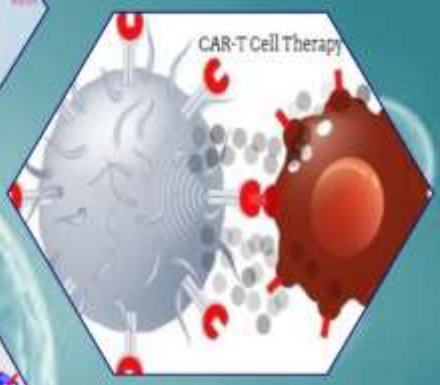

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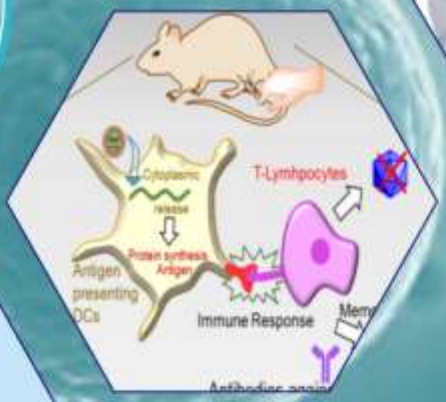


CENTRE FOR STEM CELL RESEARCH

(a unit of inStem, Bengaluru)

Christian Medical College Campus, Bagayam, Vellore

CONTENTS PAGE No.

Centre for Stem Cell Research (CSCR)

(a unit of inStem, Bengaluru)

Christian Medical College Campus, Bagayam, Vellore

The Beginnings

The establishment of the Centre for Stem Cell Research (CSCR) at the Christian Medical College (CMC) Vellore campus was approved by the Department of Biotechnology (DBT) of the Ministry of Science and Technology, Government of India, in December, 2005.

In July, 2011, CSCR (www.cscr.in) was integrated with the Institute for Stem Cell Science and Regenerative Medicine (inStem), Bengaluru (www.instem.res.in) and exists as the translational research unit.

Mandate

The mandate of CSCR is to bring stem cell science and regenerative medicine to management of human diseases with unmet needs. This is to be done by developing research along clearly defined themes addressed at enhancing understanding of disease biology to help create innovative diagnostics and therapeutics that are relevant to the needs of the country. It also aims to develop human resource for this field through doctoral programs as well as other training opportunities. An important goal will also be to share its facilities and expertise with other institutions and scientists working in this field in the country.

Governance: 2005 – 2010

Even though it was initiated as a project by the DBT, CSCR had 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 management of CSCR on a regular basis, both from the administrative as well as the scientific aspects. These included a Core Committee of faculty from CMC and CSCR who met regularly to resolve all matters at CSCR and a Steering Committee, chaired by the Director, CMC, Vellore along with other administrative officers to provide policy guidance for CSCR in the early stages of its establishment

CSCR – the translational unit of the inStem, Bengaluru from 2011

After completion of the project period of CSCR in 2011 it integrated with inStem from 1st July, 2011 through an MOA between DBT, inStem and CMC, Vellore. It continues to function at the Bagayam campus of CMC, Vellore with its emphasis on translational stem cell research and regenerative medicine. It is now governed by the CSCR committee chaired by the Director, CMC with the Director, inStem as the Co-Chair and includes the Principal, CMC, Vellore along with the Head, CSCR the member secretary / Convenor is member of the inStem both of which report to the inStem Governing Body, chaired by the Secretary, DBT. Given the predominantly translational nature of the research at CSCR, it also has a separate Scientific Advisory Committee.

Image Credit: Sonam Pandey, RDO, CSCR

CORE SCIENTIFIC ACTIVITIES AND INITIATIVES

THEMATIC RESEARCH PROGRAMS

It is the goal of scientist 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 is coordinated by Vrisha Madhuri. The other investigators in this group include, Srujan Marepally, Mohan Kumar, Nihal Thomas, Vikram Mathews, Dolly Daniel, Lilly Verghese, Alok Srivastava.

The major focus is 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 Karolinska Institute, Sweden, we have an ongoing phase I/II clinical trial for the treatment of osteogenesis imperfecta using fetal liver derived mesenchymal stem cells. In parallel we are exploring the paracrine and immunogenic effects of multiple infusions of MSCs via intraosseous and intravenous routes. Another phase I/II trial where the culture expanded muscle derived stem cells is used for the treatment of urinary sphincter incontinence. 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 have identified suitable biomaterials with kinetics for 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 also generating in vitro data to convert autologous chondrocyte therapy for physis or articular repair to a single step procedure bypassing the cell expansion step.

Another research program under the same theme is coordinated by Elizabeth Vinod. The other co-investigators in this group include Upasana Kachroo, Solomon Sathish Kumar, Soosai Amirtham Manickam, Abel Livingston, Viju Daniel Varghese, Alfed Job Daniel. The major focus is on the characterization of cartilage-derived progenitors and studying their potential implications for cartilage regeneration using in-vitro and in-vivo conditions. Their work also involves the characterization of soluble factors derived from these progenitors and assessing their potential for cultivating directly injectable therapeutic molecules.

They also work towards the creation and validation of osteoarthritic models in animals and the development of novel histological processing techniques for the assessment of chondrogenesis.

2. Gene therapy program

This program is coordinated by Alok Srivastava. The other investigators in this group include RV Shaji, Mohankumar Murugesan, Saravanabhavan Thangavel, Srujan Marepally, Sanjay Kumar and Gurbind Singh. This involves two major areas at present – The first is directed towards haemophilia where two programs are being pursued. First, a clinical trial for AAV vector-based gene therapy for haemophilia B in collaboration with Emory University, Atlanta, USA and the University of Florida, Gainesville, USA. Given the success of AAV based gene therapy reported in recent years, we have developed a novel transgene and vector combination for gene therapy of haemophilia B. The challenge with manufacturing the GMP grade vector is now being met through academic collaborations for transfer of technology from our collaborators in a major initiative for the first time in India as this technology does not currently exist in the country. Big bottle neck in gene therapy is viral vector production, towards this CSCR is partnering with National and international collaborators to bring AAV vector manufacturing. Dr. Kumar is coordinating AAV8-hFIX-Padua gene therapy for patients with haemophilia B. AAV-based state-of-the-art gene therapy technologies for the monogenic disease are to attempt finding a solution for lingering challenges in the Gene and Cell Therapy fields such as AAV-based therapeutic strategies.

The second component is a clinical trial of a lentiviral vector mediated haematopoietic stem cell based first in human gene therapy for haemophilia A. Here the lentiviral vector is ready and the final gene therapy product with the autologous stem cells will be manufactured at site in India. This has received approval of the CDSCO and should be initiated very soon. The second part of the gene therapy program involves gene therapy for the major haemoglobin disorders. Here there are two approaches being developed – a lentiviral vector-based gene addition as well as gene modulation technologies as well gene editing approaches using CRISPR-Cas9 and base editing technologies which have all been tested in cellular and animal models and are now getting close to clinical translation. This program also involves close collaboration with the Emory University, USA as well as other collaborators at University of Florida College of Medicine, USA. Other non-vector mediated gene transfer technologies are also being explored for nucleic acid transfers for gene therapy including the development of a mRNA-based vaccine against for SARS-CoV2 virus infection.

3. Cellular reprogramming and its applications - Disease modeling and Haplobanking

The area of cellular reprogramming technology is coordinated by R. V. Shaji at CSCR along with Dolly Daniel. This is now being applied to two areas: disease modelling and haplobanking.

Towards the former, reprogramming technology has been applied to the develop disease models of various bone marrow failure syndromes – Fanconi anemia, Diamond Blackfananemia and congenital dyserythropoieticanemia. The models are being used to evaluate disease phenotypes and mechanisms as well as evaluation of gene correction strategies. A major translational application has been the development of a "haplobank" – cells from HLA haplotype homozygous individuals whose mononuclear cells are being converted into iPSC lines for potential use in regenerative medicine.

The field and clinical aspects of procuring these peripheral blood samples through our collaborators, the DATRI unrelated donor registry, represented by NezihCereb, isbeing coordinated by Dolly Daniel. So far 15 GMP cell lines have been produced – one of the largest such collections in the world. This is also being done in collaboration with the international consortium for this effort – Global Alliance for iPSC Therapies (GAiT).

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 component at the National Centre for Biological Sciences (NCBS), Institute for Stem Cell and Regenerative Medicine (inStem), and the National Institute for Mental Health and Neurosciences (NIMHANS) at Bengaluru focuses on neurological disorders.

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. In addition, there is a community outreach component within NAHD related to developing a model for 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. The details are presented in the reports of individual scientists or other faculty involved.

The major components of this program are:

Clinical Trials for Gene Therapy of Haemophilia
»» Hemophilia B (see report of Alok Srivastava)
»» Hemophilia A (see report of Alok Srivastava)
»» AAV Antibody Assays (see report of Asha Abraham)

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 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 reports of the individual scientists.

RESEARCH PROJECTS

Given the translational mandate at CSCR, the unmet clinical needs in medicine and the interests as well as expertise available at the Christian Medical College, Vellore, there are several other areas of translational research that are also being pursued at CSCR. These include such as AAV based gene therapy, Immune cell Therapy, Cell Therapy for Ocular Disorder, Cancer Stem Cells in Endometrial cancer, etc.

1. Project title: Therapeutic applications of genetically manipulated human term-placenta-derived mesenchymal stem Cells (PD-MSCs) as drug cells for treating acute radiation sickness (ARS) and/or radiation-induced cutaneous damages

   Name of the Investigator: Dr Sanjay Kumar

   The study aims to provide best possible experimental combinations of mesenchymal stem cells (MSCs)-based therapy for acute radiation-accident victims including in the events of catastrophic

incidences. They propose to use genetically-manipulated human term-placenta derived mesenchymal stromal cells (MSCs) to promote hematopoietic recovery after lethal irradiation exposure. Placenta-derived mesenchymal stem Cells (PD-MSCs) are immune-privileged without the need for HLA matching even after repeat injections and have been documented to significantly home to radiation-injured tissues. MSCs have been shown to secrete an abundance of therapeutic proteins including anti-inflammatory angiogenic cytokines and hematopoietic growth factors that are involved in the prevention and treatment of acute radiation syndrome (ARS) by reducing cellular apoptosis/secondary tissue-damages.

2. Project title: Immune cell therapy
   Name of the Investigator: Dr. Sunil Martin

   Project I: CAR-T cells to target refractory or relapsed B cell Acute Lymphoblastic leukemia (r/r B-ALL):
   Immune cell therapy program is the generation of cGMP grade CAR construct. Sunil Martin's lab has generated the cGMP CAR construct by deleting the WPRE, GFP and P2A from the parental construct.

   Project II: Expansion and engineering of peripheral blood derived γ9δ2 T cells to treat blood cancers:
   They are also focused on optimizing a cGMP compatible methodology for expanding γ9δ2 T cells on serum-free media with minimal contamination of αβ T cells.

3. Mechanisms of Diseases

I. Project title: Cell Therapy for Ocular Disorder
   Name of the Investigator: Dr. Jeyanth Rose

   Ocular stem cell Lab is focused on the therapeutic use of Mesenchymal Stem Cell (MSCs) not only in treating ocular disorders but also in mitigating the mast cell response in severe allergies related to the conjunctiva.

II. Project title: Cancer Stem Cells in Endometrial cancer
   Name of the Investigator: Dr. Muthuraman N

   Muthuraman and his team are working on Endometrial cancer, which is one of the most common gynecological malignancy. They focused to study the effect of aspirin on genes of invasion and stemness in cancer stem cells, and its effect on sphere forming ability which will be useful to understand the effect in the treatment of chemo resistant cancer stem cells.

III. Project title: Biology of iron in RBC regeneration: upcoming players of regenerative medicine

Name of the Investigator: Dr. Eunice Sindhuvi

Eunice Sindhuvi is working on characterization of human erythroblasts at distinct stages and its implications on iron regulatory mechanism during normal erythropoiesis in vitro. They hypothesize that by studying the behaviour of HSCs in 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 regeneration and differentiation of Haematopoietic Stem Cells.

IV. Project title: Modulating chemoresistance in acute and chronic myeloid leukemia stem cells

Name of the Investigator: Dr. Poonkuzhali Balasubramanian

Project I: Modulation of NRF2 and downstream targets to overcome acquired chemoresistance in acute myeloid leukemia

Poonkuzhali Balasubramanian's laboratory has previously reported the role of Nrf2 in mediating inherent resistance to chemotherapeutic drugs using inherently resistant AML cell lines and primary patient samples. In this study, they are exploring the Nrf2 mediated factors of chemoresistance in acquired chemoresistant AML cell lines and leukemia stem cells (LSCs).

Project II: Generation of xenograft transplantation chronic myeloid leukemia mouse model

They are also focusing on the generation xenograft transplantation chronic myeloid leukemia mouse model. They transduce luciferase lentivirus into K562 and KCL22 cell line (chronic myeloid leukemia) and transplant into NSG mice.

Project III: Human chronic myeloid leukemia (CML) derived induced pluripotent stem cells (iPSCs) as a model to study the effect of Nuclear Hormone Receptor ligands in CML Stem Cells:

Their lab also generates induced pluripotent stem cells (iPSCs) from CML CD34+ cells and differentiates them into CML LSC like cells (lin-CD34+CD38-). They also test the effect of NHR ligands on CML LSC like cells in combination with Imatinib in-vitro and in-vivo.

A major strength of the scientific work at CSCR is its core laboratory facilities including the state-of-the-art laboratory animal facility. These facilities at CSCR provide the platforms on which many of the research programs are built. They are also available to a range of scientists from CMC, Vellore and from other institutions.

Scientists from nearly 10-15 departments in CMC use the molecular biology and flow cytometry facilities at CSCR as also several other institutions from Vellore and other parts of the country. A special mention should be made of the cGMP facility at CSCR which has allowed the initiation of early phase clinical trials in cell therapy and soon likely to support the first gene therapy clinical trial in the country. After about 12 years, since it was first built, it has been extended to include two additional suites with a separate air handling system to allow use of viral vectors and make gene modified cells for therapy.

Training is an important goal at CSCR through different programs. There is a PhD program affiliated to the following universities - the Sree Chitra Tirunal Institute of Medical Sciences and Technology, Thiruvananthapuram, the Thiruvalluvar University, Vellore and the Manipal Academy of Higher Education (MAHE). There is also short-term training for JRFs and MSc students from different universities. An important educational and scientific activity is also the annual cell and gene therapy symposium which brings all Indian scientists as well as invited international experts together to present their work and move the field forward in the country.

A very significant advance in terms of translation is the conceptualization of a special purpose vehicle that will focus on translating and advancing research products and services developed at CSCR.

There is now a pipeline of such products and services awaited that creates a need for such an entity. This has been discussed between the major stakeholders in CSCR – inStem, Bengaluru and CMC, Vellore with the knowledge of the Department of Biotechnology, Government of India and the process for its incorporation as a Section 8 company has been initiated.

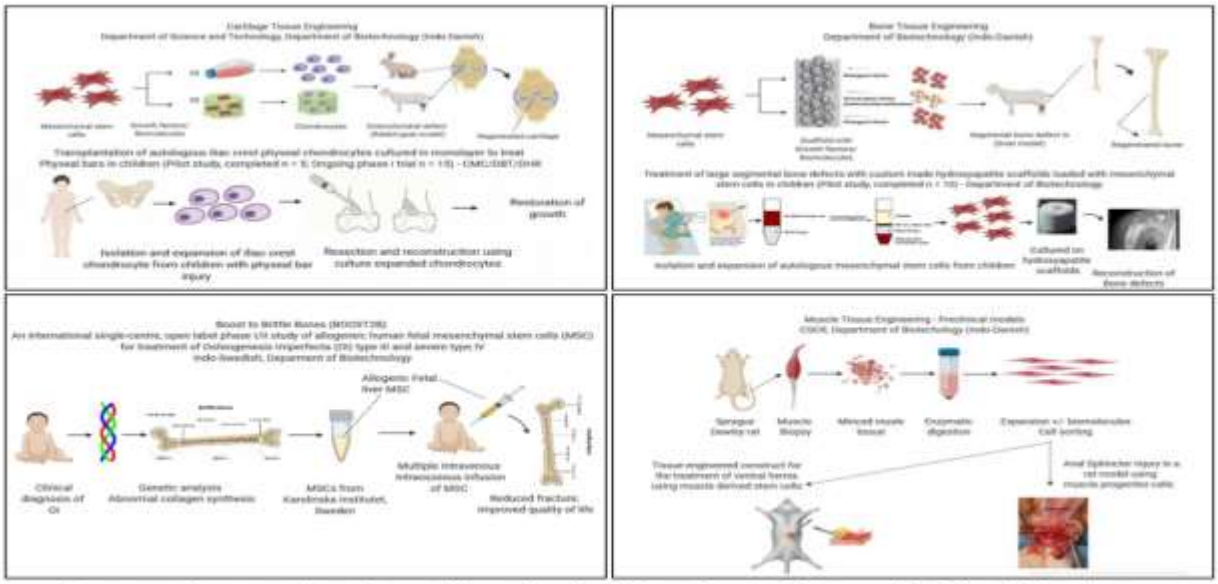
Research at CSCR has advanced significantly in several novel areas of science and medicine to move to clinical translation which will address unmet needs in medical practice in keeping with the mandate for which it was created.

Alok Srivastava
Head, CSCR

Scientific Research Profile

1. MUSCULOSKELETAL REGENERATION PROGRAM

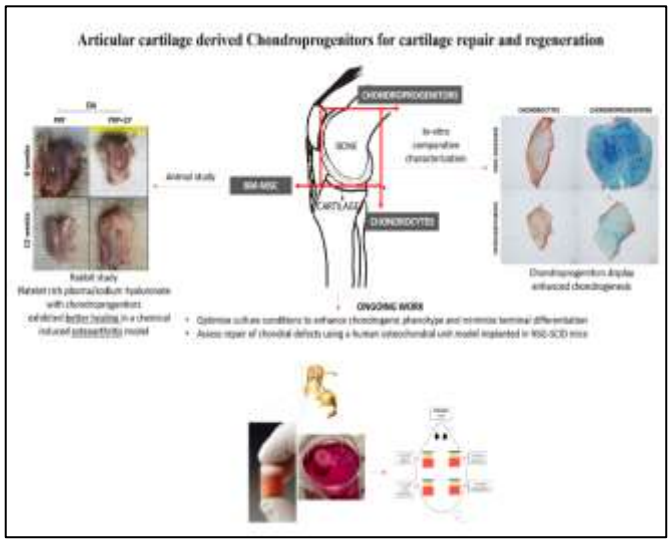
I. The major focus of ***VRISHA MADHURI's*** research group is on clinical and preclinical translation related to physis, articular cartilage, bone, and muscle regeneration. We have established small and large animal models to study the effect of mesenchymal stem cells seeded on the indigenous scaffold for the treatment of articular cartilage. Also, they have been successful in establishing physeal regeneration in a large animal model using a hydrogel scaffold loaded with culture-expanded autologous chondrocytes. This is now extended to a phase I/II clinical trial where the outcomes are promising after chondrocyte transplantation in children with physeal bar injury. In the field of bone regeneration, a new phase I/II clinical trial has been initiated in collaboration with Karolinska Institute for the treatment of osteogenesis imperfecta using fetal liver mesenchymal stem cells. The patient has received three doses of MSC injection and jumped the growth curve from <5th percentile to 5-10th percentile with no adverse effects and improved quality of life; subsequent follow-up will allow us to understand the paracrine effects of MSC administration via intraosseous and intravenous routes. In parallel, they are exploring the newer possibility of establishing cell-free therapy for cartilage and bone tissue regeneration. They are generating preliminary data on fabricating functionalized scaffolds, which can be targeted to recruit and differentiate the endogenous stem cells. This is currently being investigated in a large animal model of the articular cartilage defect and critical-size segmental bone defect as part of Indo-Danish. Under the ICMR funding, they have optimized and standardized cGMP protocols for expansion of muscle-derived stem cells for the treatment of urinary incontinence, a phase I/II clinical trial. Preclinical studies using muscle-derived stem cell for anal sphincter repair continues under the international collaboration, where they are trying novel reparative strategies to reduce inflammation and scarring to generate a functional muscle.



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

II. The focus of ***ELIZABETH VINOD's*** research group is on the characterization of cartilage-derived progenitors and their potential implications for cartilage regeneration using in-vitro and in-vivo conditions.

Hyaline cartilage regeneration remains a challenge in the field of cartilage repair. We also work towards the creation and validation of osteoarthritic models in animals and the development of novel histological processing techniques for the assessment of chondrogenesis. We hypothesize that a better understanding of these progenitors in comparison to other cell types will enable us in creating a detailed biological profile and developing better approaches towards the treatment of cartilage pathologies. In a proof of principle experiment, we observed that chondroprogenitors when compared to bone marrow mesenchymal stem cells and chondrocytes, possess a higher chondrogenic potential and lower propensity for hypertrophy, both indispensable in the field of cartilage repair.



Results from our ongoing experiment show that sorting chondrocytes based on a combinatory surface marker profile help isolate a subset of cells with progenitor-like properties. Assessing their potential for repair of chondral defects using in-vivo studies is currently underway. Another aspect we study involves assessing the potential of soluble factors derived from these progenitors and their potential for cultivating directly injectable therapeutic molecules.

PROJECT IN PROGRESS

1. Comparison of chondrogenic potential between chondrocytes, cell sorted chondroprogenitors, fibronectin assay derived and migratory chondroprogenitors derived from human articular cartilage. (Institutional Fluid Research Grant).
2. To compare the repair of chondral defects using human bone marrow mesenchymal stem cells, articular cartilage derived chondrocytes, cell sorted chondroprogenitors and fibronectin adhesion derived chondroprogenitors suspended in platelet rich plasma, in a human osteochondral unit model implanted in NSG-SCID mice. (Department of Biotechnology).
3. Evaluate the therapeutic potential of exosomes secreted by human chondroprogenitors, on the repair and regeneration of an osteoarthritic model in Wistar rats. (Major Fluid Research Grant).

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INVESTIGATORS

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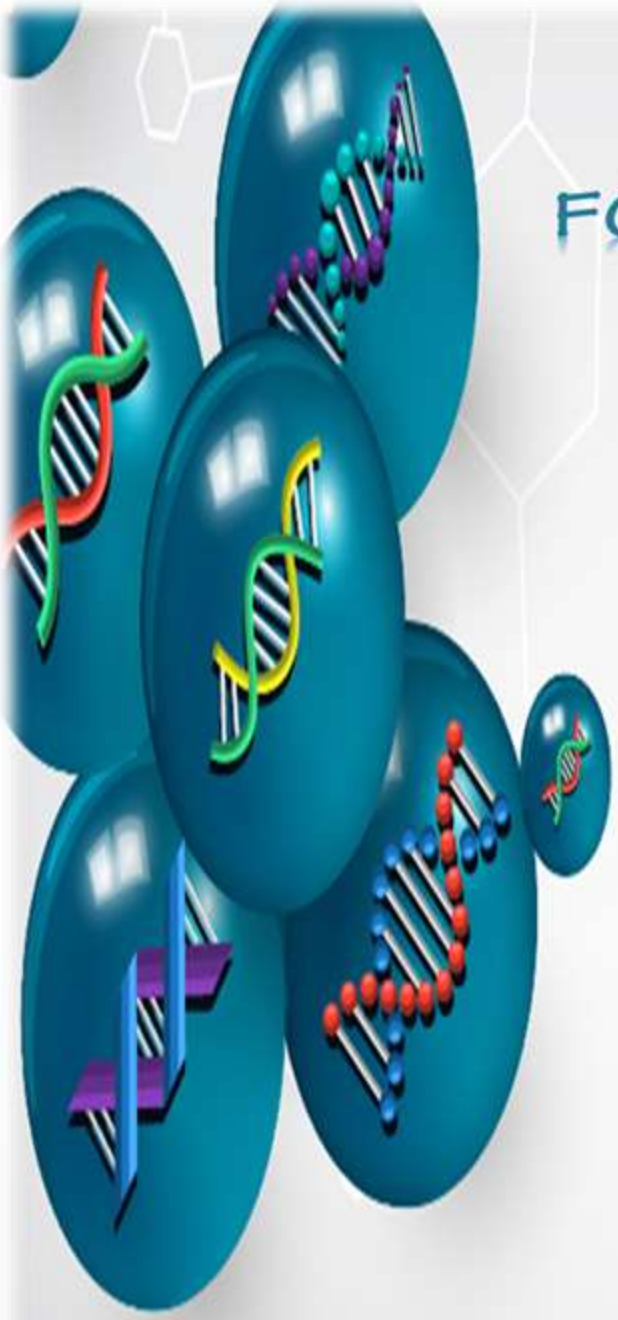
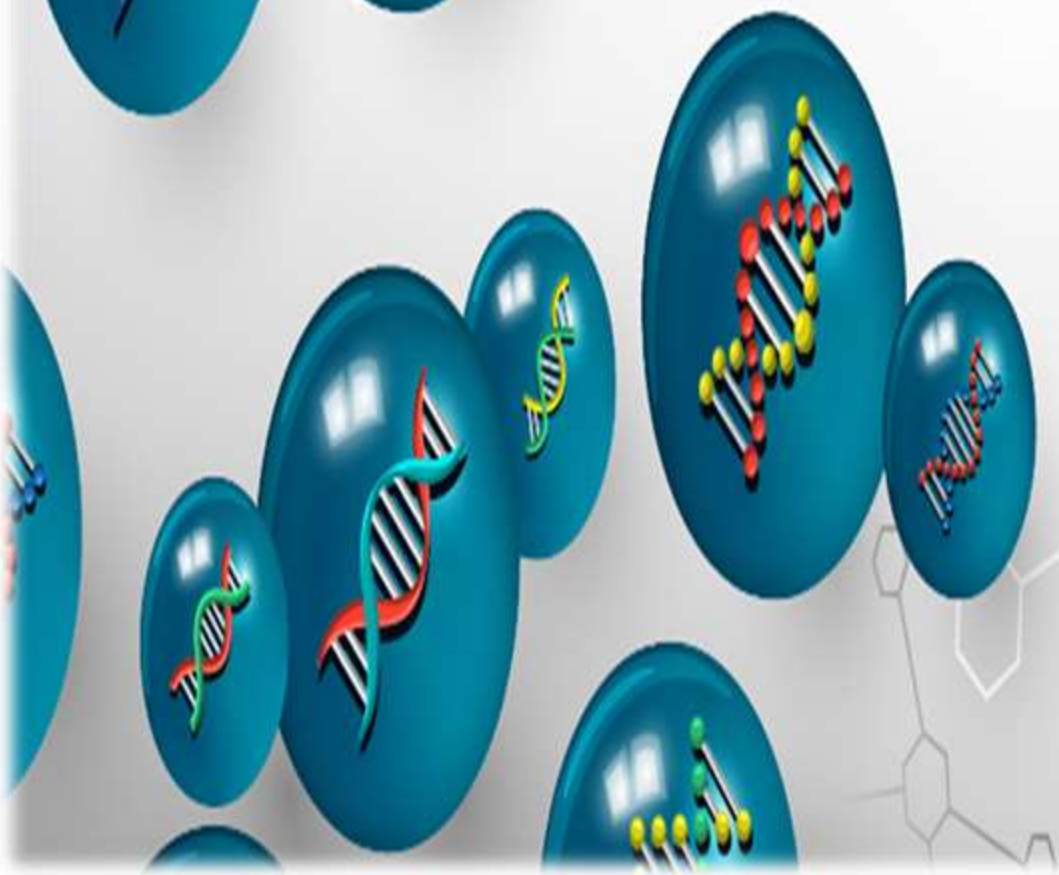
GENE THERAPY FOR HEMATOLOGICAL DISORDERS





2.1 AAV BASED GENE THERAPY

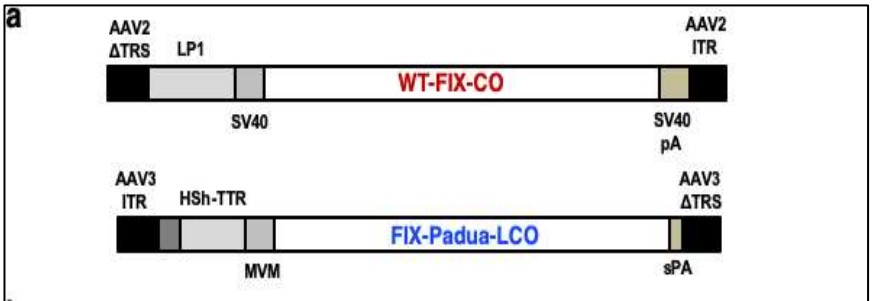
1. AAV Vector-Based Gene Therapy of Hemophilia B

A. AAV3 Vector-Based

This program is coordinated by ***ALOK SRIVASTAVA.*** As mentioned in the previous year's report, a unique transgene was designed for this clinical trial. The proof of preclinical animal model data was recently published [Brown H et al Human Gene Therapy, Aug 17, 2020]. This data established the in-vivo functionality of this transgene and allowed us to proceed towards further development of a clinical product.

The delay in initiating the clinical trial has been due to our inability so far to get cGMP grade vector manufactured at a cost that we could afford through academic collaborations. We now have a very exciting new possibility of manufacturing this in India at CSCR itself in an extended cGMP facility which has been custom extended for this purpose. Our collaborators at Emory University have established a new GMP facility through their company - Expression Therapeutics [ET]. They have acquired a team of senior GMP scientists who will do part of the process development and some of the engineering runs and then transfer the technology to at CSCR/CMC. This process has already been initiated for about 3 months now. This means that the funds requested for paying for the manufacture of this product in USA can now be used for establishing infrastructure and manufacturing in India. A suitable team has been put together to take responsibility for this manufacturing within the CSCR GMP facility. The construction of state of art cGMP facility is at the verge of completion within a month, depending to some extent on the current COVID19 pandemic situation and regulatory approvals.

Figure 1. Schematic structures and salient features of AAV-FIX vectors.



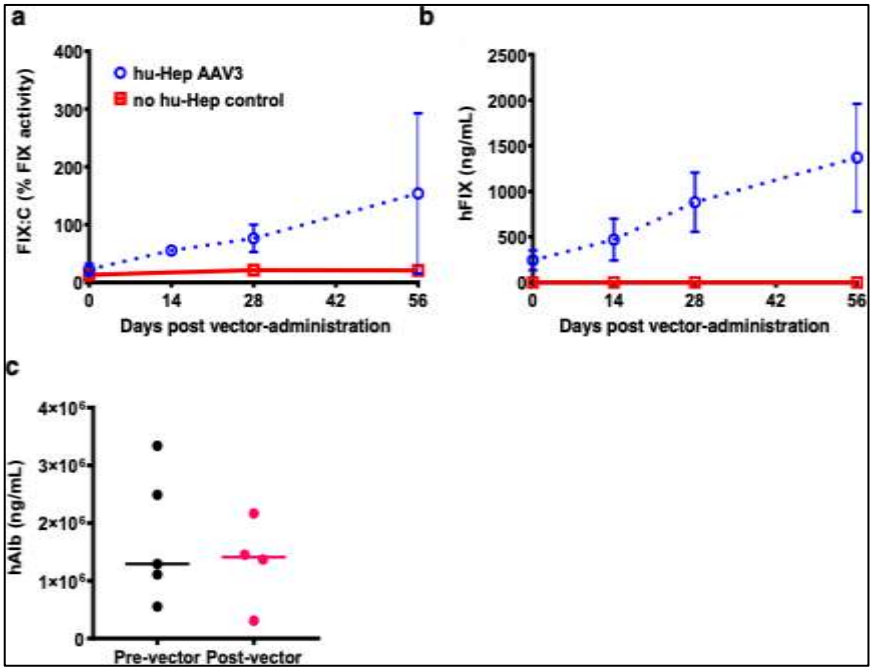
b

Feature	LP1-WT-FIX-CO	HSh-TTR-FIX-Padua-LCO
5'-ITR	AAV2-ITRΔTRS	WT-AAV3-ITR
Promoter	LP1	HSh-TTR
Intron	SV40	MVM
FIX	FIX-CO	FIX-Padua-LCO
Poly A	Late SV40	Synthetic rabbit β-globin
3'-ITR	WT-AAV2-ITR	AAV3-ITRΔTRS
Total size	~2,271 bps	2,136 bps

(a) Schematic structures of FIX expression cassettes used in the first successful clinical trial for hemophilia B (scAAV8-LP1-FIX-WT-CO), and the one used in the current studies (scAAV3-HSh-TTR-Padua-FIX-LCO).

(b) Salient features of various components of the two vectors are detailed. AAV2-ITR: adeno-associated virus serotype 2 inverted terminal repeat; AAV3-ITR: AAV3 serotype ITR; LP1: Human apolipoprotein enhancer/ ·1 anti-trypsin-based liver-specific promoter; HSh- TTR: modified variant of human SERPINA1 enhancer/transthyretin promoter; SV40: simian vacuolating virus intron; MVM: minute virus of mice intron; FIX-CO: codon-optimized, CpG-depleted human factor IX; Padua-FIX-LCO: FIX gene containing the Padua mutation, CpG-free, human liver codon-optimized, 148T isoform; SV40 pA: SV40 polyadenylation signal; s—globin pA: synthetic -globin polyA signal; AAV2- and AAV3-ITR ·trs: terminal resolution site-deleted AAV2- and AAV3-ITRs.

Figure 2. Transduction efficiency of scAAV3-FIX vectors in "humanized" mice.



Five TK-NOG mice transplanted with primary human hepatocytes and were injected with 5x1011 vgs of the scAAV3-HSh-TTR-Padua-FIX-LCO vector via the tail-vein. (a) Plasma FIX activity was determined prior to and 2, 4, and 8-weeks post-vector administration in vector treated mice and control mice that did not receive human hepatocytes nor vector. (b) Plasma FIX levels were also determined by ELISA prior to and 2, 4, and 8-weeks post-vector administration in vector treated mice and control mice that did not receive human hepatocytes nor vector. (c) Human albumin levels in plasma were measured by ELISA prior to and at the end of study to evaluate the stability of human hepatocytes in TK-NOG mice. Control mice were included to provide background FIX activity resulting from murine FIX protein.

B. Industrial collaborations for AAV vector development and manufacturing

This program is coordinated by ***SANJAY KUMAR***. We have an ongoing collaborative program with INTAS Pharmaceuticals, Ahmedabad, Gujrat for AAV8-hFIX-Padua based vector development for Hemophilia B. The other investigators in this INTAS collaborative group include Lakshmikanth Gandikota, Charnitkaur Jashal, and Alok Srivastava.

For the AAV3-hFIX-Padua vector manufacturing process (AAV3 vector packaging, AAV3 Purification, AAV3 characterization, and related preclinical studies); we have forged a collaboration with Expression therapeutics, our International collaborative lead for developing a GMP Standard Operating Procedure (SOP) for AAV3-drug product production at CSCR and established collaboration with Professor Arun Srivastava, the University of Florida for overall design and review of the preclinical work leading to the IND filing; this AAV3 manufacturing group also includes Cartikeya Reddy, Ex VP Dr. Reddy's laboratory, and Gurbind Singh, Augustine Thambaiah. P, Alok Srivastava from CSCR.

AAV gene therapy program on AAV-based state-of-the-art gene therapy technologies for the monogenic disease is to attempt finding a solution for lingering challenges in the Gene and Cell Therapy fields such as AAV-based therapeutic strategies, Next-Gen AAV-based gene-delivery systems, AAV vector design and construction for assessing pre-existing immunity against various AAV serotypes, AAV mediated transgene expression and in vivo AAV biodistribution analysis related to AAV preclinical studies.

I. rAAV3-hFIX-Padua vector manufacturing at CSCR-cGMP facility

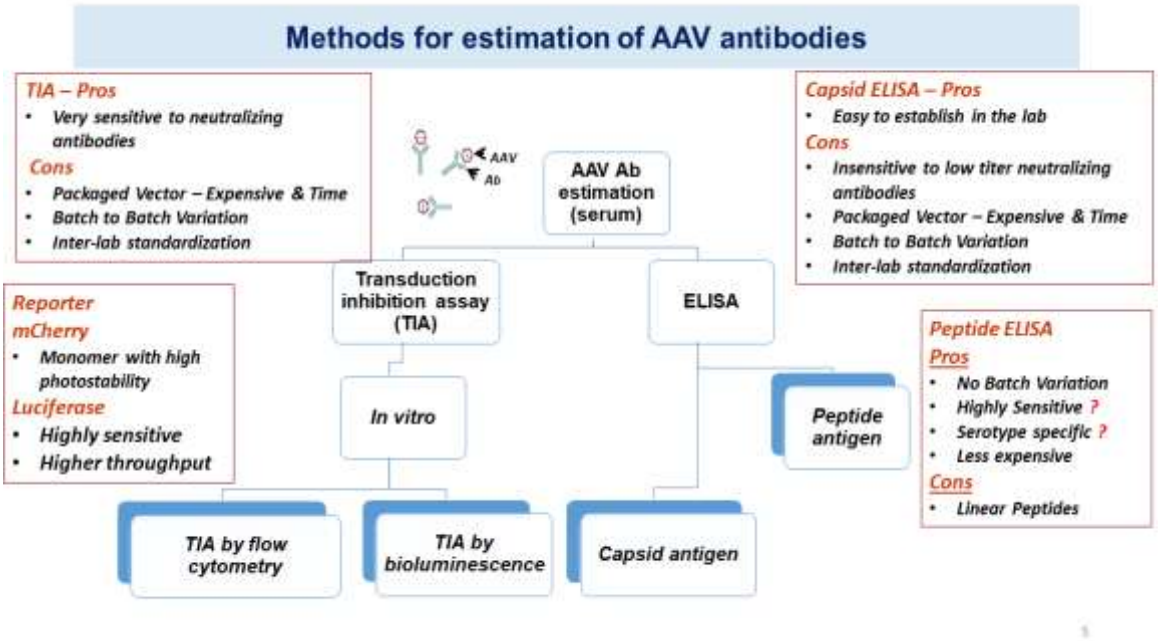
Novel AAV3 vector and hFIX-Padua transgene for gene therapy of hemophilia B developed and proof of concept (POC) preclinical studies have established the safety and efficacy of gene therapy vector. For the first time in India, the AAV3-hFIX-Padua vector production process at CSCR is being organized, following cGMP vector production technology transfer from our external collaborators. We are putting every component of this AAV3 vector production project in its place to achieve the desired goal of AAV3 gene therapy drug product production.

II. Preclinical AAV-gene therapy work:

Ongoing work mainly focuses on AAV-based preclinical gene therapy studies, which help develop AAV-based gene therapy technologies, including newer methods to determine AAV-directed pre-existing immunity in collected human samples including healthy individuals and patients with hemophilia. In collaboration with our industry partner (Intas Pharmaceuticals), we have successfully evaluated the safety and efficacy of adeno-associated virus-8 vector expressing human factor IX (scAAV8-hFIX vector) in hemophilia B transgenic mice model showing the fact that gene therapy treatment of hemophilia B mice with AAV8-hFIX-padua vector has rescued the mice from bleeding phenotype.

2. AAV Antibody Assays

This work is coordinated by ***ASHA M. ABRAHAM*** and includes Hubert Daniel and Rajesh Kannangai from the Department of Clinical Virology and Sanjay Kumar from CSCR and Alok Srivastava from CSCR and the Department of Haematology, CMC Vellore.



The use of adeno-associated virus (AAV) as a gene therapy vector is promising because of their lack of pathogenicity and persistent expression of the transgene. AAV is classified into 13 serotypes that differ in their capsid structures. AAV serotypes exhibit diverse tissue tropism which can be utilized to deliver the transgene to specific tissue

types. Various serotypes of AAV have been used in gene therapy with AAV3, AAV5 and AAV8 showing high liver tissue tropism. Gene therapy using AAV5 and AAV8 vectors has been shown to be successful in preclinical and clinical studies for hemophilia A and hemophilia B. However, the presence of naturally occurring anti-AAV antibodies in humans against the capsid antigen is a major impediment to the success of using AAV as a gene therapy vector. The prevalence of neutralizing antibody (NAb) to the different serotypes of AAV varies from 3.2% and 89% in different populations. Assessment of the AAV serotypes prevalent in the region is crucial for the effective use of AAV as a vector for gene therapy. The goal of this project is to standardize a robust method for assessment of anti-AAV antibody through different assays to allow appropriate selection of patients for gene therapy.

A comprehensive approach has been taken to address this problem as there are major challenges in standardizing these assays at the international level. Binding anti-AAV antibodies are being assessed by ELISAs utilizing capsid and peptide antigens. Neutralizing antibodies are assessed using transduction inhibition assays (TIA). The capsid and peptide ELISAs have been standardized for AAV serotypes 3, 5 and 8. TIA by mCherrybased flow-cytometry had been standardized for AAV 3 and 5 screening for AAV 3, 5 and 8 total and AAV3 and 5 neutralizing antibodies was carried out in healthy individuals and individuals with haemophilia A or B.

PUBLICATIONS

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- Sandeep R.P. Kumar, Jun Xie, Shilang Hu, Jihye Ko, Qifeng Huang, Harrison C. Brown, **Alok Srivastava**, David M. Markusic, Christopher B. Doering, H. Trent Spencer, Arun Srivastava, Guangping Gao2,7, Roland W. Herzog. Coagulation Factor IX Gene Transfer to Non-human Primates Using Engineered AAV3 Capsid and Hepatic Optimized Expression Cassette. Molecular Therapy – Methods & Clinical Development, 2021 (in press).
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- Daniel HDJ, Kumar S, Kannangai R, Lakshmi KM, Mavis Agbandje-MckennaM, Coleman K, Srivastava A, Srivastava A, **Abraham AM**. Prevalence of AAV serotypes 3 capsid binding and neutralizing antibodies in healthy and individuals with hemophilia B from India. Human Gene Therapy: 2021, May;32 (9-10):451-457.
- Sinha S, Biswas M, Chatterjee SS, **Kumar S,** Sengupta A. Pbrm1 Steers Mesenchymal

Stromal Cell Osteolineage Differentiation by Integrating PBAF-Dependent Chromatin Remodeling and BMP/TGF-b Signaling. Cell Reports, 2020 Apr; 31 :1-17.

OUTREACH AND TALKS

- Every year teaching Stem Cell Biology module and taking practical classes for M.Sc. Biotechnology students from Thiruvalluvar University, Serkadu, Vellore, Tamil Nadu 632112.
- Mesenchymal Stem Cells (MSCs)-based therapeutic approaches in mice models. Vellore Institute of Technology (VIT), Tamil Nadu.
- M.Sc. Biotechnology Course (Stem Cell Biology talk)- "Engineering Mesenchymal Stem Cells for Bone-targeted homing". Vellore Institute of Technology (VIT), Tamil Nadu.

INVESTIGATORS

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2.2 LENTIVIRAL VIRAL VECTORS FOR GENE THERAPY

1. Lentiviral Vector-Based Gene Therapy for Hemophilia A

This programme is coordinated by ***ALOK SRIVASTAVA***. Our ongoing collaboration with Emory University has also led to the development of an alternative gene therapy product - a haematopoietic stem cell based lentiviral vector mediated gene therapy product for the treatment of haemophilia A.This is a novel approach - first in human proposed clinical trial of gene therapy for haemophilia A (factor VIII deficiency) where the FVIII transgene is packaged in a lentiviral vector to transduce the haematopoietic stem cell (HSC) for stable integration and lifelong expression is similar to the principles being applied in the gene therapy for the major haemoglobin disorders. The product has been tested in pre-clinical mouse models and shown to be safe and effective (Doering *et al* Human Gene Therapy. 2018; 29:1183).

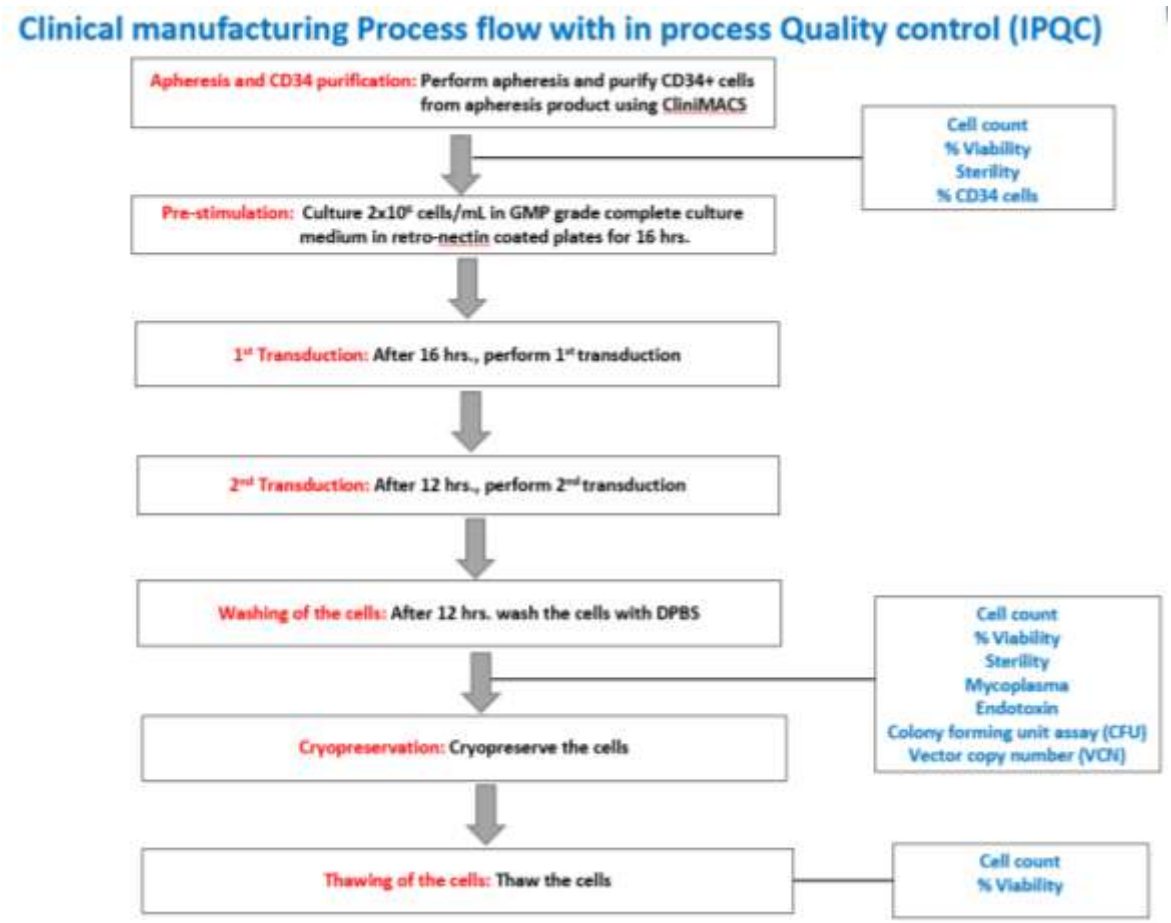
For this proposed clinical trial, all pre-clinical experiments have been completed including transfer of technology to CSCR / CMC, Vellore for the final product to be administered to the subjects in this trial – the autologous haematopoietic stem cells harvested from three patients with severe haemophilia A were transduced with the lentiviral vector with the FVIII gene. This novel work was carried out as required by the RCGM and CDSCO and with appropriate approval of the institutional committees. The data from these experiments is shown in the table below.

TABLE: Haemopoietic Stem Cells Post-Transduction with Lentiviral Vector with FVIII Transgene

PARAMETERS	SUBJECT 1	SUBJECT 2	SUBJECT 3
% Viability of control cells	98.4 %	96.5 %	97 %
% Viability of transduced cells	97.9 %	95 %	96 %
Vector copy number by RT-PCR	0.68	0.62	1.21

An investigational new drug (IND) proposal had therefore been filed in both India and USA for this clinical trial to be undertaken in August, 2018. This proposal has been under review with the CDSCO since then but has finally been approved for a phase 1 clinical trial last month in July, 2021.

We will now proceed to import the necessary clinical quantities of the cGMP lentiviral vector for the manufacture of the final drug product – the transduced autologous haematopoietic stem cells from the patient (subject) for the clinical trial to start as outlined below.



2. Lentiviral vector-based gene therapy for β – globinopathies

This programme is coordinated by ***R.V. SHAJI***. Induction of foetal haemoglobin (HbF) in sickle cell disease (SCD) and β–thalassemia is a promising approach to ameliorate the disease phenotype. B-cell lymphoma/leukaemia 11A (BCL11A) is a transcription factor that represses γ globin gene (HBG) expression in adults. Down regulation of BCL11A induces HbF levels. BCL11A is therefore a prime candidate for targeted therapy aimed at induction of HbF in the patients with β-globin disorders. As depletion of BCL11A in hematopoietic stem cells can result in impaired B-cell growth and render aging like changes in HSCs, it is important that the knock out or knock down of BCL11A should be in erythroid specific cells. We generated two novel lentiviral shRNA vectors (H23B and H234B) for the knock down of BCL11A in human erythroid cells. The H234B has three DNA hypersensitivity sites, HS2, HS3 and HS4 whereas the H23B lacks HS4. Both carry a polycistronic ZsGreen-IRES-puromycin-shBCL11a cassette cloned downstream of the HBB promoter. For efficient RNAi, the shRNA was designed using the Sherwood algorithm and miR30a scaffold for more effective knock down of the target gene. We measured the efficiency of these lentiviral vectors to downregulate BCL11A and upregulate HbF in an erythroid progenitor cell line, HUDEP-2. We observed that >90%

knock down of BCL11A by western blot and real time PCR analysis in the transduced ZsGreen+HUDEP cells. HbF analysis by flow cytometry in the HUDEP cells differentiated to the later stages of erythropoiesis showed that HbF expressing cells was 50%, while the cells transduced with scrambled shRNA showed only in 12% erythroid cells, suggesting that knockdown of BCL11A caused an increase in HbF expressing cells by nearly 40% for both H234B and H23B. This experiment was then repeated in CD34+ hematopoietic stem and progenitor cells (HSPCs), which, after transduction, were differentiated to erythroid cells. The transduction efficiency was again noted to be 30-40%. The ZsGreen+ HSPCs were flow sorted and differentiated to erythroid cells using an erythroid culture system supplemented with cytokines, SCF, EPO and IL3. BCL11A expression was analysed by real time PCR and western blot analysis on day 10 after obtaining 97% of CD71+CD235a+ erythroid cells, which showed >80% downregulation of BCL11A. Similar to the erythroid cell line data, these vectors increased the HbF expressing cells by 50% in the primary erythroid cells. We also observed significant upregulation of HBG transcripts with both H234B and H23B respectively. Interestingly, consistent with the recent reports, the H23B vector lacking HS4 appears to be produced at higher titers. Higher titers resulted in increased mean fluorescence intensity of GFP for the H23B vector.

Since it is important to study the long-term effects of knockdown of BCL11A in erythroid cells using this vector, we used the NBSGW mouse model to study the knock down in long term HSCs. We slightly improved the vector We transduced the H23B shBCL11A vector and shscrambled (Fig 1B) in CD34+ HSPCs at a MOI ~10-15 to ensure single copy integration. The transduced cells were transplanted in NBSGW mice (Fig 1B). After 16 weeks we could observe about 70-90% human CD45+(hCD45) cells in the transplanted mice bone marrow which was similar among all the groups (Fig 1C). Further, using an ex-vivo erythropoiesis culture system, we differentiated the total bone marrow cells into erythroid cells. We did not observe any significant difference in the kinetics of erythropoiesis during the erythroid culture and terminal differentiation which shows that knockdown of BCL11A did not affect the normal erythropoiesis of the long-term HSCs. The ZsGreen+ cells cell sorted and analysed for downstream experiments. We observed ~54-64% (59±5.03) knockdown of BCL11A in the sorted ZsGreen+ cells in the shBCL11A group compared to the shscrambled or untransduced groups (Fig 1E). We analysed the vector copy number (VCN) in the sorted ZsGreen+ cells using qPCR and we observed a VCN of 0.4-0.7 (0.53±0.18) in shBCL11A group vs 0.56-0.82 (0.697±0.18) in the shscrambled group (Fig 1F). After terminally differentiating of the ZsGreen+ cells we analysed the %F+cells and Gγ+Aγ globin chains. We observed that there was a robust increase of 35-36% (33.8±2) F+ cells (Fig 1G) and 30-60% (41±17.3) elevation in the Gγ+Aγ globin chains (Fig 1H). Thus, we were able to generate a lentiviral vector that provided efficient knockdown of our targeted BCL11A protein in erythroid cells in NBSGW mice.

The knockdown of BCL11A using H23BshBCL11A lentiviral vector did not affect the long-term repopulation of the transplanted HSPCs. Therefore, this vector can provide an opportunity to enhance HbF expression in clinical applications for SCD and β-thalassemia.

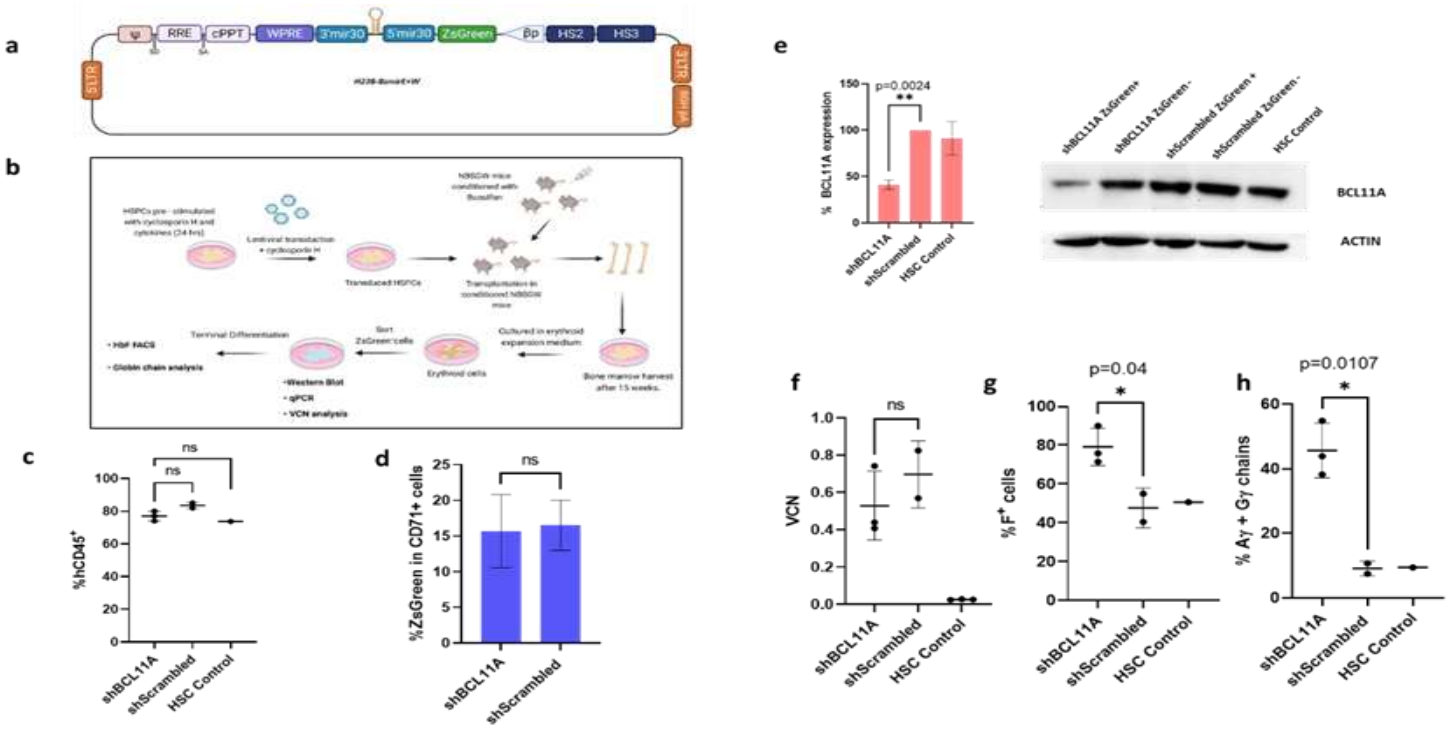


Figure: a) Vector map of H23B lentiviral vector with hypersensitivity sites: HS2, HS3; βP: β – promoter, Zsgreen: fluorescent reporter gene; 5'mir30 and 3'mir30: mir30 scaffold; WPRE: woodchuck post transcriptional regulation element; cPPT: Central polypurine tract; RRE: rev response element, Ψ: packaging signal. b) Schematic outline of the experiment performed in NBSGW mouse model. C) %hCD45 in bone marrow of NBSGW mice after 16 weeks of transplant. d) %ZsGreen^{+} cells in the CD71^{+} cells after 7 days of erythroid culture. e) Western blot analysis of BCL11A knockdown in the sorted ZsGreen^{+} cells; knockdown was compared to Zsgreen- cells of each group. f) VCN analysis in ZsGreen^{+} sorted cells g) % F^{+}cells and h) %Aγ and Gγ chains in differentiated ZsGreen^{+} cultured erythroid cells.

PUBLICATIONS

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INVESTIGATORS

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2.3 GENOME-EDITING BASED GENE THERAPY

SARAVANABHAVAN THANGAVEL and *MOHANKUMAR MURUGESAN* work collaboratively to employ different gene editing approaches for gene therapy.

I. For β-hemoglobinopathies, Dr. Thangavel's group uses NHEJ based and HDR based gene editing approaches for recapitulating beneficial mutations that are associated with HbF reactivation. In parallel they also correct the mutations causing β-Thalassemia and Sickle cell disease to produce normal levels of functional beta-globin. In a similar approach, Dr. Thangavel's group introduces beneficial CCR5 mutation to generate HIV resistant cells towards HIV-1 gene therapy. In vitro characterization of gene edited hematopoietic stem cells, functional validation, in vivo transplantation and characterization of gene edited cells are completed for both the disease targets. For gene therapy of Wiskott-Aldrich syndrome, to compensate the deficient production of WAS in hematopoietic lineage, they precisely insert the WAS transgene into a specific locus of the HSPCs. In addition, Dr. Thangavel's groupwork on various technologies to improve the efficiency of generation of gene-modified haematopoietic stem cells. The application of gene editing for HSPC gene therapy is hampered by the low frequency of gene modified long-term repopulating hematopoietic stem cells. Dr. Thangavel's group have developed an ex vivo culture system that generates high-frequency of gene modified stem cells in comparison to conventional culture conditions.

II. Research work in Dr. Mohankumar's lab is focused on using different genome editing strategies for the treatment of beta hemoglobinopathies and hemophilia. Currently, they are engaged in three different research approaches designed towards therapeutic induction of fetal globin level. The identification of key mutations associated with increased gamma-globin gene expression is of therapeutic relevance for beta-hemoglobinopathies patients. The current nuclease-based mutagenesis is limited by the imprecise repair outcomes of NHEJ and by frequent deletion of the region intervening the highly homologous sequences of the duplicated gamma globin promoter. Through systematic tiling across the gamma globin proximal promoter using two different base editors, they identified multiple novel regulatory mutations, in addition to naturally occurring mutations, that substantially elevated the fetal globin level. Further, they also demonstrated that introduction of these novel HPFH like mutations drives gamma-globin expression by creating a de novo binding site for the different master erythroid regulators. Our study demonstrates the utility of Cas9 base editors for mapping gene regulatory elements, highlighting key advantages over similar mapping using nuclease technologies. For the targeted editing of fetal globin repressors, they explored the functional consequences of editing of erythroid specific

BCL11A enhancer and LRF/ZBTB7A in mobilized hematopoietic stem and progenitor cells (HSPCs) from normal donor using the CRISPR/Cas9 system. They determined the effect of BCL11A enhancer and LRF/ZBTB7A disruption on cell growth, erythroid differentiation, fetal globin induction and BCL11A and LRF expression in erythroid cells derived from the normal donor mobilized HSPCs and also analyzed the long term mulitineage engraftment potential in NBGSW mice. These work highlights an effective approach for the targeted disruption of gamma globin repressors and regulatory elements to elevate the fetal globin levels using genome editing tools could be an efficient therapeutic strategy for beta hemoglobinopathies. Considering the current limitations in gene therapy for Hemophilia, they developed a novel approach for targeted integration of FVIII/FIX under an endogenous promoter using CRISPR/Cas9 system to drive the expression of FVIII/FIX in specific lineage of HSPCs. Towards this end, they optimized the genome editing conditions for the targeted integration of transgene in the human erythroleukemia line. Transgene integration at the lineage specific promoter through the donor delivery of DNA template using the AAV6 vector showed better HDR efficiency with no significant decrease in cellular viability. These preliminary results from the human erythroleukemia line will enable them for integrating FVIII/FIX under the endogenous promoter in human hematopoietic stem cells. Thus, the establishment of a novel ex vivo based genome editing approach for site specific integration into endogenous lineage specific promoter in HSPCs would represent a safer, permanent and functional cure for Hemophilia- A and B.

PUBLICATIONS

SARAVANABHAVAN THANGAVEL

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- Christopher A, Venkatesan V, Karuppusamy K, Srinivasan S, Babu P, Alagiri M, Karthik C, Bagchi A, Rajendiran V, Ravi N, Kumar S, Marepally S, Mohankumar M, Srivastava A, Velayudhan S and **Thangavel S**. Preferential expansion of human CD34+CD133+CD90+ hematopoietic stem cells enhance gene-modified cell frequency for gene therapy. Human gene therapy (Manuscript accepted).
- Azhagiri M, Bapu P, Venkatesan V and **Thangavel S.** Homology-directed gene-editing approaches for hematopoietic stem and progenitor cell gene therapy. Stem Cell Research & Therapy (Manuscript accepted).

MOHANKUMAR MURUGESAN

- Ravi, NS.; Wienert, B.; Wyman, SK.; Vu, J.; Pai, AA.; Balasubramanian, P.; Nakamura, Y.; Kurita, R.; Marepally, S.; Thangavel, S.; Velayudhan, SR.; Srivastava, A.; DeWitt, MA.; Corn, JE.; **Mohankumar KM.** Identification of Novel HPFH-like Mutations by CRISPR Base Editing That Elevates the Expression of Fetal Hemoglobin. bioRxiv 2020, 2020.06.30.178715. https://doi.org/10.1101/2020.06.30.178715.
- Prasad K, George A, Ravi NS, **Mohankumar KM**. CRISPR/Cas based gene editing: marking a new era in medical science. MolBiol Rep. 2021 May; 48(5):4879-4895.
- Kupp R, Ruff L, Terranova S, Nathan E, Ballereau S, Stark R, Sekhar Reddy Chilamakuri C, Hoffmann N, Wickham-Rahrmann K, Widdess M, Arabzade A, Zhao Y, Varadharajan S, Zheng T, **Murugesan MK,** Pfister SM, Kawauchi D, Pajtler KW, Deneen B, Mack SC, Masih KE, Gryder BE, Khan J, Gilbertson RJ. ZFTA-translocations constitute ependymoma chromatin remodeling and transcription factors. Cancer Discov. 2021 Sep: 2216-29 (Epub ahead of print)
- Bagchi A, Nath A, Thamodaran V, Ijee S, Palani D, Rajendiran V, Venkatesan V, Datari P, Pai AA, Janet NB, Balasubramanian P, Nakamura Y, Srivastava A, **Mohankumar KM**, Thangavel S, Velayudhan SR. Direct Generation of Immortalized Erythroid Progenitor Cell Lines from Peripheral Blood Mononuclear Cells. Cells. 2021 Mar 1;10(3):523.
- Devaraju N, V Rajendiran, Ravi NS and **Mohankumar KM**. Genome engineering of hematopoietic stem cells using CRISPR-Cas9 system. Methods in Molecular Biology 2021 (in press).

RESEARCH TALKS

SARAVANABHAVAN THANGAVEL

- Gene editing technologies for gene and cell therapy, Department of Biotechnology, Garden City university, Bangalore.
- Stem cells biology and applications. Thiruvalluvar university, Vellore.
- Gene editing for gene therapy. AICTE Training and Learning (ATAL) Academy-Faculty development programme, Bharath Institute of Higher Education and Research.

PATENTS

SARAVANABHAVAN THANGAVEL

- "A Cocktail formulation for selective enrichment of gene modified cells "Patent No. 202141000921. Investigators: Saravanabhavan Thangavel, Srujan Marepalley, Alok Srivastava.

MOHANKUMAR MURUGESAN

- "Compositions and Methods for Reactivating Developmentally Silent Genes" Application number 20204102016. *Investigators:* Mohankumar Murugesan and Alok Srivastava

INVESTIGATORS

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MOHANKUMAR MURUGESAN, Ph.D.
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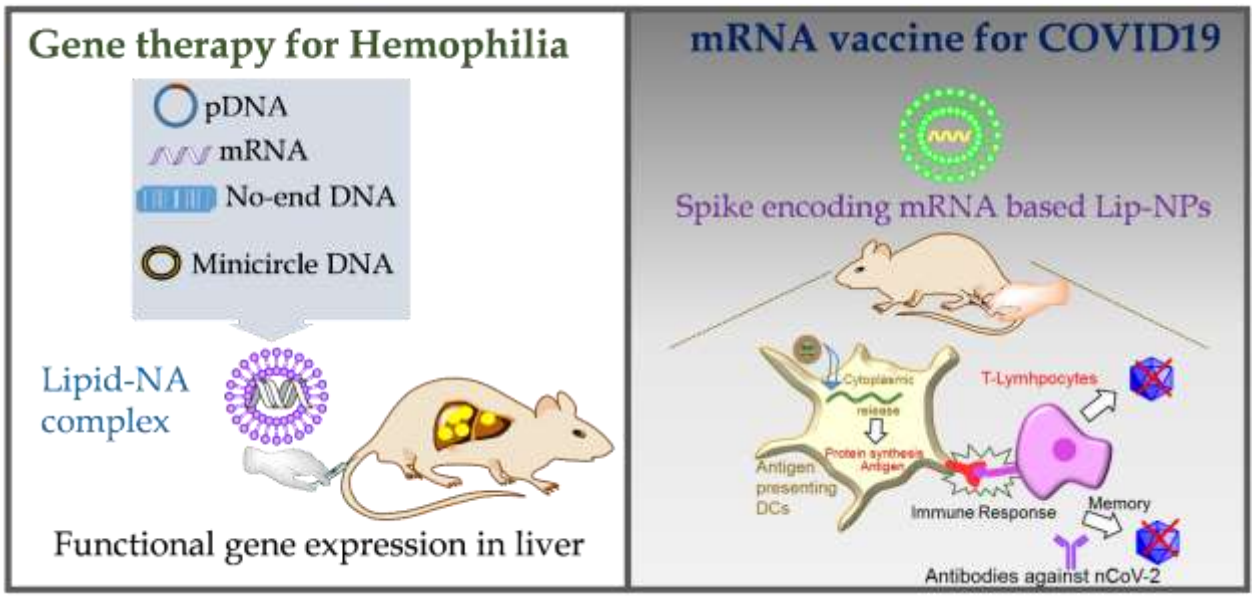
2.4 NON-VIRAL VECTOR BASED NUCLEIC ACID TRANSFER



This programme is coordinated by ***SRUJAN MAREPALLY***. His research group is on interdisciplinary area with an interface of chemistry and biology, emphasis on lipid-mediated nucleic acid therapeutics. To this end, we have been developing cationiclipids to deliver nucleic acids including pDNA, mRNA, siRNA and microRNAs for protein replacement therapies and for synthetic vaccines.

Our lab has developed a lipid nanoparticle-based platform technology to transfer nucleic acids specifically into liver to treat several monogenic liver disorders, and currently, focusing on developing nucleic acid therapeutics for Hemophilia A&B. We are also developing gain-of-function variants of Factor IX and delivering as pDNA, No-End DNA and mRNA. Pre-clinical studies are in progress for Hemphilia A gene therapy.
Towards developing protein replacement therapeutics for Hemophilia, we are working on developing mRNA-based approach. We have optimized based modifications in mRNA to evade immune responses and increase protein expression. Currently optimizing the in vivo formulations for therapeutic delivery of Factor VIII and Factor IX mRNAs.

Our lab has developed a novelnon-viral strategy to deliver gene-editing tools into Hematopoietic Stem Cells (HSCs) without affecting the stem-ness of HSCs. This approach can be useful in therapeutic gene-editing of HSCs for treating β-Hemoglobinopathies. We have also developed 3D transfection reagent to deliver nucleic acids into Human Mesemchymal Stem Cells (MSCs), which can be explored in treating osteoarthritis and osteoporosis.

Stable, Mannose Receptor Targeting (SMART) Nanoparticles evaluated in vivo delivery efficiency. We also synthesized chemically modified spike mRNA and validated functionally. Intriguingly, our

lyophilized mRNA-lipid nanoparticles found to be stable at 4oC up to a week that addresses critical challenge in transportation of mRNA vaccines. We have successfully demonstrated that the vaccinated animal could produce strong immune responses against spike protein with endpoint titer 105-106, and could neutralize SARS-CoV2 pseudovirus. As a contribution to the COVID-19 pandemic, the lab has developed an mRNA-based vaccine for SARS-CoV2. To deliver spike encoding mRNA, we have developed.

PUBLICATIONS

- Muripiti, V., Lohchania, B., Ravula, V., Manturthi, S., **Marepally, S.**,Velidandi, A., & Patri, S. V. Dramatic influence of the hydroxy functionality of azasugar moiety in the head group region of tocopherol-based cationic lipids on in vitro gene transfection efficacies. New J. Chem., 2021; 45(2), 615–627.
- Gokulnath M, Aruna M, Porkizhi A, Ajay K D, Kanimozhi S, Yogapriya P, **Srujan M**, Lipid nanoparticle enabled delivery of chemically modified mRNA into mammalian cells, JOVe (accepted).

RESEARCH TALKS

- Development of lipid-based non-viral gene delivery for therapeutic applications (Invited seminar) "Muscle & Diseases" held on November 6-7, 2020, jointly organized by Ashoka University-IISER,Tirupati – CCMB, Hyderabad.
- Development of lipid based nucleic acid therapeutics: Understanding the structural parameters of the lipids towards improving the transfection efficiencies. (Award lecture) Science beyond boundary: Invention, Discovery, Innovation and Society, on 30-4-2021, CRS-India

PATENTS

- Substituted lithocholic acid and methods thereof. Indian Patent Application number 202041047355.Investigators: PorkizhiArjunan, Gokulnath Mahalingam, Praveen Kumar Vemula, Alok Srivastava, Srujan Marepally.

INVESTIGATOR

SRUJAN MAREPALLY, Ph.D.
Scientist, CSCR

3. CELLULAR REPROGRAMMING AND ITS APPLICATIONS

I. Applications of Induced Pluripotent Stem Cell (iPSC)Technology: Disease Modelling for Erythroid Disorders for Gene correction

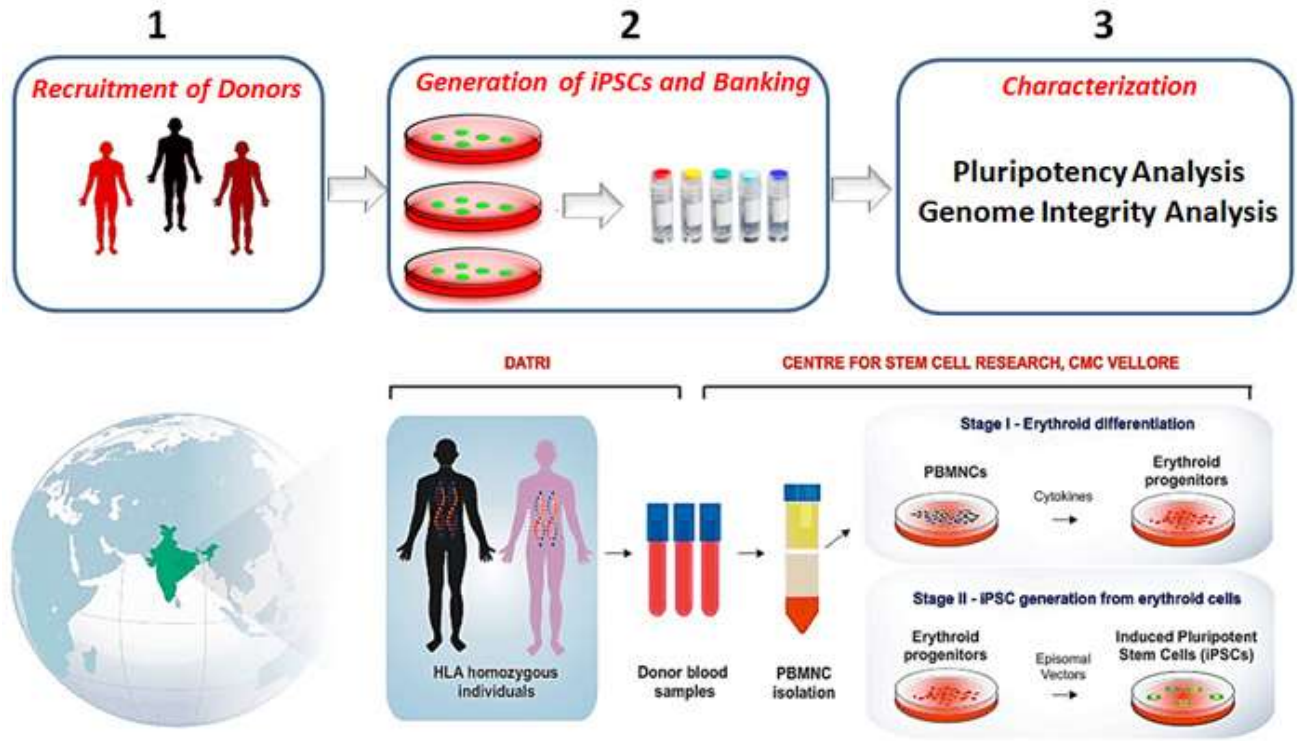
This programme is coordinated *by* ***R.V. SHAJI***. With the use of induced pluripotent stem cells (iPSCs) our lab is currently working on disease modelling of Diamond Blackfananaemia (DBA), congenital dyserythropoietic anemia (CDA) and Fanconi anaemia (FA). DBA is caused by haploinsufficient mutations in ribosomal genes, and other erythropoiesis associated genes resulting in the absence or decreased number of erythroid progenitors in the bone marrow. CDA is caused by mutations in Codanin 1 (CDN1) and SEC23B genes majorly. FA is a DNA repair disease caused by mutations in the genes in the FA DNA-repair pathway. We generate disease After genotyping of the patients by exome sequencing, we reprogram the fibroblasts to generate induced pluripotent stem cells (iPSCs). Alternatively, we use CRISPR-Cas9 based gene editing to create mutations in rare genes in a normal well-characterized iPSC line. This helps in the generation of isogenic wildtype and mutant iPSC lines for disease modelling. We established gene editing strategies CDN1, RPS19, RPL5 and SEC23B genes using lentiviral vectors to express Cas9 and gRNAs. For creating biallelic mutations, we generated an iPSC line with tetracycline inducible Cas9 expression from the AAVS1 safe harbor site, which allows temporal control of editing of the genes of interest at a specific time window during haematopoietic differentiation. Several genes were edited robustly using the AAVS1-Tet-ON-Cas9 iPSC line. Recently, we applied base editing strategies to create mutations in the target genes. We obtained a very high gene editing efficiency in the genes associated with CDA and could perform disease modelling of this disease. For modelling FA, we generated iPSCs from patients with mutations in 6 different genes. As generation of iPSCs from FA patients is challenging we used a doxycycline inducible lentiviral vector to express the wild-type cDNA of the genes defective in these cells. After generation of iPSCs, the cells were cultured in the absence of doxycycline for disease modelling. We established a modified robust protocol for differentiation of iPSCs to haematopoietic progenitor cells (HSPCs). We identified two different populations of iPSC-HSPCs with difference in the expression of surface markers and their erythroid differentiation potential. RNA sequencing of these two types of iPSC-HSPC populations showed significant different in gene expression and identified specific genes associated with increased erythroid differentiation of iPSC-HSPCs.

We are currently performing experiments to understand the functions of the genes and the pathways that we identified in these two different types of HPSCs. For obtaining more primitive iPSC-HPSCs with enhanced erythroid differentiation, we transduced iPSCs with 5 transcription factors (ERG, HOXA9, RORA, SOX4, and MYB). This method has the potential to generate large numbers of engraftable patient-specific cells for modelling haematological diseases. We used this strategy for generating transplantable HSPCs with the erythroid lineage potential. We modified Daley's protocol by transducing iPSCs with these transcription factors to avoid transduction of HSPCs, and doxycycline was supplemented when the HSPCs are cultured in HSPC expansion medium.

PUBLICATIONS

- Benjamin ESB, Ravindra N, Rajamani BM, Anandan S, Kausalya B, Veldore V, Mathews V, **Velayudhan SR,** Balasubramanian P. BCR-ABL1 kinase domain mutation analysis by next generation sequencing detected additional mutations in chronic myeloid leukemia patients with suboptimal response to imatinib. Leuk Lymphoma. 2021 Jun; 62(6):1528-1531. Epub 2021 Jan.
- Thamodaran V, Rani S, **Velayudhan SR.** Gene Editing in Human Induced Pluripotent Stem Cells Using Doxycycline-Inducible CRISPR-Cas9 System. Methods Mol Biol. 2021 Apr. Epub ahead of print.
- Bagchi A, Nath A, Thamodaran V, Ijee S, Palani D, Rajendiran V, Venkatesan V, Datari P, Pai AA, Janet NB, Balasubramanian P, Nakamura Y, Srivastava A, Mohankumar KM, Thangavel S, **Velayudhan SR**. Direct Generation of Immortalized Erythroid Progenitor Cell Lines from Peripheral Blood Mononuclear Cells. Cells. 2021 Mar ;10(3):523.
- Singh G, Manian KV, Premkumar C, Srivastava A, Daniel D, **Velayudhan SR.** Derivation of Clinical-Grade Induced Pluripotent Stem Cell Lines from Erythroid Progenitor Cells in Xenofree Conditions. Methods Mol Biol. 2021 May. Epub ahead of print.

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



Schematic representation of Haplobanking from donors with homozygous HLA

This programme is coordinated by *DOLLY DANIEL* and *R.V. SHAJI*. 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. Pre-selecting the specific donors for the generation of induced pluripotent stem cell (iPSC) lines enables to develop 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, which are HLA typed and screened for infectious diseases at the Christian Medical College, Vellore. A robust method has been established at Centre for Stem Cell Research to generate iPSC lines from cultured erythroid 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 Global Alliance of iPSC Therapies (GAiT) for haplobanking of iPSCs from normal donors of Indian origin. First, we generated a bank of blood cells from 235 donors with homozygous haplotypes from various regions of the country. We established a highly efficient feeder-free, xeno-free and integration-free protocol to generate GMP grade iPSCs. So far, we generated iPSCs from 15 donors with top 10 HLA haplotypes. The isolated clones were analyzed for pluripotency marker expression and in-vitro differentiation to three germ layers. In future, we will generate more iPSC lines from the donors with rarer haplotypes to have representation of all the top 20 HLA haplotypes.

INVESTIGATORS

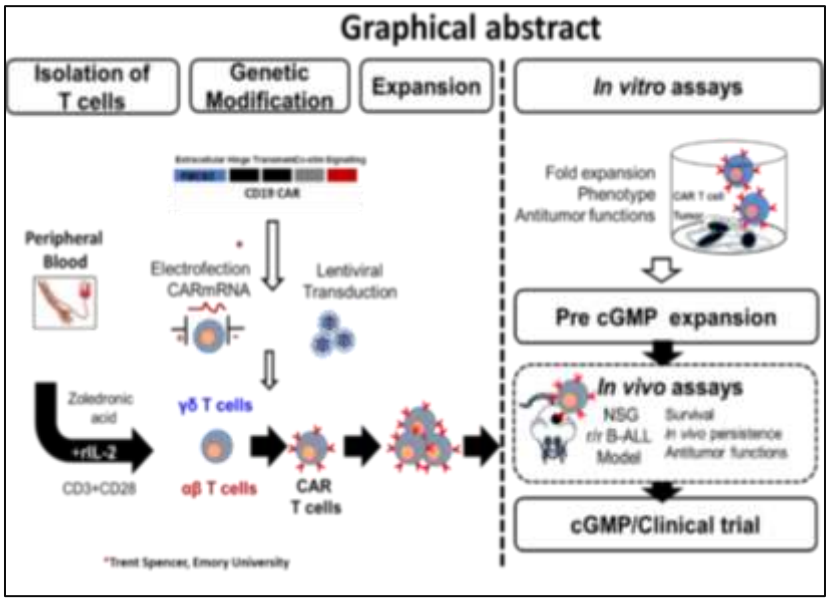
DOLLY DANIEL, M.D.
Professor, Department of Transfusion and Immunohaematology, CMC
Adjunct Scientist, CSCR

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

4. IMMUNE CELL THERAPY

This programme is coordinated by ***SUNIL MARTIN***

Schematic representation of the theme: Broadly the immune cell therapy program involves isolation, expansion, engineering, *in vitro* and *in vivo* characterization, cGMP and Clinical trial. The T cells are isolated from the peripheral blood mononuclear cells (PBMCs). The αβ T cells are stimulated and expanded by CD3+CD28 beads in presence of IL-2 whereas γδ T cells are activated by Zoledronic acid in presence of IL-2. Ours is a CAR construct of the second generation which is linked to the GFP by a ribosome skipping P2A. The whole cassette is driven by human UBC promoter. The CAR transgene is introduced into T cells by lentiviral transduction or electroporation. The transduced T cells are evaluated *in vitro* and *in vivo* before proceeding with cGMP level production and clinical trials.



I: CAR-T cells to target refractory or relapsed B cell Acute Lymphoblastic leukemia (r/r B-ALL)

Background: Chimeric Antigen Receptor (CAR) T cells made unprecedented progress in immune-oncology; yet the research in this space is in its infancy in India. CARs are synthetic receptors with ectodomains from the single-chain variable fragment (scFv) of an antibody with a co-stimulatory domain (such as CD137 or CD28) and an endodomains of CD3ζ. CARs allow HLA independent, robust response against cognate antigens to counter tumor-HLA downregulation and survive within the patient as 'living drug'. We developed αβ CAR T cell targeting CD19; a pan B cell malignancy antigen to treat patients with refractory or relapsed B cell Acute lymphoblastic leukemia (r/r B-ALL). Moreover, our CAR construct has hinge and transmembrane domain from CD8a instead of CD28 to reduce the cytokine release syndrome (CRS), which is one of the major clinical side effects of CAR therapy. We have previously evaluated αβ CAR T cells targeting CD19(+) malignancies. One of the key components of the immune cell therapy programme is the generation of cGMP grade CAR construct. We have generated the cGMP CAR construct by deleting the WPRE, GFP and P2A from the parental construct. The transmembrane domain is exchanged with CD8 for reduced cytokine toxicity. The whole construct is driven by hUBC promoter. The construct is sequence confirmed before testing the surface expression. The T cells lentivirally transduced with cGMP CAR construct was stained against CD19-PE (CAR stains reagent). In the future, we are planning to complete the antigen specific cytokine profiling and antigen specific

expansion before proceeding to in vivo experiments. The data encourage us to further the CAR-T Cell generation programme to antigen specifically targets r/r B-ALL cells in the humanized NSG Mouse model before proceeding to cGMP grade expansion

II. Expansion and engineering of peripheral blood derived γ9δ2 T cells to treat blood cancers

Background: γδ T cells are multivalent immune cells that recognize a broad spectrum of tumor associated stress patterns by germ-line encoded receptors and lyse them directly without prior potentiation in an HLA independent manner. Two major halogens to the clinical level expansion of γ9δ2 T cell subset is the serum of xenogenic origin in the media and inadvertent expansion of αβ T cells in the high IL-2 media. Therefore, we have optimized a protocol to expand cGMP compatible protocol to expand γ9δ2 T cells in a serum-free media with minimal contamination of αβ T cells. We also tested the antitumor functions of γ9δ2 T cells stored in multiple cryopreservation media. Cryopreservation of γ9δ2 T cells in serum-free media: Cryopreservation is an important step towards generating allogeneic γ9δ2 T cells for adoptive immunotherapy. Towards this effect, we have tested the phenotype and cytotoxicity of γ9δ2 T cells before and after cryopreservation in DMSO+20% human serum Cryostar and SynthFreze. We have showed that there is no change in the phenotype of the γ9δ2 T cells before and after cryopreservation. However, there is at least 50% reduction in the cytotoxicity with all the tested Cryoprotectants. Therefore, fresh γ9δ2 T cells may have preserved cytotoxicity. Further optimizations are required to identify a suitable cryopreservation media to store γ9δ2 T cells without losing viability and function.

RESEARCH TALKS

Advances in Immune cell Therapy: 2nd workshop in Immunology, Department of clinical immunology and rheumatology, Christian Medical College, Vellore, India on 28th-29thSep.,2020

a. Engineering Natural Killer (NK) cells/ γδ T cells with antiCD19 chimeric antigen receptor (CAR) for the adoptive immunotherapy.

Collaborating institutes: CSCR and CMC Vellore

- Dr. Srujan Kumar Marepally, CSCR
- Dr. Mohankumar Murugesan, CSCR
- Mr. Augustine Thambaiah, CSCR,
- Dr. Aby Abraham, CMC
- Dr. Alok Srivastava, CMC/CSCR

b. Engineering Natural Killer (NK) cells/ γδ T cells with antiCD19 chimeric antigen receptor (CAR) for the adoptive immunotherapy.

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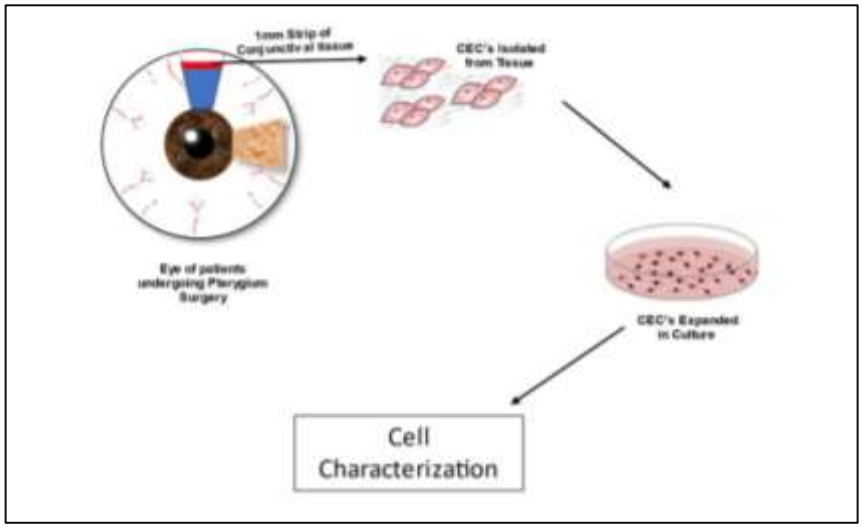
- Mr. Augustine Thambaiah, CSCR
- Dr. Aby Abraham, CMC
- Dr. Alok Srivastava, CMC/CSCR

INVESTIGATOR

SUNIL MARTIN, Ph.D.
Scientist, CSCR

5. Cell Therapy for Ocular Disorder



Schematic representation of Isolation and culture of Conjunctiva Epithelial Cells (CEC's)

This programme is coordinated by ***JEYANTH ROSE***. In the ocular stem cell Lab, we are working on translating Cell based therapies for various corneal and ocular surface disorders. Our research primarily focuses on the therapeutic use of Mesenchymal Stem Cell (MSCs) in treating ocular disorders. Our focus has been in the areas of corneal scarring, dry eye and allergic conjunctivitis. Our previous study evaluated the efficacy of placenta-derived MSCs in improving corneal transparency, in an ex-vivo organ culture model of corneal scarring. An intrastromal injection of Placenta-derived MSCs Vs placebo, showed a significant difference in the levels of scarring. The transparency was optically quantified by a tool that we made and standardized using laser light passed through an equally pressurized artificial anterior chamber. This proof of principal study showed optimistic results and formed the first phase of testing this hypothesis.

Another interest of our lab is to assess the role of MSC's in mitigating the mast cell response in severe allergies related to the conjunctiva. Our current study is involved in developing an in-vitro model for the same. The culture of conjunctival epithelial cells (CEC) from humans, can be used as an in-vitro model to test the effect of drugs &cell-based therapies in the lab. These models can also be used to study the pathogenesis of various ocular surface diseases like dry eye and allergic conjunctivitis in a simple and cost-effective way. The current study aims to harvest, isolate, culture and characterize human conjunctival epithelial cells, which will be used as an in in-vitro model for testing in further stem cell-based studies. The trimmed graft from the donor site, during pterygium surgery serves as the source of CEC'S. We are comparing the PDT and PDN of the cell

cultures acquired from various sources including – cadaver samples, brush cytology and impression cytology. Optimizing protocols to provide the most efficacious model for in-vitro testing of MSC's is being developed.

PUBLICATIONS

- Amritanand A, Lyngwa D, Thomas N, Eapen A, Immanuel J, Prabhu T, Srivatsava S, Kachroo U, Charles AS, Subramanian R, Mathew AJ, Joshua A, Paul J, Rose JS. Efficacy of two vertically integrated teaching–learning methods–The ocular basic science workshop versus lecture in enhancing knowledge and skills of ocular examination techniques and underlying principles in second clinical year medical students: A prospective interventional study. TNOA Journal of Ophthalmic Science and Research. 2021 Apr; 59(2):152.
- Rose JS, Lalgudi S, Joshua R A, Paul J, Susanne M A, Phillips A C, Jeyaraj C, Abraham G, Joshua R, Vinay S, Paul P. A validated audio-visual educational module on examination skills in ophthalmology for undergraduate medical students in the COVID-19 season-An observational longitudinal study. Indian Journal of Ophthalmology. 2021 Feb; 69(2):400.

INVESTIGATOR

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

6. MECHANISMS OF DISEASES

1. Cancer Stem Cells in Endometrial cancer:

This programme is coordinated by *MUTHURAMAN N*. Endometrial cancer is one of the most common gynecological malignancy, the prevalence of which is increasing globally. As per Bokhman's classification, endometrial cancer can be classified into type I and type II. Type I tumor occurs at younger age and includes FIGO grade I and grade II endometroid histology. Type II tumor includes FIGO grade III endometroid, serous or clear cell histology. In general, type II endometrial cancer are more aggressive and carries poor prognosis as compared to type I endometrial cancer. Cancer stem-like cells (CSCs) present in the tumor tissue are considered to be an important cause for tumor recurrence and poor prognosis. Chemoresistance and radio resistance of solid malignancies can be attributed to cancer stem-like cells. We speculate that the difference in the characteristics of type I and type II endometrial cancer could be partly attributed to the difference in the nature of their cancer stem cells. We have standardized the process of isolating cancer stem cells from HEC-1B cell line (Representative of type II endometrial cancer) using CD 133 as a marker. Our preliminary analysis shows that CD 133 positive cells (CSC) are more efficient in forming tumor spheres as compared to CD 133 negative cells. Also, there is a difference in the expression of genes related to stemness and invasion between CD 133 positive and negative cells. The same experiments would be tried in patient samples of both type I and type II endometrial cancer and analyzed to study the nature of cancer stem cells. We are interested in studying the expression and behavior of cancer stem cell population in type I and type II endometrial cancer.

Recent evidence suggests that aspirin can target cancer stem-like cells (CSCs) seen in breast, pancreatic, prostate and colorectal cancers. However, the effect of aspirin on endometrial cancer stem cells has not been investigated yet. The effect of aspirin in combination with cisplatin will be studied on the CD133 positive cancer stem cells obtained from HEC-1B cell line. It would be interesting to see if aspirin can suppress the stemness and potentiate the action of cisplatin on these drug resistant cancer stem cells of type II endometrial cancer. The effect of aspirin on genes of invasion and stemness in cancer stem cells, and its effect on sphere forming ability will be studied to understand its usefulness in treating the chemo resistant cancer stem cells

INVESTIGATOR

MUTHURAMAN N., M.D.

Associate Professor, Department of Biochemistry, CMC, Vellore

Adjunct Scientist, CSCR

2. A. Role of Iron in erythroid lineage:

Following research projects are coordinated by ***EUNICE SINDHUVI***.

I. Biology of iron in RBC regeneration: upcoming players of regenerative medicine

Differentiation of Hematopoietic stem cells (HSCs) could be an effective substitute for transfusions as it differentiates into erythroid lineage and generate red blood cells (RBCs). Many researchers have attempted to develop in vitro system for RBC regeneration in large scale. However limited proliferation ability of HSCs, late maturation and enucleation of erythroid cells restricted the usage of these cells.

During pregnancy, HSCs are regulated in such a way that it is involved in increased RBC regeneration for the developing placentofoetal compartment. In such a scenario, enhanced iron levels are required to sustain haemoglobin synthesis. Studying the behaviour of HSCs in this increased erythropoietic state of pregnancy will lead to a better understanding of RBC regeneration. Further this study of erythropoietic lineage fate will be compared with the pathological increased state of erythropoiesis in Polycythaemia Vera (PV). Physiological and pathological upregulation of erythropoiesis in pregnancy and PV would serve as a good model to understand how increased RBCs are synthesised from the HSCs.

II. Characterization of human erythroblasts at distinct stages: implications to understand iron regulatory mechanism in normal erythropoiesis in vitro

CD34 cells enrichment was processed invitro consenting donor samples using easy sep magnetic bead system, according to manufacturer's protocol. Purity of isolated cells was analyzed using flow cytometry and achieved >95%. The cells were then cultured in 3 phases. The cells were proliferated and differentiated from early basophilic to polychromatophilic stage which was morphologically examined by staining. Third phase lasted till day 24, and terminal erythroid differentiated cells were observed. Erythroid differentiation is being carried out in healthy stem cell donors and PV patients. The cells in erythroid differentiation stages will be processed for gene expression of iron regulatory and erythroid genes which will throw light on the mechanisms of increased erythropoiesis.

2. B. Stem cells in bone marrow failure syndromes:

I. Elucidating the role of Mesenchymal Stem Cells, Immune and Telomere Biology in regeneration and differentiation of Haematopoietic Stem Cells

The proliferative potential of HSCs reduces with differentiation and age in line progresses to shortening of telomeres. Also, certain antigens stimulate immune responses that induce the production of inflammatory cytokines which can terminate the growth of HSCs; and eventually truncate cell cycling and cause cell death by apoptosis of HSCs. Mechanisms involved in the loss of HSCs self-renewal, differentiation property and immune response needs to be explored. Recent studies suggest that mesenchymal stem cells (MSCs) are critical for forming a niche that maintains and directs HSCs self-renewal and differentiation. The precise mechanisms by which MSCs exert their functions are still unclear. We aim to investigate the comprehensive role of MSCs, molecular and immune mechanism in supporting HSCs regeneration in bone marrow failure condition.

II. Study the mesenchymal stem cells which provide microenvironment for HSCs, in conditions of HSCs loss

MSCs were cultured from bone marrow nuclear cells of patients with aplastic anaemia until passage 2. MSCs at P2 are used for further analysis. Differentiation potential of AA-MSCs towards osteogenic and adipogenic lineage is also being evaluated. DNA and RNA is extracted from MSCs and used for telomere length measurements and gene expression analysis respectively. Gene expression specific to osteogenic (Osteocalcin, ALP, RUNX2) and adipogenic lineage (FABP4, PPAR, LPL) will be analysed using qPCR. Telomere length was analyzed in MSCs and their respective peripheral blood samples. The median (range) MSC rTL and PB rTL were 1.34 (0.60-3.03) and 0.68 (0.37-1.70) respectively. MSC-TL in patients with AA showed significance difference in comparison to PB-TL ($p=0.00048$). Studies to measure the immuno suppressive ability of MSCs are also being planned.

INVESTIGATOR

EUNICE SINDHUVI, Ph.D.

Professor, Department of Haematology, CMC, Vellore

Adjunct Scientist, CSCR

3. Modulating chemoresistance in acute and chronic myeloid leukemia stem cells:

Following research projects are coordinated by ***POONKUZHALI BALASUBRAMANIAN.***

I. Modulation of NRF2 and downstream targets to overcome acquired chemoresistance in acute myeloid leukemia

Acute myeloid leukemia is a hematopoietic malignancy that is characterized by blocked myeloid lineage differentiation that leads to the accumulation of myeloid blast cells in the bone marrow.

The current treatment of AML starts with a 7+3 induction chemotherapy consisting of a nucleoside analog cytarabine and an anthracycline – daunorubicin, followed by high dose cytarabine or hematopoietic stem cell transplantation (HSCT). Though, 90% of those who go for induction chemo enter remission, eventually, a majority of those relapse. AML has the lowest 5-year survival among other leukemias, which can be attributed to the presence of drug-resistant clones and LSCs. Several mechanisms are known to be associated with chemoresistance, and one such mechanism is redox adaptation by which a tumor gains resistance to chemotherapy. Among the several transcription factors that regulate redox adaptation, Nrf2 is known to be an important transcription factor involved in mediating chemoresistance through transcription of its downstream target genes.

Our laboratory has previously reported the role of Nrf2 in mediating inherent resistance to chemotherapeutic drugs using inherently resistant AML cell lines and primary patient samples. Here in this study, we plan to explore the Nrf2 mediated factors of chemoresistance in acquired chemoresistant AML cell lines and LSCs.

Objectives:

- To develop & characterize chemoresistant AML cell lines as a model to study acquired chemoresistance
- To determine the function of NRF2 and its downstream targets in drug-resistant AML cell lines, primary AML patient samples, and AML LSCs.
- To establish the role of selected target genes (drug-metabolizing genes, ABC transporters, stemness genes), if any, in mediating chemoresistance.
- To screen for a suitable inhibitor(s) of NRF2 and its downstream targets in vitro.
- To determine the effect of inhibitor(s) of NRF2 and its downstream targets in combination with standard chemotherapeutic drugs in-vivo (using AML mouse model).

Molecular inhibition of Nrf2 and its downstream target genes using lentiviral knock down

To see the effect of molecular inhibition of Nrf2 and its downstream target genes, shRNA mediated lentiviral knockdown was employed. shRNA for Nrf2 and its downstream target genes were designed based on published literature, cloned into vector PLKO. The resistant cells transduced with Nrf2/its downstream target genes were selected with markers present in the vector, expanded. The knockdown in the transduced cells were confirmed by qPCR and western blot techniques

II. Generation of xenograft transplantation chronic myeloid leukemia mouse model

CML cell lines (KCL22, Lama84 and K562) transduced with luciferase containing virus by spinfection. We confirmed the presence of luciferase expression in the K562 and KCL22 cell line in-vitro. After transduction of luciferase lentivirus into K562 and KCL22 cell line, transplanted 1*106 cells into NSG mice via tail vein. CML disease development was confirmed by monitoring luciferase expression by IVIS live animal imaging in KCL22 cell line injected mice. Animals developed CML disease by 5-6 weeks. We treated mice with Imatinib, acitretin and in combination on day-7 post transplantation for 21days. We did in-vivo image to check leukemic burden after treatment and monitored survival of the mice. Our data suggested that, acitretin in combination with Imatinib reduces leukemic burden (spleen size and luciferase expression) and increased survival (median survival: control -41.5 days (n=6), imatinib alone - 42 days (n=9), acitretin alone – 42 days (n=6) and Imatinib+ acitretin -57 days (n=6)) in the mice model of CML.

III. Human chronic myeloid leukemia (CML) derived induced pluripotent stem cells (iPSCs) as a model to study the effect of Nuclear Hormone Receptor ligands in CML Stem Cells.

Objectives:

- To generate induced pluripotent stem cells (iPSCs) from CML CD34+ cells, differentiate them into CML LSC like cells (lin-CD34+CD38-)
- To test the effect of NHR ligands on CML LSC like cells in combination with Imatinib in-vitro and in-vivo.

Work done: CD34+ cells enrichment, purity check and CD34+ cells expansionCD34+ cells were enriched from the CML patient bone marrow sample and cryopreserved. 2X10^6 CD34+ cells were expanded using stem span SFEMII medium supplemented with CD34+ expansion supplement and UM729 for 10 days. CD34+ cells were proliferated for 6 - 7 days of incubation with SFEMII medium along with supplements, after that cell attain plateau phase or decrease. CD34+ purity was checked using flowcytometry, cells showed more than 90% purity after expansion. We established culture condition to expand pure population CD34+ cells. This culture condition helps to maintain CML CD34+ cells and generate CML iPSCs. Further, we transfected GFP plasmid in CML CD34+ cells using neon nucleofection system. After 24 hours transfection efficiency was checked using flow cytometry. We observed very less GFP positivity (7-14% GFP) after transfection in CML CD34+ cells. Hence, we are planning to use Sendai virus to increase the transfection efficiency in primary CML CD34+ cells.

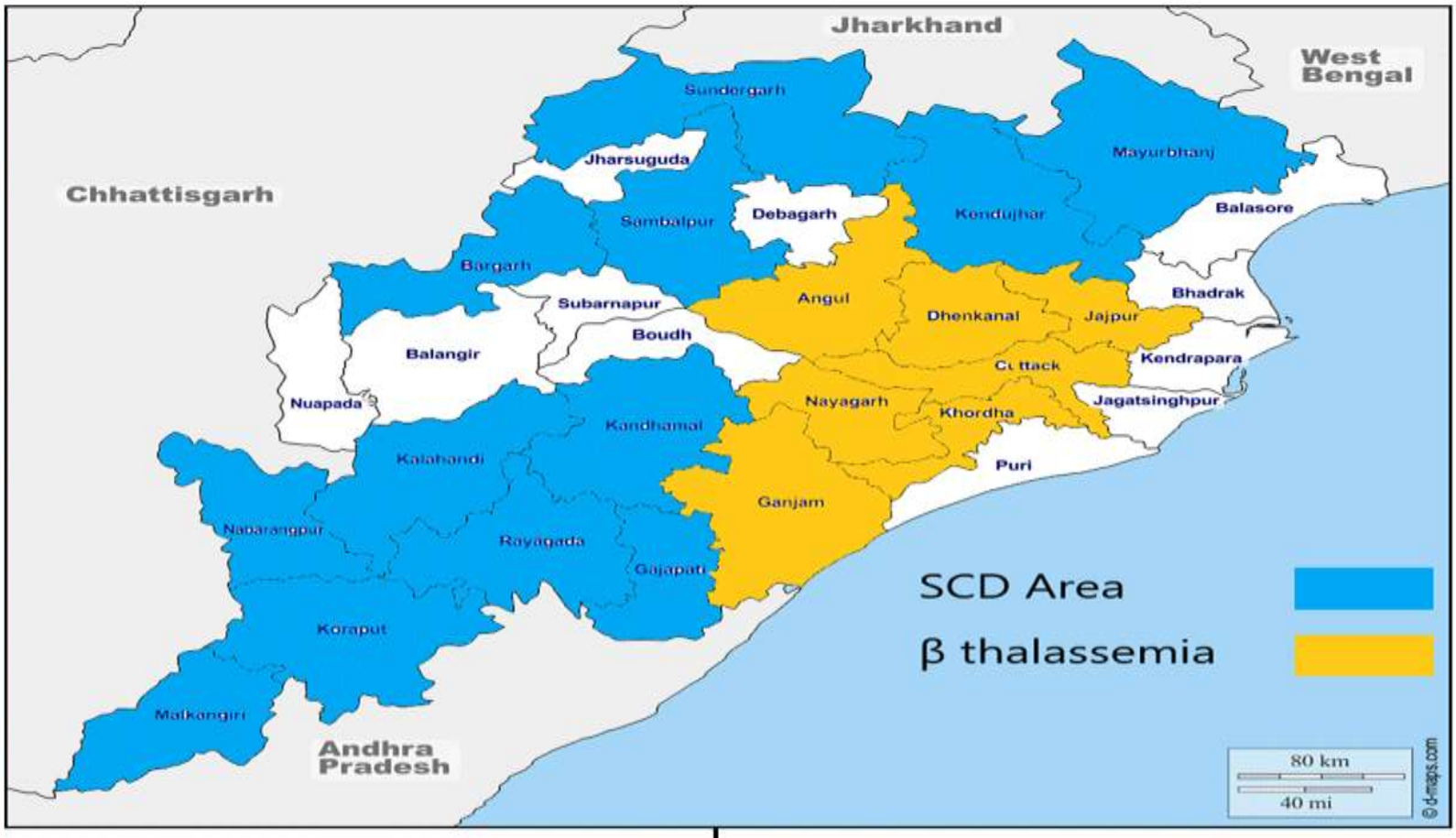
INVESTIGATOR

POONKUZHALI BALASUBRAMANIAN, Ph.D.

Professor, Department of Haematology, CMC, Vellore

Adjunct Scientist, CSCR

Community Outreach Programme



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Supported by

National Health Mission
Department of Health & Family Welfare, Government of Odisha

Supported by

Programme for Control of Thalassemia and Sickle Cell Disease in Odisha

The project focuses on Control of major hemoglobin disorders (MHD), which are beta thalassemia major and sickle cell disease, a significant public health issue in the country. In Odisha about 10-20% of the population are estimated to be either carriers or have disease.

The project aims to reduce the burden of these diseases in the affected populations in Odisha through he combined effort of Ministry of Health, Odisha, Christian Medical College, Vellore and Centre for Stem Cell Research (a unit of inStem, Bengaluru), with the support of Department of Biotechnology of the Ministry of Science and Technology, Government of India.

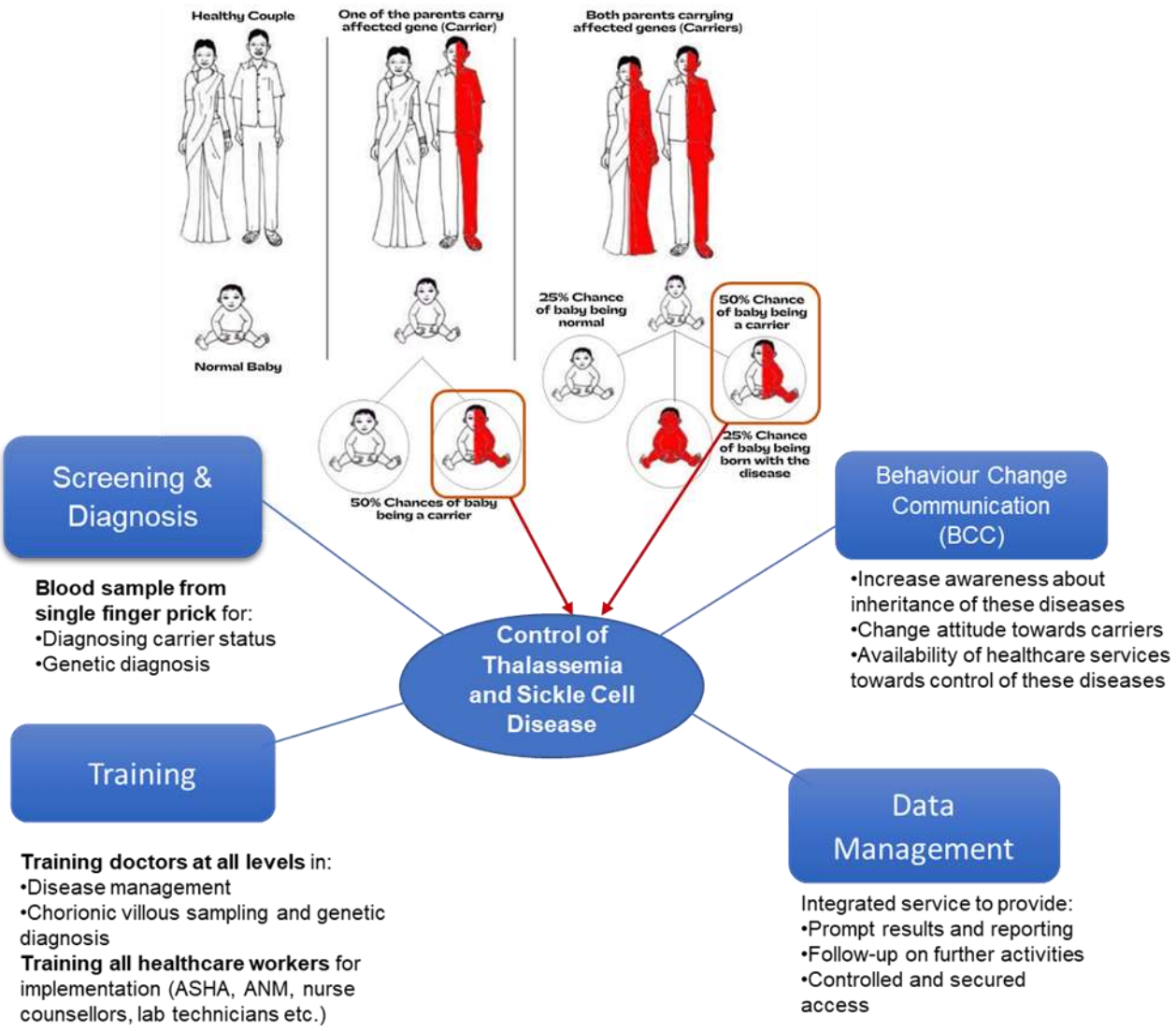
This is the first comprehensive program for the control of these major haemoglobin disorders in India to be carried out at this scale. Novel technologies have been developed for screening these haemoglobin disorder and genetic analysis of these diseases.

Priorities of project

1. To increase awareness on MHD, its genetic transmission and options for preventing it
2. To increase access to testing, counselling and pre-natal diagnosis
3. To counsel and screen individuals and couples; give relevant information and connect them to appropriate additional services;
4. Provide training to healthcare workers for improved diagnosis and treatment of MHD
5. Support partnerships among different government agencies, non-governmental organizations and civil society for this program.

The project will be implemented with the following components

1. Screening & Diagnosis of risk population including pre-natal diagnosis
2. Behavioural change & communication (BCC) program for the community
3. Training for healthcare professionals and field workers in the state health service
4. Data Management of reports obtained and for qualitative analysis.



Pictorial representation of the program (Control of Thalassemia and Sickle Cell Disease in Odisha)

1. Screening & Diagnosis

This component is being supervised by Dr. Sukesh Nair and Dr. R.V. Shaji. Blood cell counters has been installed in 5 districts (Koraput, Bargarh, Sambalpur, Balasore and Cuttack). HPLC instruments have been installed in SCB Medical college, Cuttack for confirmation of diagnosis. As of May 2021, 1500 samples were collected for screening. Out of those screened, 10 couples were found positive. In addition, with the installation of RT-PCR instrument in SCB medical college, 5

samples were collected for CVS, of which, 4 were affected with major haemoglobin disorders. Sentinel surveillance through cord blood sample collection and testing involved sampling 1597 samples till May 2021, of which, 234 were identified positive. The COVID19 pandemic has affected the implementation of the field program. The pace of screening will be increased as the implementation of labs extends to other districts in Odisha.

2. Behavioural change and communication (BCC)

This component is supervised by Dr. Shantidani Minz. The deliverables include development of media plan, development and production of BCC material, pilot of media and material, identification and training of district implementing agencies. In the past one year, BCC has reached up to 83 towns and 779 villages, 1410 panchayats, 65 blocks and 335 peripheral health facilities in 5 districts. Several agencies such as SAB agencies, EmertechRnD Solution Pvt. Ltd, Samperk Adv. Pvt. Ltd and G-Mantra were hired to complete the BCC activities. Although the BCC activities were severely hampered by the COVID-19 lock down situation, the BCC activities are in full pace to cover the rest of 25 districts in Odisha.

3. Training

The training component is supervised by Dr. Jiji Mathews together with Dr. Kuryan George and Dr. Alok Srivastava. In the past one year, training of field functionaries in sample collection and data management system were completed in four blocks of Koraput district. A district level orientation for all MOIC and administrators, as well as a separate training of trainers (ToT) were conducted for all districts of the first phase. ANM Orientation on need-based counselling services was held at Laxmipur CHC, Koraput. Hands on Training on CVS was conducted in SCB Medical College Hospital (March, 2021) and in AIIMS, Bhubaneswar (April,2021). District workshop for district and block administrators &ToT for LT and Counsellors were completed in Bargarh, Sambalpur, Cuttack and Balasore (April, 2021). Data management training was provided to1055NHM staffs under various categories in 6 districts. The training is continually being handled in order to spread the sample screening and collection in all districts of Odisha.

4. Data Management

Data management is supervised by Dr. Venkata Raghava. Android app and web-based application to facilitate data management were developed this year. Training for field staff to use the app and enter the data is completed. Purchase of Data Management equipment for 6 districts completed. Training to lab technicians and administrative officers to enter details for

ANM's completed for Koraput. In addition, installation of the android application for Data Managements in 30 Districts of Odisha was completed. Initiated data capturing from Narayanpatna, Dasmathpur and Bandhugaum blocks. Installation of project data management interphase completed at SDH Kuchinada, Sambalpur, DHH Bargarh, SDH Banki, Cuttack. Data management system for sentinel surveillance has also been completed within this year. Now, the data management system is being prepared ahead to manage sample load from all 30 districts of Odisha.

THEME COODINATORS

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Adjunct Scientist / Head, CSCR

Core Facilities and Instrumentation

8. Core Facilities and Instrumentation

Core Facilities

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 Core Facility

The Molecular Core Facility under the supervision of Dr. Shaji, is actively involved in providing the high-end molecular biology services for the users (in house and off campus). The facility currently has a 3130 4-capillary DNA sequencer from Applied Biosystems, and an Applied Biosystems QuantStudio 12K Flex Real-time PCR for high throughput analysis.

I. Genetic Analyzer 3130

Genetic Analyzer 3130 is a 4 capillary series system with Electro-osmotic flow suppression polymers (EOF). This system gives you all the advance automation with hands free operation and superior performance. This system provides compatibility with the existing application software systems, long-term reliability, automated polymer delivery system, enhanced thermal control, and optimized for multiple application.

II. Quant Studio 12 K Flex Real-Time PCR

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

III. Quant Studio 6 K Flex Real-Time PCR

The Quant Studio 6 Flex Real-Time PCR System is ideal for laboratories with multiple applications and end users on a limited budget. With a planned upgrade path to a Quant Studio 7 Flex System that accommodates automation or TaqMan Array Cards, the Quant Studio 6 Flex System is an ideal qPCR platform to accommodate changing future needs.

IV. Ultracentrifuge

Optima L 100 XP

The Optima L-XP ultracentrifuge is used to generate centrifugal forces for the separation of particles. The Optima L-100 XP has a maximum rotational speed of 100,000 RPM; the Classified S, it can be used with all currently manufactured Beckman Coulter preparative rotors. The microprocessor-controlled Optima L-XP provides an interactive operator interface, using a screen and keypad, with the eXPert operating software. Both manual and programmed operations are available. In manual operation, you enter the individual run parameters and begin the run. In programmed operation, we can create, save, recall, modify, and/or print a program, and then automatically run the ultracentrifuge via the program.

V. High Speed Centrifuge

Avanti J-30I

Achieve the fastest separations possible in the shortest amount of time with the AvantiJ-30I high performance centrifuge Swinging-bucket and fixed-angle rotors provide maximum separation forces in excess of 100,000 x g at speeds up to 30,000 rpm. Unmatched acceleration/deceleration rates 4.0L max capacity High-Throughput Processing four-liter batch throughput for bacteria and cell membrane isolation using the JLA-9.1000 J-LITE rotor at 16,800 x g DNA sample prep in up to ten microplates with the JS-5.9 rotor.

Applications Versatility

- Quickly and easily process protein separations with a fixed-angle rotor.
- Separate sub-cellular organelles with rate zonal centrifugation.

Sample Protection

- Maintain sample integrity by customizing acceleration and deceleration rates.
- Samples spend more time at full force and less time in the centrifuge.

Time and Efficiency

- Reduce total time spent on a separation protocol
- Conduct consecutive runs based on acceleration and deceleration profiles
- Low-heat output and low-energy consumption

VI. SpectraMax i3x Multi-Mode Microplate Reader

The SpectraMax i3x Multi-Mode Detection Platform from Molecular Devices is a monochromator-based, multi-mode detection platform. An external computer running the SoftMax Pro Microplate Data Acquisition and Analysis Software provides integrated instrument control, data display, and statistical data analysis.

The built-in read modes include:

- UV and Visible Absorbance (ABS)
- Absorbance Read Mode
- Fluorescence Intensity (FL)
- Luminescence (LUM)

The read capabilities of the SpectraMax i3 Instrument can read endpoint, kinetic, multi-point well-scan, and spectrum microplate applications can be set up and run with the SoftMax Pro Software. Depending on the application, the instrument can read 6, 12, 24, 48, 96, and 384-well microplates. The SoftMax Pro Software can collect data from one or more microplates and store it in a single data file, using the same or different instrument settings for different microplates. Assays requiring a read in two or more read modes or read types can be combined in a single experiment and run with a single command in the software, by defining separate microplate reads and enabling Auto Read.

The high sensitivity and flexibility of the SpectraMax i3 Instrument make it appropriate for applications in the fields of biochemistry, cell biology, immunology, molecular biology, and microbiology. Typical application includes ELISA, nucleic acid, protein, enzymatic type

homogeneous and heterogeneous assays, microbial growth, endotoxin testing, and pipettor calibration.

VII. ExPERT™ GTx Flow Transfection System

MaxCyte's GTx cell engineering platform is based on flow electroporation technology, which is suitable high-efficiency gene editing of any cell type at any scale. It has the unique ability to transfect primary cells, stem cells and cell lines with minimal cell disturbance and transfection efficiencies routinely >90% for a variety of cell types. Flow electroporation Technology provides scientists with the freedom to use the most physiologically relevant system facilitating the identification, development and manufacturing of cell therapies, bio therapeutics and small molecule candidates of the highest quality.

b. Tissue Culture core 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 core tissue culture facility, located in ground floor and first floor, houses the basic equipment required for cell culture experiments. Users from within the centre and adjunct scientist 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 easy cleaning of the facility. The users are also provided with lint free lab coats for use within the facility.

The lists of equipment in the TC facility are the following:

a. Biosafety cabinet
b. CO2 incubator
c. Refrigerated high speed centrifuge
d. Inverted phase contrast microscope and fluorescent microscope
e. Water bath
f. Cell counters (Vicel Blu and Cell drop)
g. Storage space for individual labs
h. Refrigerator, -20°C freezer & Liquid Nitrogen sample storage container

c. Radioactivity Core Facility

The Radioactivity Core Facility provides researchers a secure access to radiolabeled isotopes and instrumentation for detecting radioactivity. The facility currently has Greiger counters, GE Storm 365 Phosphor imager and a Perkin Elmer Tricarb Liquid Scintillation Counter.

d. Histopathology Core 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. With the CM1900's overall engineering and ergonomic concept, the system provides accurate results for any cryostat application.

The CM1900 is equipped with an ergonomically positioned handwheel for extremely smooth movement and easy locking in the upper position. The model also provides a functional control panel, which includes self-explanatory single-function keys and easily readable LEDs to prevent operating errors. The motorized coarse advance is ergonomically positioned in the arm rest at the left and operated via push buttons. Together with the Leica CM1900's speedy specimen Temperature control, sectioning of various different kinds of specimens can be done rapidly and easily. This helps clinics in the improving overall productivity. Other features of the Leica CM1900 cryostat include: a high-precision microtome enclosed in a special housing to protect it from contamination, a quick freeze shelf for rapid freezing, and a spacious open-top cryochamber with separate specimen cooling.

II. Embedding system

EG 1150H

The Leica EG1150 H is a heated, paraffin dispensing module with 3-liter capacity and a spacious, heated work surface with storage areas for both cassettes and molds.

All functions of the EG1150 H are controlled via an easy-to-read LED display, including the temperature settings for left- and right-hand warming trays, paraffin reservoir, and working surface.

Working days and times can be programmed for automatic instrument operation. Cassette and mold warming trays are interchangeable to accommodate changes in embedding workflow.

III. Tissue Processor

TP1020:

The Leica TP1020 tissue processor is available in four configurations: the basic instrument, the basic instrument with vacuum, the basic instrument with a fume control system and the basic instrument with both vacuum and fume control.

Gentle specimen processing and a high level of specimen safety at all stages of the processing run are supported by the robust design based on precision mechanics in conjunction with a modern user interface.

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. Choose between conventional, full-hand-wheel rotation, manual sectioning or "rocking mode", where the hand-wheel is turned back and forth over a short distance. The instrument has been specially designed for the experienced user who prefers manual over motorized sectioning and meets the many requirements of modern laboratories.

IV. Cytospin

The cytospin centrifuge gains all the advantages of the ultimate thin-layer cell preparation system with the Thermo ScientifiCytospin Cytocentrifuge.
This reliable benchtop centrifuge provides economical thin-layer preparations from any liquid matrix, especially hypocellular fluids such as spinal fluid and urine. It processes 12 specimens at one time and accepts all protocols from Cytospin 1, 2 and 3.
It allows for one-handed opening and closing with a redesigned lid-release mechanism, enables viewing of the sealed head through the polycarbonate window during operation. It protects mechanical and electronic components from damage due to accidental fluid spills Designed for easy disinfection.

TEAM MEMBERS

Molecular Biology Core Facility

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Histopathology Core Facility

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  Technician, CSCR

Flow Cytometry Core

The CSCR Flow Cytometry and Cell Sorting Laboratory provide 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 11 colors, and 13 parameters. Additionally, a wide variety of cell analysis services (up to 19 colors, 21 parameters) are offered. Currently, the facility offers one cell sorter (BD FACS Aria III) and 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 III

The BD FACS Aria III flow cytometer is a high-speed fixed-alignmentbenchtopCell sorter. With its fixed-optics design and digital electronics, the BDFACS Aria 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 FACS Aria III cell sorter with a five laser (Near UV-375nm, Violet-405nm, Blue-488nm, Yellow-Green-561nm, Red-633nm) and 11 color setups 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.

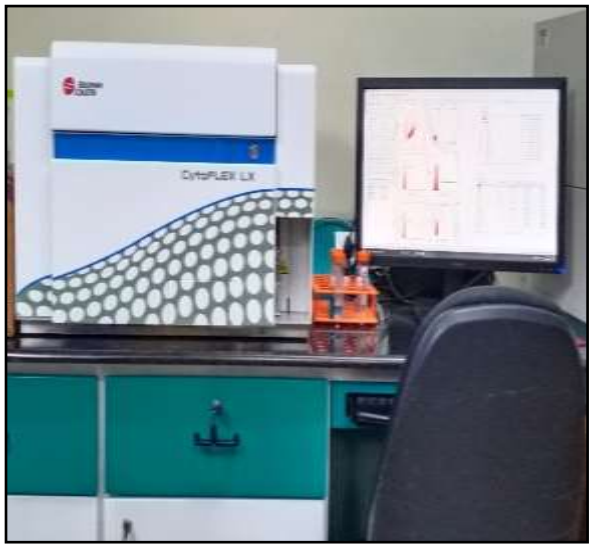
II. BD FACS Celesta

BD FACS celesta is a multi-laser flow cytometer with 3 lasers (blue-488nm, violet-405nm and yellow-green-

561-nm) and 12 color setups for delivering high sensitivity and performance. In the BD FACSCelesta, the optical and electronics system—lasers, filters, detectors, optical paths, and signal processing technologies—have been engineered to get the most out of BD Horizon Brilliant™ dyes.

III. 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 Superior Acquisition rate of 30,000 events per second and Superior Signal Processing digital system with 7 decades dynamic range.



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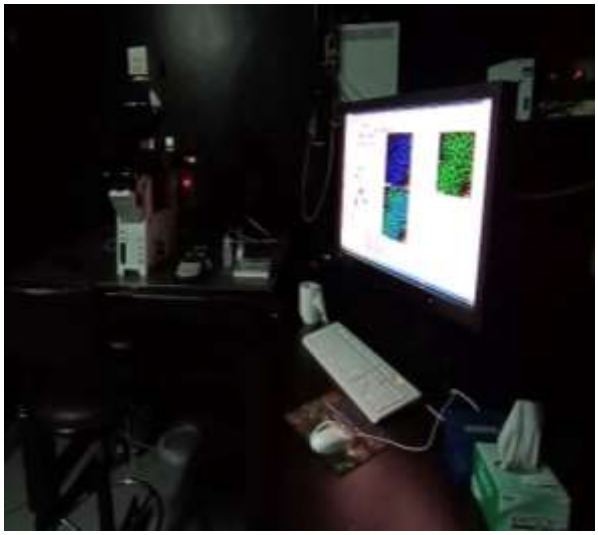
Imaging Core Facility

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.

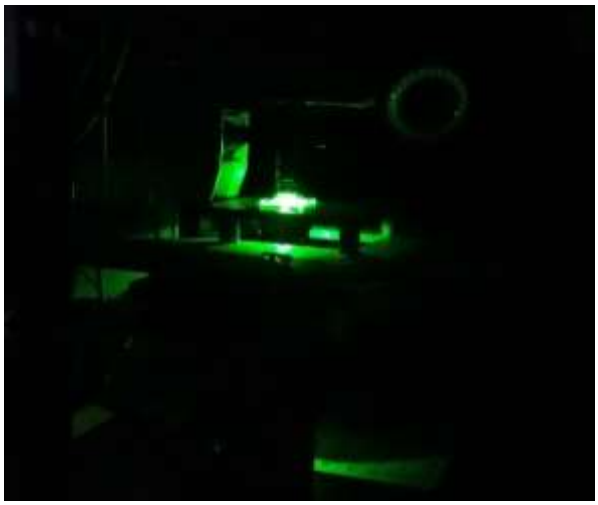
The CSCR Microscopy Core is a full-service facility serving the research community. Our aim is to provide personalized assistance on all aspects of imaging, from tips on sample preparation to training on our microscopes to processing and analysis of image data. Our facility currently houses one Multiphoton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

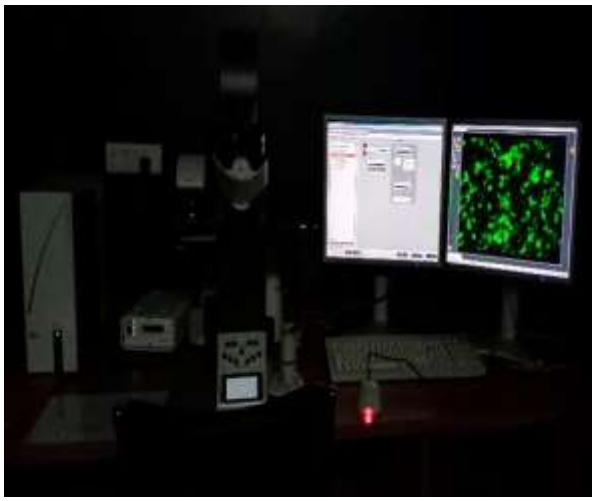
II. Laser Scanning Multi Photon Microscope (Olympus FV1000MPE)



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 specimen. Dynamic biological processes can be imaged hundreds of micrometers within living cells and tissues. Provides support for applications where phototoxicity/photobleachingare 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 of 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.

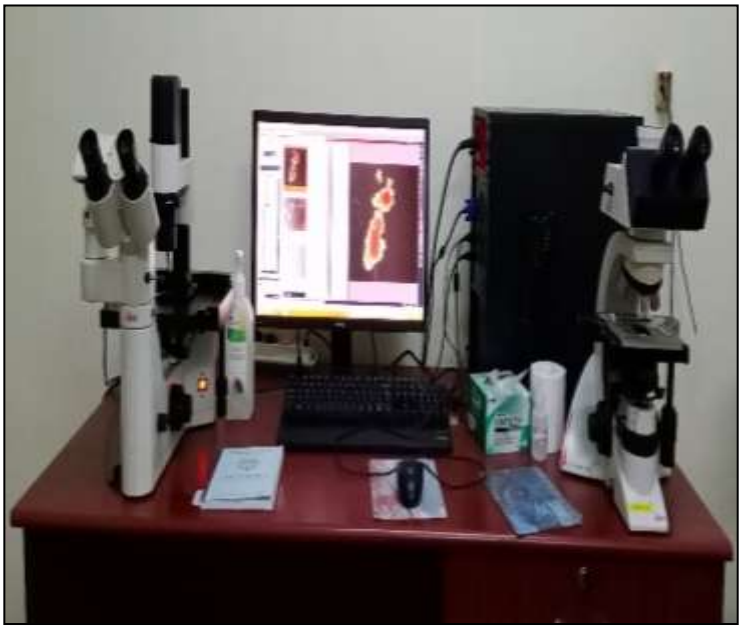
IV. EVOS FL Auto Imaging System

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 enables 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.

V. 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.

VI. Training Sessions

The Imaging Core Facility conducts training sessions regularly for both first time and experienced users. The training sessions comprise of specifically designed modules which include theory and practical sessions. The final authorization is given to the user upon successfully completing the required modules. The hands-on training sessions are tailored to the specific application requirement 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.

Team Members

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CSCR Laboratory Animal Facility

The aim of the laboratory animal facility at CSCR is to ensure humane and ethical treatment of animals, while facilitating legitimate scientific research involving experiments on animals.

Objective

The goal of the CSCR-Laboratory Animal Facility is to promote the humane care and use of laboratory animals by providing information that will enhance animal wellbeing, the quality of research, and the advancement of scientific knowledge that is relevant to both humans and animals as per the sanction from the Institutional Animal Ethics Committee (IAEC). The laboratory animal facility is registered with the 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA) for breeding and conducting experiment on small laboratory animals vide registration no. Reg. 88/PO/RcBi-S/Rc-L/1999/CPCSEA. All activities and protocols of the CSCR-LAF are carried out as per standard operating procedures (SOPs) approved by Institutional Animal Care and Use Committee (IACUC).

Infrastructure

Quality animal management and human comfort and health protection require separation of animal facilities from personnel areas. For that reason, the CSCR Laboratory Animal Facility (CSCR-LAF) is located in the basement of the CSCR building in a total floor space area of 5000 sq. ft with 6 animal rooms. The facility has got double corridor system to facilitate unidirectional movement of personnel. The clean corridor is for the movement of the animal facility staff and animal users only. The dirty corridor is for the movement of unsterile bedding, cages, trolleys. Animals are maintained within individually ventilated micro-isolator caging (IVC) system for breeding, holding and experimentation. The IVC-systems in which the animals are kept ensures that animals are breathing HEPA-filtered air (High Efficiency-Particulate Air) that defends them from most of airborne micro-organisms.

Temperature, humidity and Ventilation

Temperature and relative humidity of the animal rooms are maintained between 20 to 25 °C and 30 to 70% respectively throughout the year. All the environmental factors are monitored round the clock through individual room sensors. Photoperiod of 12 hrs. light and 12 hrs. dark maintained with automatic timers. Light intensity (300 Lux) and noise level (< 85db) are maintained as per the CPCSEA guidelines.

Individually Ventilated Cages (IVC)

Cage changing station

Veterinary care

Qualified veterinarian supervises all the animal health concerns, and provides all necessary veterinary care to ensure that healthy animals are available for research. Ad-libitum supply of UV treated autoclaved R.O water and autoclaved rodent feed imported from the France (SAFE diets) is provided to the animals. To enrich the micro environment of mice and rat enrichment devices such as playing, hiding and gnawing devices and nesting materials are provided within the cages. The Veterinary and technical staffs of the CSCR-LAF are also supporting and facilitating all animal facility users (including PhD students and Project Assistants) on mouse and rat bio-methodologies, principles of three R's, ethics, IAEC laws and guidelines on the regulation of scientific experiments on animals, haematological parameters, husbandry and care, animal identification techniques, sex differentiation, handling and restraining, anaesthesia, drug administrations, blood collection, and euthanasia.

Specialized equipments

The CSCR-LAF is equipped with In vivo small animal imaging system, animal blood counter, zoom stereo microscopes, multi photon microscope and small animal irradiator with Co-60 as source in addition to a couple of Isoflurane anesthesia machines, induction and heating chambers.

In vivo Small Animal Imaging System (PerkinElmer IVIS Spectrum CT)

The IVIS Spectrum CT supports low dose microCT for longitudinal imaging. It features 3D optical tomography for fluorescence and bioluminescence imaging and has sensitive detection for real time distribution studies for both fluorochromes and PET tracers. This instrument is used to study metastasis and engraftment level in mice models used in the stem cell research.

Blood irradiator (BI 2000)

Blood irradiator is used to irradiate mice prior to stem cells transplantation. Users are allowed to use the instrument after getting TLD batch from radio therapy department to monitor the safety of radiation. Application for the renewal of license (authorization for possession and operation of GIC) along with registration and endorsement of the security plan has been filed to the Atomic Energy Regulatory Board (AERB). Our license has been renewed and it is valid till April 2024.

Stereo Microscope

Zoom stereo microscopes are used for dissection and organ collection in rat and mice embryos and microsurgeries in mice.

Animal Blood Counter

Horiba Micros ESV60 3part animal blood cells counter is used for the hematological investigations of blood samples collected from rat and mice.

In vivo small animal imaging system(IVIS CT spectrum)

Animal blood counter

Zoom stereo microscope

Blood irradiator (BI 2000)

Strains available

The CSCR-LAF maintains several different strains of mice - including wild type, transgenic, knock out and SCID strains and SD rat. The majority of rodent strains are bred under strictly inbred conditions (Please refer to website for details; http://www.cscr.res.in/laboratory-animal-facility/).

Quality control (QC)

A quality control program for environmental microbiology, clinical pathology, genetic monitoring has been followed for monitoring the health status of laboratory rodents. Reporting of the QC tests is done in standard formats and QC reports are maintained in the Animal Facility.

Routine/ Conventional Microbiology

Routine sentinel animal sampling (culture analysis of oral swab and faecal sample) is being done in every four months to ensure the health status of breeding and experimental animal's stock. Animal skin and hair samples are checked for ectoparasites. Environmental microbiological examination of animal room air and cage air are also being carried out every month.

Animal utilization details

No. of Projects: 12
No. of Strains: 7
No. of animals sanctioned: 968
No. of animals issued: 611

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  Technician, CSCR
- S. ASHOK KUMAR, D.M.L.T
  Technician, CSCR

Current Good Manufacturing Practice (cGMP) Facility

Clinical grade cells manufactured for clinical trials

- Autologous culture-expanded iliac crest physeal chondrocytes
  - No. of samples processed: 2

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, viral vector manufacturing and genetic modification of cells required to conduct Phase I/II clinical trials in the fields of cell and gene therapy.

cGMP facility has two separate sections 1) cell therapy product manufacturing facility 2) viral vector and genetically modified cells manufacturing facility. Cell therapy product manufacturing facility has a total area of 1200 square feet. The cleanroom area is divided into four independent ISO Class 7 (Class 10,000) manufacturing suites and a common staging room. Each manufacturing suite is fitted with a dynamic pass box. The facility maintains a separate unidirectional flow for personnel and materials. Each suite is equipped with a Class II biological safety cabinet, CO2 incubators, refrigerated high-speed centrifuge, and an inverted phase-contrast microscope. The cryopreservation room is equipped with a control rate freezer for cryopreservation of the cellular product.

The facility also has raw material storage room equipped with various freezers and product storage cryopreservation room with controlled rate freezer and liquid nitrogen containers for

storage of cryovials and cryo bags in vapour phase of liquid nitrogen. The newly constructed viral vector and genetically modified cells manufacturing facility has total area of ~2800 sq.ft.. Clean rooms are divided into two independent suites. Each suit has ISO class 7 for cell and ISO class 6 area for production and final filling of the product. Each suite will be equipped with necessary equipment required for upstream and downstream processing of the product.

The trained staffs directly interact with investigators and help in process development and in the manufacturing of clinical-grade cell and gene therapy products for use in early phase clinical trials.

Authorities from Central Drugs Standard Control Organisation (CDSCO), New Delhi, and the Office of Director of Drug Control (State licensing authority), Chennai, Tamil Nadu, inspected the cell therapy product manufacturing facility. In 2019, the facility was granted license in Form 29 to manufacture "Autologous culture-expanded iliac crest physeal chondrocytes" for a Phase I/II clinical trial in 15 patients.

1. Facility maintenance

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

2. Services

There are four independent production suites capable of handling four different projects at a time. The following are the services provided by the cGMP facility for users:

- Provides clean-room suites for manufacturing of clinical-grade products under cGMP conditions for clinical applications
- Cryopreservation and storage of cell therapy products
- Endotoxin testing using the Charles River Endosafe PTS system
- Mycoplasma testing using ATCC universal mycoplasma detection kit
- Provides support in the regulatory approval process - Evaluate and interpret regulations and standards for cell-based therapy from relevant agencies to determine its applicability to a Principal Investigator's clinical trial or study

3. Current scientific projects

The cGMP facility is involved in the derivation, expansion, and banking of clinical-grade iPSC lines using the latest reprogramming technologies. Peripheral blood samples are collected from homozygous HLA haplotyped donors. Mononuclear cells are isolated and used as a starting material for the derivation of iPSC lines. The iPSC lines are characterized and cryopreserved for future studies.

<u>The cGMP facility is also involved with the following research project:</u>

Gamma delta T cell-based immunotherapy for blood cancers. Centre for Stem Cell Research, CMC Campus and Department of Haematology, CMC. The protocol for the culture and expansion of gamma delta T cells from peripheral blood mononuclear cells in serum and serum-free conditions has been established.

4. Training and Access

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

For any assistance and service-related queries, please contact *Mr. Augustine Thambaiah*

Contact details
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Email: cscrcpf@cmcvellore.ac.in

TEAM MEMBERS

Faculty In-Charge: GURBIND SINGH, Ph.D.
Scientist, CSCR

AUGUSTINE THAMBAIAH, M.Sc.
Technical Officer, CSCR

ALEYA TABASUM, M.Sc.
Gr. Technician, CSCR

9. Annual Cell & Gene Therapy Symposium

SYMPOSIUM ON CELL AND GENE THERAPY (Virtual)
3rd& 4th September, 2020

The Centre for Stem Cell Research (CSCR), a unit of inStem, Bengaluru, managed by Christian Medical College (CMC), Vellore organized the 5th Annual Symposium on Cell and Gene Therapy on 3rd&5th September, 2020. This symposium brought together scientists, physicians and all others interested in and responsible for developing this field in the country. Dr. Renu Swarup, Secretary, DBT addressed the participants through 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. About 350 participants from across the country and 25 speakers from around the world took part in the symposium.

The first day of the symposium was devoted to gene editing, iPSC technology applications, and technological advancements. 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 advances in the CRISPR/Cas9 based genome editing as well.

The key note address of the symposium was delivered by Dr. Marina CavazzanafromPaediatric Immunology at Necker-Enfants Malades Hospital and Institut Imagine, France. She delivered the keynote address titled "Gene therapy for hemoglobin disorders - What it has been and what it will be?" in the context of gene therapy.The second day of the symposium featured a variety of discussions on IMMUNE CELL THERAPY, as well as Cell Therapy in Musculoskeletal Disorders. A session on Mesenchymal Stromal Cell-Based Therapy was also held. 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

Participating institutes

International:

1. Molecular and Cell Biology at University of Orléans, France
2. Necker-Enfants Malades Hospital and Institut Imagine, France
3. Center for Genetics at Children's Hospital Oakland Research Institute, USA
4. International Stem Cell Banking Initiative, UK

5. National Eye Institute, National Institutes of Health, USA
6. San Raffaele University, Milan, Italy
7. University of Florida College of Medicine, USA
8. St. Jude Children's Research Hospital, USA
9. Institute of Biomedical Engineering, University of Toronto, Toronto
10. Expression Therapeutics, USA
11. Process Development and Manufacturing at Stanford University, USA
12. Andrew F. Anderson Emergency Center at Rhode Island Hospital, USA.
13. Coast General Teaching and Referral Hospital, Kenya
14. University of Caligornia, Irvine, California
15. University of Lincoln, UK
16. Emory University School of Medicine, Atlanta, USA
17. 17.San Raffaele Telethon Institute for Gene Therapy, Milano, Italy
18. UniversitiSains Malaysia, Malaysia
19. Thermo Fisher Scientific, USA

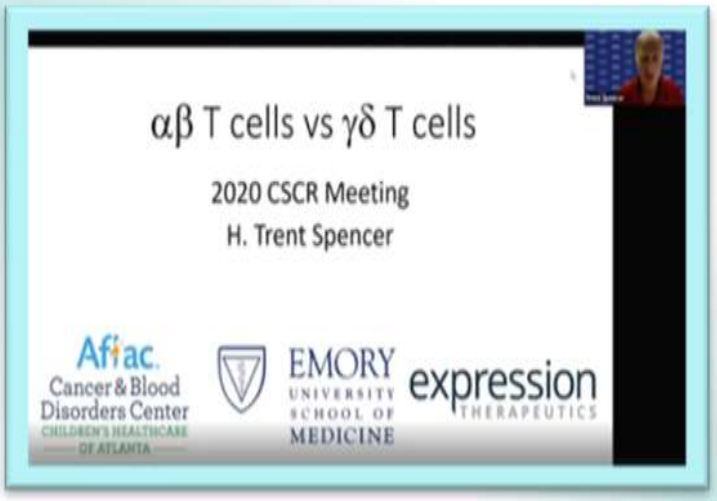
National:

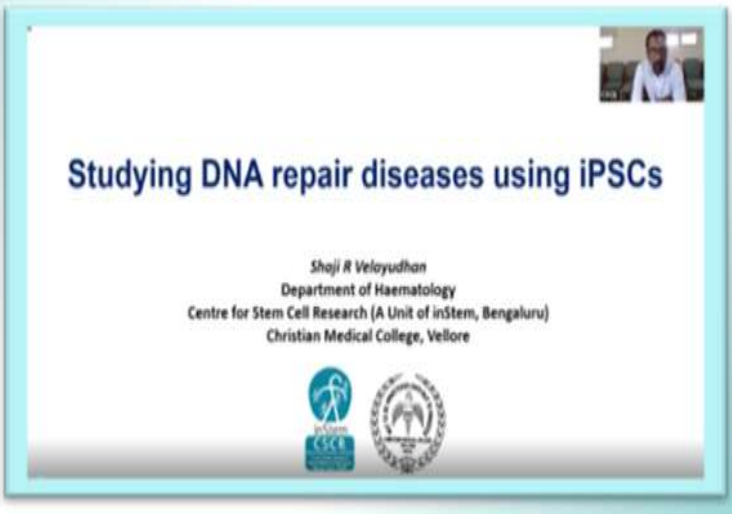
1. Eyestem Research Pvt. Ltd., India
2. Intas Pharmaceuticals, Ahmedabad
3. Indian Institute of Technology, Bombay
4. Biological E Limited, Hyderabad
5. Indian Institute of Technology, Guwahati
6. CSIR-Institute of Genomics and Integrative Biology, New Delhi
7. Centre for Stem Cell Research, Christian Medical College, Vellore
8. Dr. Rela Institute & Medical Centre, Chennai
9. Global Institute of Stem Cell Therapy and Research, Ahmedabad
10. North-Eastern Hill University, Shillong, ML
11. Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, KA
12. Immuneel Therapeutics Pvt Ltd, Bengaluru, KA
13. Biological E Limited, Hyderabad
14. National Centre for Cell Science (NCCS), Pune, MH
15. Manipal Institute of Regenerative Medicine, Manipal
16. National Institute of Technology, Calicut, KL
17. Symbiosis School of Biological Sciences, Symbiosis International (Deemed University), Pune, MH
18. Stempeutics Research, Bengaluru, KA
19. Centre for DNA Fingerprinting and Diagnostics, Hyderabad
20. CSIR-Indian Institute of Chemical Technology, Hyderabad
21. Manipal Institute of Regenerative Medicine, Manipal Academy of Higher Education, Manipal, KA
22. Sri Shakthi Institute of Engineering and Technology, Coimbatore, TN
23. Anna University, Chennai, TN
24. Vellore institute of technology, Vellore, TN
25. Indian Institute of Science (IISc), Bangalore, KA
26. Indian Institute of Technology, Kanpur, UP
27. Karpagam Faculty of Medical Sciences and Research, Othakalmandapam, TN
28. Immuno lab, Coimbatore, TN

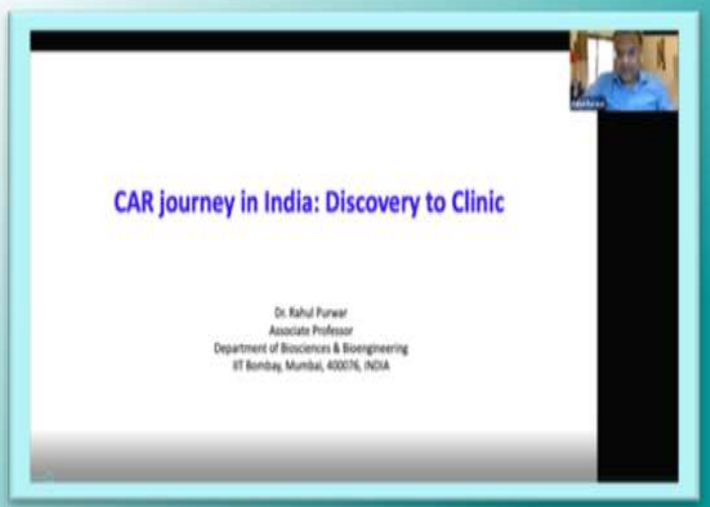
29. All India Institute of Medical Sciences, Nagpur, MH
30. All India Institute of Medical Sciences, New Delhi
31. OncoStem Diagnostics Pvt. Ltd., Bengaluru, KA
32. Banasthali Vidhyapith, Rajasthan
33. Tata Institute of genetics and society, Bengaluru, KA
34. Acranno life Genomics, Chennai, TN
35. University of Madras, Chennai, TN
36. Indian Institute of Science Education and Research, Kolkata, WB
37. St. Xavier's College, Mumbai, MH
38. Quaid-e-Millath College for Women, Chennai, TN
39. Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, UP
40. Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, UP
41. Institute of liver and biliary Sciences, New Delhi
42. S. S. Patel Nootan Science and Commerce College Sankalchand Patel University, GJ
43. NCBS-CCAMP, Bengaluru, KA
44. Uka Tarsadia University, Bardoli, GJ
45. Shree Pramukh swami Medical College, Karamsad, GJ
46. Dr. Indravadan P. Patel institute of medical technology and Research, Anand, GJ
47. Sarojini Naidu govt. Girls PG college, Bhopal, MP
48. Shree P. M. Patel college of paramedical science and technology, Anand, GJ
49. CMC, Ludhiana, PB
50. Chandan Diagnostics, Lucknow, UP
51. ACTREC, Tata Memorial Centre, MH
52. Pondicherry University, PY
53. National Center for Cell Sciences and SPPU, Pune, MH
54. St. Xaviers College, Calcutta, WB
55. Kakatiya Institute of Technology and Science, Warangal, TS
56. Regional Centre for Biotechnology, Faridabad, HR
57. Ramnarain Ruia Autonomous College, Mumbai, MH
58. Dumdum Motijheel College, Kolkata, WB
59. BJB Autonomous college, Utkal university, Odisha
60. Sahyadri Speciality Hospital, Pune, MH
61. Narayana Nethralaya, Bengaluru, KA
62. Institute of Bioinformatics and Applied Biotechnology, Bengaluru, KA
63. Ramakrishna Mission Vivekananda Centenary College, Kolkata, WB
64. Gautam Buddha university, Greater Noida, UP
65. Ahmedabad University, Ahmedabad, GJ
66. University Of Kalyani, WB
67. Madras Veterinary College, Chennai, TN
68. Sri Ramachandra Institute of Higher Education and Research, Chennai, TN
69. Trident academy of creative technology, Bhubaneswar, Odisha
70. Guru Nanak dev University, Amritsar, PB
71. Amity university, Kolkata, WB
72. Amity University, Haryana
73. University of Caligornia, Irvine, California
74. PlasmaGenBioSciences Pvt. Ltd., Bengaluru, KA
75. St. Xavier's College, Ahmedabad, GJ
76. Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, KL
77. Cancer Institute, Adyar, Chennai, TN
78. Bangalore University, Bengaluru, KA
79. Dr. ALMPGIBMS, University of Madras, Chennai, TN
80. Maharishi Dayanand University, Rohtak, Haryana
81. Cancer Institute WIA, Chennai, TN

82. Bioinformatics center-Savitribai Phule Pune University, Pune, MH
83. SRM Institutes for Medical Science, Kattankulathur, TN
84. Swami Rama Himalayan University, Dehradun, UK
85. National Brain Research Cente, Manesar, Gurgaon, Haryana
86. Shri Dharmasthala Manjunatheshwara University, Dharwad, KA
87. Indian Institute of Technology Delhi, New Delhi
88. Career College Bhopal, Bhopal, MP
89. Jadavpur University, Kolkata, WB
90. Andhra University, Visakhapatnam, AP
91. Himt Group of Institutions, Greater Noida, HR
92. Guru Nanak Institute of Pharmaceutical Science and Technology, Kolkata, WB
93. Madurai Kamaraj University, Madurai, TN
94. University of Kashmir, Srinagar, J & K
95. Kalyani Mahavidyalaya, Kalyani, WB
96. Aligarh Muslim University, Aligarh, UP
97. Institute of applied medicine and research, Ghaziabad, UP
98. Ramadevi Women's University, Bhubaneswar, Odisha
99. Ramaiah Institute of Technology, Bengaluru, KA
100. University of Allahabad, Allahabad, UP
101. IIT Gandhinagar, GJ
102. Kalinga University, Raipur, CG
103. Indian institute of technology, BHU, UP
104. Cochin University of Science and Technology, Cochin, KL
105. National Institute of Technology, Rourkela, Odisha
106. BITS-PILANI, Hyderabad
107. Katihar Medical College, Katihar, Bihar
108. Central University of Kerala, KL
109. Thiruvalluvar University, TN
110. Tata Medical Center, Kolkata, WB
111. OPFORD Foundation, Bengaluru, KA
112. Stem Cell Research Centre, Govt Stanley Hospital, Chennai, TN
113. Amity Institute of Biotechnology, Jaipur, RJ
114. Bharathiar university, Vellore, TN
115. Acharya Narendra Dev College, University of Delhi, New Delhi
116. Indian Institute of Science Education and Research, Thiruvanathapuram, KL

Glimpses of 5th Annual Cell & Gene Therapy Symposium - 2020







Education and Training

10. 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.

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	Mr. M Sathyanarayanan	Jan- June 2021	MSc - Biotechnology	University of Madras, Chennai, Tamil Nadu	Dr. Sanjay Kumar
2	Nishka Bhalla	Jan- March2021	M. Tech Biotechnology	Rajalakshmi Engineering College,Chennai, Tamil Nadu	Dr. Mohankumar Murugesan
3	Irine Briny Hepzibh	Jan- Mar2021	M. Tech Biotechnology	Rajalakshmi Engineering College, Chennai, Tamil Nadu	Dr. Mohankumar Murugesan
4.	Ms. Janaani Sri B	Jan- Mar 2021	B. Tech	Bharathidasan University,Tiruchirappalli, Tamil Nadu	Dr. Srujan Marepally

11. PERSONNEL AT CSCR

Scientific / Technical Staff

Dr. Alok Srivastava	Head / Adjunct Scientist
Dr. Mohankumar Murugesan	Assistant Investigator
Dr. Saravanabhavan Thangavel	Assistant Investigator
Dr. Srujan Kumar Marepally	Scientist
Dr. Sanjay Kumar	Scientist
Dr. Sunil Martin	Scientist (Ramalingaswamy Fellow)
Dr. Gurbind Singh	Scientist
Dr. Vrisha Madhuri	Adjunct Scientist
Dr. R. V. Shaji	Adjunct Scientist
Dr. Asha Mary Abraham	Adjunct Scientist
Dr. Dolly Daniel	Adjunct Scientist
Dr. Jeyanth Rose	Adjunct Scientist
Dr. Poonkuzhali Balasubramanian	Adjunct Scientist
Dr. Geetha Chacko	Adjunct Scientist
Dr. Elizabeth Vinod	Adjunct Scientist
Dr. Muthuraman N.	Adjunct Scientist
Dr. Alo Sen	Adjunct Scientist
Dr. Eunice Sindhuvi	Adjunct Scientist
Dr. Md. Manzoor Akheel	Scientist, Research Development Office (Resigned on 2nd January 2021)
Dr. Sonam Pandey	Scientific Program Manager (Resigned) Research Development Officer (from 1st April 2021)
Dr. Sandya Rani	Scientific Officer
Dr. Vigneshwar R.	Veterinary Officer
Mr. Augustine Thambaiah	Technical Officer
Mr. Rajesh A.	Technical Officer
Dr. Immanuel Dhanasingh	Scientific Program Manage (NAHD Program)
Dr. Vasanth Thamodaran	Post-Doctoral Fellow (Resigned 1.11.2020)
Dr. Lakshmi	Scientist B(Resigned)
Dr. Venkatesh	Research Associate
Ms. Sowmya R.	Research Associate

Mr. Abhirup B.	Senior Research Fellow
Ms. Smitha I.	Senior Research Fellow
Ms. Abisha Crystal	Senior Research Fellow
Ms. KrittikaNandy	Senior Research Fellow
Ms. Sonam Rani	Senior Research Fellow
Mr. Franklin Jebaraj Herbert	Senior Research Fellow
Mr. Nithin Sam	Senior Research Fellow
Ms. Thamizhselvi	Senior Research Fellow
Mr. Ashis Kumar	Senior Research Fellow
Ms. Dhivya Bharathi	Senior Research Fellow
Mr. Vigneswaran V	Senior Research Fellow
Mrs. Renita Raymond	Senior Research Nurse (Resigned)
Mr. Kartik C	Senior Research Fellow
Ms. Prathiba Babu	Senior Research Fellow
Ms. Sevanthy	Senior Research Fellow
Ms. Nivedhitha D	Senior Research Fellow
Ms. PorkizhiArujunan	Senior Research Fellow
Mr. Muthuganesh	Senior Research Fellow
Mr. Vignesh R	Senior Research Fellow
Mrs. Vatchala Dennis Newton	Staff Nurse
Ms. Sushasini G	Research Coordinator
Mr. Karthik V.K.	Junior Research Fellow
Mr. Manoj Kumar	Junior Research Fellow
Ms. Anila George	Junior Research Fellow
Ms. Kriti Prasad	Junior Research Fellow
Ms. Annelin Jacob	Junior Research Fellow
Mrs. Agnes Selina	Junior Research Fellow
Ms. Aruna	Junior Research Fellow (Resigned on 1.4.2021)
Mr. Ajay Kumar Dhyani	Junior Research Fellow (Resigned on 11.02.21)
Ms. Aleya Tabasum	Graduate Technician
Ms. Dhavapriya B.	Graduate Technician
Ms. Pavithra R.	Graduate Technician
Ms. Esther Rani J.	Technician
Mr. Ashok Kumar	Technician
Mr. Joshua Paul	Graduate Technician

Ms. Chitra P.	Graduate Technician
Mr. Abdul Muthallib	Graduate Technician
Ms. Praveena	Graduate Technician
Mr. Vighesh Kumar	Graduate Technician
Ms. Immani Job	Graduate Technician
Ms. Frazana	Graduate Technician
Mr. Joseph Joel	Staff II Graduate Technician
Ms. Mohana Priya	Graduate Technician (resigned)
Ms. Sumathy	Graduate Technician

Working in the project titled NAHD-Thalassemia and SCD Program, Odisha)

Mr Surendra Pradhan	Associate Project Director
Dr Sreeya Das	Pathologist
Dr. Chinmayee Panda	Project Coordinator
Mr. Brundaban Sahoo	Project Coordinator (BCC and Training)
Dr. Midhun Rajiv	Project Coordinator (Vellore)
Mr. Sangram Keshari Sarangi	Technical Officer (Training & Counselling)
Ms. Gomathi S.	Technical Officer (Data Management)
Ms. Sarika Nayak	Technical Officer (Laboratory)
Mr. Rashmi Rajan Swain	Graduate Technician
Mr. Solomon Ekka	Graduate Technician
Ms. Ratipragya Das	Graduate Technician
Ms. Sidheshwari Senapati	Graduate Technician
Mr. Debaprasad Pattanaik	District Coordinator
Mr. Panchanan Pasayat	District Coordinator (Resigned)
Mr. Prabodha Sundaray	District Coordinator
Ms. Monalisa Das	District Coordinator
Mr. Utkal Debasis	District Coordinator
Mr. John D	District Coordinator
Mr. Satyabrata Prusty	District Coordinator
Ms. Taraka Nayak	Nurse Counselor
Ms. Bhagyashree Rout	Nurse Counselor
Ms. Salomi Nivedita Singh	Nurse Counselor

Admin, Finance and Support Staff

Mrs. Anupama Nambiar	Assistant Manager
Mrs. Shirley Anandanathan	Secretary
Mrs. Selvi P.	Clerk Typist (transferred)
Mr. Muthukrishnan J.	Multi-tasking personnel
Mr. Tamil Vanan J.	Librarian
Mr. J Pinto	Sr Finance (Consultant)
Mr. Balakrishnan	Consultant Accountant
Mr. Venugopal	Consultant Accountant
Ms. Sanju Bhargavi	Accountant & Cashier
Ms. Geetha	Accountant
Mr. Silambarasan	Driver
Mr. Nithyanand	Attendant
Mr. Arun Kumar	Attendant
Mr. Ramraj	Attendant
Mr. Shankar	Attendant
Mr. Augustin Vasanthakumar	Attendant
Mr. Vigash	Attendant
Mr. Vijay	Attendant
Mr. Sakthivel	Housekeeping Attendant
Mrs. Renuka Devi	Housekeeping Attendant

12. GOVERNANCE OF CSCR

inStem Governing Council

Secretary, DBT	Dr. Renu Swarup	Chairperson
Joint Secretary- Administration, DBT	Dr Shri Chandra Prakash Goyal	Member
Additional Secretary & Financial Adviser	Sh. Vishvajit Sahay	Member
DBT Coordinator for inStem	Dr. Alka Sharma	Member
Programme Officer for inStem at DBT	Dr. Niloo Srivastava	Member
Director, inStem	Dr. Apurva Sarin	Member
Director NCBS	Dr Satyajit Mayor	Member
Centre Director/Dean, NCBS	Dr. Upinder S. Bhalla	Member
Director, CMC, Vellore	Dr. J V Peter	Member
Head- CSCR, CMC, Vellore	Dr. Alok Srivastava	Member
Director, TIFR	Dr. Sandeep Trivedi	Member
One or more Senior Faculty, inStem	Dr. Colin Jamora, Dr. S Ramaswamy	Member(s)
One expert from the field of Clinical Translation and Applied Research	Dr. Gagandeep Kang, CMC Vellore	Member
External experts Member(s)	Dr. Soniya Nityanand, Executive Registrar SGPGI, Lucknow; Dr. Dinakar M Salunke, Director ICGEB, New Delhi; Dr. B. S. Ramakrishna, Director SIMS Institute for Medical Science, Chennai Dr. Jyotsna Dhawan, CCMB, Hyderabad	Member(s)
Scientist nominated by Chairperson	Dr. Mammen Chandy, Director Tata Medical Centre, Kolkata	Member
Head, Admin and Finance inStem	Shri Pawan Kumar Pahwa	Non-Member Secretary

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

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

CSCR Scientist Review Committee (appointed by Principal, CMC)

Dr. Prasad Mathews, Medical Superintendent	Chair
Dr. B. S. Ramakrishna, SIM Institute of Medical Science, Chennai	Member (External)
Dr. Molly Jacob, Department of Biochemistry, CMC Vellore	Member
Dr. Nihal Thomas, Department of Endocrinology, CMC Vellore	Member
Dr. Asha Mary Abraham, Department of Virology, CMC Vellore	Member
Dr. Alok Srivastava, Head, CSCR	Member Secretary

CSCR Scientific Team

















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