dr. R. V. Shaji	Adjunct Scientist
Dr Vrisha Madhuri	Former Professor and Adjunct Scientist
Dr. Elizabeth Vinod	Adjunct Scientist
Dr. Susan Jehangir Homi	Adjunct Scientist
Dr. Jeyanth Rose	Adjunct Scientist
Dr. Eunice Sindhuvi	Adjunct Scientist
Dr. Poonkuzhali Balasubramanian	Adjunct Scientist
Dr. Sonam Pandey	Scientist (Research Development Office)
Dr. Thiyagaraj Mayuranathan	DBT-Ramalingaswami Re-entry Fellow

Veterinary /Technical Officers

Dr. Vigneshwar R.	Veterinary Officer
Mr. Mohanashankar	Technical Officer
Mr. Rajesh A.	Technical Officer

Research Associates

Dr. Dinesh Babu	Research Associate- III
Dr. Vinu R	Research Associate -III

Dr. Praveen Kumar L	Research Associate -III
Dr. Gokulnath	Research Associate- I
Mr. Ashis Kumar	Research Associate- I
Dr. Arjun J K	Project Manager
Dr. Subbarayuda S.	Project Manager

Senior Research Fellows

Mr. Vigneswaran V	ICMR- Senior Research Fellow
Mr. Karthik C	DBT-Senior Research Fellow
Ms. Anila George	CSIR- Senior Research Fellow
Ms. Prathiba Babu	CSIR- Senior Research Fellow
Mr. Lokesh Panigrahi	CSIR- Senior Research Fellow
Mrs. Ramya	CSIR- Senior Research Fellow
Ms. Kriti Prasad	ICMR- Senior Research Fellow
Ms. Sevanthy	Senior Research Fellow
Ms. Nivedhitha D	Senior Research Fellow
Mr. Manoj Kumar	Senior Research Fellow
Mrs. Agnes Selina	Senior Research Fellow
Mr. Joshua Paul	Senior Research Fellow
Ms. Keerthiga A	Senior Research Fellow

Junior research fellows

Ms. Jyothis Mary Mathew	CSIR- Junior Research Fellow
Mr. Abhijit K	DBT- Junior Research Fellow
Mr. Malewar Shiva Santhosh	UGC- Junior Research Fellow
Mr. Anant Kumar	DBT- Junior Research Fellow
Ms. Madugula Shabareesh	Junior Research Fellow
Ms. Ayshath Ruksana C	Junior Research Fellow
Ms. Harrshani K	Junior Research Fellow
Ms. Asha Kiran Lima	Junior Research Fellow
Ms. Swathi K	Junior Research Fellow
Ms. Johnseena NM	Junior Research Fellow
Mr. Adityakumar Lanka	Junior Research Fellow

Project Associates

Ms. Porkizhi Arjunan	Project Associate II
Mr. Riswan N	Project Associate II

Technical Staffs

Ms. Aleya Tabasum	Sel. Gr. II Graduate Technician
Ms. Dhavapriya B.	Sel. Gr. III Graduate Technician
Ms. Pavithra R.	Sel. Gr. III Graduate Technician
Ms. Chitra P.	Staff I Graduate Technician
Mr. Abdul Muthallib	Staff I Graduate Technician

Ms. Praveena	Assistant Graduate Technician
Ms. Farzana	Assistant Graduate Technician
Mr. Joseph Joel	Graduate Technician Trainee
Ms. Esther Rani J.	Sel. Gr. II Technician
Mr. Ashok Kumar	Sel. Gr. III Technician
Ms. Poornasree Sadanandan	Lab Technician
Mrs. Sandhiya	Lab Technician
Ms. Faaikha Kounain	Lab Technician
Ms.Tharanya. p	Lab Technician
Ms..Jayashree. k	Lab Technician
Ms. Ramya a	Lab Technician

Project Staffs: NAHD and Control Program of Thalassemia and Sickle Cell Disease in Odisha

Dr. Kuryan George	Project Director
Dr Sreeya Das	Pathologist
Dr. Chinmayee Panda	Project Coordinator
Dr. Muhammed Basheer K M	Project Coordinator (Vellore)
Mr. Deepak Kumar Panda	Project Coordinator
Mr. Sangram Keshari Sarangi	Technical Officer (Training & Counselling)
Ms. Gomathi S.	Technical Officer (Data Manager)
Mr. Rashmi Rajan Swain	Graduate Technician
Mr. Solomon Ekka	Technical Officer
Ms. Ratipragya Das	Graduate Technician
Mr. Utkal Debasis	District Coordinator
Mr. John D	District Coordinator
Mr. Satyabrata Prusty	District Coordinator
Mr. Subash Chandra Hazra	District Coordinator
Mr. Bhibudatta Nayak	District Coordinator
Mr. Arun Maharana	District Coordinator
Ms. Korra Sandhya	Nurse Counsellor
Ms. Jyotirmayi Padal	Nurse Counsellor
Mr. Sushanta Kumar Sahu	District Coordinator
Ms. Diptimayee Ray	Office Secretary
Mr. Raj Kishore Panda	District Coordinator
Ms. Namrata Mohanta	District Coordinator
Mr. Litun Kumar Dora	Lab Technician
Ms. Samaleswari Senapati	Lab Technician
Mr. Subhabrata Barik	Lab Technician
Ms. Swayamprava Gouda	Lab Technician
Mr. Amaresh Behera	Lab Technician

Admin, Finance, and Support Staffs

Mrs. Anupama Nambiar	Assistant Manager
Mrs. Jenny John Thomas	Senior Agri. Officer
Ms. Vineetha	Secretary

Mr. Tamil Vanan J.	Librarian
Mr. Jaganathan	Administrative Assistant
Mr. Cladston	Sr. Finance (Consultant)
Mrs. Geetha	Accountant
Ms. Anisha	Accountant

Other Support Staffs

Ms. Priya	Receptionist
Mr. Muthukrishnan J.	Multi-tasking personnel
Mr. Silambarasan	Driver
Mr. Nithyanandham	Housekeeping staff
Mr. Saran Raj	Housekeeping staff
Mr. Arun Kumar	Housekeeping staff
Mr. Vijay	Housekeeping staff
Mr. Ramraj	Housekeeping staff
Mr. Shankar	Housekeeping staff
Mr. Augustin	Housekeeping staff
Mr. George	Housekeeping staff
Mrs. Renuga Devi	Housekeeping staff

15. GOVERNANCE OF CSCR

GOVERNANCE OF CSCR

CSCR Committee (as per CMC, inStem, and DBT MoU)

Director, CMC (Ex-officio)	Chairperson
Director, inStem (Ex-officio)	Co-Chairperson
Dean, inStem (Ex-officio)	Member
Principal, CMC (Ex-officio)	Member
Head, CSCR (Ex-officio)	Member Secretary/ Convener
Admin & Finance - CMC & inStem	DBT representative

CSCR- Scientific Advisory Committee

Dr. Mammen Chandy	Professor of Haematology, Former Director, Tata Medical Centre, Kolkata, West Bengal	Chairperson
Dr. Shiv Kumar Sarin	Senior Professor and Head, Department of Hepatology Director, Institute of Liver and Biliary Science, New Delhi	Member
Dr Harikrishna Varma	Head, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Poojappura, Trivandrum, Kerala	Member
Dr. Mahendra S Rao	Eminent scientist, Institute for Stem Cell Science and Regenerative Medicine, Bangalore, Karnataka	Member
Dr. Thangarajan Rajkumar	Professor and Head, Cancer Institute, Department of Molecular Oncology, Adyar, Chennai, Tamil Nadu	Member
Dr. Soniya Nityanand	Director, DrRML Hospital Lucknow, Uttar Pradesh	Member
Dr. Ramakrishna B S	Head, Dept. of Gastroenterology, SRM Institutes for Medical Science, Vadapalani, Chennai, Tamil Nadu	Member
Dr Alka Sharma	Senior Advisor, Department of Biotechnology, Ministry of Science & Technology, Government of India, New Delhi	Member

Dr. Jyotsna Dhawan	Emeritus Scientist, CSIR - Centre for Cellular & Molecular Biology, Hyderabad, Telangana	Member
Dr Maneesha Inamdar	Director, Institute for Stem Cell Science and Regenerative Medicine, Bangalore, Karnataka	Member
Dr Alok Srivastava	Professor of Medicine Department of Haematology Head, Centre for Stem Cell Research (a unit of inStem Bengaluru), Christian Medical College campus, Bagayam Vellore, Tamil Nadu	Member

Special Invitees

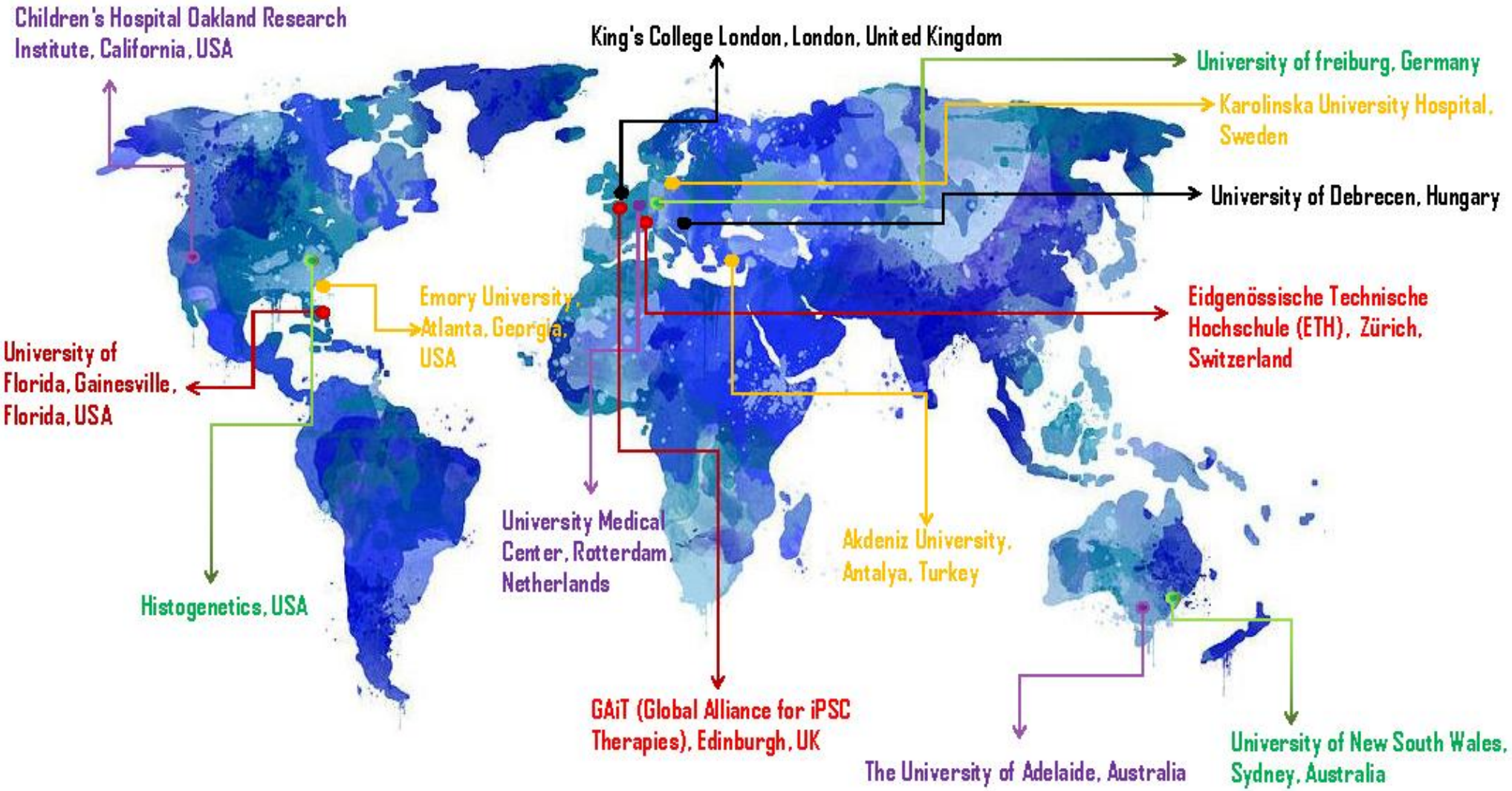
Dr. Vijay Chandru	Co-Founder and Director Strand Life Sciences Pvt. Ltd., Bangalore, India	Member
Dr. Cartikeya Reddy	Director, Open Platform for ORphan Diseases (OPFORD), Bangalore, India	Member

InStem Governing Body

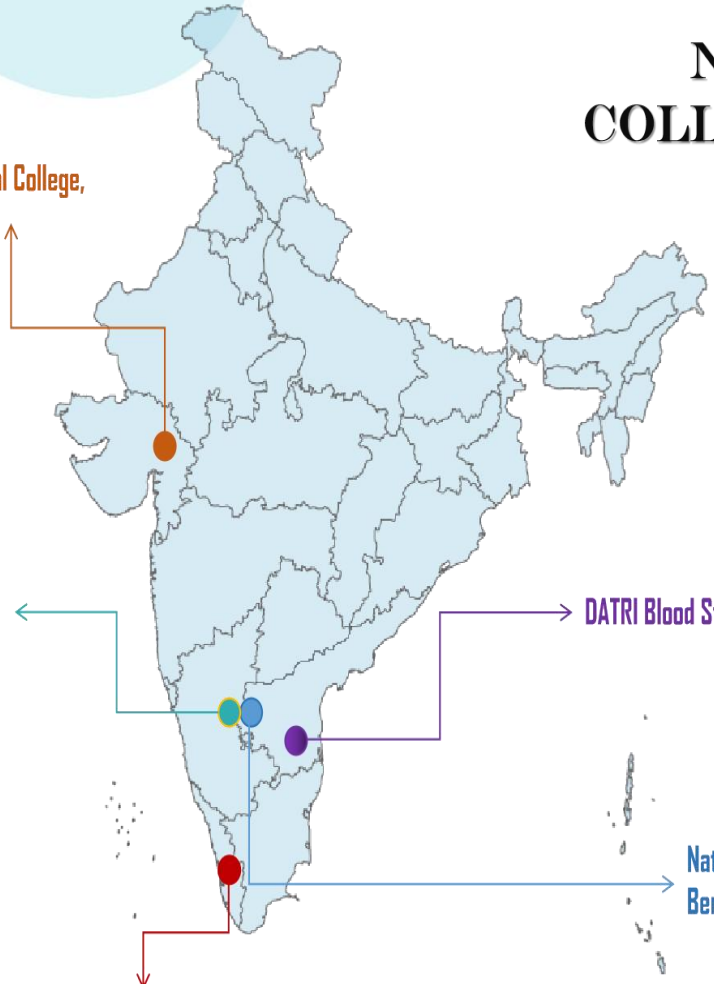
Secretary, DBT	Dr Rajesh Gokhale	Chairperson
Joint Secretary- Administration, DBT	Shri Sunil Kumar	Member
Additional Secretary & Financial Adviser, DBT	Shri Vishvajit Sahay	Member
Senior Adviser / Scientist 'H', DBT	Dr. Alka Sharma	Member
Director, inStem	Dr. Maneesha Inamdar	Member
Head Research, inStem	Dr. Arvind Ramanathan	Member
Scientist 'F', inStem	Dr. Dasaradhi Palakodeti	Member
Scientist 'F', DBT, Nodal Officer	Dr. Sangita M Kasture	Member
	Prof. Gagandeep Kang	Member
	Prof. Soniya Nityanand	Member
	Dr Dinakar Salunke	Member
	Prof. Vidita Vaidya	Member
Centre Director NCBS	Prof. L.S. Shashidhara	Member (Ex-Officio)
Director CMC, Vellore	Dr. Vikram Mathews	Member (Ex-Officio)
Director TIFR, Mumbai	Prof. Jayaram N Chengalur	Member (Ex-Officio)
Head, Administration inStem	Mr. Ramanathan K	Non-Member Secretary

External experts Member(s)	Dr. Soniya Nityanand, Director, RML Hospital Lucknow, UP, India; Dr. Dinakar M Salunke, Director ICGEB, New Delhi, India; Prof. Vidita Vaidya, Department of Biological Science, TIFR, Mumbai, India	Member(s)
Head, Admin and Finance inStem	Mr. Ramanathan K	Non-Member Secretary

CSCR INTERNATIONAL COLLABORATIONS



CSCR NATIONAL COLLABORATIONS



Smt. NHL Municipal Medical College, Ahmedabad, Gujarat

Institute For Stem Cell Science and Regenerative Medicine (inStem), Bengaluru, Karnataka

DATRI Blood Stem Cell Donors Registry, Chennai

National Centre for Biological Sciences (NCBS), Bengaluru, Karnataka

Sree Chitra Tirunal Institute for Medical Sciences & Technology,Trivandrum, Kerala

Annual Report
2024

CSCR Administration, Finance and Technical Team Members

CSCR Houskeeping Staff

To know more about CSCR, please scan the QR code or contact us **cscr@cmcvellore.ac.in**

Designer: Dr Sonam Pandey
Content Curation: Research Development Office

2024 ANNUAL REPORT

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