CSCR

ANNUAL REPORT
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CENTRE FOR STEM CELL RESEARCH
A unit of inStem, Bengaluru
Christian Medical College Campus, Bagayam
Vellore, Tamil Nadu

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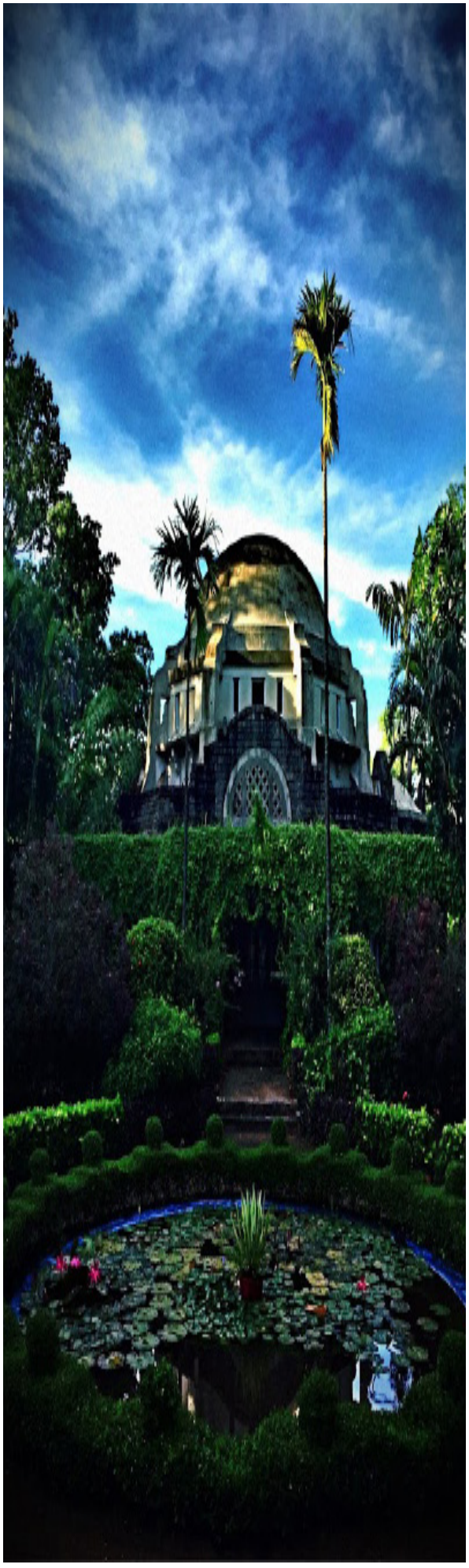

Centre for Stem Cell Research (CSCR)
(a unit of inStem, Bengaluru)
Christian Medical College Campus, Bagayam, Vellore

The Beginnings: 2005 - 2010

The Center for Stem Cell Research (CSCR) in Vellore was sanctioned by the Department of Biotechnology (DBT) of the Ministry of Science and Technology, Government of India, to be established in collaboration with the Christian Medical College (CMC), Vellore in December, 2005.

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

Mandate

The mandate of CSCR is to bring stem cell science to management of human diseases with unmet needs. This is to be done by developing research along clearly defined themes which will help enhance understanding of disease biology or help create innovative diagnostics and therapeutics that is relevant to the needs of the country. It will also aim 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 was governed by a Governing Body, chaired by the Secretary DBT and also had a Finance Committee. There also was a DBT designated Scientific Advisory Committee that reviewed the work done at CSCR every year. In addition, there were two committees appointed by the 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 meet regularly to resolve all matters at CSCR that require discussion 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 – A unit of the Institute for Stem Cell Biology and Regenerative Medicine (inStem), Bengaluru from 2011

After completion of the sanctioned period of CSCR as a project, CSCR has 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 egenerative medicine. It is now governed by a CSCR committee chaired by the Director, CMC and includes the Principal of CMC, Vellore along with the Director and Dean of inStem. It also has a Finance Subcommittee which is part of Finance Committee of 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.

CORE SCIENTIFIC ACTIVITIES AND INITIATIVES

THEMATIC RESEARCH PROGRAMS

1. Musculoskeletal regeneration program

This program is coordinated by Vrisha Madhuri with her team. The major focus is on clinical translations related to physis, articular cartilage and bone regeneration. For articular cartilage regeneration small and large animals studies have been completed with differentiated MSCs on indigenous scaffolds with successful outcome. Osteoarthritis prevention is another area that is being explored. There is a new focus on using biomolecules on scaffold for regeneration with in vitro studies completed and ongoing large animal studies. The continued follow up for pilot human physeal regeneration with culture expanded autologous chondrocytes has shown success at 5 years and a phase 1 clinical trial has been initiated. The group has also achieved success in physeal regeneration using hydrogel scaffolds in large animal model. A first of its kind pilot study on human bone defect regeneration study has been completed and further work is ongoing in the area of bone regeneration using biomolecules. A new phase I/II clinical trial is initiated in collaboration with Karolinska Institutet for treatment of osteogenesis imperfecta using fetal liver mesenchymal stem cells. Under international collaboration the work on non-invasive manipulation of physeal cartilage and muscle derived stem stem cell for sphincter repair continues.

2. Gene therapy program

This program is coordinated by Alok Srivastava with RV Shaji, Saravanabhavan Thangavel, Mohankumar Murugesan and Srujan Marepally and involves two major areas at present – The first is directed towards a clinical trial for AAV vector based gene therapy for haemophilia B in collaboration with Emory University, Atlanta, USA and the Powell Gene Therapy Centre as well as scientist at the University of Florida, Gainesville, USA. Given the success of AAV based gene therapy reported in the last 4 years, the plan here is to apply a similar yet innovative approach to initiate a clinical trial in India with a novel AAV. Towards this end, apart from these scientific elements, regulatory processes are being established through ICMR, CDSCO and DBT in India. The possibility of vector production at an industrial level is also being explored through a pharmaceutical partner in India. The second part of the gene therapy program involves preclinical models for lentiviral vector based gene therapy through hematopoietic stem cell for the major haemoglobin disorders. This is in collaboration with the Emory University, USA. Lentiviral vectors carrying the beta globin gene are tested in human ex-vivo erythropoietic systems developed at CSCR. Work towards using genome editing technologies towards therapeutic gene corrections in stem cells has also been initiated. Other non-vector mediated gene transfer technologies are also being explored.

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

The area of cellular reprogramming technology is coordinated by R. V. Shaji at CSCR. This is now being applied to two areas of disease modeling and haplobanking. Towards understanding the mechanisms of reprogramming, a shRNA library is being used to investigate the role of epigenetic factors in different stages of reprogramming. Results so far have identified specific histone methylases and protein arginine methylases involved in the late stages of reprogramming.

The reprogramming technology is also being applied to the development of disease models of various bone marrow failure syndromes – Fanconi anemia, Diamond Blackfan anemia and congenital dyserythropoietic anemia. A major translational effort has also been initiated towards establishing a “haplobank”, where the field and clinical aspects are
being coordinated by Dolly Daniel and Alok Srivastava. This involves obtaining blood mononuclear cells from HLA haplotype homozygous normal individuals and creating a bank of these cells from which iPSCs are generated in a GMP compliant manner. This is part of an international consortium called the Global Alliance for iPSC Therapies (GAiT) for potential use in regenerative medicine in the future.

NOVEL APPROACHES TO HEMATOLOGICAL DISEASES (NAHD) PROGRAM

In 2016, the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India launched a major project titled ‘Accelerating the application of Stem cell technology in Human Disease’ or ASHD program. This program involves leading Indian research institutions engaged in cutting edge research and technology – The Christian Medical College (CMC) with the Centre for Stem Cell Research (CSCR), a unit of inStem, at Vellore, 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) from Bangalore – in a massive collaborative effort to use stem cells in research, diagnostics and therapeutics.

In addition, the ASHD program collaborates with the Centre for iPS Cell Research and Application (CiRA), Kyoto University, Japan, under the leadership of Prof. Shinya Yamanaka, a pioneer and Nobel Prize winner in stem cell technology. The program at NCBS, inStem, and NIMHANS - The Accelerator program for Discovery in Brain disorders using Stem cells (ADBS) – encompasses research to unravel complex problems in brain disorders / mental illnesses by exploiting the advances in modern human genetics, stem cell technology and clinical investigations. The program at CSCR / CMC - Novel Approaches to Hematological 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. To ensure maximum impact on hereditary hemoglobin diseases in the population at risk in India, this collaborative initiative blends these efforts with a community outreach program for the control of major haemoglobin disorders.

The major components of this program are:

»»Clinical trial for gene therapy of Hemophilia B (see report of Alok Srivastava)

»»AAV antibody screening (see report of Asha Abraham)

»»Lentiviral (see report of R V Shaji) and gene editing (see reports of Saravanabhavan / Mohankumar) approaches for treatment of major hemoglobin disorders

»»Applications of iPSC technology - Haplobanking (see reports of Dolly Daniel / R V Shaji)

»»Population-based control program for major hemoglobin disorders (see report of Alok Srivastava)

The components of this program are within the thematic research programs that are ongoing in CSCR. More details of this program are shown in individual reports as mentioned above.

RESEARCH PROJECTS

Given the translational mandate at CSCR and the clinical needs and interests at the Christian Medical College, Vellore, there are several other areas of translational research that are also being pursued at CSCR. These include work on human mesenchymal stromal cells (hMSCs), with its immense possibilities of translational applications. This work in Sanjay Kumar's laboratory is aimed at exploring the biology of hMSCs from different sources with regard to their isolation, expansion, and manipulation for therapeutic use which are being evaluated in mouse models. Neuronally differentiated cells have shown promising results in a spinal cord injury model. Given the wide possibilities for immune cell therapy, particularly CAR T cells, Sunil Martin's laboratory is working to develop this technology for applications in human cancers along with Aby Abraham as a clinical partner who is also working towards developing gamma delta T-cell based therapies.

The core facilities at CSCR continue to support scientific activities not only within CSCR but also for several scientists from CMC, Vellore and from other institutions. Scientists from nearly 15 departments in CMC use the molecular biology and flowcytometry facilities at CSCR as also several other institutions from Vellore and outside. Training continues at CSCR through the PhD programs affiliated to the Sree Chitra Tirunal Institute of Medical Sciences and Technology, Thiruvananthapuram and the Thiruvalluvar University, Vellore. Short term training programs are also offered to MSc students from different universities. CSCR continues to evolve and attempts to fulfill the mandate for which it was created.

Alok Srivastava
Head, CSCR

Scientific Research Profile

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

LABORATORY HIGHLIGHTS

Our lab focuses on regenerative strategies using cell-based therapy for musculoskeletal disorders. This year's highlights include the following:

a. Boost to Brittle Bones (BOOST2B): This phase I/II trial (n=15) aims to evaluate to study the safety and efficacy of repeated transplantation of human fetal liver MSC to ameliorate severe OI in comparison to an untreated control group. We have standardized the baseline clinical, laboratory protocols and recruited 3 patients. The infusion will begin early August 2020.

b. Physeal regeneration: As an extension to our pilot study this phase I trial aims to evaluate the safety of autologous iliac crest physeal chondrocytes to treat physeal bars in children (n=15). We have transplanted 3 cases so far and the preliminary outcome is satisfactory.

c. Genetic Heterogeneity in patients with OI: This study aims to create a molecular diagnostic NGS panel in Indian patients with osteogenesis imperfecta. We have performed targeted sequencing and phenotyping for 95 probands with 32 novel variants.

d. Musculoskeletal stem cell targeting (MUSTER):

1. Muscle-derived stem cells in the treatment of anal sphincter injury in a rat model – We have isolated satellite cells using a single cell surface marker and established rat anal sphincter injury model. As a next step, we will inject cells with factors that can accelerate muscle regeneration with minimal scarring.

2. Treatment of osteochondral and segmental bone defects using functionalised scaffold with or without MSC- We have standardized the animal model by creating critical size defects in a goat model (n=6 each); functionalized scaffolds will be used for further testing.

e. Effect of shock wave treatment on growth plate cartilage: In vivo studies rabbit suggest that high-energy shock wave treatment may stimulate bone growth while in vitro we demonstrated rescuing effects of shock wave in the presence of small molecule inhibitors.

f. Differentiation of mesenchymal stem cells into chondrocytes by sustained delivery of miRNAs using chitosan hydrogel: A combination of miRNAs effectively induced chondrogenesis of MSCs without any growth factors. A new (liposome-based) method of transfecting plasmid, mRNA and siRNA/miRNA in a 3D condition has been identified and preliminary patent filing is under process.

g. Treatment of urinary incontinence in postpartum women using muscle derived stem cells: Under this phase I clinical trial we have standardized the GMP culture protocols and in the process of obtaining DCGI clearance.

h. Culture expanded satellite cells/myoblasts for the treatment of fecal incontinence in patients with anal sphincter insufficiency: Based on our preclinical data, a pilot study in 5 patients is planned under this study. We are in the process of obtaining DCGI clearance.

Ongoing studies & funding:

Sl. No.	Project Title	Funding Agency	Budget (INR)
1	Transplantation of autologous iliac crest physeal chondrocytes cultured in monolayer to treat Physeal bars in children	DHR	74.93 lakhs
2	BOOST2B (Indo-Swedish)	DBT	480.02 lakhs
3	MUSTER (Indo-Danish)	DBT	99.77 lakhs
4	Molecular genetic analysis of Osteogenesis imperfecta in Indian Children	ICMR	69.00 lakhs
5	In vivo effect of shockwave on rabbit growth plate	CMC-CSCR	10.00 lakhs
6	Treatment of urinary incontinence in postpartum women using muscle derived stem cells	ICMR	75.00 lakhs
7	Treatment of fecal incontinence due to anal sphincter injury using muscle derived stem cells	CMC-CSCR	15.00 lakhs

Honors and Awards:

Venkatesh S - 2nd place – Poster – In vitro culture and characterization of human chondrocytes from iliac physeal cartilage – Annual Research Day (Basic Science Category)

Sowmya Ramesh - 1st place - Oral - To Grow or Not to Grow: The Rescuing Effects Of High Energy Radial Shock Wave Treatment - Annual Research Day (PhD Students category).

Sowmya Ramesh – International Travel Support, DST SERB, 2019

Publications:

1. Shyamasundar, L.G., Loganathan, L., Kumar, A. et al. MATN3 Mutation Causing Spondyloepimetaphyseal Dysplasia. Indian J Pediatr 87, 227–228 (2020).

2. In vivo cartilage regeneration in a multi-layered articular cartilage architecture mimicking scaffold. Karthikeyan Rajagopal, Sowmya Ramesh, Noel Malcolm Walter, Aditya Arora, Dhirendra S Katti, Vrisha Madhuri- Accepted, May 2020.

3. Case report of a PRDM5 linked Brittle Cornea Syndrome type 2 in association with a novel SLC6A5 mutation. Agnes Selina, Deepa John, Lakshmi Loganathan, Vrisha Madhuri. Indian Journal of Ophthalmology – Accepted, June 2020.

4. Ramesh S, Zaman F, Madhuri V, Sävendahl L. Radial Extracorporeal Shock WaveTreatment Promotes Bone Growth and Chondrogenesis in Cultured Fetal Rat Metatarsal Bones. Clin Orthop Relat Res. 2020 Mar.

5. Rajagopal K, Ramesh S, Madhuri V. Early Addition of Parathyroid Hormone- Related Peptide Regulates the Hypertrophic Differentiation of Mesenchymal Stem Cells. Cartilage. 2020 Jan 2.

6. Madhuri V, Ramesh S, Varma H, Sivadasan SB, Sahoo B, John A, Fernandez F, Rajagopal K, Mathews V, Balakumar B, Dinesh VD, Chilbule SK, Gibikote S, Srivastava A; Bone Defect Study Group. First Report of a Tissue-Engineered Graft for Proximal Humerus Gap Non-union After Chronic Pyogenic Osteomyelitis in a Child: A Case Report. JBJS Case Connect. 2020 Jan-Mar;10.

Collaborations

International Collaborations:

1. Henrik Daa Schrøder, University of Southern Denmark, Denmark
2. Jorgen Kjems, Department of Molecular Biology, University of Aarhus, Denmark
3. Moustafa Kaseem, Endocrinology, University of Southern Denmark, Denmark
4. Lars Savendahl, Pediatric Endocrinology, Karolinska University Hospital, Sweden
5. Cecilia Gotherstrom, Division of Obstetrics and Gynecology, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.

National collaborations (Both Inter and intra institutional collaborations):

1. Jyotsna Dhawan, Centre for Cellular & Molecular Biology, Hyderabad
2. Prabha D. Nair, Tissue Engineering and Regeneration Technologies Division, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum
3. Harikrishna Varma, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum.
4. Nihal Thomas, Department of Endocrinology, CMC, Vellore
5. Sukriya Nayak, Department of General Surgery, Unit -4, CMC, Vellore
6. Vikram Mathews, Department of Haematology, CMC, Vellore
7. Madhavi. K., Department of Radiology, CMC, Vellore
8. Shyam, Department of Radiology, CMC, Vellore
9. Thomas Paul, Department of Endocrinology, CMC, Vellore
10. Alok Srivastava, CSCR / Department of Haematology, CMC, Vellore
11. Srujan Marepally, CSCR, Vellore
12. Dolly Daniel, CSCR / Department of Transfusion medicine & Immunohematology, CMC, Vellore
13. Antonisamy, Department of Biostatistics, CMC, Vellore
14. Sumita Danda, Department of Clinical Genetics, CMC, Vellore

ELIZABETH VINOD, MD

Assistant Professor, Department of Physiology, CMC, Vellore
Adjunct Scientist, CSCR

PROJECT-1

Project title: Chondroprogenitor cells in Platelet Rich Plasma for treatment of osteoarthritis and osteochondral defects in rabbit knee model.

Funding source: CSCR Core Grant

Duration: March 2018 - June 2020

Articular cartilage, an avascular tissue has low potential for self-repair. Cell-based therapy forms the mainstay treatment for cartilage regeneration. The two commonly used cells are BM-MSCs and chondrocytes which display varied results in terms of hyaline cartilage regeneration. Use of articular cartilage derived chondroprogenitors has garnered interest due to its inherent chondrogenic potential and low propensity for hypertrophy. The use of platelet rich plasma (PRP) has achieved recognition in its applications towards the treatment of local cartilage defects and osteoarthritis improving the quality of cartilage repair. The rationale for its application is largely dependent on its growth factors and scaffolding effect. The primary objective of our study was to isolate chondroprogenitor cells from rabbit knee articular cartilage, culture, expand, label, and characterize them. Next, was to assess the efficacy of chondroprogenitors suspended in PRP vs PRP alone in the treatment of experimentally created osteoarthritis and critical chondral defect. Currently analysis/histological scoring is underway. The results obtained thus far, show comparable healing between the test and control arm in the OA study group.

Publications:

1. Amritham SM, Ozbey O, Kachroo U, Ramasamy B and Vinod E. Optimization of immunohistochemical detection of collagen type II in osteochondral sections by comparing decalcification and antigen retrieval agent combinations. Clin Anat 2019.

2. Elizabeth Vinod, Jithu James Varghese, Upasana Kachroo, Solomon Sathishkumar, Abel Livingston and Boopalan Ramasamy. Comparison of incremental concentrations of micron-sized superparamagnetic iron oxide for labelling articular cartilage derived chondroprogenitors. Acta Histochemia. 2019.

3. Vinod E, Vinod Francis D, Manickam Amirtham S, Sathishkumar S, Boopalan PRJVC. Allogeneic platelet rich plasma serves as a scaffold for articular cartilage derived chondroprogenitors. Tissue Cell. 2019 Feb;56:107–13.

PROJECT-2

Project title: 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.

Funding source: Department of Biotechnology, Govt. of India

Duration: June 2020 - 2022

In this study we aim to isolate, expand, and characterize human BM-MSCs, chondrocytes and fibronectin adhesion derived chondroprogenitors from normal knee joints (requiring above knee amputation following a traumatic event). The cultured chondrocytes will also be subjected to sorting based on a combination of markers. Chondral defects will be fashioned in osteochondral units obtained from knee joints. The aforementioned cells will be reconstituted with PRP and delivered into the chondral defect and placed in NSG-SCID mice. At specified timepoints healing will be evaluated using histological scoring systems and immunohistochemical quantifications.

PROJECT-3

Project title: Comparison of chondrogenic potential between cell sorted chondroprogenitors, fibronectin assay derived chondroprogenitors and chondrocytes derived from human articular cartilage.

Funding source: CMC Fluid Research Grant

Duration: December 2019 - December 2021

Cell based therapy optimization for regeneration of genuine hyaline cartilage is constantly underway. Extensive work on chondrocytes has afforded valuable information to their use in cartilage repair, although questions pertaining to their behavior in culture remain unanswered. Results from our previous study was the first attempt, where characterization was performed between chondrocytes and chondroprogenitors derived from the same human articular cartilage samples using flow cytometry, gene expression studies, growth kinetics and tri-lineage differentiation. Our results suggest that sorting chondroprogenitors based on a specific combination of surface markers yield a population of cells primarily composed of progenitors. In the present study we aim to isolate and culture chondrocytes and chondroprogenitors cells from normal articular cartilage of human knee joints and subject chondrocytes to sorting. The chondrogenic potential of cell sorted chondroprogenitors, fibronectin assay derived chondroprogenitors and chondrocytes will be compared using FACS, gene expression and differential studies. We are currently procuring non-diseased knee joint samples for the experiments.

Publications:

1. Elizabeth Vinod, Roshni Parameswaran, Soosai Manickam Amirtham, Abel Livingston, Boopalan Ramasamy and Upasana Kachroo. Comparison of the efficiency of laminin versus fibronectin as a differential adhesion assay for isolation of human articular cartilage derived chondroprogenitors. Connective Tissue Research. April 2020.

2. Upasana Kachroo and Elizabeth Vinod. Comparative analysis of gene expression between articular cartilage derived cells to assess suitability of fibronectin adhesion assay to enrich chondroprogenitors. The Knee. April 2020.

3. Upasana Kachroo, Boopalan Ramasamy and Elizabeth Vinod. Evaluation ofCD49e as a distinguishing marker for human articular cartilage derived chondroprogenitors. The Knee. April 2020.

4. Elizabeth Vinod, Boopalan Ramasamy and Upasana Kachroo. Comparison of immunogenic markers of human chondrocytes and chondroprogenitors derived from non-diseased and osteoarthritic articular cartilage. Journal of Orthopaedics, Trauma and Rehabilitation. April 2020.

5. Upasana Kachroo, Shikha Mary Zachariah, Augustine Thambaiah, Aleya Tabasum, Abel Livingston, Grace Rebekah, Alok Srivastava and Elizabeth Vinod. Comparison of human platelet lysate versus fetal bovine serum for expansion of human articular cartilage derived chondroprogenitors. Cartilage. March 2020.

6. Elizabeth Vinod, Upasana Kachroo, Soosai Manickam Amirtham, Boopalan Ramasamy and Solomon Sathishkumar. Comparitive analysis of fresh chrondrocytes, cultured chondrocytes and chondroprogenitors derived from human articular cartilage. Acta Histochemia. November 2019.

Support from CSCR for these projects: Funding, lab space and core lab facilities

External Collaborations:

1. Boopalan Ramasamy, Department of Orthopaedics, Royal Darwin Hospital, NT, Tiwi, Australia
2. G.J.V.M Van Osch, Erasmus MC, University Medical Center, Rotterdam, Netherlands
3. Ozlem Ozbey, Akdeniz University, Antalya, Turkey
4. Sabareeswaran Arumugam, , Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram
5. Anjali Goyal, Smt NHL Municipal Medical College, Ahmedabad

Internal Collaborations:

1. Soosai Amirtham Manickam, Department of Physiology, CMC, Vellore
2. Upasana Kachroo, Department of Physiology, CMC, Vellore
3. Solomon Sathishkumar, Department of Physiology, CMC, Vellore
4. Abel Livingston, Department of Orthopaedics, CMC, Vellore
5. Viju Daniel Varghese, Department of Orthopaedics, CMC, Vellore

ALOK SRIVASTAVA, MD, FRACP, FRCPA, FRCP
Professor, Department of Haematology, CMC, Vellore
Adjunct Scientist / Head, CSCR

Scientific Areas of Research

The major focus during 2019-20 continued on the gene therapy program for haemophilia and the major haemoglobin disorders. I also continue to support the on-going work related to the development of assays for AAV antibodies as well as the program on banking of iPSCs from HLA haplotype identical individuals. The field outreach program for the control of thalassemia and sickle cell disease in Odisha has also progressed well. The details are outlined below:

A. The gene therapy program

1. AAV VECTOR-BASED GENE THERAPY OF HEMOPHILIA B

As mentioned in the previous year's report, a unique transgene was designed for this clinical trial. This 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.

As also reported last year, there was a setback in the plans for this clinical trial as the collaborating vector manufacturing facility in USA was unable to produce the vector at the right titers to allow for cost-effective production of the required quantity of the vector for the proposed clinical trial. Two challenges have made this very difficult - at the academic sites, which have the capability for producing this vector, there is waiting list of products lined up for gene therapy clinical trials (in fact, this is the biggest single challenge in the development of gene therapy products at present in the world) and the cost of production at the contract manufacturing organizations is way beyond the budget available to us in this clinical trials, in the range of US$ 1-1.5 million for the requirements of this trial.

We now have a very exciting new possibility. 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 from another facility and have agreed to do the 'engineering' / standardization runs in their facility with the highest priority and then transfer the know-how / technology to us at CSCR/CMC. A letter of support to this effect has been obtained. This means that the funds requested for paying for the manufacture of this product in USA [about Rs. 3.5 crores / USD 0.5 million] will now be used for manufacturing in India. A suitable team is being put together to take responsibility for this manufacture within the CSCR GMP facility. If this succeeds, it will help establish this technology in the country. We will contract a suitable biotech technical consultant in India to interact with the ET team and take full responsibility for the manufacturing and regulatory processing this product at the cGMP facility at CSCR. As soon as this process is established, RCGM review will be requested and the remaining pre-clinical study on the non-human primates conducted at Emory University.

2. LENTIVIRAL VECTOR-BASED GENE THERAPY FOR HEMOPHILIA A

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 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) (Figure 1). The GMP grade vector is also ready for use in a clinical trial - jointly applied for conduct in USA and India. The principle here is to use mobilized HSC from the patient, ex-vivo transduce them with the lentiviral vector and then administer them back to the patient after suitable conditioning as an autologous HSC transplantation procedure. Within 3-4 weeks after transplantation production of FVIII is expected from these cells in clinically significant quantities.

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 undergone review of the CDSCO as well as several rounds of review by the RCGM. The RCGM has finally approved the import of this vector for establishing the HSC transduction protocol in our laboratory and show adequate efficiency and batch consistency. The product has been imported and is now in CSCR. The transduction experiments are planned to be done over the next 3-4 months - depending to some extent on the state of the COVID19 pandemic situation and our access to suitable patients for collecting the peripheral blood stem cells. If that data is as required, then we will be ready to submit the final details to the CDSCO for initiating a Phase 1 clinical trial before the end of this year.

B. Haplobanking

This novel and unique project aimed at creating a bank of induced pluripotent stem cells (iPSCs) from normal individuals with homozygous HLA haplotypes. It is part of an international consortium working in this area. http://www.gait.global/. The major advance in this year is the completion of a large number of donors which cover the top 20 haplotypes in India and the generation of a significant number of iPSCs clones under cGMP conditions. Please see report of Dolly Daniel and R.V. Shaji for details of this program in collaboration with DATRI stem cell donor registry.

C. Other areas of work related to stem cell transplantation / gene therapy

My engagement with clinical haematopoietic stem cell transplantation (HSCT) and related research continues with particular emphasis on HSCT for thalassemia major. I continue to coordinate the Indian Blood and Marrow Transplant Registry for haematopoietic stem cell transplantation done in India. My position as vice chair of the Asia-Pacific Blood and Marrow Transplant [APBMT] Group continues. We will be organizing the APBMT 2020 meeting this October on a virtual platform with many colleagues from the ISBMT.

My task as the chair of The Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis should be completed soon with a final recommendation from the group.

D. Community outreach – Creating a model for control of thalassemia and sickle cell disease

I provide overall coordination to this program which is led along with Profs. Kuryan George and Shantidani Minz along with several other senior colleagues from the departments of Community Health, Haematology, Transfusion Medicine and Immunohaematology, and Obstetrics and Gynecology at CMC, Vellore in collaboration with the National Health Mission of the Government of Odisha. This program was launched in August 2020 in the district of Koraput. It is unique in terms of scale and complexity in this field.

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

In the first phase, this program will be implemented in six districts of Odisha (Balasore, Bargarh, Cuttack, Jharsuguda, Koraput, Sambalpur). Other districts will be covered in a phased manner over a period of 3-4 years. The major field components of this program are: comprehensive testing of the population at risk for carrier status with a single blood sample right up to genetic diagnosis and a major component of Behaviour Change Communication (BCC) targeted activities using all forms of media to increase knowledge and awareness about these disease among the general population particularly towards prevention as well as treatment for those affected. Towards these goals, laboratories are being established at selected community health centres for assessing carrier status of the individuals. A robust data management system has been developed to ensure data security. Towards increasing capacity and capability for treatment of major haemoglobin disorders in Odisha State health system, training workshops were arranged at different levels (State / Regional levels) for doctors / other healthcare workers of Odisha to train them on different aspects of management and prevention of sickle cell disease and thalassemia in the State. So far, over 300 medical doctors from the district hospitals and CHC / PHCs have been trained from across Odisha. Specialized training was also provided to physicians from two selected medical colleges in chorionic villous sampling for prenatal diagnosis. Training of other healthcare workers is being done for counselling and disseminating information regarding the screening and testing activities for implementing the program.

Two new technologies are being developed within this field program - a proteomic (MALDITOF) based method for high throughput low cost analysis of variant haemoglobins and a low-cost novel technology genetic diagnosis of globin gene defects. Both these are with overseas industry collaboration.

Selected publications

1. Vrisha Madhuri, Sowmya Ramesh, Harikrishna Varma, Suresh Babu Sivadasan, Bibhudatta Sahoo, Annie John, Francis Fernandez, Karthikeyan Rajagopal, Vikram Mathews, Balakumar B, Vivek Dutt Dinesh, Sanjay Kashinath Chilbule, Sridhar Gibikote, Alok Srivastava. First report of a tissue-engineered graft for proximal humerus gap non-union following chronic pyogenic osteomyelitis in a child. Journal of Bone and Joint Surgery Case Connector – Accepted for publication, July 1, 2019

2. Doering CB, Denning G, Shields JE, Fine EJ, Parker ET, Srivastava A, Lollar P, Spencer HT. Preclinical Development of a Hematopoietic Stem and Progenitor Cell Bioengineered Factor VIII Lentiviral Vector Gene Therapy for Hemophilia A. Hum Gene Ther. 2018 Oct;29(10):1183-1201

3. Sullivan S, Stacey GN, Akazawa C, Aoyama N, Baptista R, Bedford P, Bennaceur Griscelli A, Chandra A, Elwood N, Girard M, Kawamata S, Hanatani T, Latsis T, Lin S, Ludwig TE, Malygina T, Mack A, Mountford JC, Noggle S, Pereira LV, Price J, Sheldon M, Srivastava A, Stachelscheid H, Velayudhan SR, Ward NJ, Turner ML, Barry J, Song J. Quality control guidelines for clinical-grade human induced pluripotent stem cell lines. Regen Med. 2018 Oct;13(7):859-866. doi: 10.2217/rme-2018-0095. Epub 2018 Sep 12. PubMed PMID: 30205750

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6. Vikram Mathews, Department of Haematology, CMC, Vellore
7. Sukesh Nair, Department of Immunohematology, CMC, Vellore
8. Asha Mary Abraham, CSCR / Department of Clinical Virology, CMC, Vellore
9. Hubert Daniel, CSCR / Department of Clinical Virology, CMC, Vellore
10. Rajesh Kannangai, Department of Clinical Virology, CMC,Vellore
11. Saravanabhavan Thangavel, CSCR
12. Mohankumar Murugesan, CSCR
13. R. V. Shaji, CSCR / Department of Haematology, CMC, Vellore
14. Dolly Daniel, CSCR / Department of Immunohematology, CMC, Vellore
15. Kuryan George, Department of Community Health, CMC, Vellore
16. Shantidani Minz, Department of Community Health, CMC, Vellore
17. J. P. Muliyil, Retd. Professor, CMC, Vellore
18. Vrisha Madhuri, CSCR / Department of Pediatric Orthopedics, CMC, Vellore
19. Mahendra Rao, inStem, Bengaluru

External Collaborations:

1. Arun Srivastava, Department of Genetics, University of Florida and Barry Byrne, Powell Gene Therapy Center, University of Florida
2. Mavis Agbandje-McKenna, Director, Center for Structural Biology, University of Florida
3. Trent Spencer, Director, Gene Therapy Program, Aflac Children's Cancer Center, Emory University, Atlanta, USA
4. Chris Doering and John Lollar, Emory University, USA
5. INTAS Pharmaceuticals, Ahmedabad, India
6. Nezih Cereb, Chief Scientific Officer, & Raghu Rajagopal, CEO, DATRI, Chennai for the haplo-banking project

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

LABORATORY HIGHLIGHTS

Our lab carries out research in two themes at CSCR, gene therapy and iPSC technology.

Lentiviral viral vectors for gene therapy:

Beta globin and gamma globin gene addition lentiviral vectors: We have generated two efficient lentiviral vectors for beta and gamma globin expression for gene therapy for haemoglobinopathies. These vectors have been evaluated sickle cell disease mouse model to show high levels of adult (HbA) and foetal haemoglobins (HbF). We are currently evaluating them further by transducing CD34+ cells from patients with sickle cell disease (SCD) with these vectors and transplanting them in NSG/NBSGW mice.

Lentiviral vectors for erythroid specific expression of BCl11A shRNA: We generated a lentiviral vector with beta globin promoter and LCR sequences to efficiently knock down the expression of BCl11A in erythroid cells. This vector has shown that it increases HbF in in-vitro erythropoiesis, and it is being tested further by transplanting the transduced CD34+ cells in NSG/NBSGW mice.

Generation of mutations in gamma globin promoter to increase foetal haemoglobin: In an alternative strategy, we are creating HPFH-like mutations in the gamma globin promoters to increase HbF, by creating in-dels using CRISPR-Cas9. Experiments carried out in HUDEP cells identified unique mutations that increase HbF. We are testing this most efficient strategy to increase HbF in NGS mice.

iPSC Technology: Disease modelling and Haplobanking:

Early pre-clinical gene therapy research - Creating disease models of Erythroid disorders for gene correction:
Towards modelling of erythroid disorders, 2 inherited monogenic diseases have been preliminarily selected, Diamond Blackfan anemia (DBA) and congenital dyserythropoietic anemia (CDA). 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. CRISPR/Cas9 mediated gene editing is used to either disrupt a gene for DBA or create a bi-allelic single nucleotide substitution for CDA in a normal, well-characterized induced pluripotent stem cell (iPSC) line followed by haematopoietic and erythroid differentiation to observe disease phenotype. When using a gene edited iPSC, the non-edited cell line serves as an isogenic control and gives the confidence that any change is phenotype is a direct result of the edited locus and not due to any background genetic changes induced during reprogramming. Hence, engineering a disease-causing mutation in a healthy iPSC line and differentiating to the erythroid lineage along the wild type line, is the best approach to model erythroid disorders and study the onset and progress of the disease as well develop treatment strategies. In the previous years, we reported gene editing strategies in the CDN1, RPS19, RPL5 and SEC23B using lentiviral vectors to express Cas9 and gRNAs. We reported in the previous years that balletic mutations in the target genes that we chose to study were found to be lethal to iPSCs.

Generation of a cell line with doxycycline inducible expression of Cas9 for disease modelling:
An inducible Cas9 expressing iPSC line that allows controllable gene editing will serve as a valuable tool for studying lineage specific gene functions, disease modelling and drug screening. We generated an iPSC line with inducible Cas9 expression from the AAVS1 safe harbor site. Gene editing to create mutations in the genes that are important for the maintenance of pluripotent stem cells and their differentiation to a specific lineage, affects functional studies. Inducible expression of Cas9 helps to knock out the gene of interest at a specific time window, and allows temporal control of gene editing. A previously reported iPSC line in our laboratory generated from a healthy Indian donor (CSCRi005-A) was used for generating the inducible Cas9 (Ind-Cas9) iPSC line. AAVS1 integration was mediated by transfection of two TALEN plasmids targeting the AAVS1 locus and a donor plasmid with PPP1R12C homology arms encompassing the Cas9 gene, which is regulated by a doxycycline-inducible promoter, and a puromycin selection marker gene that is expressed under endogenous AAVS1 promoter after integration. After puromycin selection, the cells were subjected to single cell sorting and the clones were screened for site specific integration by PCR analysis and doxycycline induced Cas9 expression was confirmed by western blotting. One of the clones (Clone 2) showed expression of Cas9 in all the cells after doxycycline as measured by immunofluorescence. For validating the gene editing potential of this line, we chose to target miRNA 182 and miR183 regions, whose function has been implicated in pluripotency and haematopoiesis. T7 endonuclease assay revealed a robust gene editing efficiency. We will use this iPSC line for creating mutations in the genes associated with DBA and CDA.

Gene expression analysis of iPSC-HSPCs: In the previous years' reports, we described a modified robust protocol for differentiation of iPSCs to haematopoietic progenitor cells (HSPCs). We reported in the previous years that the HSPCs collected from these two different days have characteristic surface marker expression, and day 17 HSPCs have more erythroid potential. We performed RNA sequencing of HSPCs collected from day 11 and day 17 of differentiation and performed RNA sequencing to understand the molecular features of these cells. We found significance difference in the expression of several pathways between these two HSPCs. We are performing further experiments to understand their role in iPSC-HSPC formation and determining the properties of iPSC-HSPCs.

Generation of iPSC-HSPCs with higher erythroid differentiation potential: For disease modelling of red cell diseases, George Daley's group showed that transduction of 5 transcription factors (ERG, HOXA9, RORA, SOX4, and MYB). Doxycycline (Dox)–regulated conditional induction of 5F expanded immature CD34+CD38− blood progenitors (CD34-5F) and removal of Dox initiated differentiation. CD34-5F cells gave rise to short-term engraftment after transplantation in immunodeficient mice, with erythroid progenitors undergoing maturation and haemoglobin switching in vivo. This system 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 We collected HSPCs from day 12 and day 17 of the differentiation. The HSPCs were cultured in liquid medium to propagate HSPCs in the presence and absence of doxycycline. We found that the 5F-HSPCs cultured in the presence of dox showed higher proliferation. Untransduced iPSCs were used as a control. Notably, the day 17 HSPCs showed proliferation only when 5F expression is induced. Methyl cellulose assays showed that the 5F-HSPCs had increased potential for erythroid colony formation.

Haplobanking - Establishing a Bank of iPS cells from individuals with homozygous HLA haplotypes
The major focus of this project this year was to generate iPSCs lines from top 10 homozygous HLA haplotype donors in the GMP facility. Blood samples from 235 donors had been collected from various regions of the country covering the top 10 haplotypes. PBMNCs were isolated by Ficoll gradient method and cryopreserved in GMP facility. High resolution 6 locus HLA typing was performed on 40 donor samples with top 10 haplotypes. There was concurrence of HLA typing with the original donor typing we had from the registry data. An observation of heterozygosity of the HLA –DPB*1 locus in 12 of the 40 samples tested raised the query as to whether HLA-DPB*1 locus homozygosity should be ensured prior to shortlisting of donors. Following a discussion with DATRI, we have included this as criteria for all subsequent donor collections. Furthermore, we will be contacting donors homozygous for any of the top 50 haplotypes during the coming year to enlarge the pool of iPSCs that can be generated.

To generate hiPSC lines, we selected top 10 homozygous HLA haplotype donors. PBMNCs from 10 donors were thawed in GMP laboratory and erythroid progenitor cells were expanded in xenofree media with GMP grade cytokines. PBMNC and erythroid progenitors were characterized at the different time point of the erythroid cell expansion with CD71, CD235 expression (erythroid progenitor marker) and CD3 and CD19 (T cell and B cell marker respectively). Erythroid progenitors were expanded for 8 days for further reprogramming experiments. We established highly efficient feeder-free, xeno-free and integration free protocol to generate GMP grade iPSCs lines from 10 homozygous HLA haplotype donors. To generate xenofree and transgene-free iPSCs from erythroid progenitors, cells from 10 donors were nucleofected with GMP grade Y4 (OCT4-p53-shRNA, SOX2, KLF4, L-MYC, and LIN28,) combination of episomal vectors in appropriate ratio. The nucleofected cells were subsequently plated on cell therapy grade biolaminin-coated plates and maintained for 3 days in erythroid expansion media. On day 3 and day 5 of reprogramming, cells were batched fed with the addition of the cell therapy system Essential 8 (CTS-E8) medium. From day 7 onwards, cells were fed every day with CTS-E8 media and the formation of iPSC colonies were observed in the culture. Three iPSCs clones showing pluripotent stem cell morphology with clear boundary and tightly packed cells were picked from each donor on day 19-22 and plated on cell therapy grade vitronectin. Three clones from each donor were expanded in xenofree conditions in GMP laboratory.

Cell bank was created at passage 10 for one clone from each donor, all the expanded clones maintained the pluripotent stem cell morphology and showed a high level of alkaline phosphatase staining. The expression of pluripotency markers (OCT4, NANOG, SSEA4) was confirmed by immunofluorescence staining. In the coming year, the expansion of erythroid cell progenitors from other homozygous HLA donors will be performed in xenofree erythroid expansion medium and the cells will be reprogramed into iPSCs lines in GMP laboratory. Detailed cellular and molecular analysis for identity, differentiation potential, karyotyping and genomic stability will be performed with the newly established GMP grade iPSC lines.

Publications:

1. Screening of genetic variants in ELANE mutation negative congenital neutropenia by next generation sequencing. Arunachalam AK, Suresh H, Edison ES, Korula A, Aboobacker FN, George B, Shaji RV, Mathews V, Balasubramanian P. J Clin Pathol. 2020 Jun;73(6):322-327. doi: 10.1136/jclinpath-2019-206306. Epub 2019 Nov 15.

2. Novel frameshift variant (c.409dupG) in SLC25A38 is a common cause of congenital sideroblastic anaemia in the Indian subcontinent. Ravindra N, Athiyarath R, S E, S S, Kulkarni U, N A F, Korula A, Shaji RV, George B, Edison ES. J Clin Pathol. 2020 Jun 30:jclinpath-2020-206647. doi: 10.1136/jclinpath-2020-206647. Online ahead of print.

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4. Alok Srivastava, CSCR / Department of Haematology, CMC, Vellore

External Collaborators:

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2. Mavis Agbandje-McKenna, University of Florida, USA
3. Christopher Doering, Emory University, USA
4. John Lollar, Emory University, USA

SARAVANABHAVAN THANGAVEL, PhD
Assistant Investigator, CSCR

LABORATORY HIGHLIGHTS

The prime focus of our lab is to develop the gene-edited haematopoietic stem and progenitor cells (HSPCs) for the Haematopoietic stem cell gene therapy applications. Our target diseases are Beta hemoglobinopathies, HIV-1 infection and Wiskott- Aldrich Syndrome (WAS).

1. The gene edited HSPCs therapy for β- haemoglobinopathies We are developing two different strategies towards the permanent correction of the phenotype of β- haemoglobinopathies.

A) One strategy involves the correction of disease-causing mutation. We target the DNA sequences near the mutation locus in the β-globin gene for site-specific cleavage and facilitate a precise correction by co-delivering a homologous recombination repair donor template. We have developed reagents and optimized the conditions for the genetic correction of disease-causing mutation in the primary hematopoietic stem cells. We are currently testing hemoglobin expression pattern in these cells.

B) Reactivation of fetal gamma-globin is a pharmacological strategy to compensate for the defective production of beta globin chains for both Thalassemia and Sickle cell disease phenotypes. Hereditary Persistence of Fetal hemoglobin (HPFH) is a genetic condition where the individuals have high fetal-gamma globin production. When a sickle cell or a thalassemia patient co-inherits the HPFH mutations, the disease severity is ameliorated. We try to recapitulate the HPFH like mutations in the hematopoietic stem and progenitor cells (HSPCs) of SCD or thalassemia patients in the second strategy. We have performed a CRISPR-Cas9 genetic screening and identified at least two targets for the HBF reactivation. We have validated these targets in HSPCs from healthy donors and we are currently characterizing the gene edited HSPCs.

2. The gene edited HSPCs therapy for Wiskott-Aldrich Syndrome (WAS). Wiskott-Aldrich syndrome is one of the very first diseases to be tested for viral vector -mediated gene therapy. The fitness advantage of gene- corrected WAS-HSPCs plays a crucial role in the success of the gene therapy. Considering the chances that small number of edited cells can reverse disease phenotype, we chose to correct WAS-HSPCs. WASP plays a key role in hematopoietic actin cytoskeleton reorganization. To compensate the deficient production of WAS in hematopoietic lineage, we precisely insert the WAS transgene into a specific locus of the HSPCs. We have Identified the locus for insertion, developed the CRISPR/Cas9 system for cleaving the locus and also constructed the HDR compatible WAS transgene. Target specific insertion of WAS transgene has been validated in the HSPCs of the healthy donor and we are currently testing the expression of the transgene.

3. The gene edited HSPCs therapy for HIV infection The HSPCs with CCR5 Δ32 genotype provides a resistance against HIV-1 infection and thus a permanent cure. However, the very limited availability of HLA matched CCR5 Δ32 donor restricts the allogenic stem cell transplantation. We have developed CCR5 gene edited HSPCs and currently characterizing them.

4. Enhanced production of gene edited HSPCs We also work 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 correction. Improving the delivery of gene editing reagents to the HSPCs and Ex vivo expansion of gene edited HSPCs are the two different strategies that we use to overcome this issue.

A) We have developed a novel platform for the ex vivo expansion of hematopoietic stem cells. The immunophenotypic profile of the expanded cells and the long-term multi-lineage repopulation potential in the NSG mice indicates that our platform preserves the primitive hematopoietic cell population during ex vivo culture. We are currently testing the suitability of this platform for gene manipulation studies.

B) Delivery of gene editing reagents to HSPCs is currently being achieved by electroporation approach which consumes high doses of HSPCs and gene editing reagents. In collaboration with Dr. Srujan Marepally, we have developed a liposomes for delivering gene editing reagents into HSPCs. Currently, we are working on improving the efficiency of the delivery.

Projects	Stage of development						
	Target selection	Optimized editing reagents	validation in cell lines	validation in primary HSPCs	validation in Patient HSPCs	Engraftment and differentiation studies in mice	Late pre-clinical studies
1. Gene editing for the gene therapy of β-haemoglobinopathies i) Mutation correction							
Gene editing for the gene therapy of β-haemoglobinopathies II) HbF reactivation							
2. Gene editing for Wiskott-Aldrich Syndrome							
3. Gene editing for HIV infection							
4. Technologies for efficient HSPC gene therapy a) HSPC ex vivo expansion							
Technologies for efficient HSPC gene therapy b) HSPC transfection							

Table summarizing the projects and the progress

Honors and Awards:

Early Carrier Research award by SERB-DST, India
Indo-U.S GETin Fellow award by Indo-US Science and Technology Forum

Publications:

1. XLF and H2AX function in series to promote replication fork stability. Chen BR, Quinet A, Byrum AK, Jackson J, Berti M, Thangavel S, Bredemeyer AL, Hindi I, Mosammaparast N, Tyler JK, Vindigni A, Sleckman BP. Journal of Cell Biology. 2019 May 23.

2. Exploring membrane permeability of Tomatidine to enhance lipid mediated nucleic acid transfections. Rangasami VK, Lohchania B, Voshavar C, Rachamalla HR, Banerjee R, Dhayani A, Thangavel S, Vemula PK, Marepally S. Biochim Biophys Acta Biomembranes. 2019 Jan;1861(1):327-334

Academic Activities:

- In-Charge: Imaging facility, Students presentation
- Stem cell gene therapy class to JRFs

Internal Collaborations:

1. R. V. Shaji, CSCR / CMC, Vellore (Gene editing blood disorders, HSPC expansion)
2. Mohankumar Murugesan, CSCR (Gene editing blood disorders, HSPC expansion)
3. Srujan Marepally, CSCR (HSPC expansion, HSPC transfection)
4. Sunil Martin, CSCR (Gene editing T cells)
5. Alok Srivastava, CSCR / CMC, Vellore (Gene editing blood disorders, HSPC expansion)
6. Rajesh Kannangai, Department of Clinical Virology, CMC, Vellore (HIV-1 gene therapy)
7. George M. Varghese, Department of Infectious Diseases, CMC, Vellore (HIV-1 gene therapy)

External Collaboration:

1. David Martin, Children's Hospital Oakland Research Institute, USA (Gene editing for Beta-haemoglobinopathies)

MOHANKUMAR MURUGESAN, PhD
Assistant Investigator, CSCR

LABORATORY HIGHLIGHTS

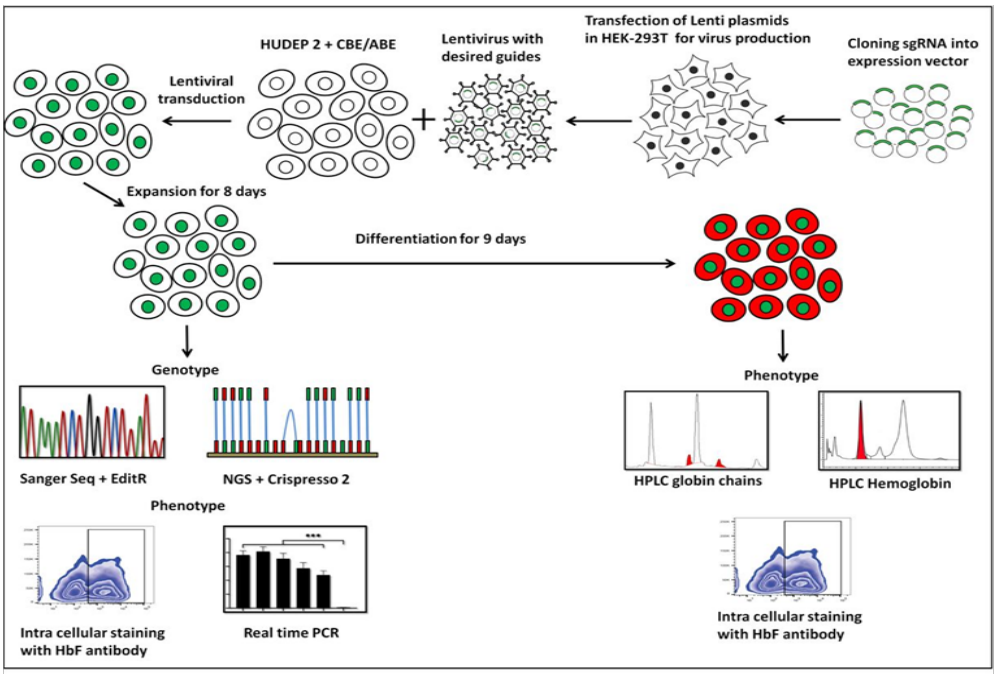
Our lab is focused on the development of novel genome editing strategies for the treatment of β - hemoglobinopathies and Haemophilia-A.

1. Preclinical genome editing approach for the treatment of beta-globin disorders

Sickle cell disease (SCD) and β-thalassemia are the most common genetic disorders in India, caused by mutations in the gene coding for the adult hemoglobin β-chain. Current curative treatment is limited by the bone marrow transplantation from HLA matched donor. Elevation of fetal haemoglobin in SCD and β-thalassemia patients offers greater therapeutic advantage by ameliorating clinical symptoms. We utilize targeted genome engineering platform based on CRISPR/Cas9 system to reactivate gamma globin by editing the two potent gamma globin regulatory regions in hematopoietic stem cells for the treatment of SCD and thalassemia.

(i) Non deletional HPFH

Non deletional HPFH mutations are caused by point mutations in the HBG1 and HBG2 gene promoters. These point mutations greatly increase the production of Aγ-globin or Gγ-globin chain, which is normally silenced in humans after birth due to the interplay of transcription regulators with the HBG promoters. We screened the proximal promoter of human HBG genes using adenine and cytosine base editors to identify key nucleotide point mutations that could potentially lead to elevated levels of fetal globin. Both the base editors efficiently and precisely edited at the target sites with a minimal generation of indels and no deletion of one of the duplicated HBG genes. Through systematic tiling across the HBG proximal promoter, we identified multiple novel target sites that resulted in a significant increase in fetal globin levels. Further, we individually validated the top eight potential target sites from both the base editors and observed robust elevation in the fetal globin levels up to 47 %, without any detrimental effects on erythroid differentiation. Our screening strategy resulted in the identification of multiple novel point mutations and also validated the known non-deletional HPFH mutations that could elevate the fetal globin expression at therapeutically relevant levels. Overall, our findings shed light on so far unknown regulatory elements within the HBG promoter that normally mediates fetal globin silencing and identify additional targets for therapeutic upregulation of fetal hemoglobin.



Lenti viral delivery of CRISPR Cas9 and gRNAs into HUDEP cells
Overview of screening of HBG promoter using base editors to identify novel point mutations to elevate HbF

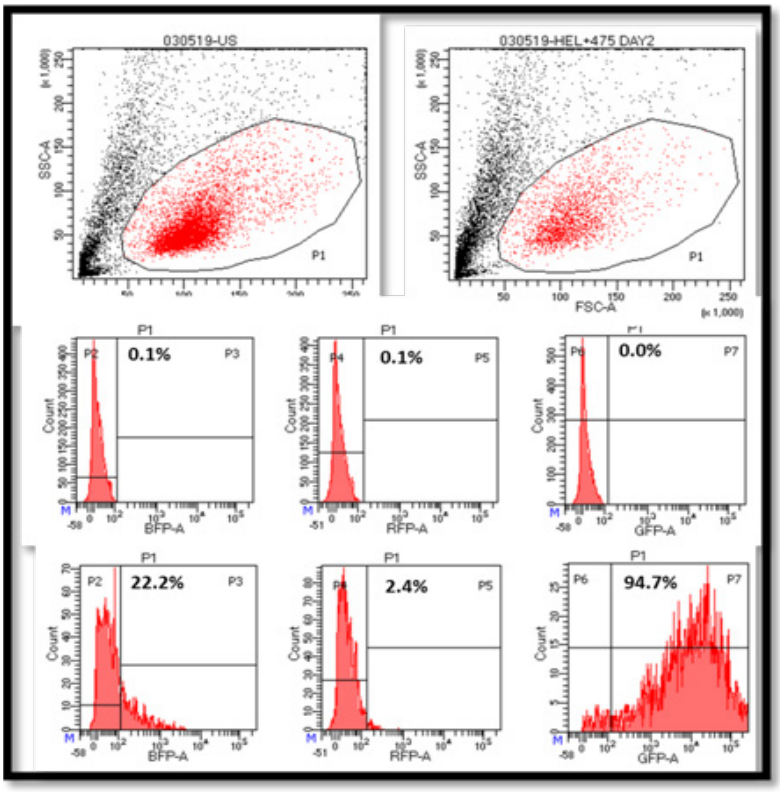
(ii) Fetal globin repressors

For the targeted editing of fetal globin repressors, we performed the editing of erythroid specific BCL11A enhancer in mobilized hematopoietic stem and progenitor cells (HSPCs) from normal donor using the CRISPR/cas9 system. We have successfully edited HSPCs with the gRNA targeting the BCL11A enhancer and confirmed the cleavage of target locus by both T7 endonuclease and sanger sequencing. Editing of the minimal sequence in BCL11A enhancer gene increased the gamma globin expression to levels that of knockout of BCL11A coding region without having any effect on erythroid differentiation and cell survival. Currently, we are evaluating the long term engraftment, repopulation efficiency ability of BCL11A enhancer edited HSPCs by using the irradiated NSG mouse model.

We also investigated the feasibility of targeting the another major fetal globin repressor ZBTB7A/LRF in the human hematopoietic stem and progenitor cells for the treatment of beta hemoglobinopathies. Towards this end, we have screened the effective guide RNA targeting the LRF gene and validated by surveyor nuclease assay. We now have at least two different guide RNAs successfully cleaving the LRF locus in HUDEP2 cells. The edited cells were differentiated and induction of fetal globin was evaluated by FACS analysis. We further validated the upregulation of fetal globin levels upon targeted disruption of LRF/ZBTB7A in erythroid cells derived from human HSPCs.

2. Genome engineering hematopoietic stem cells for the treatment of Hemophilia A

Hemophilia A (HA) is an X-linked monogenic congenital bleeding disorder due defective FVIII in the bloodstream. To improve the current approaches of gene therapy for Hemophilia A, we developed a novel ex vivo gene therapy strategy for targeted integration of FVIII in hematopoietic stem cells for the treatment Hemophilia A. To this end, we have made several advances in building a gene editing platform for targeted integration of transgene under the endogenous lineage specific promoter. For the targeted integration of lineage specific promoter, we designed the different guide RNAs targeting different region of endogenous locus and cloned these guide RNAs into lentiviral vector co-expressing GFP. These vectors were then transduced into target cell line that expresses cas9 protein intrinsically. The validation of these guide RNAs were achieved through T7- endonuclease assay. After confirming the cleavage of target locus, donor plasmid containing promoterless BFP gene was designed and co-transfected with respective guides using Lonza nucleofector for site-specific homologous recombination. We were able to achieve targeted integration of fluorescent reporter expressed via endogenous promoter through HDR pathway. This approach will enable us for integrating FVIII in the endogenous locus in near future.



Targeted integration of transgene under endogenous locus using CRISPR/Cas9 nucleases. FACS analysis for HDR targeting endogenous locus, 2 days after nucleofection.

Honors and Awards:

Indo-U.S GETin Fellow award by Indo-US Science and Technology Forum

Publications:

1. Identification of novel HPFH-like mutations by CRISPR base editing that elevates the expression of fetal hemoglobin. Preprint: https://doi.org/10.1101/2020.06.30.178715

Patent:

Compositions and methods for reactivating developmentally silent genes". Application No.: TEMP/E-1/21886/2020-CHE

Academic activities:

- Organizing Committee member of the 3rd Annual Symposium on Cell and Gene Therapy, September 6-7, 2019
- In-Charge: JRF review process
- Stem cell gene therapy class to JRFs

Internal Collaborations::

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External Collaborations:

1. Jacob Corn, Innovative Genomics Institute, UC Berkeley, USA

SRUJAN K MAREPALLY, PhD
Scientist, CSCR

LABORATORY HIGHLIGHTS

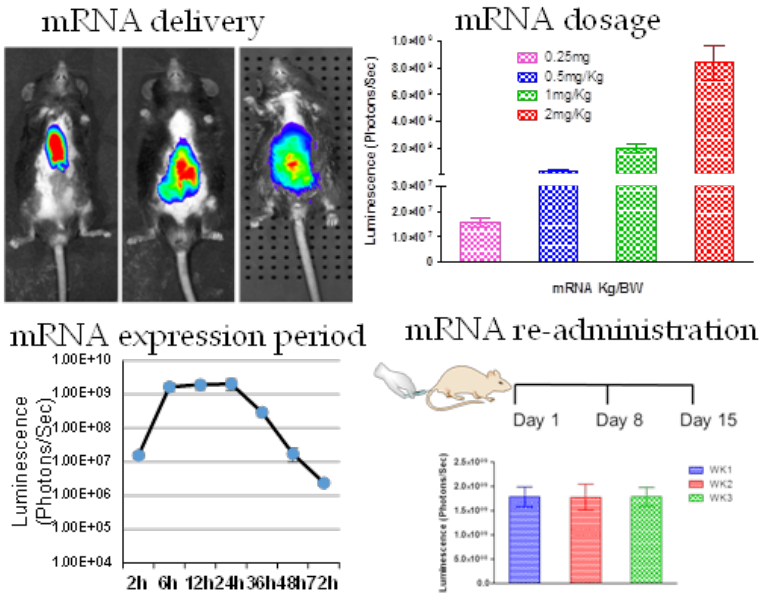
My current research focus involves developing lipid mediated nucleic acid delivery systems for gene therapy, vaccination and genome editing applications. Primary focus on gene therapy with pDNA and mRNA for blood disorders including β-thalassemia, sickle cell anemia and hemophilia. Vaccination project includes in vivo delivery of antigenic mRNA with lipid nanoparticle system. To overcome translational hurdles such as poor transfection efficiency and cytotoxicity, we are probing the mechanisms involved in transfections.

Lipid enabled nucleic acid therapy for Hemophilia

Towards developing lipid nanoparticle mediated gene therapy for hemophilia, liver specific lipid nanoparticle system to deliver functional genes and design of the functional nucleic acids are prerequisite.

Development of liver specific lipid nanoparticle system:

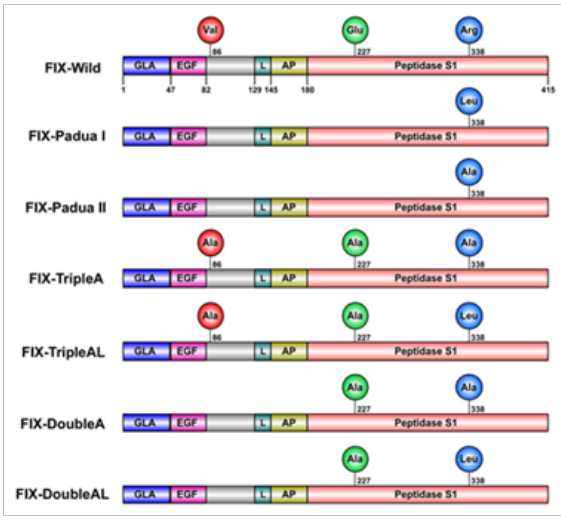
We developed asialoglycoprotein receptor (ASGPRs) targeting β-D-Galactose tethered lipid nanoparticle system (Galactosylated Lipid Nucleic Acid system (GALINAs)) that could selectively deliver nucleic acids to liver, in particular to hepatocytes. We validated the efficiency of our liver targeting nanoparticles with luciferase reporter system in both plasmid and mRNA forms in vivo. The luciferase expression was found specifically in liver. (Fig. 1) mRNA expression was observed as early as in 6hr. We further demonstrated that the lipid nanoparticle system could be re-administered without compromising the transgene expression. This could potentially overcome one of the major challenges with viral vectors, where development of antibodies against the vector limits the re-administration and prevents early stage intervention. Toxicity studies showed that these nanoparticles are safe even after 7 administrations, there is no detectable histological changes both in H&E staining and MT staining of the major organs. Liver and kidney functions found to be normal and no genotoxicity was observed. Overall, the novel liver specific lipid nanoparticle system found to be safe and efficient to deliver nucleic acids into the liver.



Luciferase mRNA expression in Balb/c mice after intravenous administration of lipid-mRNA complexes

Development of hyper functional variants:

Prior clinical studies demonstrated that supplementing gene encoding natural mutation in FIX protein at 338 position from arginine to leucine (R338L) increased FIX activity to 5-10 folds compared to wild type. Other efficient variants including R338A, Triple mutant (FIX-V86A/E277A/R338A) and TripleL mutant (FIX-V86A/E277A/R338L) have been identified as gain-of-function variants. Towards improving the efficacy of gene therapy, these gain-of-function variants were cloned in our lab. (Fig.2) We also cloned the plasmids containing gain-of function variants of Factor IX with liver specific promoters including LP-1 and Albumin. Currently, we are evaluating the efficacy of these plasmids in HepG2 cells.



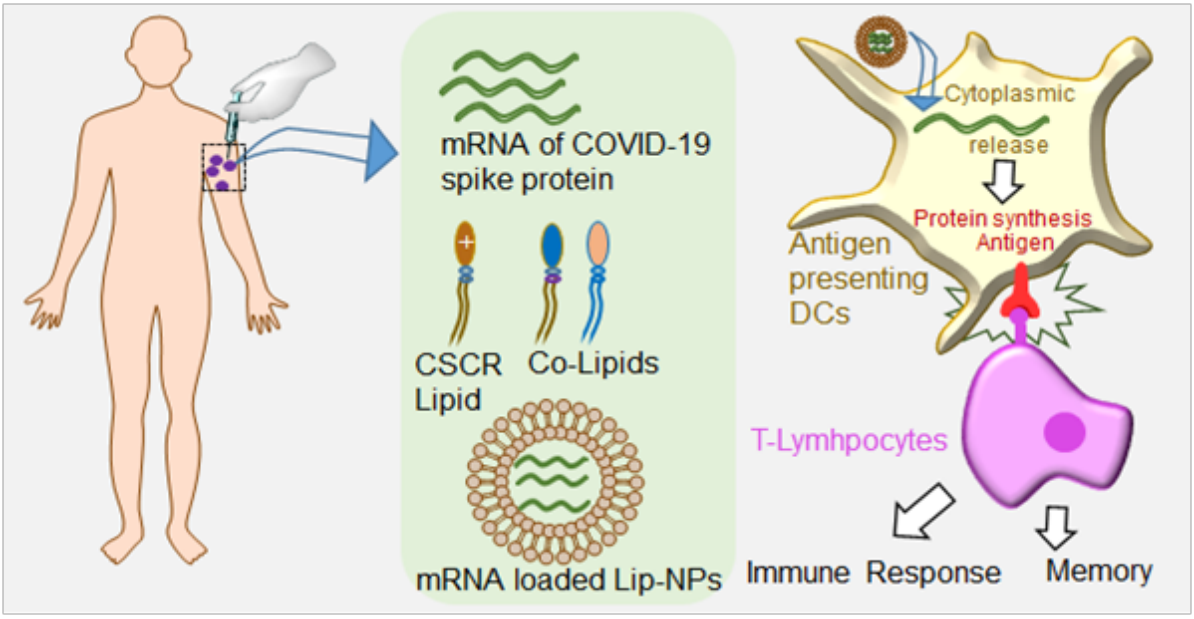
Recombinant FIX	Amino acid residue modified	Codon modified
FIX-wild	V86 E277 R338	GTA GAA CGA
FIX-Padua I	R338L	CTG
FIX-Padua II	R338A	GCA
FIX-TripleA	V86A E277A R338A	GCA, GCA, GCA
FIX-TripleAAL	V86A E277A R338L	GCA, GCA, CTG
FIX-DoubleA	E277A R338A	GCA, GCA
FIX-DoubleAL	E277A R338L	GCA, CTG

Cloning of hyperfunctional variants of Factor IX

Towards developing an mRNA based therapeutic approach, we have optimized and validated IVT (in vitro transcription) of Factor VIII and FIX mRNAs of wild type and variants with pseudouridine (ΨU), N1 Methyl pseudouridine (N1M ΨU modification) and M6 Adenine for longer mRNA stability. Currently, we are evaluating transfections in SK-Hep-1 and HepG2 respectively.

Development of a lipid encapsulated mRNA based vaccine for SARS-CoV-2 virus

Rapid and large-scale deployment of vaccines is challenging with conventional vaccines for pandemics such as SARS-COV-2. Antigen encoding mRNA vaccines offer great advantage as they are non-integrative and less expensive. The antigenic chemically modified mRNA can be delivered with the lipid nanoparticles. Surface glycoprotein of the virus (Spike protein) mediates intracellular entry of the host cells through binding to ACE-2 receptor (Angiotensin converting enzyme receptor-2) in turn induces the viral membrane fusion of lung epithelial cells. Current investigational vaccine candidates are aimed at developing antibodies against Spike protein to neutralize the virus. Taking cues from prior findings, we designed and developed 3 fusion spike mRNAs with optimized 5' and 3' UTRs and chemical modifications for higher expression and stability.



Schematic of mRNA based vaccine for SARS-COV-2. mRNA encoding spike protein of SARS-COV-2 virus encapsulated in lipid nanoparticles and injected intramuscularly. DCs process mRNA and present the antigen on surface. T- Cells recognizes the antigen and trigger immune responses both direct and long-term memory

Further, we have developed and validated an ionizable lipid nanocarriers system, which can efficiently deliver nucleic acids into dendritic cells. We also demonstrated that the spike mRNA encapsulated lipid nanoparticle could express on the surface of HEK 293 cells. To test the efficacy of our vaccine candidate, we are evaluating IgM and IgG levels against spike protein in Balb/C mice. Encouraging pre-clinical data would enable us to initiate clinical trials.

Publications:

1. N Sharma, AK Dhyani, S Marepally, DA Jose, Nanoscale lipid vesicles functionalized with a nitro-aniline derivative for photoinduced nitric oxide (NO) delivery Nanoscale Advances 2020, 2 (1), 463-469
2. Nithin Sam Ravi, Beeke Wienert, Stacia K Wyman, Jonathan Vu, Aswin Anand Pai, Poonkuzhali Balasubramanian, Yukio Nakamura, Ryo Kurita, Srujan Marepally, Saravanabhavan Thangavel, Shaji R Velayudhan, Alok Srivastava, Mark A DeWitt, Jacob E Corn, Kumarasamypet Murugesan Mohankumar Identification of novel HPFH-like mutations by CRISPR base editing that elevates the expression of fetal hemoglobin bioRxiv, 2020.

Patent: Srujan Marepally, Saravanabhavan Thangavel, Alok Srivastava, Compact liposomal vehicle for delivery of large molecules Indian Patent Application No. 202041010160.

Academic activities:

- Organizing Committee member of the 3rd Annual Symposium on Cell and Gene Therapy, September 4-5, 2019
- Taught nanotechnology in stem cells applications for doctoral students

Internal Collaborations:

1. Alok Srivastava, CSCR / CMC, Vellore
2. Vrisha Madhuri, CSCR / CMC, Vellore
3. R. V. Shaji, CSCR / CMC, Vellore
4. Poonkuzhali Balasubramaniam, CSCR / CMC, Vellore
5. Sanjay Kumar, CSCR
6. Saravanabhavan Thangavel, CSCR
7. Mohankumar Murugesan, CSCR
8. Mahesh Moorthy, CMC, Vellore
9. George Varghese, CMC, Vellore

External Collaborations::

1. Arun Srivastava, University of Florida, USA
2. Chantal Pichon, University of Orleans, France
3. Rajkumar Banerjee, CSIR-IICT, Hyderabad
4. Srilakshmi V. Patri, NIT, Warangal
5. V. G. M. Naidu, NIPER, Guwahati
6. Amilan Jose, NIT, Kurukshetra

ASHA MARY ABRAHAM, MD
Professor, Department of Clinical Virology, CMC, Vellore
Adjunct Scientist, CSCR

Project title: Standardizing methods for detection of AAV antibodies in humans

Brief description of the project:

Adeno-associated virus (AAV) is a small non-enveloped virus which requires a helper virus for active replication. In the absence of a helper virus AAV establishes a latent infection either by integrating into the host genome or remain as episomal form. AAV is classified into 12 serotypes and over 100 isolates. AAV is used as a gene therapy vector because they are not pathogenic, they persistently express the transgene in the transduced cells, and they can transduct into both dividing and non-dividing cells. However, the major obstacle to gene therapy is the generation of immune response against AAV capsid antigens. Humoral immune response against AAV vector is classified into neutralizing and binding antibodies. Studies have shown that neutralizing antibodies bind against a serotype of AAV vector can neutralize the vector and reduce the efficiency of the gene delivery of the vector. Reports show that pre-existing neutralizing antibodies against a serotype of AAV do not neutralize other serotypes. Hence serotype prevalence and detection of neutralizing antibodies of AAV is very important for the effective use of AAV as a vector for gene therapy. Several methods have been used for the detection of antibodies against AAV. Some of the methods detect total antibodies while others detect neutralizing antibodies by in vivo or in vitro methods. Total antibodies have been detected mostly by ELISA and Western blot. The presence of different serotypes and the vast advancement in gene therapy requires tests which can detect neutralizing antibodies against specific serotypes. Screening of AAV serotype specific antibodies is done mainly by transduction inhibition assay (TIA) thus far. However, TIA is expensive, cumbersome and has longer assay duration than ELISA. ELISA is being used for screening of total antibodies against AAV serotypes. However, this format (using VLP) does not give information on serotype-specific detection of AAV antibodies. AAV Peptides which can detect neutralizing antibodies against different AAV serotypes will be cheaper, easier to perform and give serotype specific information for screening individuals before gene therapy.

Overall Objective:

The overall objective is to establish an efficient method of screening for AAV antibodies to different serotypes in individuals for potential gene therapy.

Specific Objectives:

1. To establish an in-house peptide ELISA for the serotype specific detection of AAV serotypes 3, 5 and 8 and compare with TIA.
2. To establish a transduction inhibition assay (TIA) for the detection of neutralizing antibodies against AAV serotypes 3, 5 and 8.
3. To screen for AAV antibodies in healthy volunteers and hemophilia A or B patients before gene therapy using the standardized in-house peptide ELISA and TIA.

Work Done:

- An in-house peptide ELISA has been established for the detection of AAV serotypes 3, 5 and 8.
- An in-house whole capsid ELISA has been established for the detection of AAV serotypes 3, 5 and 8.
- Transduction inhibition assay (TIA) for AAV serotypes 3 and 5 has been standardized using mCherry flow cytometry.
- Blood samples were collected from 564 individuals with haemophilia A and 119 individuals with hemophilia B and 250 individuals who gave blood for pre-operative screening and are negative for blood borne viral pathogens (HIV, HBsAg and HCV-Ab).
- Serum samples from 130 healthy individuals and 90 individuals with hemophilia B were screened for AAV 3, 5 and 8 total antibodies using the standardized capsid ELISA. Peptide binding antibodies were detected using AAV capsid epitope specific peptides and the results compared with capsid ELISA.
- Screening for neutralizing antibodies for AAV3 was performed on 90 individuals with haemophilia B and 130 healthy individuals serum samples using TIA by mCherry flow cytometry.

Specific highlights of the project:

- Design peptides for the serotype specific detection of AAV 3, 5 and 8.
- Establish an in-house peptide ELISA for the serotype specific detection of antibodies against AAV 3,5 and 8.
- Establishing transduction inhibition assay (TIA) for quantitation of antibodies against different serotypes of AAV using mCherry flow cytometry and 96 well format luciferase detection system.

Support from CSCR: Lab infrastructure

Collaborations:

Internal:

1. Sanjay Kumar, CSCR
2. Hubert Daniel, CSCR / Department of Clinical Virology, CMC, Vellore
3. Rajesh Kannangai, Department of Clinical Virology, CMC, Vellore
4. Alok Srivastava, CSCR / Department of Haematology, CMC, Vellore

External:

1. Mavis Agbandje-Mckenna, University of Florida, USA
2. Arun Srivastava, University of Florida, USA

DOLLY DANIEL, MD

Professor, Department of Transfusion Medicine and Immunohaematology, CMC, Vellore
Adjunct Scientist, CSCR

Project title: Creating a bank of cells homozygous for HLA haplotypes

Funding source: Department of Biotechnology, Government of India

Duration: December 2015 – 2020

Brief description of the project:

A major limitation to the use of stem cell therapeutics is the immunological barrier, contributed largely to the diversity of the HLA system. Developing individual personalized cell lines is expensive and labour intensive. Identifying individuals who are most "immune-compatible" with the largest number of potential recipients and creating a bank of iPSC lines from these individuals, thus creating a haplobank is a model being trialed worldwide). Centre for Stem Cell Research (CSCR), through the Department of Biotechnology, Government of India aims to establish harmonized approaches to generate iPSCs for regenerative medicine applications. Considering the diversity and uniqueness of the Indian population, it is important that we identify individuals homozygous for the most common haplotypes in the Indian population. The Haplobanking project primarily involves the following stages:

- Identifying the most common HLA haplotypes from India / Asia using published and unpublished data available
- Collaboration with DATRI – the stem cell registry in Chennai and identifying individuals with those haplotypes
- Donor recruitment - counseling and sample collection
- Banking of peripheral blood mononuclear cells (PBMNC) for the generation of iPSC lines from the cultured donor cells in the laboratory, through Good Manufacturing Practice (GMP)
- Banking of iPSCs generated in the GMP facility

Peripheral blood samples collected from donors after consent are screened for infectious diseases at the Blood Bank, CMC, Vellore. So far, 235 blood samples have been collected through DATRI and PBMNCs have been isolated and cryopreserved. Donors with the most common 10 haplotypes are now being shortlisted from Tamil Nadu and the neighbouring states. Samples collected so far represent the top 20 haplotypes in the Indian population (from data available). The donors are from Tamil Nadu covering Chennai, Thiruvannamalai, Coimbatore, and Thiruppur. Also, sample collection was expanded to Bangalore, Karnataka. Generation and banking of iPSCs are shown in the report of R V Shaji. Ten (10) iPSC lines were generated and characterized for the expression of pluripotency markers. Karyotyping analysis was also carried out on all the iPSC lines. For the haplobanking program, CSCR has been part of the international consortium - The Global Alliance for iPSC Therapies (GAiT).

Internal Collaborations::

1. R. V. Shaji, CSCR / CMC, Vellore
2. Alok Srivastava, CSCR / CMC, Vellore

External Collaborations::

1. Raghu Rajagopal, CEO, DATRI, Chennai
2. Nezih Cereb, Chief Scientific Officer, DATRI, Chennai

ABY ABRAHAM, MD
Professor, Department of Haematology, CMC, Vellore
Adjunct Scientist, CSCR

Project title: Gamma delta T cell-based immunotherapy for blood cancers

Funding source: CSCR core fund

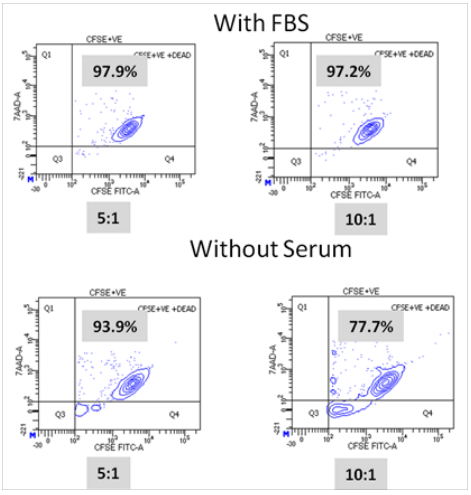
Duration: July 2017 - June 2019

Objectives:

1. To establish a protocol for the culture and expansion of γδ T cells from human peripheral blood mononuclear cells using serum-free medium and serum-rich medium and to compare fold expansion with IL-2 and Zoledroinc acid as supplements.
2. To check if the expansion of αβ T cells can be minimized by delayed addition of IL-2 and IL-15 in culture.
3. To assess the stability and functionality of the cells post-cryopreservation, cells expanded in three different conditions will be compared i.e. fresh cells, cells post cryopreservation and cells cultured post cryopreservation

Work Progress:

A total of 30 samples have been processed to establish the cell culture protocol and the flow cytometry analysis. The samples were cultured in both serum and serum free conditions. In all the samples cultured, there was an increase in the fraction of γδ T cells from the initial cell population (MNC) to the cells cultured for 14 days. In one of the sample we have been able to achieve γδ cell purity of >95%. We have noticed variation between samples in terms of Gamma delta T cell purity and fold expansion. Following this a protocol for cytotoxicity assay was established to assess the cytotoxic potential of the expanded γδ T cells in vitro. It is a flow cytometry based assay.



Representative image of cytotoxic killing of two week cultured γδ T cells in both serum and serum free conditions against CFSE labelled K562 cells at 5:1 and 10:1 effector:target cell concentration.

Publication (poster):
Augustine Thambaiah Prabakumar, Thamizhselvi Ganapathy, Aleya Tabasum, Mohankumar Murugesan, Trent Spencer, Alok Srivastava, Aby Abraham & Sunil Martin. Expansion of human γ9δ2 T cells to target hematologic malignancies. Cytotherapy, May 01, 2020; Volume 22, Issue 5, Supplement , S130-S131

Support from CSCR: Funding and lab space

Collaborations:

1. Sunil Martin, CSCR
2. Mohankumar Murugesan, CSCR
3. Augustine Thambaiah, CSCR
4. Alok Srivastava, CSCR / CMC, Vellore

POONKUZHALI BALASUBRAMANIAN, PhD
Professor, Department of Haematology, CMC, Vellore
Adjunct Scientist, CSCR

Project title: Identification of novel nuclear receptors (NHR) drug targets in myeloid leukemia

Funding source: Department of Biotechnology, Govt. of India (part of the Centre of Excellence Grant)

Duration: November 2015-2020

Objectives:

1. To screen for basal RNA expression of NHR genes in AML and CML cell lines, normal CD34+ cells and primary human CML/AML CD34+ cells.
2. To assess the effect of knock down/overexpression of differentially expressed NHRs in AML cell lines with different mutations on cell proliferation, apoptosis and RNA expression after treatment with ara-C/Dnr/both.
3. To assess the effect of knock down/overexpression of differentially expressed NHRs in CML cell lines representing different disease stages on cell proliferation, apoptosis and RNA expression after imatinib treatment.
4. Validation of the findings by overexpression / knock down experiments / AML/CML mouse model

Work Done:

We had established upto primary transplantation and harvesting of splenocytes from these mice (as submitted in the previous annual report). These splenocytes (1 x 10^6 cells) were injected into sub-lethally irradiated mice. CML disease development was confirmed by RT-PCR for detecting BCR-ABL fusion transcript. Despite testing positive for BCR-ABL transcript, the mice did not develop CML disease as observed in the primary transplantation. This could possibly be due to the low frequency of haematopoietic stem and progenitor cells in the primary transplanted mice. Hence we planned to enrich Sca-1 positive cells and transduced with BCR-ABL and transplanted into recipient mice to get high frequency of haematopoietic stem and progenitor cells from the primary transplanted mice.

Sca-1 enrichment for the primary transplantation: Bone marrow cells were harvested from the donor C57Bl/6 6-8 weeks old mice and sca1 cells were enriched form the BM cells using Easy sep sca1 positive selection kit. After 24 hours incubation with stimulation media, these Sca-1 enriched cells (1*106 cells) were transduced with BCR-ABL containing virus and were injected to the lethally irradiated recipient mice. CML disease burden was evaluated by checking the GFP+ Gr-1+ cells in the peripheral blood. We observed an increase in GFP+ Gr-1+ cells in peripheral blood between 2-3 weeks after transplantation. We euthanized the mice and checked for the phenotypic changes. We observed the splenomegaly, hepatomegaly & pulmonary infiltration showing the signs of CML disease. Secondary transplantation with these splenocytes is ongoing.

Generation of xenograft transplantation chronic myeloid leukemia mouse model (Additionally funded by CMC Fluid grant, 2019-2021): 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 imaging 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.

Support from CSCR: Lab space, animal facility, imaging and flow cytometry facilities

Collaborations:

1. R. V. Shaji, CSCR / CMC, Vellore

GEETA CHACKO, MD
Professor, Department of Pathology, CMC, Vellore
Adjunct Scientist, CSCR

Project title: C11orf95-RELA fusions in supratentorial ependymomas: Relevance in prognostication

Funding source: Department of Science and Technology, Govt. of India

Duration: June 2017 - 2020

Brief description of the project:

Ependymomas are tumors of the brain and spinal cord, occurring in both, children and adults. The mainstay of treatment of these rare tumors is surgery, though some are treated with adjuvant radiotherapy as well. These tumors are unresponsive to chemotherapy and about 40-45% are incurable. The recent characterization of a C11orf95-RELA translocation in supratentorial ependymomas and the impact of this oncogenic driver on NFkB signaling pathway has generated significant interest. Interestingly, these translocations are not associated with the other subtypes of ependymomas, providing a unique molecular alteration that could be investigated for its role towards prognostication and would perhaps provide a good therapeutic target for anti-NF-kB therapy.

This study is being undertaken with the hypothesis that Supratentorial ependymomas with the C11orf95-RELA translocations are associated with a worse prognosis. The following key questions are being investigated:

Are these translocations restricted to particular sites in the supratentorial compartment?

Do samples that harbor the translocation demonstrate aberrant NF-kB signaling?

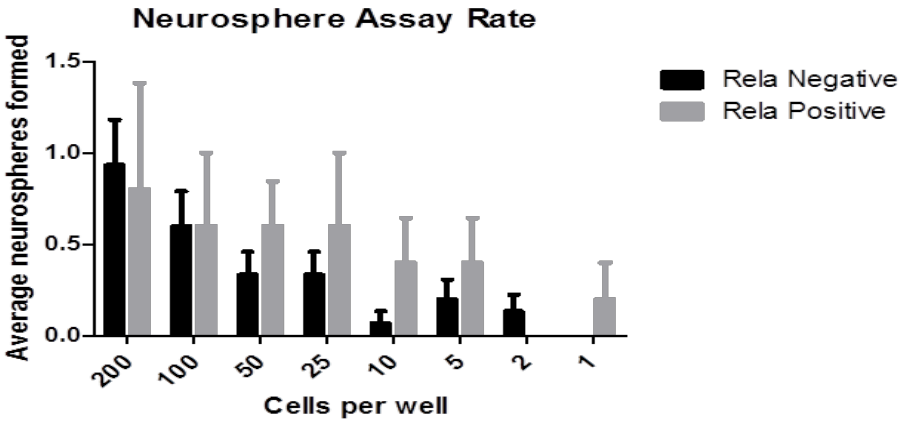
Do such tumors also have a population of stem cells that have increased expression of the C11orf95-RELA translocation?

What is the prognosis in patients with tumors that demonstrate the C11orf95-RELA translocation?

Work done: A total of eighteen supratentorial ependymoma tumor samples were collected from Neuro unit I, Neuro unit II and Neuro unit III of Christian Medical College and transported to Centre for Stem Cell Research (CSCR) in ice cold conditions. The samples were cultured using Neural Basal Media with supplements and growth factors. Among the eighteen samples, twelve samples formed neurospheres, two samples underwent necrosis after forming neurospheres and four samples did not form any neurospheres.

Identification of CSC population using FACS Analysis: The brain tumor stem cell population in one RELA positive (Hosp. no.207497H) and two RELA negative (Hosp.Nos. 435704H, 585643H) supratentorial ependymomas, were determined by the analysis of expression profiles of CD133 marker in cultured cells by flow cytometry within seven days of primary neurospheres formation. Briefly, primary neurospheres were harvested from the culture, mechanically dissociated into single cell suspension (cell concentration of 1 million cells/ml) and centrifuged at 1000rpm for 5min to pellet it down. Cells were washed once with 1XPBS to remove residual media. Then the cells were resuspended in 100ul of FACS buffer and incubated with5µg of FITC labelled CD133 antibody for 30 minutes at 4°C in the dark. After washing, cells were resuspended in 100µl of fixing buffer and cells were evaluated by BD FACS AriaIII. The unstained cells served as negative control to eliminate any background fluorescence generated by cells. Flow cytometric quantification of CD133 expression of RELA negative and RELA positive supratentorial ependymomas ranged from 11.3 – 14.6 % and 52.1% respectively which indicates higher expression of CD133 marker in RELA positive as compared to RELA negative supratentorials ependymomas.

Neurosphere Assay: The sphere formation rate was performed for analyzing the proliferative and self-renewal potential of neurospheres within the RELA positive and RELA negative supratentorial ependymomas. Samples with primary neurosphere were mechanically dissociated into single cell suspension and seeded in 96 well plates with serial dilution ranging from 200 cells/well to 1 cell/well in five technical replicates. After 7 days, the generation of secondary sphere was counted and the mean number of spheres per 400 cells was calculated which is around 3.4 spheres/400 cells for RELA positive and 2.4-3.2/400 cells for RELA negative supratentorials ependymomas. The difference in sphere forming ability between the RELA positive and RELA negative supratentorial ependymomas was found to be without significance.



Western blotting: Protein expression of C11ORF95-RELA fusion and CCND1 in neutrospheres isolated from twelve supratentorial ependymoma (one RELA positive and eleven RELA negative (by RT PCR) and one glioma samples, cells from one RELA positive tumor (no neutrosphere), cells from one RELA positive tumor (no neurosphere) were analyzed by western blot with NF-kB P65 and CCND1 antibodies respectively. In one of the RELA positive samples, we observed the expression of the RELA fusion protein that corresponded to the appropriately sized ~120 kDa (c11orf95-RELA fusion-2) and also CCND1 protein. Sample from one of the patient with RELA positive tumor who underwent radiotherapy did not generate any neutrosphere and the cells isolated from this tumor failed to show the positive for any of the RELA fusion protein. In three of the RELA negative and one of the glioma samples showed the band of corresponding molecular weight of different RELA fusion proteins. Among these four tumors, other than one sample, the remaining samples showed the expression of CCND1.

Support from CSCR: Infrastructure, lab space, core lab facilities, imaging, cryopreservation

Internal Collaborations: :

1. Rekha Pai, Department of Pathology, CMC, Vellore
2. Mohankumar Murugesan, CSCR
3. Ari G Chacko, Department of Neurosurgery, CMC, Vellore
4. V. Rajshekhar, Department of Neurosurgery, CMC, Vellore
5. Leni G. Mathew, Department of Paediatric Oncology, CMC, Vellore
6. Sunitha S. Varghese, Department of Radiotherapy, CMC, Vellore
7. Thambu David S, Department of Medicine, CMC, Vellore

CHRISTHUNESA CHRISTUDASS, PhD
Professor, Department of Neurological Sciences, CMC, Vellore
Adjunct Scientist, CSCR

Project title: Isolation of Cancer stem cells from high grade primary and secondary gliomas: their response to microenvironmental cues and Notch signalling blockade.

Funding source: Department of Biotechnology, Govt. of India

Duration: August 2016 – December 2019

Brief description of the project:

Cancer Stem Cells (CSCs) are considered as the driving force of cancer formation and are more resistant to treatment. Gliomas are the most common tumors of CNS and glioblastoma multiforme (GBM) are the most malignant tumors of the brain. The prognosis for patients with GBM remains dismal, largely due to the highly invasive nature of this disease and inadequate treatment strategies. On the basis of clinical presentation, GBMs have been further subdivided into primary or secondary GBMs and there is also evidence that CSCs in primary and secondary glioblastomas may also be different. Brain CSCs are characterized by their ability to form neurospheres, undergo self-renewal and differentiate into other cell lineages. Our objectives in this study are to: (i) identify and characterize CSCs in high grade gliomas using neurosphere formation and expression of cell markers CD133, A2B5 and/or Nestin, (ii) establish primary or secondary nature of gliomas based on age, mutation(s) in IDH1 and overexpression EGFR, (iii) study CSCs response to microenvironmental cues by measuring VEGF, HIF-1α,HIF-2α, MMP-9 and CCL-3 levels before and after pretreatment with HIF & VEGF inhibitors, (vi) study CSCs capacity to differentiate into endothelial cells (ECs), and (v) study the role of Notch signaling pathway in both primary and secondary CSCs by Notch pathway blockade. Till date we have successfully isolated and characterized GBM derived CSCs using neurospheres and A2B5 (21-24% positivity) and nestin (70-90%) markers in 27 primary and one secondary gliomas. We also have developed a FACS- acquisition protocol for GBM derived CSCs. We are currently involved in studying: (i) the IDH1 mutation(s) in glioma tissues, (ii) the microenvironmental cues after HIF1α inhibition and Notch signaling blockade in CSCs using variations in specific gene expressions and proteins.

Specific highlights of the project:

Developed a FACS- acquisition protocol for GBM derived CSCs using A2B5 and Nestin markers. MACS sorting could not improve CD133 positivity in these samples. CSCs derived from GBM samples have been analysed by real time PCR to see the expression of specific genes in response to microenvironment inhibition by GN44028 hypoxia inhibitor, Notch signalling inhibition by DAPT notch inhibitor, cryptotanshinone (STAT3) inhibitor. We also studied EGFR overexpression and IDH1 mutation status in these samples. Final analysis is being carried out to know the CSCs response to microenvironmental cues and Notch signalling blockade.

Support from CSCR:

Regularly using CSCR core facility for FACS analysis, nanodrop, cDNA synthesis and Real-time PCR. As the project aims to analyse the gene expression levels of the different markers for cancer stem cells for microenvironment and Notch signalling inhibition by using specific inhibitors, we utilized CSCR facilities for these studies.

Internal Collaborations: :

1. Ari George Chacko, Department of Neurosurgery CMC, Vellore
2. Geeta Chacko, Department of Pathology, CMC, Vellore

SANJAY KUMAR, PhD
Scientist, CSCR

LABORATORY HIGHLIGHTS

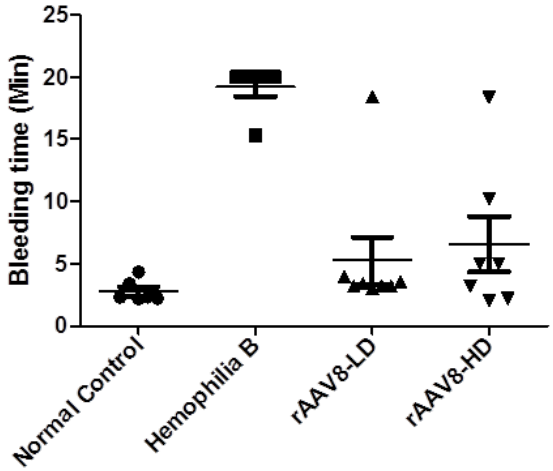
Lab scientific contributions in AAV gene therapy program on AAV-based state-of-the-art gene therapy technologies for the monogenic disease are to attempt finding a solution for lingering challenges in the Gene and Cell Therapy fields such as AAV-based therapeutic strategies, Next-Gen AAV-based gene-delivery 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, mesenchymal stem cells (MSCs)-based therapies, novel imaging approaches, reporter gene expression towards gene and cell therapy technologies.

Preclinical AAV-gene therapy work:

Ongoing work is mostly focussed on AAV based preclinical gene therapy studies, which helps in developing AAV-based gene therapy technologies, including newer methods to determine AAV-directed pre-existing immunity in collected human samples from healthy individuals and patients with hemophilia. Also, participate in the current and future AAV projects of generating different AAV serotype vectors and related distinctive AAV-based assay protocols utilizing AAV-specific expertise. The lab has substantial involvement in each of the ongoing AAV projects and making real-time practical solutions to each of the assays vital for the studies. E.g., a) A transgene comprising AAV vector's in vitro therapeutic testing in various hepatocyte cell lines and primary cells; b) in vivo scAAV8-hFIX vector therapeutic efficacy testing in suitable Hemophilia-B transgenic mice models; c) Troubleshooting in the AAV vector-based ELISA and transduction inhibition assay (TIA) assays based on AAV technical facts and AAV-specific knowledge; d) Different serotypes of AAV vector production (AAV3, AAV5, and AAV8) and improving the AAV genome containing viral particles during AAV purification steps.

Contribution towards Affordable Excellence in AAV gene therapy program:

Co-ordinating a scAAV8-hFIX-Padua vector-based in vivo study in Hemophilia B transgenic mice model at CSCR in collaboration with Intas Pharmaceuticals, Ahmadabad and helping Intas vector core developing team in every step of the rAAV vector packaging, rAAV purification, testing rAAV transduction efficiency, rAAV quality control testing by electron microscopy to identify the percentage of genome filled versus empty capsid and eventual in vivo human FIX transgene expression testing in Factor IX null Hemophilia B transgenic mouse model.



Tail bleeding-time Assay in Hemophilia-B Transgenic Mice: scAAV8-hFIX treatment cohort mice showed a significant improvement in bleeding time. Groups were as follows: Normal C57BL6 Positive Control Group; Control Hemophilia B Group; Low dose (LD) scAAV8-hFIX treatment Group; High dose (HD) scAAV8-hFIX treatment Group.

Aiming PTEN, seeking pulse, finding Dox: A targeted, transient in vivo approach to facilitate functional repair in mice spinal cord injury: In Spinal cord injury (SCI), the primary injury/assault is only the tip of the iceberg; the real threat being the secondary injury associated with a cascade of molecular events that follow - inflammation, macrophage type switch, cytokine outburst, ROS, apoptotic signals, infiltration of inflammatory cells, glial scarring, demyelination, axonal dieback, and fibrosis to name a few. Unattended, it progresses to an irreversible chronic phase and ultimately to paraplegia - an economic burden to and social death of the individual. Although many targets have been previously implied in SCI, due to the complexity in pathophysiology and therapeutic interventions required, it remains a "Molecular Disease," where standard clinical, cell and rehabilitation therapy has had minimal impact on augmenting motor function. Over the past decade, PTEN deletion showed some potential. Still, long-term tumorigenicity, toxicity, and immunogenicity employing shRNA/viral vectors, the fate of the demyelinated axons, and the inhibitory glial scar/lesion are greater challenges to be addressed. Combinatorial therapies introduce more variations in the mode of delivery, optimization of dosage and period of intervention/assessment – thus arises the quest for a "magic bullet" in SCI.

We have developed a novel, targeted, inducible, virus-free, localized yet safe approach by in vivo electroporating engineered DOX-inducible miR-E constructs carrying a GFP reporter into the injured spinal cord to modulate PTEN in mice models during the therapeutic window period. Among the randomly divided Dox+/Dox- groups, our results show that DOX+ PTEN modulated mice were consistently associated with a marked increase in spinal cord tissue sparing, reduced cavity/lesion size and glial scarring, improved BMS scores displaying functional motor recovery, alleviated astrogliosis in lesions, increased re-myelination of the spared axons, and significant motor evoked potentials when compared to DOX- group where evoked potentials were absent altogether in the hindlimb. Our findings collectively suggest that targeted therapy by transient expression of sh-PTEN-miR during the therapeutic window period is a promising therapeutic strategy to augment functional repair in spinal cord injury.

Publications:

1. Pbrm1 Steers Mesenchymal Stromal Cell Osteolineage Differentiation by Integrating PBAF-Dependent Chromatin Remodeling and BMP/TGF-b Signaling. Sinha S, Biswas M, Chatterjee SS, Kumar S, Sengupta A. Cell Reports, volume- 31, 107570, April 28, Page:1-17; 2020.
2. Impact of Induced Pluripotent Stem Cells in Bone Repair and Regeneration. Rana D, Kumar S, Webster TJ, Ramalingam M. Curr Osteoporos Rep. 2019 Aug;17(4):226-234.
3. Heterogeneity of Mesenchymal Stromal Cells in Myelodysplastic Syndrome-with Multilineage Dysplasia (MDSMLD). Abbas S, Kumar S, Srivastava VM, M MT, Nair SC, Abraham A, Mathews V, George B, Srivastava A. Indian J Hematol Blood Transfus https://doi.org/10.1007/s12288-018-1062-6. Published online on 01 January 2019.
4. ssGreen synthesis, characterization, and antibacterial activity of silver nanoparticles by Malus domestica and its cytotoxic effect on (MCF-7) cell line. Mariadoss AVA, Ramachandran V, Shalini V, Agilan B, Franklin JH, Kumar S, Alaa YG, Tawfiq MA, Ernest D. Microb Pathog. 2019 Jun 24;135:103609.

Internal Collaborations:

1. R. V. Shaji, CSCR / CMC, Vellore
2. George Tharion, CMC, Vellore
3. Antony Devasia, CMC, Vellore
4. Suresh Devhsayam, CMC, Vellore
5. Rajdeep Ojha, CMC, Vellore
6. Alok Srivastava, CSCR / CMC, Vellore
7. Sukesh C Nair, CMC, Vellore
8. Paul M. J., CMC, Vellore
9. Asha Abraham, CSCR / CMC, Vellore
10. Vrisha Madhuri, CSCR / CMC, Vellore
11. Ashish Gupta, CMC, Vellore
12. Margaret Shanti, CMC, Vellore
13. Jeyanth Rose, CSCR / CMC, Vellore

External:

1. Selvarangan Ponnazhagan, UAB School of Medicine, USA
2. Arun Srivastava, University of Florida College of Medicine, USA

SUNIL MARTIN, PhD
Scientist, CSCR

LABORATORY HIGHLIGHTS

Immune Cell Engineering and Therapy (iCET) Laboratory

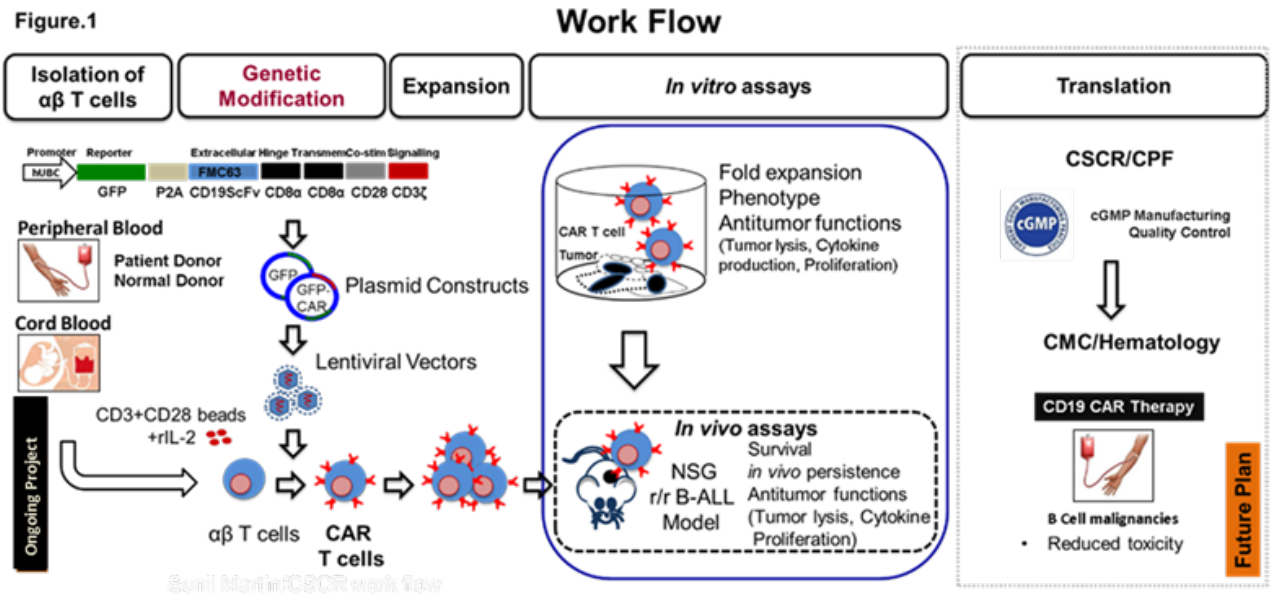
The primary goal of our lab is to expand or modify NK cells, γδ T cell, and αβ T cells to target hematological malignancies. We are currently focussing on generating and characterizing Chimeric Antigen Receptors (CARs) expressing αβ and γδ T cells CARs are artificial receptors with an extracellular antigen-binding domain of an antibody with the intracellular domains of T Cell Receptor/ CD3ζ often incorporating co-stimulatory domains. HLA independent target recognition and modular design of CARs makes them an attractive approach to target refractory or relapsed B cell malignancies.

Recently, CD19 CAR T cells demonstrated remarkable antitumor functions in the preclinical and clinical studies against B cell acute lymphoblastic leukemia (r/r B-ALL). Although CAR Therapy provided outstanding results against aggressive B Cell malignancies, the therapy is available for - at least an expensive $402 647 per patient- in the USA. In contrast, neurologic toxicities due to hypercytokinemia are the major side effect of CAR Therapy which can be mitigated by incorporating CD8α hinge and transmembrane domains in the CAR design. Therefore, our primary objective is to generate indigenous anti CD19 CAR T cells containing CD8α hinge and transmembrane region with CD28 co-stimulatory domain from peripheral blood and cord blood and to characterize them in vitro and in vivo. An alternative approach is to expand γ9δ2 T cells with multivalent immunity to lyse the tumor cells with reduced toxicity as the γ9δ2 T cell receptor recognizes malignancy-associated patterns. We are also generating CD19 CAR-expressing γ9δ2 T cells to confer the adaptive specificity and robust activation of CAR.

I. CD19 CAR T cells to target refractory or relapsed B cell Acute Lymphoblastic Leukemia (r/r B-ALL)

Our strategy towards generating CAR T cells at CSCR is summarized in figure.1. Our CAR construct has Single-Chain Variable Fragment (scFv) derived from FMC63 (a hybridoma of mouse anti-human CD19) with a transmembrane domain from CD8α. The cytoplasmic domain consists of the co-stimulatory domain from CD28 and CD3ζ (Fig.1). First, we venture to expand conventional αβCART cells from peripheral blood. The cells will then be stimulated with a Second-generation CAR construct that was packed into lentiviral vectors and the pre-stimulated αβT cells were transduced in the presence of IL-2. These lentiviral vectors transduced the αβT cells with remarkable efficiency across three donors (Mean 25.8, ±5.8 on day 12, cGMP criteria being more than 20%). The CAR T cells thus generated lyse the CD19+ NALM-6 cell line but not the CD19- NALM-6 cells.

Figure.1



We also generated CAR T cells from the T cells derived from cord blood by lentiviral transduction (26.3±2.2%). We are proceeding to further characterize the antigen-specific proliferation, cytokine production, and cytotoxicity.

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

Currently, we are focussing on transducing and expanding engineered γδ T cells. These innate-adaptive cells have natural antitumor functions with germline-encoded receptors. The expression of high-affinity Fc receptors facilitates ADCC (Antibody-dependent cell-mediated cytotoxicity) which would allow combination therapy with already approved therapeutic antibodies further enhancing tumor clearance. γδ T cells evoke reduced GvHD (Graft versus Host Disease) and remarkable GvL (Graft versus Leukemia) due to the sensing of stress ligands associated with malignant transformation. γδ T cells are also capable of presenting antigens to the αβ T cells. Furthermore, γδ T cells have reduced propensity to induce cytokine storm - a caveat of the αβ CART cell infusion

CARs can confer an adaptive specificity and robust activation, which is reported to boost the tumor lysis capacity of γδ T cells. Overall, γδ T cells are emerging as a potential candidate effector cells to host CARs in the adoptive immune-oncology program. An FDA approved drug zoledronic acid (ZA) is a known inhibitor of farnesyl diphosphate synthase and its upstream product Isopentenyl pyrophosphate (IPP) accumulate in the cell. IPP is a potential ligand of γδ TCR. Most of the protocols for in vitro expansion of γδ T cells involve ZA stimulation of γδ T cells in the presence of high rhIL-2

An effective expansion protocol is essential for the generation of therapeutic CAR γδ T cells. In collaboration with Dr. Aby Abraham (CMCHaematology) / Augustine (cGMP-CSCR), we are establishing a protocol for in vitro expansion and functional validation of peripheral blood-derived γδ T cells with ZA and rhIL-2. These γδ T cells lyse established leukemic cell lines NALM-6 and K562 in various effector to target ratios.

Furthermore, we have validated the plasmid constructs encoding gene cassettes for chimeric antigen receptor (CAR) targeted against CD19; a leukemic antigen. We are fine-tuning the lentiviral transduction (MOI, time of transduction, Media, etc.) for optimal transduction efficiency in line with cGMP standards. Our preliminary results indicate that higher lentiviral titers would significantly improve lentiviral transduction. Our preliminary ongoing experiments on electroporation indicate that the transducted γδ -T cells can be cultured for 04 days without the loss of GFP transgene expression (Fig.2). Our goal is to generate CD19 CAR γδ T cells from multiple donors and test its tumor lysis capacity in vitro. We will then test its antitumor functions against the patient-derived sample before proceeding with in vivo animal studies and cGMP level expansion.

Publication:

Abstract: A.T. Prabakumar, G. Thamizhselvi, A. Tabasum, M. Murugesan, H.T. Spencer, B. Ryan, A. Srivastava, A. Abraham and S. Martin. Expansion of human γ9δ2 T cells to target hematologic malignancies. Cytotherapy, Vol. 22, Issue 5, S130–S131. DOI:https://doi.org/10.1016/j.jcyt.2020.03.253.

Invited Talks:

"Targeting B cell malignancies with CD19 CAR" - Annual Conference of Indian Association of Cancer Research. Thiruvananthapuram, 5-7 February 2020.

Academic Activities:

- Organizing Committee member of the 4th Annual Symposium on Cell and Gene Therapy, September 5-6, 2019
- JRF review process

Internal Collaborations:

1. Alok Srivastava, CSCR / CMC, Vellore
2. Aby Abraham, CSCR / CMC, Vellore
3. Mohankumar Murugesan, CSCR
4. Saravanabhavan Thangavel, CSCR
5. Srujan Marepally, CSCR
6. Augustine Thambaiah, CSCR

External Collaborations:

1. Trent Spencer, Emory University School of Medicine, USA
2. Sunil Raikar, Emory University School of Medicine, USA

MUTHURAMAN N, MD
Assistant Professor, Department of Biochemistry, CMC, Vellore
Adjunct Scientist, CSCR

Project title: Does aspirin inhibit cancer cell stemness in endometrial cancer?

Funding source: CMC Fluid Reseach Grant

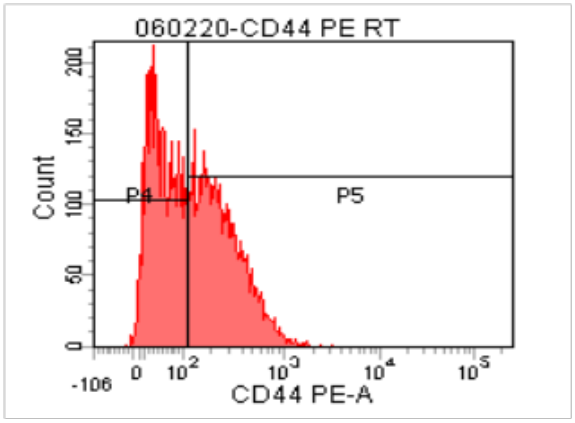
Duration: April 2019 to March 2021

Brief description of the project:

The prevalence of endometrial cancer (EC) is increasing globally. Cisplatin is one of the most commonly used chemotherapeutic agents in patients with EC. However, its efficiency remains a major problem. Cancer stem-like cells (CSCs) are thought to be an important cause of chemotherapy-resistance and radio-resistance, ultimately leading to treatment failure in patients with advanced disease. With the emergence of the CSC theory, there is a growing need for developing therapeutic agents which can potentially target them. Cancer stem cells are increasingly preferred as a target to prevent disease recurrence or to circumvent therapeutic resistance. Recent evidence suggests that aspirin may be able to 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. In the present study, we plan to investigate the effect of aspirin on cancer stem-like populations derived from endometrial cancer cell line. The effect of aspirin on potentiating the antitumour effect of cisplatin will also be analysed.

Work done:

We have identified the presence of cancer stem like population in HEC-1B cancer cell lines by means of their sphere forming capability and also by their positivity for CD44, which is a marker of cancer stem like cells.



CD44 positive cells in HEC-1B cell line identified by flow cytometer

Support from CSCR: Lab space.

Collaborations:

1. Premila Abraham, Department of Biochemistry, CMC, Vellore
2. Mohankumar Murugesan, CSCR

JEYANTH ROSE, MS

Associate Surgeon, Department of Ophthalmology, CMC, Vellore
Adjunct Scientist, CSCR

Project Title: Standardized approach to isolate and culture - Conjunctival epithelial cells from Conjunctival impression cytology.

Funding Source: CMC Major Fluid Research Grant

Duration: July 2019 to August 2021

Objectives:

1. To isolate conjunctival epithelial cells from unused conjunctival impression cytology specimen.
2. To expand these cells on appropriate culture media.
3. To characterize these cells after a 2-weeks period using IHC antibodies markers - E-cadherin, CK19 and CK7 for epithelium, MUC5AC for goblet cells and Vimentin and FSP-1 for stromal cells and proliferative marker Ki67
4. To perform cell cycle analysis to identify the phase of the cell cycle.

The aim of the study is to standardize the protocol for isolation and culture of conjunctival epithelium cells from the conjunctival impression cytology. Conjunctival impression cytology is a noninvasive procedure to acquire superficial conjunctival epithelium. Cell will be isolated from impression cytology to culture and characterize.

Work done:

- Impression cytology protocol to isolate epithelial cells were standardized according to the lab's requirement.
- The modified staining protocol to stain the impression cytology acquired cells were standardized in the lab.
- Isolation and culture of epithelium cells from the impression cytology was attempted with three technique including, mechanical agitation of the filter paper in the media, direct incubation of filter paper in culture plate containing media and centrifuging the media along with filter paper.
- Two different coating for the culture plate were tested to check for the attachment of the cells.
- Brush cytology sample were acquired and attempted to isolate cells for culture.

Support from CSCR: Lab space and infrastructure

Internal Collaborators:

1. Sanita Korah, Department of Ophthalmology, CMC, Vellore
2. Sanjana Shanmugam, Department of Ophthalmology, CMC, Vellore
3. Alo Sen, Department of Ophthalmology, CMC, Vellore.
4. Sanjay Kumar, CSCR

ALO SEN, MS
Assistant Professor, Department of Ophthalmology, CMC, Vellore
Adjunct Scientist, CSCR

Project Title: Standardized approach to isolate and culture - Conjunctival epithelial cells from Conjunctival specimens.
(Co-PI: Jeyanth Rose)

Funding Source: CMC Major Fluid Research Grant / CSCR Core Grant

Duration: November 2018 to October 2021

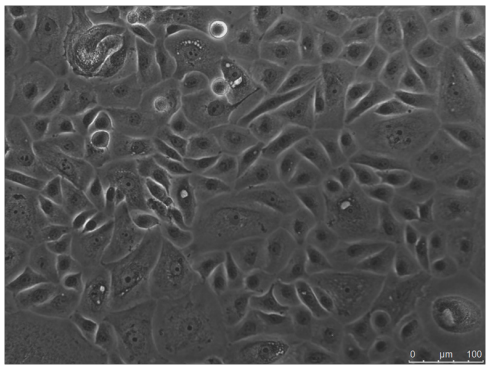
Objectives:

1. To isolate conjunctival epithelial cells from unused conjunctival specimens of patients undergoing pterygium surgery
2. To expand these cells on appropriate culture media.
3. To characterize these cells after a 2-weeks period using IHC antibodies markers - E-cadherin, CK19 and CK7 for epithelium, MUC5AC for goblet cells and Vimentin and FSP-1 for stromal cells and proliferative marker Ki67
4. To perform cell cycle analysis to identify the phase of the cell cycle.

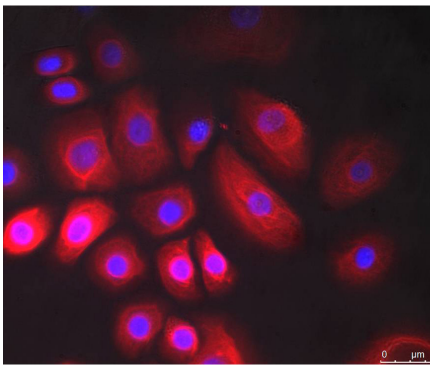
The aim of the study is to standardize the protocol for isolation and culture of conjunctival epithelium cells from the discarded conjunctival specimens of patients undergoing pterygium surgery. Briefly, the conjunctival epithelial cells from the conjunctiva of patient undergoing pterygium surgery will be isolated and cultured in 6-well plate. The cells will be characterized when confluence of cell is achieved.

Work done: Conjunctival tissue acquisition from patients undergoing pterygium surgery has been standardized.
Suitable media for culture of the conjunctival epithelial cells was tested with three different media and supplement. The samples were acquired from three participants for this standardization. After standardizing the media and supplements, samples from three participants were cultured for over 21 days up to passage 3. (Fig 1)

One sample from a conjunctiva acquired from enucleated cadaver eye was cultured up to passage 2.
One six well plate of cells was cell counted and cryopreserved during each passage. This was later revived to check for cell reviving property and to assess the yield of cells after reviving.
The culture plate coating for cell attachment was testing using 1-hour coating, 2-hour coating and overnight coating to test the best duration for maximum cell attachment. This was done during passage 1.
The cells were stained using CK19 antibody and Immunofluorescence imaging was done to characterize the cells were epithelial cells.(Fig 2)



1. Image of the cultured conjunctival epithelial cells



2. Immunofluorescence image of conjunctival epithelial cells against CK19 antibody.

Support from CSCR: Lab infrastructure and funding.

Internal Collaborators:

1. Sanita Korah, Department of Ophthalmology, CMC, Vellore
2. Sanjana Shanmugam, Department of Ophthalmology, CMC, Vellore
3. Alo Sen, Department of Ophthalmology, CMC, Vellore.
4. Sanjay Kumar, CSCR

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

PROJECT-1

Project Title: Biology of iron in RBC regeneration

Funding source: Department of Science and Technology, Govt. of India

Duration: 2020-23

Brief description of the project:

Blood transfusion is an essential therapy for various clinical conditions. Due to deficient number of blood donors and potential contamination with undetectable pathogens, it is crucial to have alternative blood sources for transfusions. Stem cells could provide a better alternative solution for blood transfusion as it has unique ability to develop into specialised cell types in the body. 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 placenta-foetal compartment. In such a scenario, enhanced iron levels are required to sustain haemoglobin synthesis. These requirements are mostly fulfilled by recycling iron through erythrophagocytosis by macrophages. Increased RBC regeneration in pregnancy occurs in a physiological manner. It involves erythropoiesis expansion causing increase in red blood cell mass and plasma volume during second and third trimester up to term. The total blood volume escalates up to 1.5 litres to support the newly developing fetus and loss recurring in delivery. Studying the behaviour of HSCs in this increased erythropoietic state of pregnancy will lead to a better understanding of RBC regeneration.

Objectives:

1. Elaborate the understanding on how iron plays important role in RBC regeneration from HSC
2. Study the differentiation of CD34 progenitor stem cells from pregnant women, PV and BT patient samples, expand them invitro into erythroid lineage, and study their behaviour.
3. Comprehensive analysis of iron homeostasis regulators in erythropoiesis during pregnancy using iron replete and iron deficient mice models.

Specific highlights of the project:

The outcome of present study would pave way for regenerating RBC from HSCs. Understanding the mechanism of accelerated RBC regeneration and iron biology in different states of erythropoiesis would provide insight into the development of exvivo experimental system for RBC regeneration.

PROJECT-2

Project Title: Elucidating the role of Mesenchymal Stem Cells, Immune and Telomere Biology in regeneration and differentiation of Haematopoietic Stem Cells

Funding source: Department of Biotechnology, Govt. of India and CMC Fluid Grant

Duration: 2019-22

Brief description of the project:

The indefinite self-renewal and potential to differentiate into other types of cells represent stem cells as frontiers of regenerative medicine. Hematopoietic stem cells (HSCs) are arguably the well-characterized tissue-specific stem cell and are in routine use clinically. Recent studies suggest that mesenchymal stem cells (MSCs) are critical for forming a niche that maintains and directs HSCs self-renewal and differentiation. Although the precise mechanisms by which MSCs exert their functions are still unclear, their immunosuppressive capacity is generally accepted. This effect appears to be largely due to suppression of T cell proliferation by inflammatory cytokines. The proliferative potential of HSCs reduces with differentiation and age in line progresses to shortening of telomeres. The propagation capacity of stem cells requires a mechanism that maintains telomere length through many cell divisions; which is carried out by telomerase enzyme complex. Insufficient telomerase function may

thrust in a quantitative deficiency decreasing the number of HSCs that maintain haematopoiesis, as well as a qualitative defect by impairing HSCs regeneration. Loss of HSCs by immunologic mechanisms is observed in aplastic anemia (AA). Certain antigens are thought to stimulate autoreactive T-cells to proliferate and attack host HSCs. Such immune responses 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. However unknown mechanisms which are involved in the loss of HSCs self-renewal and differentiation property needs to be explored

Objectives:

1. Study the mechanism of mesenchymal stem cells which provide microenvironment for HSCs, in condition of HSCs loss.
2. Investigate on the role of telomere biology in maintenance of HSCs self-renewal property.
3. Understanding the immune mechanism in aplastic anemia that leads to the loss of HSCs regeneration.
4. Outline a model by modifying the factors which will help in HSCs regeneration, from the data generated.

Work done:

MSC culture has been standardized from the bone marrow - mononuclear cells (BM-MNC) from patients with Aplastic Anemia. CD34 cells enrichment standardization was done using EasySep magnetic bead separation method from Bone marrow donor apheresis sample. Characterisation of these CD34 cells are being undertaken. We are standardizing methods for other assays and work is in progress.

Specific highlights of the project:

Destruction of HSCs by various causes lead to bone marrow failure. One such scenario is loss of HSCs by immunologic mechanisms as observed in aplastic anemia (AA). The mechanisms which are involved in the loss of HSCs self-renewal and differentiation property are not clear in AA and needs to be explored.

Support from CSCR: Lab infrastructure.

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.

Molecular Biology Core Facility

- Technical Officer: A. Rajesh
- Technical Staff: Abdul Muthallib
- Faculty support: R. V. Shaji

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 Avanti J-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.

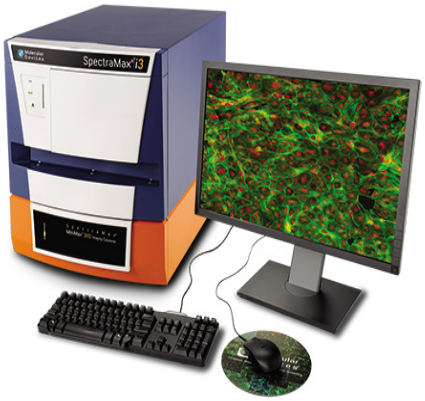
Sample Protection

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
y, 4-way sorting and single cell sorting.

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 include ELISA, nucleic acid, protein, enzymatic type homogeneous and heterogeneous assays, microbial growth, endotoxin testing, and pipettor calibration.

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 list of equipment in the TC facility are the following:

Biosafety cabinet
CO2 incubator
Refrigerated high speed centrifuge
Inverted phase contrast microscope and fluorescent microscope
Water bath
Cell counter
Storage space for individual labs
Refrigerator, -20°C freezer & Liquid Nitrogen sample storage container

c. Radioactivity Core Facility

The Radioactivity Core Facility provides researchers a secure access to radiolabelled isotopes and instrumentation for detecting radioactivity. The facility currently has Greiger counters, GE Storm 365 Phosphor image -r 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.

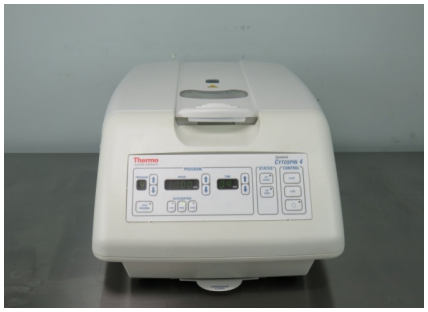
Choose between conventional, full-hand-wheel rotation, manual sectioning or “rocking mode”, where the hand-wheel is turned back and forth over a short distance. The instrument has been specially designed for the experienced user who prefers manual over motorized sectioning and meets the many requirements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

V. Cytospin:

The cytospin centrifuge gains all the advantages of the ultimate thin-layer cell preparation system with the Thermo Scientifi Cytospin 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.



Imaging Core Facility

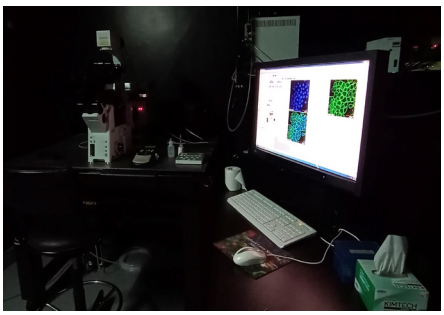
Scientific Officer: B. Sandya Rani
Technical Officer: A. Rajesh
Faculty In-Charge: Saravanabhavan Thangavel

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.

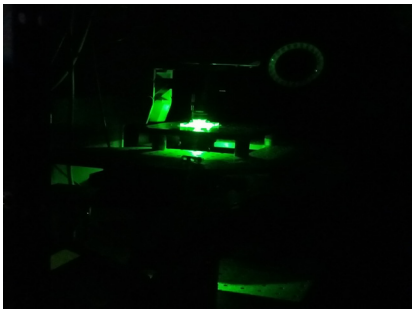
The CSCR Microscopy Core is a full-service facility serving the research community. Our aim is to provide personalized assistance on all aspects of imaging, from tips on sample preparation to training on our microscopes to processing and analysis of image data. Our facility currently houses one Multiphoton 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.

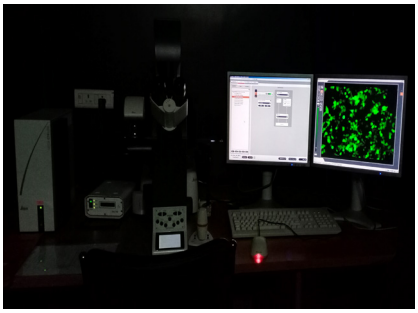


II. Laser Scanning Multi Photon Microscope (Olympus FV1000MPE)



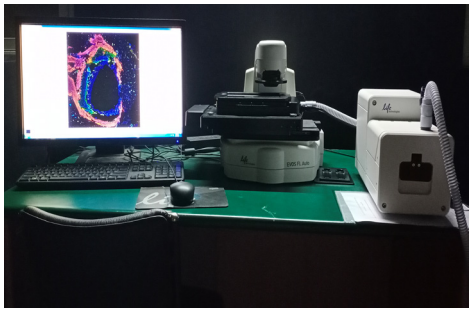
The FV1000MPE is an upright multiphoton laser-scanning microscope coupled with a Mai Tai HP-Deep See–OL laser with automated broadband wavelength tuning from 690 to 1040nm for deep tissue imaging.
The systems supports multicolor fluorescent studies for imaging of living, whole mount or thickly sliced specimen. Dynamic biological processes can be imaged hundreds of micrometers within living cells and tissues. Provides support for applications where phototoxicity/photobleaching are 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



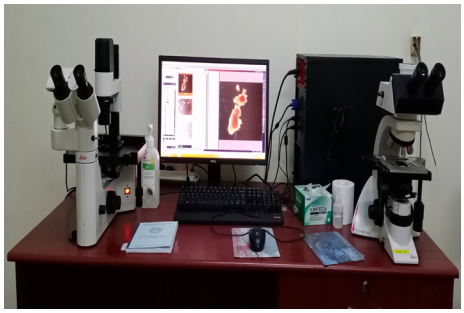
The Leica DMI6000B is an inverted fluorescence microscope comprising of 6 interchangeable filters for detecting various fluorochromes. It has two independent cameras – DFC295 for high resolution bright-field imaging and DFC360 FX for high frame rate fluorescence imaging. It is also equipped with a fluorescence 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 and Olympus. In the current year, 2019-2020, a total of 40 users have been trained in Brightfield and fluorescence microscopes.

Flow Cytometry Core Facility

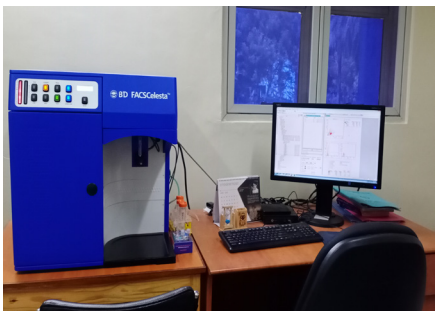
Scientific Officer: B. Sandya Rani
Technical Officer: A. Rajesh
Technical Staff: T. Abdul Muthallib, Immani Job
Faculty Support: Sanjay Kumar

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:

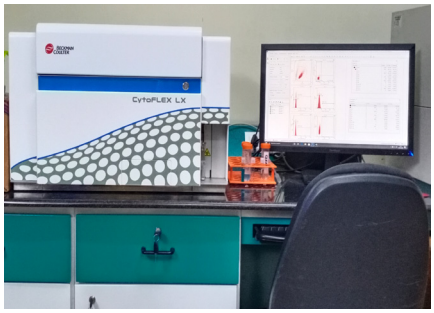
The BD FACSAria III flow cytometer is a high-speed fixed-alignment benchtop Cell sorter. With its fixed-optics design and digital electronics, the BD FACSAria III flow cytometer measures up to 11 colors simultaneously and supports a wide range of applications in immunology, genomics, cancer, and stem cell research. A patented flow cell with a gel-coupled cuvette and patented octagon and trigon detection system allow the system to achieve unrivaled sensitivity and resolution. BD FACSAria III cell sorter with a five laser (Near UV-375nm, Violet-405nm, Blue-488nm, Yellow-Green-561nm, Red-633nm) and 11 color setup has a throughput of 70,000 events per second and can do one-way, two-way, three-way, 4-way sorting, and single-cell sorting.

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 setup 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.

Laboratory Animal Facility

- Veterinary Officer: R.Vigneshwar
- Technical Staff: R. Pavithra, J. Esther Rani & S. Ashok Kumar
- Faculty support: Srujan Kumar & Sanjay Kumar

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, and 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 lab animals are breathing HEPA-filtered air (High Efficiency-Particulate Air) that defends them from most of airborne micro-organisms.

Individually Ventilated Cages (IVC)

Cage changing station

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 CPCSEA guidelines.

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 are 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, and IACUC approved techniques for anaesthesia, drug administrations, blood collection, humane euthanasia etc.

Specialized equipments

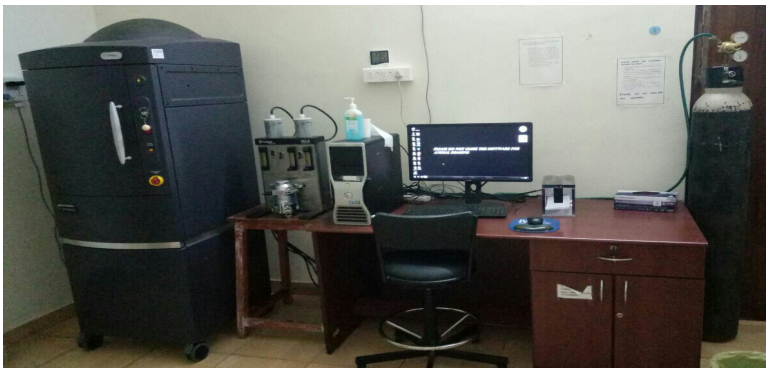
The CSCR-LAF is equipped with In vivo small animal imaging system, Multi photon microscope and Small animal irradiator with Co-60 as source in addition to a couple of Isoflurane anesthesia machines, induction and heating chambers, animal blood counter and zoom stereo microscopes.

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.

Blood irradiator (BI 2000)

Irradiation of blood & blood products by gamma rays is a proven and safe method to inhibit T-lymphocyte proliferation and eliminate the risk of post transfusion graft versus host disease (T-GVHD). We are using the same instrument to irradiate animals 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. Inspection by the radio safety officer from the Atomic Energy Regulatory Board (AERB), Radiological Safety Division, Government of India was held on 16.12.2019 and all the safety measures were found to be satisfactory.



In vivo small animal imaging system (IVIS CT spectrum)

Rodent isofulrane anaesthesia system

Animal blood counter

Blood irradiator (BI 2000)

Leica zoom stereo microscope

Stereo Microscope

Stereo microscope is an optical microscope variant designed for low magnification observation of a sample, typically using light reflected from the surface of an object rather than transmitted through it. This arrangement produces a three-dimensional visualization of the sample being examined. We are using this instrument for dissection and organ collection in rat and mice embryos.

Strains available

The CSCR-LAF maintains eleven different strains of mice - including normal, transgenic, knock out and SCID strains and SD rats. The majority of rodent strains are bred under strictly inbred conditions. Two new mice strains, NBSGW and Sickle mice were imported from the Jackson Laboratory, US on 05.12.2019. The animals were quarantined for a period of 30 days and health monitoring was carried out, all the animals were free from pathogens. They have been included in our breeding colony and breeding was initiated after getting the clearance from the Animal Quarantine and Certification Services (AQCS), Chennai.

S.No	Strain	Description	Disease Model	Source
1	C57BL/6J	Inbred strain	Multi- Purpose model	Jax Lab, US
2	BALB c/J mice	Inbred strain	Cancer biology and toxicology studies	Jax Lab, US
3	FVB/NCrl mice	Inbred strain	Chronic myeloid leukemia model	Charles River, UK
4	CD-1	Out bred strain	Hemophilia B Gene therapy studies	Charles River, UK
5	B6.129S4-F8tm1Kaz/J	Knock-out strain	Double immunodeficient Transplantation studies	Jax Lab, US
6	B6.129P2-F9tm1Dws/J	Knock-out strain	Hemophilia B Gene therapy studies	Jax Lab, US
7	B6.CB17-Prkdcscid/SzJ	B6 SCID	Double immunodeficient Transplantation studies	Jax Lab, US
8	C.B-17/Icr-Prkdc<Scid>Ic-rIcoCrl	FOX-Chase SCID	Double immunodeficient Immunology and xeno graft research	Charles River, UK
9	NOD.CgPrkdcscid Il2rgtm1Wjl/SzJ	NSG SCID	Triple immunodeficient strain; Immunology & Transplantation studies	Jax Lab, US
10	NOD.Cg-Kit<W-41J> Tyr<+> Prkdc<scid> Il2rg<tm1Wjl>/ThomJ	NBSGW SCID	Triple immunodeficient strain; Transplantation studies	Jax Lab, US
11	B6;129Hbb<tm2 (HBG1,HBB*)Tow>/ Hbb <tm3(HBG1,HBB) Tow>Hba<tm1	Sickle mice	Sickle cell anaemia Gene therapy studies	Jax Lab, US
12	Sprague Dawley	Rat-Outbred strain	Surgical models Orthopaedic research	Jax Lab, US

Quality control (QC): A quality control program for environmental microbiology, clinical pathology, genetic analysis is being implemented for monitoring the health 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 animals 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 in every month.

ELISA based Microbiology

Furthermore randomly selected serum samples are screened microbiologically by ELISA-based kits for selected rodent pathogens such as Mouse Pneumonia virus, Lymphocytic Choriomeningitis virus, Mouse Noro virus, Mouse Hepatitis virus, Hanta virus, Sendai virus, Adeno virus (FL/K87), rat corona virus, Kilham rat virus, Mycoplasma pulmonis and Cilia Associated Respiratory Bacillus (CARB).

Genetic Monitoring

Genetic monitoring (Genotyping) of mutant and SCID strains is conducted periodically by using molecular biology techniques such as standard PCR, RT-PCR and gene sequencing.

Training sessions:

First "Laboratory animal handling and bio-methodologies - A hands on orientation", six days training programme was held from 14.10.19 to 19.10.19. Research scholars of the CSCR from different laboratories were participated. They were trained for animal handling, blood collection, anesthesia, tail vein injection, euthanasia, dissection and organ collection. Theory sessions have been conducted on rodent biology, bio-methodologies, animal ethics and CPCSEA/IAEC guidelines.

Second "workshop on the use of laboratory animals in biomedical research" for the research scholars and junior faculty of CMC, in collaboration with the Institutional Animal Ethics Committee (IAEC) of Christian Medical College, has been conducted on 12.07.2019 to 13.07.2019.

Projects in progress

S. No.	Project title	Name of PI
1	Personalizing conditioning regimen in HSC transplantation- Animal study to evaluate the damage to endothelial cells and to test the effect of inhibitors in reversing the effect	Dr. Poonkuzhali
2	Identification of novel nuclear receptor drug targets in myeloid leukemia (To identify the effect of selected NHR ligands in modulating drug resistance in AML mouse model)	Dr. Poonkuzhali
3	Pre-clinical studies for the gene therapy for Wiskott-Aldrich Syndrome (WAS)	Dr.Saravanabhavan
4	To study the effect of shock wave on rat metatarsal organ culture	Dr. Vrisha Madhuri
5	Muscle derived stem cells in the treatment of anal sphincter injury in rat model – an interventional study	Dr. Vrisha Madhuri
6	Lipid nanocarrier guided chemically modified factor IX messenger RNA therapy for Hemophilia B	Dr. Sanjay Kumar
7	Identification of novel nuclear receptor drug targets in myeloid leukemia (To identify the effect of selected NHR ligands in modulating drug resistance in CML mouse model)	Dr. Poonkuzhali
8	Pre-clinical model for gene therapy for Thalassemia and Sickle Cell disease	Dr. Mohan kumar
9	Liver targeting lipid nanocarrier guided No-end DNA gene therapy for haemophilia A and B	Dr. Srujan Kumar
10	In vivo efficacy and safety studies of CSCR-ST04, the gene-edited autologous hematopoietic stem cells, for the gene therapy of □-hemoglobinopathies	Dr. Saravanabhavan

Future direction: In the near future, apart from continuing our current activities, our goal is to accredite our facility with Good Laboratory Practices (GLP) for the pre-clinical toxicity studies and implements the OECD guidelines. Applications have been filed to the Department of Science Technology (DST), and Central Drugs Standard Control Organization (CDSCO), Government of India.

Current Good Manufacturing Practics (cGMP) Facility

- Technical Officer: Augustine Thambaiah
- Technician: Aleya Tabasum
- Faculty support: Gurbind Singh & Alok Srivastava

About the facility

The facility is designed to develop and manufacture cellular and tissue engineered products for clinical applications. It provides the infrastructure for large scale expansion of stem cells required to conduct Phase I/II clinical trials in the fields of cell therapy and regenerative medicine.

GMP facility has total area of 1200 square feet. The clean room area is divided into four independent ISO Class 7 (Class 10,000) manufacturing suites and one common staging room. Each manufacturing suite is fitted with active pass boxes. The facility maintains separate unidirectional flow for personnel and materials. Each suite is equipped with biological safety cabinet, CO2 incubators, refrigerated high speed centrifuge and inverted phase contrast microscope. Staging room is equipped with control rate freezer for cryopreservation of the cellular product. Facility also have a cryopreservation room with high capacity liquid nitrogen container for storage of cryovials and cryobags in vapour phase.

The trained staff, directly interact with investigators and help in process development and manufacturing of clinical grade products for use in early phase clinical trials. The facility was inspected by authorities from CDSCO, New Delhi and the Office of Director of Drug Control, Tamil Nadu. In 2019, facility was granted license in Form 29, for the manufacturing of "Autologous culture expanded iliac crest physeal chondrocytes" for a Phase I/II clinical trial in 15 patients.

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 QC checks for door pressure, temperature, humidity.
- Liquid nitrogen monitoring and scheduled filling of the storage tanks

Services

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

- Provides clean-room suites for manufacture of clinical grade products under cGMP conditions for clinical applications
- Cryopreservation and storage of cell therapy products
- Bacterial 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 PI's clinical trial or study.

Current scientific activities

The cGMP facility is currently involved in the derivation, expansion and banking of clinical grade iPSC lines by using 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 molecularly characterized and cryopreserved for future studies.

The cGMP facility is also involved with the following research projects:

1. Gamma delta T cell-based immunotherapy for blood cancers. The protocol for the culture and expansion of gamma delta T cells from peripheral blood mononuclear cells in both serum and serum free condition has been established. (Centre for Stem Cell Research, CMC Campus and Department of Haematology, CMC)
2. Establishing a protocol for expansion of Natural Killer cells (Centre for Stem Cell Research, CMC Campus and Department of Haematology, CMC)

Training and Acces

GMP staff are trained in carrying out product development and manufacturing of various clinical grade cellular products. Access to the facility is limited only to GMP trained staff. The services are available for investigators from Christian Medical College, Vellore and other non-profit organizations.

FOURTH ANNUAL SYMPOSIUM ON CELL AND GENE THERAPY
5 & 6 SEPTEMBER, 2019

The Centre for Stem Cell Research (CSCR), a unit of inStem, Bengaluru, managed by Christian Medical College (CMC), Vellore organized the 4th Annual Symposium on Cell and Gene Therapy on 5th & 6th September, 2019. This symposium brought together scientists, physicians and all others interested in and responsible for developing this field in the country. The symposium was supported by the Department of Biotechnology (DBT), Govt. of India and Science and Engineering Research Board (SERB), Govt. of India. Dr. Renu Swarup, Secretary, DBT addressed the participants through video link.

The program this year focussed on immune cell therapy, cell and gene therapy in ocular disorders, gene editing, GMP manufacturing and technology advances in cell and gene therapy and applications of iPSC technology. About 150 participants from across the country and 22 speakers from around the world took part in the symposium.

The first day of the symposium focussed on immune cell therapy, applications of iPSC technology and technology advances. The industry participants also made a presentation on the advanced technologies that have been developed for 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 Prof. David Gottlieb from Westmead Hospital, University of Sydney, Australia. He discussed the development immune cell therapy in the world and delivered the keynote address titled 'Evolution of T- cell therapy for infections' in the context of hematopoietic stem cell transplantation.

The second day of the symposium had various discussions on gene therapy in haemophilia and muscular dystrophy including gene editing approaches towards developing a treatment of beta-hemoglobinopathies. There was also a session on cell therapy in ocular disorders. The symposium was much appreciated by all participants as a unique 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. University of Florida College of Medicine, Florida, USA
2. Emory University School of Medicine, Atlanta, USA
3. Children's Hospital Oakland Research Institute, USA
4. University of Cincinnati, USA
5. Seattle Children's Research Institute, USA
6. Westmead Hospital Sydney, Australia
7. Free University of Brussels & University of Leuven, Belgium
8. International Stem Cell Banking Initiative, UK
9. Global Alliance for iPSC Therapies, UK
10. Karolinska Institutet and Karolinska University Hospital, Sweden
11. Thermo Fisher Scientific, Switzerlan

National:

1. ACTREC, Tata Memorial Cancer Centre, Mumbai
2. Adyar Cancer Institute, Chennai
3. Armed Forces Medical College, Pune
4. Centre for Cellular & Molecular Biology (CCMB), Hyderabad
5. Cellsure Biotech & Research Centre, Thane
6. Centre for Stem Cell Research, Vellore
7. Christian Medical College, Ludhiana
8. Christian Medical College, Vellore
9. CSIR-Indian Institute of Chemical Technology (IICT), Hyderabad
10. CSIR-Institute of Genomics and Integrative Biology, New Delhi
11. Dr. Reddy's Laboratories Ltd., Hyderabad
12. Eyestem Research Private Limited, Bengaluru
13. Immuneel Therapeutics, Bengaluru
14. Institute for Stem Cell Science and Regenerative Medicine, India
15. Indian Institute of Technology Bombay, Mumbai
16. Indian Institute of Technology Delhi, New Delhi
17. Institute of Liver and Biliary Sciences, Delhi
18. Intas Pharmaceuticals, Ahmedabad
19. Kakatiya Institute of Technology and Science, Warangal
20. L. V. Prasad Eye Institute, Hyderabad
21. Manipal Institute of Regenerative Medicine, Bengaluru
22. National Centre for Biological Sciences (NCBS), Bengaluru
23. Narayana Health, Bengaluru
24. Narayana Netralaya, Bengaluru
25. Sahyadri Hospital, Pune
26. Sathyabama Institute of Science and Technology, Chennai
27. Sri Ramachandra Medical College and Research Institute, Chennai
28. Stempeutics Research Pvt. Ltd. Bengaluru
29. Strand Life Sciences, Bengaluru
30. Tata Medical Centre, Kolkata
31. Terumo Penpol Private Limited, Trivandrum

The 5th Annual Symposium on Cell and Gene Therapy (Virtual) is scheduled on 3 & 4 September, 2020.

Education and Training

EDUCATION AND 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	Duration	Qualification	University	PI /Lab
1	Mr. Annamalai. N	Jan - Jun 2020	MSc - Biochem	PSG College of arts & science, Coimbatore	Dr. Sanjay / Lab-3
2	Mr. Aravind M	Jan - Jun 2020	MBBS	MGR University, Chennai	Dr. Sanjay / Lab-3
3	Ms. Akshaya C	Jan - Jun 2020	MSc - Biotech	VIT University	Dr. Sunil / Lab-6
4	Mr. Rajalingam R	Jan - Jun 2020	B.Tech - Biotech	Anna University	Dr. Sunil / Lab-6
5	Ms. Umesh R	Jan - Jun 2020	MTech - Biopharma. Tech.	Anna University	Dr. Saravana / Lab-7
6	Ms. Dhakshanya P	Jan - Jun 2020	MTech - Biotech	Rajalakshmi Engineering College, Chennai	Dr. Saravana / Lab-7
7	Ms. Elamathi K	Jan - Jun 2020	MSc - Life Sciences	Bharathidasan University	Dr. Saravana / Lab-7
8	Ms. Kanimozhi S	Jan - Jun 2020	MTech - Biotech	Periyar Maniammai University, Thanjavur	Dr. Srujan / Lab-8
9	Ms. Yoga Priya M	Jan - Jun 2020	MTech - Biotech	Periyar Maniammai University, Thanjavur	Dr. Srujan / Lab-8
10	Ms. Ragavi R	Jan - Jun 2020	BTech - Biotech	VIT University	Dr. Mohan / Lab-9

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 Marpally	Scientist
Dr. Sanjay Kumar	Scientist
Dr. Sunil Martin	Scientist
Dr. Vrisha Madhuri	Adjunct Scientist
Dr. R. V. Shaji	Adjunct Scientist
Dr. Aby Abraham	Adjunct Scientist
Dr. Asha Mary Abraham	Adjunct Scientist
Dr. Christunesa Christudass	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
Dr. Sandya Rani	Scientific Officer
Dr. Vigneshwar R.	Veterinary Officer
Mr. Augustine Thambaiah	Technical Officer
Mr. Rajesh A.	Technical Officer
Dr. Gurbind Singh	Scientist (NAHD - Haplobanking)
Dr. Sonam Pandey	Scientific Program Manager (NAHD Program)
Dr. Chinmayee Panda	Project Coordinator (NAHD-Thalassemia and SCD Program, Odisha)
Mr. Brundaban Sahoo	Project Coordinator (NAHD-Thalassemia and SCD Program, Odisha)
Dr. Midhun Rajiv	Project Coordinator (NAHD-Thalassemia and SCD Program, Odisha)
Mr. Sangram Keshari Sarangi	Technical Officer (Training & Counselling)
Ms. Gomathi Technical Officer	Technical Officer (Data Management)
Ms. Sarika Nayak	Technical Officer(Laboratory)
Dr. VasanthThomodaran	Post Doctoral Fellow
Dr. Lakshmi	Scientist B
Dr. Venkatesh	Research Associate
Ms. Sowmya R.	Senior Research Fellow
Mr. Abhirup B.	Senior Research Fellow
Ms. Smitha I.	Senior Research Fellow
Ms. Abisha Crystal	Senior Research Fellow
Ms. Kritika Nandi	Senior Research Fellow
Ms. Aneesha Nath	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. Balasubramanian S.	Senior Research Fellow
Mr. Ashis Kumar	Senior Research Fellow
Mr. Karthikeyan R	Senior Research Fellow
Ms. Dhivya Bharathi	Senior Research Fellow
Mr. Vigneswaran V	Senior Research Fellow
Mrs. Renita Raymond	Senior Research Nurse
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Mr. Karthik V.K	Junior Research Fellow
Mr. Vignesh R	Junior Research Fellow
Ms. Prathiba Babu	Junior Research Fellow
Mr. Manoj Kumar	Junior Research Fellow
Ms. Anila George	Junior Research Fellow
Ms. Kriti Prasad	Junior Research Fellow
Ms. Sevanthy	Junior Research Fellow
Mrs. Agnes Selina	Junior Research Fellow
Ms. Nivedhitha D	Junior Research Fellow
Ms. Porkizhi Arujunan	Junior Research Fellow

Mr. Ajay Kumar Dhyani	Junior Research Fellow
Ms. Aruna	Junior Research Fellow
Mr. Muthuganesh	Junior Research Fellow
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	Gr 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 Gr Technician
Ms. Mohana Priya	Graduate Technician
Mr. Rashmi Rajan Swain	Graduate Technician (NAHD-Thalassemia and SCD Program, Odisha)
Mr. Ratipragya Das	Graduate Technician (NAHD-Thalassemia and SCD Program, Odisha)
Mr. Solomon Ekka	Graduate Technician (NAHD-Thalassemia and SCD Program, Odisha)
Mr. Debaprasad Pattanaik	District Coordinator (NAHD-Thalassemia and SCD Program, Odisha)
Mr. Panchanan Pasayat	District Coordinator (NAHD-Thalassemia and SCD Program, Odisha)
Mr. Prabodha Sundaray	District Coordinator (NAHD-Thalassemia and SCD Program, Odisha)
Ms Monalisa Das	District Coordinator (NAHD-Thalassemia and SCD Program, Odisha)
Mr Utkal Debasis	District Coordinator (NAHD-Thalassemia and SCD Program, Odisha)

Admin, Finance and Support Staff

Mrs. Anupama Nambiar	Assistant Manager
Mrs. Shirley Anandanathan	Secretary
Mrs. Selvi P.	Clerk Typist
Mr. Muthukrishnan J.	Multi-tasking personnel
Mr. Tamil Vanan J.	Librarian
Mrs. Geetha R.	Accountant
Mr Simal Tudu	Accountant (NAHD-Thalassemia and SCD Program, Odisha)
Mr. Silambarasan	Driver
Mr. Nithyanand	Attendant
Mr. Arun Kumar	Attendant
Mr. Ramraj	Attendant
Mr. Shankar	Attendant
Mr. Augustin Vasanthakumar	Attendant
Mr. Vikas	Attendant
Mr. Vijay	Attendant
Mr. Sakthivel	Housekeeping Attendant
Mrs. Renuka Devi	Housekeeping Attendant

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»»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, THSTI*	Member
»»External experts	*Dr. Soniya Nityanand, SGPGI, Lucknow;* *Dr. Dinakar Salunke, ICGEB, New Delhi;* *Dr. BS Ramakrishna, SIMS Hospital Chennai* *Dr. Jyotsna Dhawan, CCMB, Hyderabad*	Member(s)
»»Scientist nominated by Chairperson	Dr. Mammen Chandy	Member
»»Head, Admin and Finance inStem	Shri Pawan Kumar Pahwa	Non Member Secretary

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»»Dr. Nihal Thomas, Department of Endocrinology	Member
»»Dr. Asha Mary Abraham, Department of Virology	Member
»»Dr. Alok Srivastava, Head, CSCR	Member Secretary

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