CSCR

CENTRE FOR STEM CELL RESEARCH

(a unit of inStem, Bengaluru)

Christian Medical College Campus, Bagayam, Vellore

ANNUAL REPORT

2018-19

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

CONTENTS

Introduction

Core scientific activities and initiatives

Scientific Research Profile

Thematic Research Programs

Musculoskeletal regeneration program

»» Vrisha Madhuri
»» Elizabeth Vinod

Gene therapy program

»» Alok Srivastava
»» R. V. Shaji
»» Saravanabhavan Thangavel
»» Mohankumar Murugesan
»» Srujan Kumar Marepally
»» Asha Mary Abraham

Cellular reprogramming and its applications

»» R. V. Shaji
»» Dolly Daniel
»» Alok Srivastava

Research Projects

»» Aby Abraham
»» Poonkuzhali Balasubramanian
»» Geeta Chacko
»» Christhunesa Christudass
»» Sanjay Kumar
»» Sunil Martin
»» Muthuraman N.
»» Jeyanth Rose
»» Alo Sen

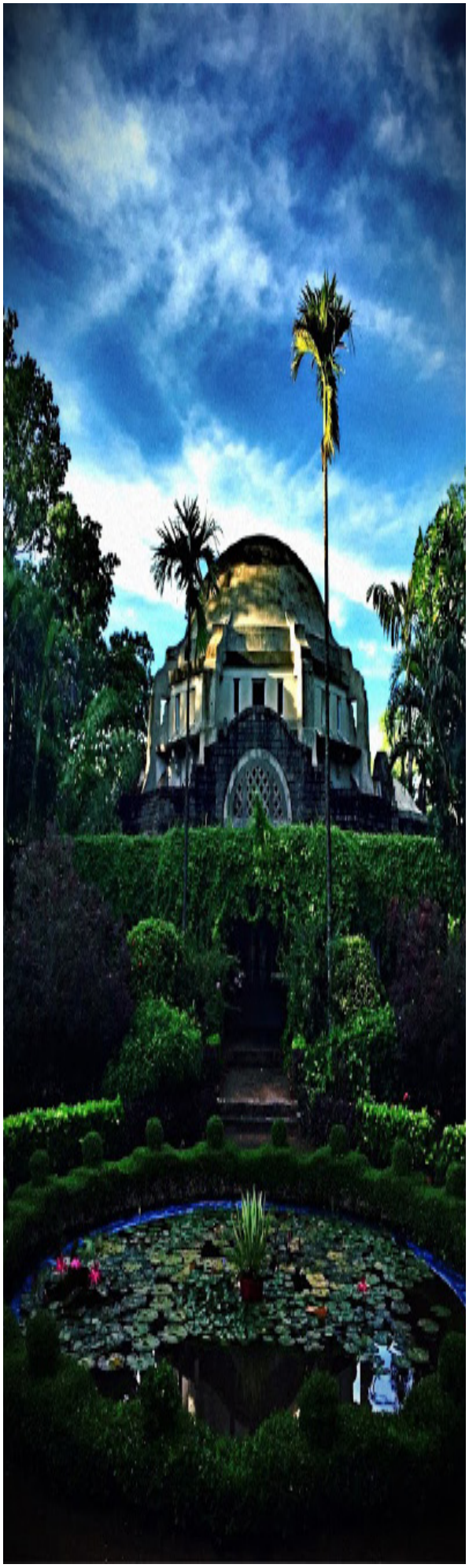
Core Facilities and Instrumentation

Annual Cell & Gene Therapy Symposium

Education & Training

Personnel at CSCR

Governance of CSCR



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.

1. Boost to Brittle Bones (BOOST2B): This phase I/II trial aims to evaluate the safety and efficacy of intravenous and intraosseous infusion of allogeneic expanded fetal mesenchymal stem cells for the treatment of severe Osteogenesis Imperfecta. We are in the process of recruiting patients for the trial (n=15).

2. Physeal regeneration: This phase I trial aims to evaluate the safety of autologous iliac crest physeal chondrocytes to treat physeal bars in children. We are in the process of recruiting patients for the trial (n=15).

3. Genetic Heterogeneity in patients with OI: Genetic profiling to screen 150 children with OI by NGS for all known genes (targeted sequencing will be performed using Illumina).

4. Musculoskeletal stem cell targeting (MUSTER):
   1. Muscle-derived stem cells in the treatment of anal sphincter injury in a rat model – We have isolated quiescent satellite cells using a single cell surface marker and established rat anal sphincter injury model.
   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).

5. Effect of shock wave treatment on growth plate cartilage: A novel model of growth retardation in rat metatarsal bone was established using a Gli1 inhibitor. Shockwave treatment may counteract the effect of inhibitor-induced growth retardation.

6. Bone regeneration: We have completed 2-4 years follow up in all 10 children treated with hydroxyapatite loaded with MSC. The response is satisfactory in an infected nonunion while the results cannot be extrapolated to bone diseases with underlying genetic conditions.

7. Differentiation of mesenchymal stem cells into chondrocytes by sustained delivery of miRNAs using chitosan hydrogel: A combination of miRNAs was effective in 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.

Ongoing studies & Funding

Project 1: Transplantation of autologous iliac crest physeal chondrocytes cultured in monolayer to treat Physeal bars in children
Funding agency: DHR
Budget: Rs. 74.93 lakhs

Project 2: BOOST2B (Indo-Swedish)
Funding agency: DBT
Budget: Rs. 480.024 lakhs

Project 3: MUSTER (Indo-Danish)
Funding agency: DBT
Budget: 99.77 lakhs

Project 4: Molecular genetic analysis of Osteogenesis imperfecta in Indian Children
Funding agency: ICMR
Budget: Rs. 69 lakhs

Project 5: In vivo effect of shockwave on rabbit growth plate
Funding agency: IRB/CSCR
Budget: Rs. 10.00 lakhs

Completed project:

Title: Differentiation of MSCs into chondrocytes by sustained delivery of miRNAs using chitosan hydrogel.
Funding agency: DST
Budget: Rs. 74 lakhs

Honors and awards:

1. Sowmya Ramesh - Travel Grant - 57th Annual European Society for Paediatric Endocrinology Meeting, Greece.
2. Sowmya Ramesh received the Svenska frimurare (Frimurare Barnhus Foundation) scholarship from the Queen Silvia of Sweden, October 10, 2018.
3. Sowmya Ramesh- Elected as Treasurer for the Tissue Engineering and Regenerative Medicine Society-Asia Pacific region (TERMIS-AP) society - SYIS.
4. Karthikeyan Rajagopal – 3rd place oral - A novel protocol to produce articular cartilage in a sustained delivery system using RNAi technology - Annual Research Day (Ph.D. Specialty).
5. Sowmya Ramesh - 1st place oral - Efficacy of a novel cell-laden dressing in the topical treatment of partial-thickness burns in a rat model- Annual Research Day (PG Surgical Specialty).
6. Ashis Kumar - Selected on merit basis for the Whole Genome Sequencing workshop organized by the Accelerator program for Discovery in Brain disorders using Stem cells-Institute of Bioinformatics and Applied Biotechnology (IBAB), Bengaluru, December 2018.
7. Dr. Vrisha Madhuri- Appointed Member 4th Programme Advisory Committee IMPRINT2 (Impacting Research Innovation and Technology Version 2), SERB, DST
8. Dr. Vrisha Madhuri - Elected National Delegate for APPOS from April 2019.
9. Dr. Vrisha Madhuri - Appointed as a member of the Reconstituted Task force of Bioengineering DBT.
10. Dr. Vrisha Madhuri - Appointed as a member of DBT Biomedical engineering and Biodesign Technical Expert Committee October 2018.

Publication:

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

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

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

Brief description of the project:

Articular cartilage is an avascular tissue with low potential for self-repair. Cell based therapeutics aim to produce tissue that closely mimic the mechanical and biochemical properties of native cartilage. Recently, the use of articular cartilage derived CP (classified MSCs) have gained popularity in its potential role for cartilage repair. Key features of these multipotent progenitors include differential adhesion to fibronectin, high replicative potential with maintained potency, ability to form large colonies from a small seeding density and importantly primed chondrogenic potential by nature. Their positive immunomodulatory properties make them amenable to allograft strategy. The use of 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 principal components of growth factors such as TGFβ known to stimulate cellular anabolism, possess anti-inflammatory properties and interact with fibrinogen contributing to its scaffolding effect.

In this study, we aim to isolate chondroprogenitor cells from rabbit knee articular cartilage, culture and characterize them, to create an allogeneic bank. Monosodium Iodoacetate induced early grade osteoarthritis will be created in bilateral hind limbs, following which labelled-chondroprogenitors with platelet rich plasma will be injected intra-articularly. Similarly, osteochondral defects will be created in the trochlear groove of both knees and labelled CPs resuspended in appropriate volume of PRP will be delivered into the defect. At the end of 6 and 12 weeks, healing in both knees will be assessed by synovial fluid analysis, immunohistochemistry studies and histologically using OARSI/ Wakitani scoring

We hypothesize that the regenerative potential of chondroprogenitor cells with its restrictive differential potential, combined with the ability of PRP to modulate proliferation and provide essential growth factors crucial for its survival will work in synergy towards achieving functional cartilage.

Work done: Cell culture and animal interventions have been completed. Knee joints have been harvested for processing. Blinded histological grading and data analysis are in progress.

PROJECT-2

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 - 2021

Brief description of the project:

Cell based therapy optimization is constantly underway since regeneration of genuine hyaline cartilage is under par. Extensive work on chondrocytes has afforded valuable information to their use in cartilage repair, although questions pertaining to their behavior in culture remain unanswered. Although single source derivation of chondrocytes and chondroprogenitors is advantageous, lack of a characteristic differentiating marker between chondrocytes and chondroprogenitors obscures clear identification of either cell type which is essential to create a biological profile

and is also required to assess cell type superiority for cartilage repair. Our previous study was the first attempt, where characterization was performed on the two cell populations derived from the same human articular cartilage samples using flow cytometry, gene expression studies were done using RT-PCR, growth kinetics and tri-lineage differentiation were also studied. Our results suggest that sorting chondroprogenitors based on a combination of surface markers instead of isolation using fibronectin adhesion assay would yield a population of cells primarily composed of chondroprogenitors.

In the present study, based on the obtained knowledge we aim to isolate and culture chondrocytes and chondroprogenitors (fibronectin adhesion assay) cells from normal articular cartilage of human knee joints and characterize them by FACS. P1 chondrocytes will be sorted using a combination of CD markers to obtain chondroprogenitors. The chondrogenic potential of cell sorted chondroprogenitors, fibronectin assay derived chondroprogenitors and chondrocytes will be compared using RT PCR studies for Chondrogenic and hypertrophic markers, FACS for markers of enhanced chondrogenesis, positive and negative MSC markers. Trilineage differentiation and staining will also be done.

Results obtained will provide us comparative information about the cell population with higher chondrogenic potential, thus translatable results in terms of enhanced chondrogenesis and reduced hypertrophy; both indispensable for the field of cartilage regeneration

Publications:

1. 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.

2. Elizabeth Vinod, Deepak Vinod Francis, Tripti Jacob, Soosai Manickam Amirtham, Solomon Sathishkumar, Praghalathan Kanthakumar, and Vinay Timothy Oommen. Autologous Platelet Rich Fibrin as a Scaffold for Chondrocyte Culture and Transplantation: An in Vitro Bovine Study. Journal of Clinical Orthopaedics and Trauma. 2019.

3. Elizabeth Vinod, Upasana Kachroo, Ozlem Ozbey, Solomon Sathishkumar, and P. R. J. V. C. Boopalan. Comparison of Human Articular Chondrocyte and Chondroprogenitor Cocultures and Monocultures: To Assess Chondrogenic Potential and Markers of Hypertrophy. Tissue Cell. 2019; 57: 42–48.

Support from CSCR: Funding support, lab space and core lab facilities

External Collaborations:

Ozlem Ozbey, Akdeniz University, Antalya, Turkey
Sabareeswaran Arumugam, , Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram
Anjali Goyal, Smt NHL Municipal Medical College, Ahmedabad

Internal Collaborations:

Soosai Amirtham Manickam, Department of Physiology, CMC, Vellore
Upasana Kachroo, Department of Physiology, CMC, Vellore
P.R.J.V.C Boopalan, Department of Orthopaedics, CMC, Vellore
Solomon Sathishkumar, Department of Physiology, CMC, Vellore

ALOK SRIVASTAVA, MD, FRACP, FRCPA, FRCP

Professor, Department of Haematology, CMC, Vellore

Adjunct Scientist / Head, CSCR

Scientific Areas of Research

There major focus during 2018-19 has been the gene therapy program for haemophilia and the major haemoglobin disorders. The field outreach program for the control of thalassemia and sickle cell disease in Odisha has also been launched. I also continue to support the on-going workrelated to the development of assays for AAV antibodies as well as the program on banking of iPSCs from HLA haplotype identical individuals. The details are outlined below:

A. The gene therapy program

1. CLINICAL TRIAL FOR GENE THERAPY OF HEMOPHILIA B

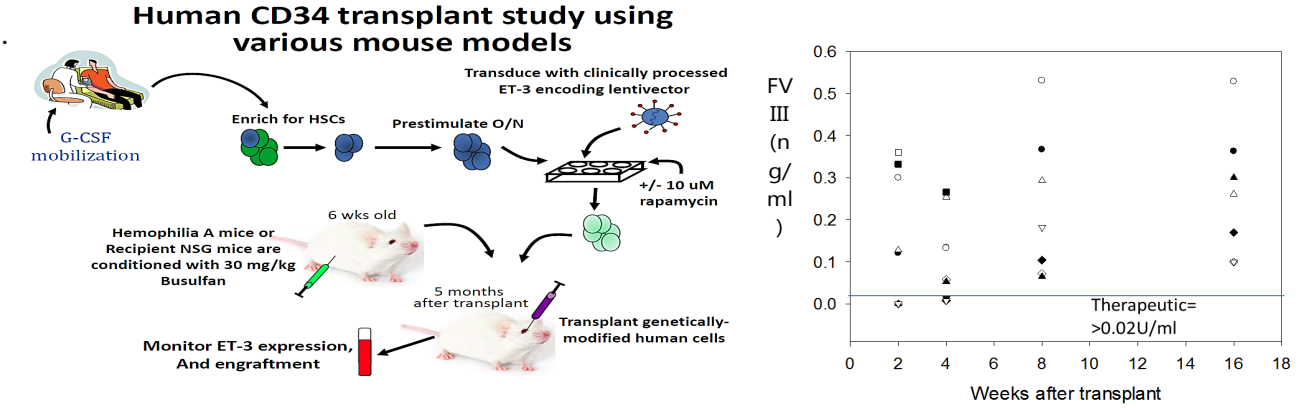
After generation of a suitably efficient transgene and packaging it in the AAV3 vector which was tested to two mouse models for in vivo efficacy, as also reported last year, there was a setback in the plans for this clinical trial as the Powel Gene Therapy Center (PGTC) at the University of Florida at Gainesville, Florida, USA was unable to produce the vector at the right titers to allow for a cost effective production of enough quantity of the vector for the IND enabling proposed non-human primate studies and the subsequent clinical trial.

Since then we have explored the option of several other sites (academic and contract manufacturing organizations) for getting this AAV production done. Two challenges have not made this possible - 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 - lack of capacity within existing GMP facilities for production of the required products) while 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 million for the requirements of this trial.

We have a in principle agreement from the GMP facility at the Cincinnati Medical Center which also has considerable experience in producing vectors for gene therapy trials, of making this vector for us by the end of 2019. If that happens, we will be able to proceed with the clinical trial plans after testing it in the NHP models at the Emory University. This is certainly a disappointment but we are hopeful that we will be able to proceed with the clinical trial later this year / early next year in 2020 with this very promising transgene and vector.

2. LENTI VIRAL VECTOR GENE THERAPY FOR HEMOPHILIA A

Apart from this, a novel lentiviral vector-based gene therapy for haemophilia A has also been developed over the last 2 years in collaboration with the scientists at the Emory University, Atlanta, USA (AS). (Hum Gene Ther. 2018, 29:1183-1201 - see figure below) A proposal for a phase 1 clinical trial has been reviewed by the CDSCO and has received technical clearance. Certain regulatory issues are to be resolved. These are being addressed and the final requiremnst will be submitted within the enxt 4-6 weeks. In the meantime, the US FDA has already approved this product for a phase 1 IND in USA in June,2019. (Annexure 2) An agreement has also been reached with our collaborators in USA for full freedom to operate in India with this product, if found successful in this trial. This product, therefore, which has been developed with joint collaborative research and is accessible to us for further licensing, manufacture and use in India, should be considered to be like an 'Indian' product for all regulatory purposes.



This approach involving transplanted of gene corrected autologous haematopoietic stem cells has become even more critical to explore for haemophilia in India as our preliminary data on the AAV serology distribution in the country is showing that >50% of the patients wimay be ineligible for current AAV-based gene therapy due to pre-existing anti-AAV antibodies. Though this approach is well established for several diseases including the major haemoglobin disorders, this is the first such proposal for haemophilia in the world. It is also the first proposal for a clinical trial of gene therapy in India.

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/ These haplo identical iPSCs could then serve as a source for cell therapy for many individuals with different organ dysfunctions. Please see report of Dolly Daniel and R.V. Shaji for details of this program in collaboration with DATRI stem cell donor registry.

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

Apart from the work described above, I also continue to be involved with clinical hematopoietic stem cell transplantation (HSCT) and research related with it particularly in the area of HSCT for thalassemia major. An Indian Society for Blood and Marrow Transplantation has been established. The Indian Stem Cell Transplant Registry for hematopoietic stem cell transplantation done in India will now be part of this scientific society's activities. I also continue the vice chair of the Asia-Pacific Blood and Marrow Transplant Group.

The Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis has established a task force for gene therapy for haemophilia. I chair this taskforce which includes members from the European Medicinal Authority and also engages with the USA FDA. The aim of this group is to provide guidance on development of products for gene therapy.

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

This program 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. I am helping getting it started by coordinating the planning of this program – a unique event in terms of scale and complexity in this field in the world.

In the last year, several aspects of this program have progressed. Six districts have been identified to implement the first phase of this program. Towards increasing capacity and capability for treatment of major haemoglobin disorders in Odisha, workshops are being arranged at different levels (State / Regional / District 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. The field program is expected to be launched within the next 2-3 months. Training in genetic diagnosis is also being initiated as a part of this project. Prenatal diagnosis for thalassemia will be initiated in the State by September, 2019. The Odisha government and NHM have also been advised to identify centres where stem cell transplantation (SCT) can be established. Once this is done, training can also be provided at CMC, Vellore to build the expertise for SCT in Odisha.

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

Internal Collaborations::

1. Aby Abraham, CSCR / Department of Haematology, CMC, Vellore
2. Fouzia N. A., Department of Haematology, CMC, Vellore
3. Eunice Sindhuvi, Department of Haematology, CMC, Vellore
4. Anu Korula, Department of Haematology, CMC, Vellore
5. Biju George, Department of Haematology, CMC, Vellore
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
2. Barry Byrne, Powell Gene Therapy Center, University of Florida
3. Mavis Agbandje-McKenna, Director, Center for Structural Biology, University of Florida
4. Trent Spencer, Aflac Children's Cancer Center, Emory University, Atlanta, USA
5. Chris Doering, Emory University, USA
6. John Lollar, Emory University, USA
7. INTAS Pharmaceuticals, Ahmedabad, India
8. Nezih Cereb, Histogenetics, USA / DATRI, Chennai
9. Raghu Rajagopal, DATRI, Chennai

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.

Gene therapy: Novel lentiviral vectors to induce therapeutic levels of HbF

In the gene therapy projects, we are currently working on developing novel lentiviral vectors for gene therapy applications. Induction of fetal hemoglobin sickle cell disease (SCD) and β–thalassemia is a promising approach to ameliorate the disease phenotype. B-cell lymphoma/leukemia 11A (BCL11A) is a transcription factor that represses gamma globin gene (HBG) expression in adults. Down regulation of BCL11A induces HbF levels. Therefore, BCL11A is a prime candidate for targeted therapy aimed at induction of HbF in the patients with haemoglobin diseases. As depletion of BCL11A in hematopoietic stem cells can result in impaired B-cell growth and render aging like changes in HSCs, it is important that the knock out or knock down of BCL11A be erythroid specific. We have generated two novel lentiviral shRNA vectors (CSCREUv1 and CSCREUv5) for the knock down of BCL11A in human erythroid cells. The CSCREUv1 has three DNA hypersensitivity sites, HS2, HS3 and HS4 and a 180 bp HBB promoter. The CSCREUv5 lacks HS4, and has a 266 bp β – globin (HBB) promoter. Both carry a polycistronic GFP-IRES-puromycin-shBCL11a cassette cloned downstream of the HBB promoter. We observed that >90% knock down of BCL11A in transduced HUDEP2 erythroid cells resulting in an increase in HbF expressing cells by nearly 40% for both CSCREU v1 and v5. This experiment was then repeated in the ex-vivo erythropoiesis model. The GFP+ HSPCs were flow sorted and differentiated to erythroid cells using an erythroid culture system. GFP+CD71+CD235a+ erythroid cells showed >80% down regulation of BCL11A, resulting in 40% of the cultured erythroid cells expressing HbF. CSCREUv5 vector lacking HS4 appears to be produced at higher titers. For developing a gene therapy vector, we removed GFP-IRES-puromycin cassette from CSCREU-v5 vector. CD34+HSPCs were transduced with the new vector (CSCREU-v6), differentiated to erythroid cells and analysed for the expression of HbF and BCL11A and HBG. The analysis showed that, compared to the control cells transduced with scrambled shRNA, there was a >70% down regulation of BCL11A resulting in 5-fold upregulation of HBG transcripts and 20% increase in the HbF expressing cells. This was achieved without selecting the transduced erythroid cells. This data shows that this lentiviral vector provides significant erythroid specific knockdown of BCL11A which can result in clinically relevant enhancement of HbF expression in the therapy of SCD and thalassemia. Furthermore, this vector can be used to clone other shRNAs targeted at any of the genes in the erythroid cells to alter their function for the study human erythropoiesis or for therapeutic purposes.

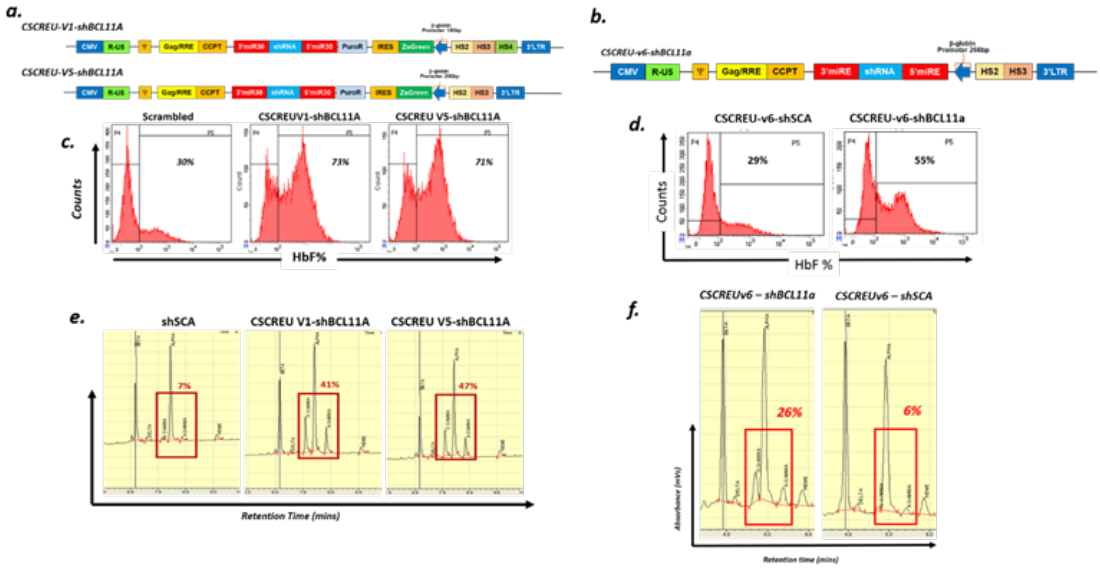


Figure: Induction of HbF expression in cultured erythroid cells after knocking down the expression of BCL11a using CSCREUv1, CSCREUv5 and CSCREU-v6 vectors A) Schematic of the CSCREU-v1 and CSCREU-v5 with shRNA inside a modified mir30 scaffold with ZsGreen-IRES-puromycin cassette, B) Modified CSCREU-v5 (CSCREU-v6) without ZsGreen-IRES-puromycin cassette. (C) and (D) show HbF expression measured by flow cytometry in the erythroid cells derived from transduced HSPCs (E) and (F) show the levels of different globins measured by HPLC. The results were compared with the cells transduced with scrambled (SCA) shRNA.

iPSC Technology: Disease modelling and Haplobanking

Disease modelling: We are currently working on iPSC based disease medlling of two erythroid diseases, Congenetal disyerythropoietic anaemia and Diamond Blackfan anaemia. DBA is caused by haploinsufficient mutations in ribosomal genes, and other erythropoiesis associated genes resulting in absence or decreased number of erythroid progenitors in the bone marrow. CDA results from mutations in CDAN1 (Type I), C15ORF41 (Type Ia), SEC23B (Type 2) and KIF23 (Type III) and KLF1 (Type IV) genes and is also characterised by ineffective erythropoiesis. Hence, engineering a disease-causing mutation in a normal iPSC line and differentiating to the erythroid lineage along with the wild type line is the best approach to model erythroid disorders and study the onset and progress of genetic diseases and develop drugs to treat these diseases.

For efficient gene editing, we first developed an iPSC line in which Cas9 is expressed in doxycycline inducible manner. Guide RNAs (gRNAs) cloned in a plasmid containing GFP as a maker for tranfection was used for targeting ribosome biogenesis genes. We have created mutant iPSC lines confirmed by in-del analusis using T7 endonuclease. We are currently performing single cell sorting to select the mutant clones and differentiating them to haematopoietic and erythroid cells to study disease biology of DBA. For disease modelling of CDA we use fluorescence assisted CRISPR/Cas9 gene editing to create missense mutations. In this approach donor DNA containing mutations and a fluorescence marker is inserted into the CDN1 and SEC23B genes and the gene edited cells are sorted by FACS. We have created missense mutations in both the genes associated with CDA. In the ongoing experiments we are performing erythroid differentiation of the mutant iPSCs for disease modelling.

Internal Collaborations::

1. Saravanabhavan Thangavel, CSCR
2. Mohankumar Murugesan, CSCR
3. Dolly Daniel, CSCR / Department of Immunohematology, CMC, Vellore
4. Alok Srivastava, CSCR / Department of Haematology, CMC, Vellore

External Collaborators:

1. Trent Spencer, Director, Gene Therapy Program, Aflac Children's Cancer Center, Emory University, Atlanta, Mavis Agbandje-McKenna, Director, Center for Structural Biology, University of Florida
2. Chris Doering and John Lollar, Emory University, USA

SARAVANABHAVAN THANGAVEL, PhD
Assistant Investigator, CSCR

LABORATORY HIGHLIGHTS

The prime focus of our lab is to develop the gene-modified haematopoietic stem and progenitor cells (HSPCs) for the autologous stem cell transplantation. Our target diseases are beta hemoglobinopathies, Wiskott-Aldrich Syndrome (WAS) and HIV infection.

Gene edited HSPC therapy for β- haemoglobinopathies:

We are developing two different strategies towards the permanent correction of the phenotype of β-haemoglobinopathies.

1) 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.

2) 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 next 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.

Gene edited HSPC therapy for 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.

Gene edited HSPC therapy for HIV infection:

The living examples; the London and the Berlin patient – both transplanted with allogeneic hematopoietic stem cells demonstrated that HSPCs with CCR5 Δ32 genotype provided a HIV resistance and thus as a permanent cure. However, the very limited availability of HLA matched CCR5 Δ32 donor restricts this approach. This year we have initiated a project to genetically modify the patient's HSPCs.

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.

i) In collaboration with Dr. Srujan Marepally, we have screened and identified a novel system for the delivery of gene editing reagents into the cell lines. We identified a reagent that efficiently delivers cargo into the HSPCs. Currently, we are working to reduce the toxicity associated with our approach.

ii) We are developing a novel platform for the ex vivo expansion of hematopoietic stem cells for its application in gene therapy. The immunophenotypic profile and various in vitro experiments have indicated that our platform preserves the primitive hematopoietic cell population and expands them in the ex vivo culture. The expanded HSPCs are currently being characterized for their repopulation potential in NSG mice.

Grants:

- Novel Strategies for promoting Gene-edited cell expansion- Improving Hematopoietic stem cell gene therapy - Funded by DBT

Publications:

- 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.
- 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:

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

Internal Collaborations:

1. R. V. Shaji, CSCR / CMC (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 (Gene editing blood disorders, HSPC expansion)

External Collaboration:

1. David Martin, Children's hospital Oakland Research Institute, USA

MOHANKUMAR MURUGESAN, PhD
Assistant Investigator, CSCR

LABORATORY HIGHLIGHTS

The current focus of my lab is to develop a novel genome editing approach for the treatment of β - hemoglobinopathies and Haemophilia-A.

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

Hemoglobinopathies such as sickle cell disease (SCD) and β-thalassemia are the most common genetic disorders in India. 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.

Fetal globin repressors

Several transcription factors including BCL11A and LRF play a vital role through modulation of fetal globin level. However, BCL11A is dispensable in non-erythroid functions such as for normal lymphoid and neural development. Functional mapping of the Bcl11A enhancer identified the minimal critical sequence that is specific for erythroid specific BCL11A expression. We have designed and cloned multiple gRNAs targeting the erythroid specific BCL11A enhancer into lentiviral vector. We have successfully edited HUDEP2 cells with these guide RNAs by using lentivirus and confirmed the cleavage of target locus by using a T7 endonuclease and sanger sequencing. The gene edited HUDEP-2 cells were differentiated into erythroid cells and the fetal globin levels was evaluated. We established purification, culture and characterisation conditions for mobilised peripheral blood HSPCs. Upregulation of fetal globin levels was observed in erythroblasts upon targeted deletion of BCL11A enhancer in HSPCs

For the targeted deletion of human ZBTB7A locus, we have designed and synthesized guide RNA targeting the LRF. We cloned these guide RNAs in a LentiCRISPR v2 vector. 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.

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. In this context, we are using CRISPR-Cas9-mediated genome editing approach to recreate the naturally occurring HPFH-associated mutation in human blood progenitors for the therapeutic induction of fetal hemoglobin level. We have designed the genome editing tools to recreate the mutations in HBG promoter that drive the production of fetal globin chain. We determined the effect of these mutation on gamma globin mRNA and fetal hemoglobin protein levels in edited cells.

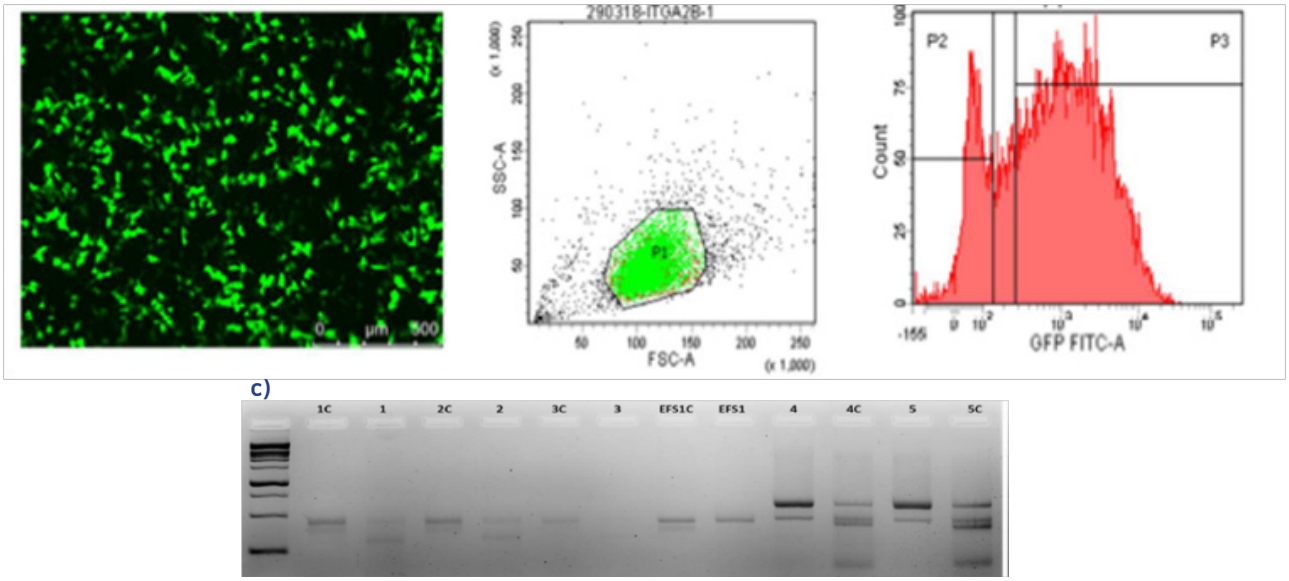
2. Genome engineering hematopoietic stem cells for the treatment of Hemophilia A

Hemophilia A (HA) is an X-linked monogenic congenital bleeding disorder due defective FVIII in the bloodstream. Currently available therapeutic options include clotting factor replacement either with functional recombinant protein or with delivering FVIII gene in viral vectors. However, they are limited with immune tolerance induction and preexisting antibodies respectively. Even a small increase in FVIII levels can significantly ameliorate disease phenotype and patient's quality of life, making this disease an optimal target for CRISPR/cas9 based genome editing system. To improve the current approaches of gene therapy for Hemophilia A, we are working on a novel ex vivo gene therapy approach 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. We established the protocol that is important for effective transfection of Cas9-RNP complex for the targeted integration of transgene in to lineage specific promoter.

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

Hemophilia A is an X-linked monogenic congenital bleeding disorder caused by the absence of functional FVIII in the bloodstream. The severity of the disease varies according to FVIII residual activity. Even a small increase in FVIII levels (above 1%) can significantly ameliorate disease phenotype and patients' quality of life, making this disease an ideal candidate for gene therapy approaches. In recent years sustained correction of Hemophilia B was achieved by systemic administration of an AAV8 containing FIX cDNA under the control of a liver specific promoter. Despite these promising outcomes for Hemophilia B, gene therapy for Hemophilia A is still an open challenge. To improve the current approaches of gene therapy for Hemophilia A, we are working on a novel ex vivo gene therapy approach for targeted integration of FVIII in hematopoietic stem cells for the treatment Hemophilia A. To this end, we have designed and synthesized guide RNA targeting the endogenous locus. We have screened the effective guide RNA targeting the endogenous locus and validated by surveyor nuclease assay. We now have at least two different guide RNAs successfully cleaving the target locus in K562 cells.

a) b)



c)

Targeted integration of transgene under endogenous locus using CRISPR/Cas9 nucleases
a) Transfection of cells with lentiviral vector expressing GFP b) FACS data transduced cells expressing GFP c) T7 endonuclease assay showing successful targeting different regions of endogenous locus

3. Preclinical evaluation of gamma delta T cells for blood cancers

Expanded γδ2 T cells from cancer patients are ideal candidate for adoptive cellular immunotherapy in comparison to other group of immune effector cells, making them the most effective immune cells in the context of anti-tumor cytotoxicity. To improve knowledge about the efficacy of expanded γδ2 T cell in malignancies, we determined the cytolytic activity of selectively expanded γδ2 T lymphocyte under different conditions and from different sources invitro by rapid flow cytometry-based assay.

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::

1. Alok Srivastava, CSCR / Department of Haematology, CMC, Vellore
2. R. V. Shaji, CSCR / Department of Haematology, CMC, Vellore
3. Srujan Marepally, CSCR
4. Saravanabhavan Thangavel, CSCR
5. Geeta Chacko, CSCR / Department of Neuropathology, CMC, Vellore
6. Premila Abraham, Department of Biochemistry, CMC, Vellore
7. Christhunesa Soundararajan, CSCR / Department of Neurochemistry, CMC, Vellore
8. Aby Abraham, CSCR / Department of Haematology, CMC, Vellore

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 bio-inspired cationic amphiphiles for gene therapy and genome editing applications, in particular, to treat blood disorders including β-thalassemia, sickle cell anemia and hemophilia. 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 B:

Current therapy for hemophilia requires repeated intravenous replacement of clotting factor concentrates (CFC). However, major patient population require treatment with by passing agents for hemostasis and immune tolerance induction. All these make current treatment challenging and significantly expensive. Due to these limitations, nearly 70-80% of hemophilic patients from developing countries do not get access to proper treatment.

Gene therapy holds a promising alternative to protein replacement therapy. It would ideally involve a single injection that would induce long-term production of the defective clotting factor, instead of repeated injections of protein. Expression at only 5% of endogenous levels can improve the disease to a mild phenotype and essentially eliminate the risk of spontaneous bleeding events as well as the need for prophylactic protein therapy. Although a variety of mechanisms to introduce the transgene have been investigated, some of the most popular are recombinant viral vectors.

Recent advances in AAV-5 vector based gene therapy found to be promising for hemophilia B. However, over 80% of patients have pre exhibiting antibodies against AAV-5. RNA-based therapeutics have some inherent advantages over DNA and viral vectors but their therapeutic use limited by poor translatability, lack of stability, inefficient delivery, and adverse immune reactions. Chemical modifications on RNAs addressed these concerns to a great extent. Lipid nanoparticles (LNPs) have been developed as a non-viral option to deliver nucleic acids and proved to be successful in clinical trials in delivering siRNAs. Liver targeted nanoparticle delivery could be a better strategy as FIX is expressed in liver.

Towards developing efficient lipid nanoparticles to deliver nucleic acids specifically to liver, we have adopted asialoglycoprotein receptor (ASGPRs) targeting strategy as they are highly expressed on hepatocytes. Previous findings, including our own, demonstrated that β-D-Galactose tethered lipids selectively deliver nucleic acids to liver. Taking cues from the previous findings including our own findings, we developed cationic amphiphiles bearing Galactose head group and develop novel galactosylated nanoparticle system that efficiently deliver nucleic acids (FIX encoding mRNA) to hepatocytes to produce functional FIX protein at therapeutically relevant level.

We developed novel galactosylated lipid nucleic acid system (GALINAs) for delivering FVIII/FIX mRNA/no-end DNA/pDNA specifically to liver. GALINAs demonstrated receptor mediated endocytosis, superior transfection efficiencies, cellular uptake of lipoplexes, serum stability and cytotoxicity profiles in ASGPR positive HepG2 and lower in ASGPR negative SK-Hep-1. In vitro transfections with GALINAs in HepG2 using eGFP mRNA demonstrated comparable transfection with commercial Lipofectamine messenger max at 5:1 lipid to mRNA charge ratio. The optimal molarity of the targeted lipid in GALINAs found to be 0.25mM. In order to understand in vivo stability of GALINAs, firstly, we labelled GALINAs with DiL dye, injected them i.v. After 12 hrs, we evaluated the biodistribution of GALINAs. In corroboration with our previous results, targeted lipid nanoparticles accumulated only in liver, where as non-targeted lipid nanoparticles accumulated in liver, spleen and kidney. Further we evaluated the expression with luciferase expressing plasmid & mRNA and found the specific expression in liver.\

Next, we are evaluating the gain-of function variants of Factor IX. The In prior clinical studies, 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 delivering these gain-of-function variants in mRNA form, we cloned these plasmids in our lab. We have optimized and validated IVT (in vitro transcription) of Factor IX mRNA of wild type and variants with psudouridine modification for longer mRNA stability. Currently, we are evaluating transfections in human hepatocytes.

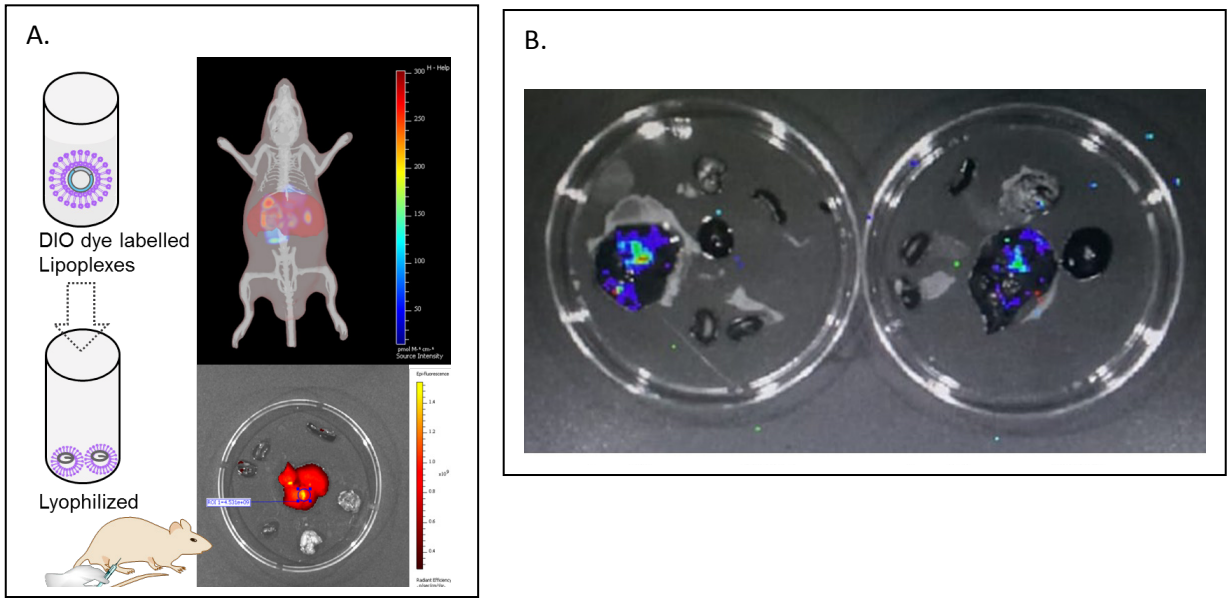


Fig: Biodistribution of GALINAs. Dil labelled GALINAs accumulated specifically in liver (A) and liver specific mRNA and pDNA expression was found (B).

Publications:

1. Venkanna Muripiti, ThasneemYoosuf Mujahid, Venkata HarshaVardhanBoddeda, Shrish Tiwari, Srujan Kumar Marepally, Srilakshmi V Patri, VijayaGopal. Structure-activity relationship of serotonin derived tocopherol lipids Int. J. Pharmaceutics 2019; 554:134-148.
2. Vignesh K. Rangasami, Brijesh Lochania, Chandrashekhar Voshavar, Harikrishna R. Rachamalla, Rajkumar Banerjee, Ashish Dayani, Saravanabhavan Thangavel, Praveen K. Vemula*, and Srujan Marepally*. Exploring membrane permeability of tomatidine in lipid mediated nucleic acid transfections BBA Biomembranes, 2019, 1861(1):327-334.
3. Venkanna MVN, Brijesh Lohchania, Srujan Marepally, Srilakshmi PV. Hepatocellular Targeted α-Tocopherol based pH sensitive Galactosylated Lipid: Design, Synthesis and Transfection Studies MedChemComm, 2018, 9, 264-274

Patent: J. Arun Kumar, Vegi Ganga Modi Naidu, Srujan Marepally, Alok Srivastava, Ch. Naveen, Nanomicellar composition of lithocholic acid tryptophan conjugate and preparation methods thereof. Indian Patent Application No. 201941022351.

Book Chapter: Chandrashekhar Voshavar, Praveen Kumar Vemula*, Srujan Marepally* Topical/Transdermal Delivery with Chemical Enhancers and Nanoparticles. Imaging Technologies and Transdermal Delivery in Skin Disorders Chapter-8, Elesevier. (in press)

Academic activities:

- Organizing Committee member of the 3rd Annual Symposium on Cell and Gene Therapy, September 6-7, 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. Mohan Kumar Murugesan, CSCR

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.
- Transduction inhibition assay (TIA) for AAV serotypes 3 and 5 has been standardized using luminometer detection method in 96 well format.
- Blood samples were collected from 650 individuals with haemophilia A or B and 335 individuals who gave blood for pre-operative screening and are negative for blood borne viral pathogens (HIV, HBsAg and HCV-Ab).
- Screening for total/binding antibodies against AAV serotypes 3, 5 and 8 using capsid ELISA and in-house peptide ELISA were performed in 198 hemophilia A or B individuals serum samples and 50 healthy individuals who are negative for blood borne viral pathogens.
- Screening for neutralizing antibodies for AAV3 was performed on 21 individuals with haemophilia A or B and 6 healthy individuals serum samples using TIA by mCherry flow cytometry.

Specific highlights of the project:

- Design peptides for the serotype specific detection of AAAV.
- Establish an in-house peptide ELISA for the serotype specific detection of antibodies against AAV.
- 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-1

Project title: Establishing a protocol for expansion of Natural Killer cells

Funding source: CSCR core fund

Duration: July 2017 - June 2019

Brief description of the project:

The project has two components, the first one is the development of the cGMP compliant protocol for the expansion of NK cells and the second component is the cytotoxicity of these expanded NK cells.

In the first part, methods for culture and expansion of NK cells suitable for clinical use will be established. NK Cells will be expanded in vitro from mononuclear cells isolated from human peripheral blood obtained from healthy volunteers. At the end, the expanded NK cells will be analysed by flow cytomery for its phenotypic characters.

The next part of the proposal, which will run in parallel, tests the ability of these expanded NK cells to cause cytotoxicity in K562 cells. This will also be assessed in-vitro. The overall results obtained from phenotypic analysis and cytotoxicity assays will be compared against NK cells obtained by a standard method of expansion.

Objectives:

1. To establish a protocol for the culture and expansion of NK Cells from peripheral blood mononuclear cells using ionomycin and IL-2
2. To compare the difference in cytotoxicity profile between the expanded NK cells which will not be pre-activated with IL-12,15 and 18 with the ones which will be pre-activated
3. To compare fold expansion of NK cells and its cytotoxicity with NK cells which are expanded with a regular method of feeder layer using modified K562 cells

Current limitations to be addressed in this project:

1. Higher number of NK cells with a modified protocol.
2. To find a cost effective method which does not require the involvement of CliniMACS for isolation of pure population of NK cells.
3. To make the NK cells more cytotoxic in nature by converting them into memory like NK cells against malignant cells.

Expected Outcome: Sufficient number of NK cells with maximum purity in the final cell culture and with enhanced cytotoxic activity against K562 cells

Highlights of the project:

In all the samples processed there is an increase in the NK cell population over the period of two weeks in culture. There is a difficulty in recovering the NK cells cultured using ionomycin. The cells are strongly adherent and the recovery of the cells from the flask surface is not complete with the use of mild detachment agents. Use of concentrated detachment agent resulted in increased cell death. We had tried different detachment agents but still the issue persists. The use of human AB serum could be one of the reasons for strong attachment of the cells to the culture surface. The use of ultra-low binding flask has also been suggested. The flasks have been procured and will culture and assess the recovery of the cells at harvest. Moreover, there are plans to try other culture medium like the NK cell xeno free culture medium (Miltenyi).

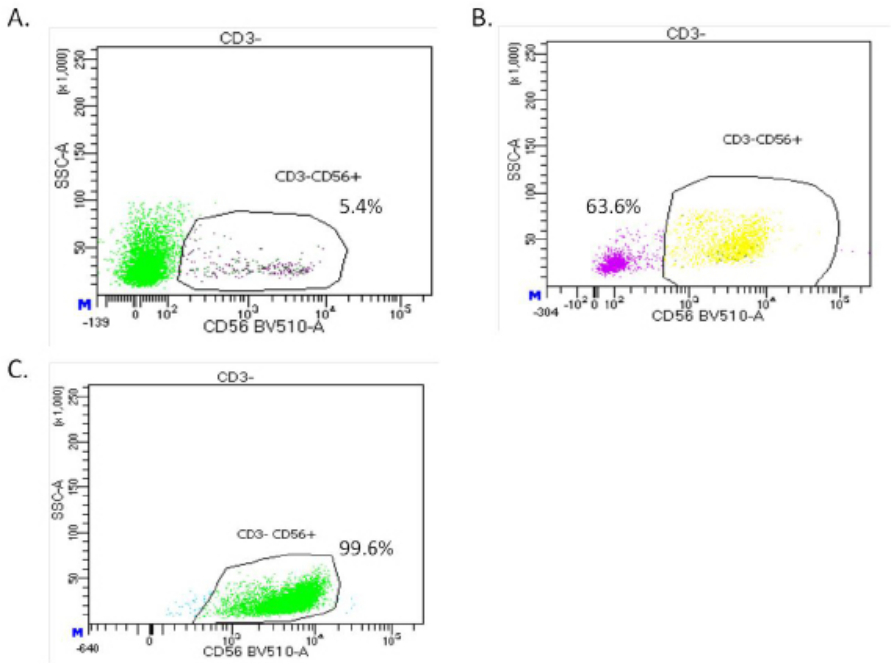


Figure: (A) In day 0 of culture, the PBMNC population had 5.4% of CD3-CD56+ NK cells. (B) On day 7 the CD3-CD56+ NK cells increased to 63.6% and (C) on day 14 it was 99.6%. This clearly shows the selective growth of only the CD3-CD56+ NK cells in culture.

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

PROJECT-2

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

Funding source: CSCR core fund

Duration: July 2017 - June 2019

Brief description of the project:

A. Cell expansion

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

B. Preclinical models

1. To investigate the antitumor activity of selectively expanded γδ2 T lymphocyte from patients with human low grade lymphoma and myeloma both in vitro and in vivo.
2. Analyse the molecular integrity of γδ2 T cells during different stages of expansion including cryopreserved cells to address suitability for its preclinical applications.
3. Establishing in vitro non-radioactive and in vivo novel bioluminescent assay to assess the cytolytic potential of γδ2 T Cells Determine the effect of cryopreservation, different culture conditions and expansion methods on γδ2 T cell cytolyic function.
4. Investigate the cytotoxic potential of autologous compared with allogeneic γδ2 T cells against human low grade lymphoma and myeloma cells.
5. Evaluate the efficacy of γδ2 T cells on the growth of human low grade lymphoma and myeloma xenografts in SCID mice.

Work Progress:

A total of 22 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.

.

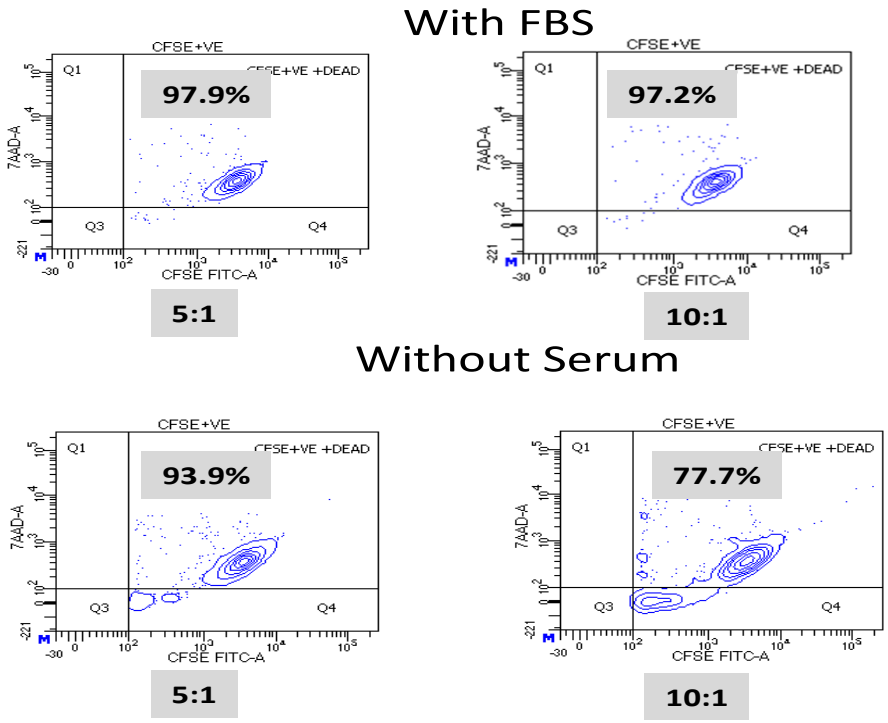


Figure: Representative image of cytotoxic killing of two week cultured γδ T cells in both serum and serum free conditions against CFSE labelled K562 cells at 5:1 and 10:1 effector:target cell concentration..

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-1

Project title: To evaluate the role of epigenetic factors in drug resistance in acute myeloid leukemia by shRNA library screening.

Funding source: Department of Biotechnology, Govt. of India

Duration: 2015-2019

Brief description of the project:

Acute myeloid leukemia (AML) is genetically and clinically heterogeneous disease characterised by accumulation of malignantly transformed immature myeloid precursors. The backbone of treatment remains a combination of cytarabine and anthracycline based regimens with allogeneic stem cell transplantation for eligible candidates. Despite the introduction of genetic testing at the initial diagnostic workup, the ability of clinicians to forecast resistance to treatment remains limited. The presence of numerous mutations involved in DNA methylation and chromatin modification, as well as the identification of new epigenetic targets by proteomic approaches or functional screens, are an exciting and rapidly expanding therapeutic area.

Objectives:

1. Basal RNA expression of all the epigenetic factors in parental as well as resistant AML Cell Lines.
2. RNAi screening in AML cell lines (parental & resistant) to identify the epigenetic factors that influence sensitivity to Ara-C / Dnr.
3. Identification of genes that are associated with drug resistance based on our results with RNAi screening of sensitive and resistance AML lines as well as from gene expression analysis of the already reported datasets.
4. Validation of the role of the target genes in AML cell lines and primary AML cells and in mouse models.

Work Done:

RNAseq was carried out in AML cell lines MV4-11, THP1 and U937 made resistant to cytarabine.

RNAseq of Ara-C Resistant Cell lines: MV4-11, the cell line harbouring MLL-translocation and FLT3-ITD mutation was made Ara-C resistant by exposing it repeatedly with increasing concentration of Ara-C, till we obtained the resistant clones in the highest most concentration. The morphology and sensitivity to drug were monitored for both the resistant and parental cell lines throughout the selection. The other two Ara-C resistant cell lines THP1 and U937 harbouring MLL-translocation were obtained from University of Kent as a kind gift from Dr. Martin Michaelis's Lab. The resistance was conferred by MTT assay for all the 3 cell lines. Transcriptome is dynamic and a good representative of the cellular state. To get an overview of the entire mechanism of resistance we performed "Single Stranded Transcriptomic Analysis". According to the RNA Sequencing data, 845+ 268 genes were differentially expressed in THP1 ara-C resistant cell line, 344+647 genes in case of U937 ara-C resistant cell line and 178+256 genes in MV4-11 ara-C resistant cell line. Further detailed bioinformatic analysis is ongoing for selecting the targets for validation among the differentially expressed genes. Epigenetic ShRNA library experiments are now planned in these paired parental and resistant cell lines.

Support from CSCR: All the Library Experiments are being carried out in Dr. Shaji's Lab, CSCR, under his guidance and supervision.

Collaborations:

1. R. V. Shaji, CSCR / Department of Haematology, CMC, Vellore

Project-2

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

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

Duration: November 2015 - October 2020

Objectives:

- 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.
- To assess the effect of siRNA mediated knock down of NHRs in AML cell lines with different mutations on cell proliferation, apoptosis and RNA expression after treatment with ara-C/Dnr/both.
- To assess the effect of siRNA mediated knock down of NHRs in CML cell lines representing different disease stages on cell proliferation, apoptosis and RNA expression after imatinib treatment.
- Validation of the findings by overexpression / knock down experiments / AML/CML mouse model.

Development of xenograft -AML mouse model in NSG mice for in-vivo investigation: Previously we have established a murine AML mouse model by transplantation of splenic cells harboring the FLT3-ITD/DNMT3a mutation, to test the in-vivo efficacy of chemotherapeutic drugs doxorubicin (DOX) and Arsenic trioxide(ATO).
We observed ATO induced differentiation in the murine AML model and confirmed this finding by Flowcytometry analysis of GR-1 marker on bone marrow and splenic cells and hematoxylin staining of the bone marrow tissue.

To assess the engraftment potential of human AML cell lines MOLM13 in an immune compromised non-obese diabetic SCID gamma mouse (NSG), initially the MOLM13 cell line were transduced with lentivirus harboring a luciferase reporter gene construct. Transduction efficiency was evaluated by bioluminescence imaging using IVIS in-vivo imaging system. The transduced cells were injected into irradiated NSG mice via tail injection. Engraftment was assessed weekly by in-vivo bioluminescence imaging. The mice were euthanized when showed signs of moribund. Investigation of the bone marrow, spleen and liver tissues showed infiltration of the leukemic cells confirming the development of AML. Further this model will be used to assess the anti-leukemic effect of retinoid X receptor ligands in combination with chemotherapeutics drugs.

Development of CML mouse model by retro-viral transduction method (additionally funded by CMC Fluid grant, 2018-2020): Donor C57Bl/6 - 9 weeks old mice were injected with 5-fluro-uracil (5FU-200mg/kg) and euthanized on day 4. Harvested bone marrow cells were stimulated using cytokine cocktail for 24 hours. BCR-ABL plasmids transfected into the plat-E cells and virus was collected. Donor bone marrow cells (1*106 cells) were transduced with BCR-ABL containing virus which were then injected into 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+ (more than 40%) cells in peripheral blood between 4-5 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. For secondary transplantation, splenic cells harvested from the primary CML mice were injected into sub-lethally irradiated mice. CML disease will be evaluated using same GFP+ Gr-1+ marker in the peripheral blood sample. Once secondary transplantation was successful this model will be used to test the anti-leukemic effect of drugs in-vivo that reduces CML disease burden.

Support from CSCR: All the animal experiments are being carried out in CSCR

Collaborations:

1. R. V. Shaji, CSCR / Department of Haematology, CMC, Vellore

Publications:

1. RBharathi M. Rajamani, Sreeja Karathedath, Raveen Stephen Illangeswaran, Esther Sathya Bama Benjamin, Vikram Mathews, Aby Abraham and Poonkuzhali Balasubramanian. Nuclear hormone receptors modulate imatinib resistance in chronic myeloid leukemia (CML). Cancer Res. DOI: 10.1158/1538-7445.AM2018-3739 Published July 2018
2. Raveen Stephen Stallon Illangeswaran, Sreeja Karathedath, Bharathi M Rajamani and Poonkuzhali Balasubramanian. Global Gene Expression Analysis Reveals Genotoxic Effect of Nrf2 Pharmacological Inhibitor Brusatol in Myeloid Leukemia Cells- a Promising Novel Anticancer Agent. Blood 2018 132:5150; doi: https://doi.org/10.1182/blood-2018-99-117829
3. Saswati Das, Stallon Ilangeswaran, Bharathi Rajamani, Sreeja Karathedath, Ajay Abraham, Poonkuzhali Balasubramanian. Gene expression changes in ara-cmetabolism and transport explains acquired ara-c resistance in AML cell lines. Indian J Hematol Blood Transfus 34(suppl 1):S266.

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

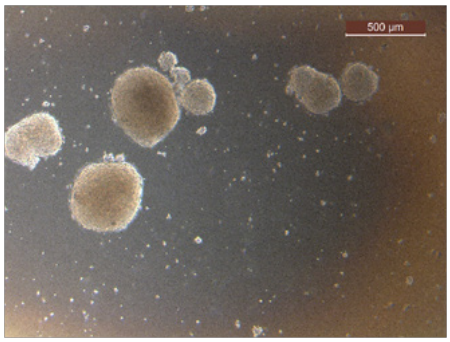
Brief description of the project:

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 (SE) 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.

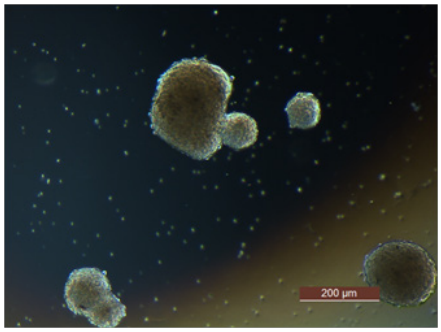
This study is being undertaken with the hypothesis that Supratentorial ependymomas (SE) with the C11orf95-RELA translocations are associated with a worse prognosis. The following key questions are being investigated:

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 twelve 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. Primary neurospheres started forming on around 6-10 days of culture in seven supratendorial ependymoma tumor samples. The supratentorial ependymomas primary neurospheres after the development of secondary neurospheres were dissociated into single cells and replated for neurosphere formation.



Case X: Anaplastic ependymoma, WHO Grade III



Case Y: Anplastic ependymoma, WHO Grade III

Work in progress: Western blotting was done with neurospheres isolated from glioblastoma sample with different protein concentrations by using NF-kB P65 and CCND1 antibodies. The 65kDa and 33 kDa prominent bands were observed in blot probed with NF-kB P65 and CyclinD1 antibody respectively. The optimized protein concentration and antibody dilutions will be used to determine the expression of RELA fusion in neurospheres isolated from supratentorial ependymoma samples.

Differentiation assay: The supratentorial ependymoma samples with neurosphere will be dissociated and seeded in 8 well chamber slide for differentiation. Subsequently, IHC staining for differentiated cells will be performed with biomarkers (Nestin, MAP2, GFAP, NG2 and galactocerebroside) and examined using fluorescence microscopy.

FACS Analysis: Neurospheres will be dissociated in to single cells and suspended in FACS buffer. Single cell suspension will be incubated with CD133 Alexa flour @ 647 and A2B5 FITC antibodies, washed and evaluated by FACS calibur flow cytometry.

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, MD

Professor, Neurochemistry Laboratory, Dept. 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:

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 and completed FACS analysis for 36 samples. MACS sorting could not improve CD133 positivity in these samples.

In order to analyse the gene expression levels of the different markers in CSCs during inhibition microenvironment and Notch signalling, we treated 17 samples with GN44028 (a hypoxia inhibitor), DAPT (a Notch inhibitor) and cryptotanshinone (a STAT3 inhibitor) and analysed the expression of specific genes. We also studied EGFR overexpression in these samples. IDH1 mutation status was also studied in 35 samples by PCR.

Support from CSCR: CSCR core facility for FACS analysis, MACS sorting, Nano drop for DNA & RNA quantification, cDNA synthesis and Real-time PCR

Internal Collaborations: :

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

SANJAY KUMAR, PhD
Scientist, CSCR

LABORATORY HIGHLIGHTS

My scientific contributions in AAV gene therapy program on AAV-based state-of-the-art gene therapy technologies for monogenic disease are to attempt finding a solution for lingering challenges in the fields such as AAV-based therapeutic strategies, AAV 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 regulation towards gene and cell therapies.

Preclinical AAV-gene therapy work:

My laboratory works mostly on AAV based preclinical gene therapy studies and helps in developing newer methods to determine AAV-directed pre-existing immunity in human samples. My lab is helping in the ongoing project of generating different AAV serotype vectors and related distinctive AAV-based assay protocols utilizing AAV-specific expertise related to the AAV projects. 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) Different serotypes of AAV vector production (AAV3, AAV5, and AAV8) and improving the AAV genome containing viral particles. b) A transgene comprising AAV vector's in vitro therapeutic testing in various hepatocyte cell lines and primary cells as well as in vivo scAAV-hFIX transduction testing in suitable Haemophilia-B transgenic mice models. c) Troubleshooting in the AAV vector-based ELISA and transduction inhibition assay (TIA) assays based on AAV based scientific facts and AAV specific knowledge.

Contribution towards Affordable Excellence in AAV gene therapy program:

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

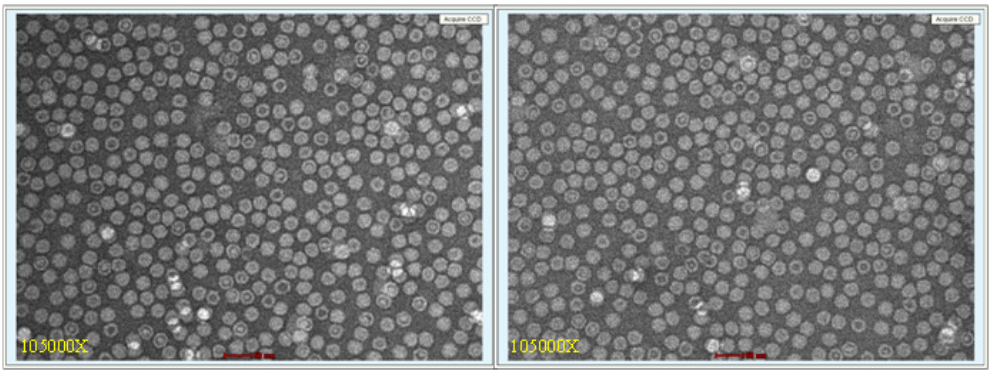


Figure: Ultrastructural characterization of rAAV8-hFIX-Padua particles: rAAV8 particles are observed as 25-nm spheres. Genome-containing AAV particles are impermeable to uranyl acetate, while empty AAV particles TEM images show differentially negatively stained empty particles, which can be distinguished based on the electron dense center within the particle.

Evaluating the safety and efficacy of adeno-associated virus vector expressing human factor IX (scAAV8-hFIX vector) in hemophilia B transgenic mice model:

- Negative stain TEM analysis of purified recombinant scAAV8-hFIX-Padua shows reasonably pure genome containing AAV particles. Several batches of AAV preparation were tested.

- scAAV8-hFIX treatment significantly reduced the tail bleeding-time in Hemophilia B transgenic mice, and ELISA assays show significantly higher hFIX antigen in scAAV8-hFIX injected cohorts (Low dose as well as high dose) than non-injected transgenic hemophilia B mice, correlating very well with the tail bleed assay.

▪ In vivo tissue Bio-distribution study of scAAV8-cohFIX-Padua shows high vector dose mice cohort had 0.172-0.582 vector genomes per liver cells (as determined by real-time qPCR), translating into liver-targeted human factor IX transgene expression.

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, but 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 constantly 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 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. Heterogeneity of Mesenchymal Stromal Cells in Myelodysplastic Syndrome-with Multilineage Dysplasia (MDS-MLD). 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
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.

Award:

International Society of Stem Cell Research (ISSCR):-Merit Award-2019 and Travel Award -2019 for title "Aiming PTEN, seeking pulse, Finding Dox: A targeted, transient in vivo approach to facilitate functional repair in mice spinal cord injury"

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 overarching goal of our lab is to redirect and expand immune cells to target hematological malignancies. We are primarily focussing on augmenting the antitumor functions of NK cells, γδ T cell and αβ T cells by conferring specificity and robustness with Chimeric Antigen Receptors (CARs).

CARs are artificial receptors synthesized by genetically fusing the extracellular antigen binding domain of an antibody or a natural ligand with the intracellular signalling domains of T Cell Receptor/CD3ζ and co-stimulatory molecules. CARs are unique because of the HLA independent target recognition integrating primary and co-stimulatory signals. This platform is designed to be upgradable, envisaging specific, durable and universal living T cell therapy to tackle the drug resistant and relapsed tumors which are often genetically unstable.

The potential for CAR therapy to be a therapy of choice for relapsed or chemotherapy resistant or intensely treated hematological malignancies is already a truism in medicine. 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). Hallmark clinical side effects such as tumor lysis syndrome, cytokine release syndrome and neurotoxicity still incur costly hospitalization. The FDA approved CAR-T formulations are processed and administered in the autologous setting, which significantly increase the expense. Graft versus host disease evoked by the endogenous αβTCR recognition of the host HLA restricts the potentially allogenic CAR-T cell transfusion. Multiple caveats of CAR therapy such as toxicity due to GvHD, batch to batch variability and cost can be mitigated by generating universal immune cells which can be engineered. Our lab ventures to address this limitation by changing the host cell of CARs from αβT cells to γδ T cells, which sense patterns of malignant stress as opposed to tumor antigens presented by HLA.

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

A parallel approach towards universal CAR-T cell generation is to switch the host cell of CARs to NK cells/ γδ T cells. 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 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 (Fig.1).

Our preliminary ongoing experiments indicate that the transducted γδ -T cells can be cultured for 06 days without the loss of the transgene as evident from GFP expression. Our goal is to generate CD19 CAR γδ T cells from multiple donors and test its tumor killing capacity in vitro. We will then test its antitumor functions against patient derived sample before proceeding with in vivo animal studies and cGMP level expansion.

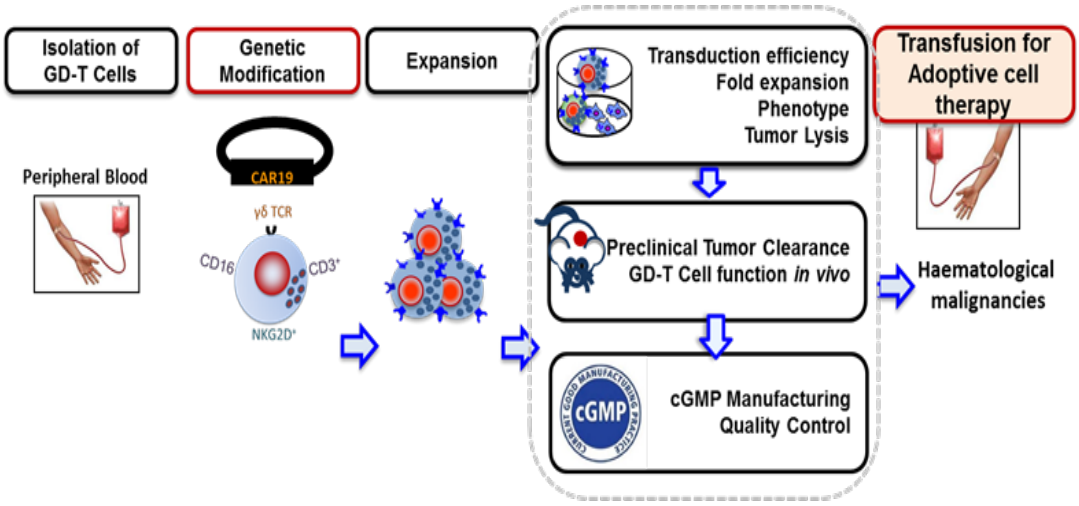


Figure-1: γδ CAR T cell generation work flow

Publication:

Hassounah NB, Malladi VS, Huang Y, Freeman SS, Beauchamp EM, Koyama S, Souders N, Martin S, Dranoff G, Wong KK, Pedamallu CS, Hammerman PS, Akbay EA. Identification and characterization of an alternative cancer-derived PD-L1 splice variant. Cancer Immunol Immunother. 2019 Mar;68(3):407- 420

Academic Activities:

- Organizing Committee member of the 3rd Annual Symposium on Cell and Gene Therapy, September 6-7, 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. 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-1

Project title: Isolation and characterization of cancer stem like cells of human primary endometrial cell culture derived from type I and type II endometrial cancer tissue from patients.

Funding source: CSCR

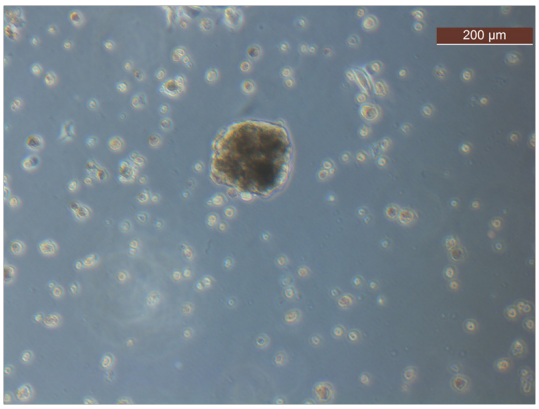
Duration: August 2018 to July 2019

Brief description of the project:

There is a global increase in the incidence of endometrial cancer. Type I endometrial cancer, which has endometroid histology has got better prognosis, whereas type II endometrial cancer which has non-endometroid histology has higher rates of treatment failure and recurrence. In this study we are interested in studying the properties of cancer stem like cells obtained from type I and type II endometrial cancer. This study will help in understanding whether there is a difference in property of cancer stem like cells obtained from these two types of endometrial cancer. If there is a difference in the property of cancer stem cells, this can be studied further to see whether this difference can be attributed to the difference in the prognosis of type I and type II endometrial cancer.

Work done:

We have standardized the procedure for establishing primary cell culture from endometrial tumour tissue. Patient's endometrial tumour sample were digested by collagenase and grown in serum free media supplemented with growth factors. We observed the formation of tumour spheres in certain samples. Also we standardized the procedure for sorting CD44+ and CD133+ cells from the tumour sample. These cancer stem cells will now be analyzed for their stemness and invasive properties. We will then compare the properties of cancer stem like cells obtained from type I and type II endometrial cancer.



Formation of tumour sphere in primary culture of endometrial tumour sample grown in serum free media.

Support from CSCR: Funding and lab space.

Collaborations:

1. Premila Abraham, Department of Biochemistry, CMC, Vellore
2. Mohankumar Murugesan, CSCR
3. Abraham Peedicayil, Department of Gynecologic Oncology, CMC, Vellore
4. Anitha Thomas,Department of Gynecologic Oncology, CMC, Vellore
5. Thomas Samuel Ram, Department of Radiotherapy, CMC, Vellore

PROJECT-2

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

Funding source: CMC Fluid Reseach Grant

Duration: February 2019 to January 2021

Brief description of the project:

Endometrial cancer (EC) is an increasing threat due to the growing prevalence of obesity. Cisplatin is one of the most commonly used agents in the post curative surgery in patients with EC. Although cisplatin is recognized as the first-line chemotherapy agent for patients with EC, its efficiency remains a major problem. Cancer stem-like cells (CSCs) are thought to be the root 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). These include 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 standardized the procedure for establishing primary cell culture from endometrial tumour tissue. Patient's endometrial tumour sample were digested by collagenase and grown in serum free media supplemented with growth factors. We observed the formation of tumour spheres in certain samples. Also we standardized the procedure for sorting CD44+ and CD133+ cells from the tumour sample. These cancer stem cells will now be analyzed for their stemness and invasive properties. We will then compare the properties of cancer stem like cells obtained from type I and type II endometrial cancer.

Support from CSCR: Lab space.

Collaborations:

1. Premila Abraham, Department of Biochemistry, CMC, Vellore

JEYANTH ROSE, MS

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

Project Title: Efficacy of placenta derived Mesenchymal Stem Cells in reducing corneal scarring, in an ex-vivo organ culture model of post-mortem human corneas.

Funding Source: CMC Major Fluid Research Grant / CSCR Core Grant
Duration: November 2016 to April 2018
Objectives:

1. To evaluate the efficacy of placenta derived Mesenchymal Stem Cells (MSCs) in reducing corneal scarring in an ex vivo organ culture model.
2. To evaluate the influence of intrastromal MSCs on corneal transparency in a ex-vivo organ culture model of evolving corneal scarring.
3. To compare the influence of MSCs on the basic histopathology of the cornea.
4. To compare the influence of MSCs on markers of fibrotic corneal scarring.

The aim of this project is to use intrastromal injection of placenta-derived Mesenchymal Stem Cells as a treatment for prevention of corneal scar. Briefly the MSCs will be extracted from the placenta of consented LSCS donor by enzymatic digestion. Five pairs of post mortem human corneas harvested for corneal transplant, excluded for clinical use, of a grade equal to fair or better will be chosen for the study. Both eyes of each pair will have a superficial keratectomy with a standardized protocol. The eyes will be randomly assigned to receive a test or sham injection. The test eye will have an intrastromal injection of 3x100000 placenta-derived MSCs. The control eye will have an intrastromal injection of carrier without cells. The eyes will be maintained in an organ culture model system at the total immersion in media for a 28 days period. All assessment will be done on the 28th day onwards.

Work done:

- Mesenchymal Stem Cells were characterized using CD73, CD90, CD105 antibody and negative control using CD 45, CD 34, CD 14 antibody.
- All five pairs of cornea underwent a standardized superficial keratectomy followed by an intrastromal injection of MSCs in one of the pair and placebo in the other.
- Laser Quantification was done and the image was processed using ImageJ software and statistically analyzed for comparing the corneal transparency between the treated and untreated corneas.
- Histopathology and Immunohistochemistry staining was done.

Support from CSCR: Lab infrastructure and funding

Publication: Richard, A. J., Rose, J. S., Korah, S., Keziah, M., Arambhan, S., Arthi, A., Jaisakthi, S. and Vijayarajan, V. (2019), Quantification of corneal transparency in post-mortem human corneas using laser scatter image analysis. Clin Exp Optom. doi:10.1111/cxo.12898

Internal Collaborators:

1. Sanita Korah, Department of Ophthalmology, CMC, Vellore
2. Thomas Kuriakose, Department of Ophthalmology, CMC, Vellore
3. Rutika Dodeja, Department of Ophthalmology, CMC, Vellore
4. Deepthi Kurien, Department of Ophthalmology, CMC, Vellore
5. Charles Immanuel, Department of Ophthalmology, CMC, Vellore
6. Srinivasan S., Department of Ophthalmology, CMC, Vellore
7. Alok Srivastava, CSCR / Department of Haematology, CMC, Vellore
8. Suresh Devasahayam, Department of Bioengineering, CMC, Vellore
9. Syrpailyne Wankhar, Department of Bioengineering, CMC, Vellore
10. Geeta Chacko, Department of Pathology, CMC, Vellore
11. Tripti Jacob, Department of Anatomy, CMC, Vellore
12. Joe Varghese, Department of Biochemistry, CMC, Vellore
13. Vinay Oomen, Department of Physiology, CMC, Vellore

External:

1. Mindy Fox, University of Cincinnati, USA
2. Winston Kao, University of Cincinnati, USA

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.

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

Duration: November 2018 to October 2020

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.

Work done: Standardization of protocol in the lab from autograft pterygium specimens.

Support from CSCR: Lab infrastructure and funding.

Internal Collaborators:

1. Sanita Korah, Department of Ophthalmology, CMC, Vellore
1. Jeyanth Rose, Department of Ophthalmology, CMC, Vellore
2. Sanjay Kumar, CSCR
3. Sanjana Shanmugam, Department of Ophthalmology, CMC, Vellore
4. Rajan Sony Raj, Department of Ophthalmology, CMC, Vellore

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

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 imager and a Perkin Elmer Tricarb Liquid Scintillation Counter.

Many departments from CMC, Vellore and outside use this core facility extensively. The molecular biology core also aims to collaborate with people outside CSCR to share expertise and knowledge on platform development and augmentation.

Flow Cytometry Core Facility

- Scientific Officer: B. Sandya Rani
- Technical Officer: A. Rajesh
- Technical Staff: T. Abdul Muthallib & Immani Job
- Faculty support: Sanjay Kumar

Flow cytometry is a pivotal tool in cell biology. Many intra and extra-cellular parameters can be analyzed and statistically evaluated with high speed and precision. The Flow Cytometry Core Facility currently houses following instruments.

I. BD FACS Aria III

The 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 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 5 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 set up 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. BD FACS Calibur

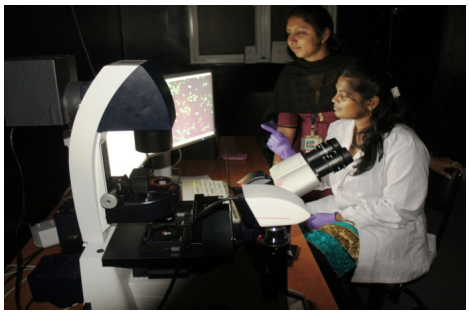
The BD FACSCalibur™ platform allows users to perform cell analysis in a benchtop system. The system supports a wide variety of research and clinical applications and is complemented by a broad suite of intuitive software solutions to streamline analysis for a wide range of applications including enumeration of lymphocyte subsets, stem cells, residual white blood cells, and reticulocytes. The flow cytometer has 2 lasers (Blue- 488nm and Red-633nm), 4 colors system and is routinely used for intracellular and surface markers analyses.

The Flow Cytometry core aims to conduct regular workshops in flow sorting and cell analysis for human resource development in flow cytometry and provides support to various departments in selecting antibody panels and experiment design. An offline workstation with a FlowJo license is also available and networked for data sharing and post-acquisition data analysis.

Imaging Core Facility

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

I. 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.

II. Light Microscopes

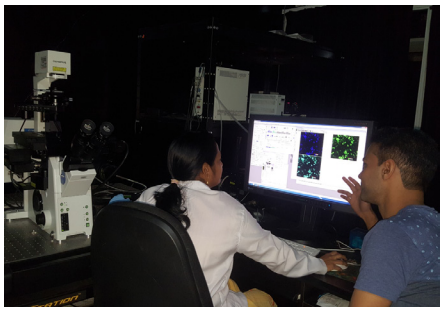
Leica DMIL (upright) and Leica DMI1000 (inverted) microscopes are available for users to perform routine light microscopy imaging. Both microscopes are provided with an interchangeable Leica DFC290 camera for high resolution bright-field imaging. The Leica DMI1000 is also installed in the tissue culture 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.

III. EVOS FL Auto Imaging System

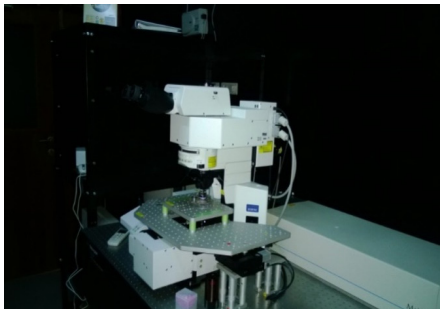
The EVOS® FL Auto Imaging System is a fully-automated, digital, inverted multi-channel fluorescence and transmitted light imaging system with outstanding workflow efficiency. Designed to meet demanding requirements over a broad range of applications, the EVOS® FL Auto system supports high-resolution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

IV. 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.

V. 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.

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.

Core Tissue Culture Facility

The tissue culture (TC) facility is a full-service cell culture shared resource at CSCR and it is the most extensively used facility in CSCR. This facility is located in the ground and the first floors and it houses all the required equipment for the tissue culture experiments. Users from CSCR and the adjunct scientists and faculty from CMC have 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

Histopathology Core Facility

- Technical Officer: A. Rajesh
- Technical staff: Esther Rani & Ashok Kumar
- Faculty Support: Noel Walter

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

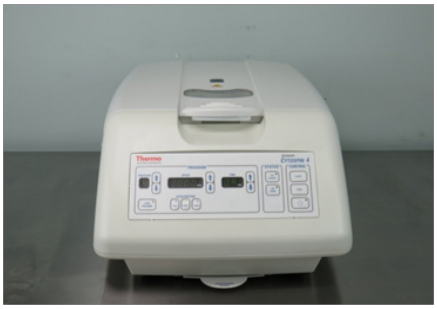
The RM2245 is a semi-motorized rotary microtome, designed for routine in histopathology.

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 gain 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.

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 were 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. The cages are constructed and designed in a specific way to ensure an absolute microparticle-free inner environment. It is also designed to allow maximum comfort for the animals and to provide a secure, chew proof environment. An external ventilation unit supplies the cages with fresh HEPA-filtered air which passes through the filtered cage lids. The ventilation-system mostly consists of two tubes for inlet and outgoing air.

Individually Ventilated Cages (IVC)

Cage changing station

Temperature, humidity and ventilation

Temperature and relative humidity of the animal rooms were maintained between 20 to 25 °C and 30 to 70% respectively throughout the year. All the environmental factors were 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) maintained as per CPCSEA regulations.

Veterinary care

Qualified veterinarians supervise all the animal health concerns, and provide all necessary veterinary care to ensure that healthy animals are available for research. Ad-libitum supply of UV treated autoclaved R.O water and autoclavable vegetable diets were given to animals.

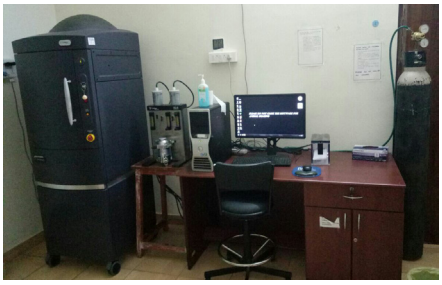
The Veterinary and technical staffs of the CSCR-LAF are also supporting and facilitating all animal facility users (including PhD students and Project Assistants) on mouse and rat bio-methodologies, principles of three R's, ethics, IAEC laws and guidelines on the regulation of scientific experiments on animals, hematological parameters, husbandry and care, animal identification techniques, sex differentiation, handling and restraint, and IACUC approved techniques for anesthesia and monitoring, drug administrations, blood collection, humane euthanasia etc.

Specialized equipments

The CSCR-LAF is equipped with Small animal live imaging system, Multi photon microscope and Small animal irradiator with Co-60 as source in addition to a couple of Isoflurane anesthesia machines, animal blood counter and Leica 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 for transplantation studies. Users are allowed to use the instrument after getting TLD batch from radio therapy department to monitor the safety of radiation. Last year we renewed the license for this instrument from Atomic Energy Regulatory Board (AERB), Radiological Safety Division, Government of India.

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. The instrument uses two separate optical paths with two objectives and eyepieces to provide slightly different viewing angles to the left and right eyes. This arrangement produces a three-dimensional visualization of the sample being examined. We are using this instrument for dissection and organ collection from embryos of rat and mice.

Blood irradiator (BI 2000)

Leica zoom stereo microscope

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

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 immunology studies	Jax Lab, US
3	FVB/NCrl mice	Inbred strain	Chronic myeloid leukemia model	Charles River, UK
4	CD-1	Out bred strain	Sentinel animals, Pseu-dopregnancy studies	Charles River, UK
5	B6.129S4-F8tm1Kaz/J	Knock-out strain	Hemophilia A	Jax Lab, US
6	B6.129P2-F9tm1Dws/J	Knock-out strain	Hemophilia B	Jax Lab, US
7	B6;129S4 Pou5f1tm1Jae/J.	Transgenic strain	OCT-GFP model (used for iPSC technology)	Jax Lab, US
8	B6.129-Adamts13tm1Dgi/J	Mutant Strain	Thrombotic Thrombo-cytopenic Purpurea and von Wille brand disease	Jax Lab, US
9	B6.CB17-Prkdcscid/SzJ	SCID	Transplantation studies	Jax Lab, US
10	C.B-17/Icr-Prkdc <Scid>Ic¬rIcoCrl	SCID	Xeno Graft Research	Charles River, UK
11	NOD.CgPrkdc^{scid} Il2rg^{tm1Wjl}/SzJ	SCID (NSG)	Most immunodeficient strain; Transplantation studies	Jax Lab, US
12	Sprague Dawley	Rat- Outbred strain	Surgical models	Jax Lab, US

Quality control (QC)

A quality control program for environmental microbiology, clinical pathology, genetic analysis is being implemented for monitoring of the laboratory rodents and animal feed. 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 is being done in every three months to ensure the health status of breeding and experimental animals stock. Animal skin and hair samples are checked for ectoparasites. Fecal samples are checked for the endoparasites by sedimentation method. Microbiological examination of animal room air, animal feed, water, bedding material, fecal samples and throat swabs 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, Hantan virus, Sendai virus, Adeno virus (FL/K87), rat corona virus, Kilham rat virus and Cilia Associated Respiratory Bacillus (CARB).

PCR based Monitoring: Blood samples of sentinel animals are checked for Mycoplasma pulmonis by PCR method.

Genetic Monitoring: Genetic monitoring (Genotyping) of mutant and SCID strains are conducted often by PCR. All reports of QC are maintained in CSCR-Laboratory Animal Facility office.

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	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)	Poonkuzhali
3	Pre-clinical studies for the gene therapy for Wiskott-Aldrich Syndrome (WAS)	Saravanabhavan
4	To study the effect of shock wave on rat metatarsal organ culture	Vrisha Madhuri
5	Muscle derived stem cells in the treatment of anal sphincter injury in rat model – an interventional study	Vrisha Madhuri
6	Genetically-engineered human umbilical cord-derived mesenchymal stem cells (UC-MSC) / engineered UC-MSC derived exosomes as therapeutic delivery vehicles for tumor-targeted therapy or maintaining tissue homeostasis	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)	Poonkuzhali
8	Safety and efficacy study of AAV based FIX gene therapy productin Hemophilia B mice	Priyanka Priyadarsini
9	Pre-clinical model for gene therapy for Thalassemia and Sickle Cell disease	Mohankumar

Rodent enrichment program

To avoid the incidence of cannibalism and to enrich the behaviour of lab animals, the rodent enrichment program has been introduced in our facility. Autoclavable wooden and plastic based enrichment devices of different structures, wooden gnawing devices and nesting material has been procured and introduced to the animals. The rate of cannibalism has reduced and the breeding performance of the animals has improved.

Training sessions

The Lab Animal Facility conducts hands-on orientation sessions regularly for both first time and experienced users. The training sessions comprise of theory and practical. The hands-on training sessions include animal handling, blood collection, anesthesia, tail vein injection, euthanasia, dissection and organ collection. The final authorization is given to the user upon successfully completing the required training.

In collaboration with the Institutional Animal Ethics Committee (IAEC) of Christian Medical College, the “Workshop on the use of laboratory animals in biomedical research” was conducted for the research scholars and junior faculty of CMC on December 7 and 8, 2018. This 2 day workshop was very first of its kind in CMC. It is planned to conduct this workshop twice a year.

Future direction

In the near future, apart from continuing our current activities, our goal is to set up and establish a mouse embryo and sperm cryopreservation facility to archive and retrieve mouse strains important for our research. We planned to standardize the animal model (cranial window model) to study the vascular engineering by using multiphoton microscopy available in our facility. We are also looking into obtaining several genetically modified transgenic and knockout mouse strains from various reputed international mouse laboratories to expand our colony and ensure animal supply for our researchers as and when required.

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.

THIRD ANNUAL SYMPOSIUM ON CELL AND GENE THERAPY
6 & 7 SEPTEMBER, 2018

The Centre for Stem Cell Research (CSCR), (a unit of inStem, Bengaluru) organized the 3rd Annual Symposium on Cell and Gene Therapy on 6th & 7th September, 2018. 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) and Indian Council of Medical Research (ICMR). Dr. Renu Swarup, Secretary, DBT addressed the participants through video link.

The program focussed on cell and gene therapy in haematological disorders, gene editing technology, non-viral gene transfer technology and cancer cell and gene therapy. Over 100 participants from across the country and 18 speakers from around the world took part in the symposium.

The first day of the symposium focussed on taking cell and gene therapy to clinic and the recent advances in this field for hemoglobinopathies. The key note address of the symposium was delivered by Prof. Arun Srivastava from University of Florida College of Medicine, USA. He discussed the evolution of gene therapy in the world and delivered the keynote address titled 'AAV: From Almost A Virus to An Awesome Vector'. The second day of the symposium had various discussions on cancer cell and gene therapy, non-viral gene transfer technology and gene editing technology. The symposium was well received by all the participants who were all actively engaged in the discussions.

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. Parker Institute for Cancer Immunotherapy,University of Pennsylvania, USA
4. Center for Molecular Biophysics-CNRS, University of Orléans, France
5. Free University of Brussels & University of Leuven, Belgium
6. National Cancer Centre & Agency for Science, Technology and Research, Singapore
7. Thermo Fisher Scientific, Switzerland
8. Miltenyi Biotec Asia Pacific, Singapore
9. International Stem Cell Banking Initiative & SSC Bio Ltd., UK

National:

1. ACTREC, Tata Memorial Cancer Centre, Mumbai
2. Adyar Cancer Institute, Chennai
3. All India Instiute of Medical Sciences, New Delhi
4. Armed Forces Medical College, Pune
5. Armed Forces Medical Services, Delhi
6. Biocon Research Limited, Bengaluru
7. Centre for Cellular and Molecular Biology, Hyderabad
8. Centre for Stem Cell Research, Vellore
9. Christian Medical College, Ludhiana
10. Christian Medical College, Vellore
11. CSIR-Indian Institute of Chemical Technology, Hyderabad
12. CSIR-Institute of Genomics and Integrative Biology, New Delhi
13. Dystrophy Annihilation Research Trust, Bengaluru
14. EyeStem Research, Bengaluru
15. Govt.Stanley Medical College, Chennai
16. Indian Institute of Technology Bombay
17. Indian Institute of Technology, Delhi
18. Institute of Liver and Biliary Sciences, Delhi
19. Intas Pharmaceuticals, Ahmedabad
20. L. V. Prasad Eye Institute, Hyderabad
21. Manipal University, Bengaluru
22. Narayana Health, Bengaluru
23. Narayana Netralaya, Bengaluru
24. National Centre for Cell Sciences, Pune
25. Sahyadri Hospital, Pune
26. Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow
27. Sri Ramachandra University, Chennai
28. Stempeutics Research Pvt. Ltd. Bengaluru
29. Strand Life Sciences, Bengaluru
30. Tata Medical Centre, Kolkata
31. Takshashila Institution, Bengaluru

The 4th Annual Symposium on Cell and Gene Therapy is scheduled on 5 & 6 September, 2019.

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 Medical Science and Technology (SCTIMST), Thiruvananthapuram or Thiruvalluvar University, Vellore.

II. Other training programs:

Short term student projects (Bi-annual)

S. No	Name	Duration	Qualification	University	Project title	PI /Lab
1	Ms. Sneha Kurian	Jan - Jun 2019	MBBS	Dr. MGR University	Invitro culture and characterization of human wharton jelly MSCs	Dr. Sanjay / Lab-3
2	Ms. Beula Nesakumari	Jan - Jun 2019	B.Tech - Biotech	Karunya University	Genetic modification of immune cells for adoptive immunotherapy	Dr. Sunil / Lab-6
3	Ms. Harshita Srinivasan	Jan - Jun 2019	B.E - Biotech	R.V College of Engineering, Bengaluru.	Analysis of the immune cells in the tumor microenvironment and assessing their cytotoxicity	Dr. Sunil / Lab-6
4	Mr. Vedha Viyas Thilagarajan	Jan - Jun 2019	M.Tech - Biomedical Instrumentation	Karunya University	Generation of endogenous TCR negative CAR-T Cells	Dr. Sunil / Lab-6
5	Ms. Swetha. A.P	Jan - Jun 2019	M.Sc., Life Sciences	Bharathidasan University, Tiruchirappalli	Comparison of HDR - donors for gene editing mediated transgene integration	Dr. Saravana / Lab-7
6	Ms. Sahana Sadhasivam	Jan - Jun 2019	M.Tech - Biotech	Bannari Amman Institute of Technology, Sathyamangalam	Gene editing mediated generation of Wiskott- Aldrich Syndrome (WAS)- Like cells	Dr. Saravana / Lab-7
7	Ms. Akshaya S	Jan - Jun 2019	M.Sc. - Biochem	Avinashilingam University, Coimbatore	Epigenome editing of BCLIIA enhancer and KLF promoter for the trageted fetal hemoglobin induction	Dr. Mohan / Lab-9
8	Ms. Nikitha N	Jan - Jun 2019	B.Tech - Biotech	Rajalakshmi Engineering College, Chennai	Perclinical genome editing approach for the treatment of beta globin disorders	Dr. Mohan / Lab-9
9	Ms. Shruti Bharadwaj	Jul - Dec 2019	B.Tech - Biotech	SRM University, Chennai	Generation of lentiviral vector to create isogenic iPSCs for disease modelling	Dr. Shaji / Lab-2
10	Ms. Akshaya C	Jul - Dec 2019	M.Sc - Biotech	VIT University, Vellore	Engineering GD-T cells for adoptive immunotherapy	Dr. Sunil / Lab-6

11	Ms. Sakshi Seth	Jul - Dec 2019	B.Tech - Biotech	Banasthali University	Generation and expansion of CART cells	Dr. Saravana / Lab-7
12	Mr. Dhakshanya P	Jul - Dec 2019	M.Tech - Biotech	Rajalakshmi Engineering College, Chennai	CRISPR-CAS9 mediated gene editing in HSPCs	Dr. Saravana / Lab-7
13	Ms. Kanimozhi S	Jul - Dec 2019	M.Tech - Biotech	Periyar Maniammai University, Thanjavur	Evaluation of liposomal formulations for nucleic acid delivery into hapetic cells / cell lines	Dr. Srujan / Lab-6
14	Ms. Yoga Priya M	Jul - Dec 2019	M.Tech - Biotech	Periyar Maniammai University, Thanjavur	Evaluation of liposomal formulations for efficient delivery of crisp tools into HSCs	Dr. Srujan / Lab-6
15	Ms. Mugi Swetha	Jul - Dec 2019	M.Sc - Biotech	SVIMS, Tirupati	Optimization of nucleofection conditions in different cell lines	Dr. Mohan / Lab-9
16	Ms. Prasanthi Chittineedi	Jul - Dec 2019	M.Sc - Biotech	SVIMS, Tirupati	Validation of editing efficiency of different cytosine deaminase base editors	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. Aniket Kumar (Study leave)	Adjunct Scientist
Dr. Aby Abraham	Adjunct Scientist
Dr. Asha Mary Abraham	Adjunct Scientist
Dr. Boopalan Ramasamy (resigned Fb 2019)	Adjunct Scientist
Dr. ChristunesaChristudass	Adjunct Scientist
Dr. Dolly Daniel	Adjunct Scientist
Dr. Inian Samarasam-(sabbatical from CMC)	Adjunct Scientist
Dr. Jeyanth Rose	Adjunct Scientist
Dr. Poonkuzhali Balasubramanian	Adjunct Scientist
Dr. Ravikar Ralph	Adjunct Scientist
Dr. Geetha Chacko	Adjunct Scientist
Dr. Elizabeth Vinod	Adjunct Scientist
Dr. Muthuraman N.	Adjunct Scientist
Dr. Alo Sen	Adjunct Scientist
Dr. Gurbind Singh	Scientist (NAHD - Haplobanking)
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. Indhumathi Vadarethinam	Scientific Program Manager (NAHD Program)
Dr. Chinmayee Panda	Project Coordinator(NAHD-Thalassemia and SCD Program, Odisha)
Mr. Theophilius Lakiang	Project Coordinator(NAHD-Thalassemia and SCD Program)
Dr. Vasanth Thomodaran	PostDoctoral Fellow
Dr. Santosh Chandar Maddila	Post Doctoral Fellow*(up to September 2018)*
Dr. Suhashini	Research Coordinator
Dr. Lakshmi	Scientist B
Dr. Venkatesh	Research Associate
Mr. Karthikeyan R.	Senior Research Fellow
Ms. Sowmya R.	Senior Research Fellow
Mr. Ashis Kumar	Senior Research Fellow
Ms. Renita Raymond	Senior Research Nurse
Mr. Balasubramanian S.	Senior Research Fellow *(up to January 2019)*
Mr. Franklin Jebaraj Herbert	Senior Research Fellow
Ms. Smitha I.	Senior Research Fellow
Mr. Abhirup B.	Senior Research Fellow

Ms. Aneesha Nath	Senior Research Fellow
Ms. Abisha Crystal	Senior Research Fellow
Ms. Sonam Rani	Senior Research Fellow
Ms. Kritika Nandy	Junior Research Fellow
Ms.Dhivya Bharathi	Junior Research Fellow
Mr. Vigheshwaran V	Junior Research Fellow
Mr. Vignesh R	Junior Research Fellow
Ms Rashmi Prakash Chowath	Junior Research Fellow
Ms Saranya Srinivasan	Junior Research Fellow *(up to July 2019)*
Mr Karthik V. K.	Junior Research Fellow
Ms Prathibha Babu	Junior Research Fellow
Mr Nithin Sam	Junior Research Fellow
Ms Thamizhselvi	Junior Research Fellow
Ms. Sarubala	Junior Research Fellow *(up to November 2018)*
Ms. Aishwarya Prasannan	Junior Research Fellow *(up to June 2019)*
Ms. Nivedhitha D	Junior Research Fellow
Ms. Sayed Syeda Mohammed Khalil	Junior Research Fellow *(up to February 2019)*
Ms. Porkizhi Arujunan	Junior Research Fellow
Mr. Ajay Kumar Dhyani	Junior Research Fellow
Mr. Muthuganesh	Junior Research Fellow
Ms. Aruna	Junior Research Fellow
Ms. Anila Geroge	Junior Research Fellow
Ms. Kirti Prasad	Junior Research Fellow
Ms Sevanthy	Junior Research Fellow
Ms. Agnes Selina	Junior Research Fellow
Ms. Aleya Tabasum	Graduate Technician
Ms. Dhavapriya B.	Graduate Technician
Ms. Kalaivani G.	Graduate Technician *(up to September 2018)*
Ms. Saranya J.	Graduate Technician *(up to January 2019)*
Ms. Pavithra R.	Graduate Technician
Ms. Esther Rani J.	Technician
Mr. Ashok Kumar	Technician
Mr. Aarwin Joshua	Optometry Technician *(up to September 2018)*
Ms. Chitra P.	Graduate Technician
Ms. Jayashree	Graduate Technician *(up to October 2018)*
Mr. Abdul Muthallib	Graduate Technician
Ms. Praveena	Graduate Technician
Mr. Giftson Joel	Staff Graduate Medical Lab Technician
Mr. Vignesh Kumar	Staff Graduate Lab Technician
Ms. Immani Job	Graduate Technician
Ms. Farzana	Graduate Technician
Ms. Mohana Priya	Graduate Technician
Ms. Nithya Jeba Princy	Project Assistant
Ms. Vatchala Dennis Newton	Senior Staff Nurse
Mr. John Welfred	Graduate Technician *(up to May 2019)*
Ms. Logeshwari	Graduate Technician Trainee
Mr. Joshua Paul	Graduate Technician
Mr. Joseph Joel	Graduate Technician

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.	Asst. Librarian
Mrs. Geetha R.	Accountant
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

GOVERNANCE OF CSCR

inStem Governing Council

»»Dr. Renu Swarup, Secretary to the Government of India, DBT	Chair
»»Prof. Apurva Sarin, Director, inStem (Ex-officio)	Member
»»Prof. Satyajit Mayor, Director, NCBS (Ex-officio)	Member
»»Prof. Jyotsna Dhawan, Senior Professor CCMB	Member
»»Prof. S Ramaswamy, Dean, inStem (Ex-officio)	Member
»»Prof. Upinder S Bhalla, Dean, NCBS (Ex-officio)	Member
»»Mr. Chandra Prakash Goyal, Joint Secretary (Admin), DBT (Ex-officio)	Member
»»Smt. Sumita Mukherjee, JS & FA, DBT(Ex-officio)	Member
»»Prof. J V Peter, Director, CMC Vellore (Ex-officio)	Member
»»Prof. Satyajith Rath, Scientist NII	Member
»»Prof. K Muniyappa, Professor & Chairman, Department of Biochemistry, IISc	Member
»»Prof. Chandrima Shaha, Director, NII	Member
»»Prof. Chittaranjan Yajnik, Director, Diabetes Unit, KEM Hospital and Research Centre	Member
»»Dr. Alka Sharma, Adviser/Scientist 'G', Medical Biotechnology DBT (Ex-officio)	Member
»»Prof. Alok Srivastava, Head, CSCR & Professor of Medicine, CMC, Vellore (Ex-officio)	Member
»»Mr. Pawan Kumar Pahwa , CAO, inStem	Non Member Secretary

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

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

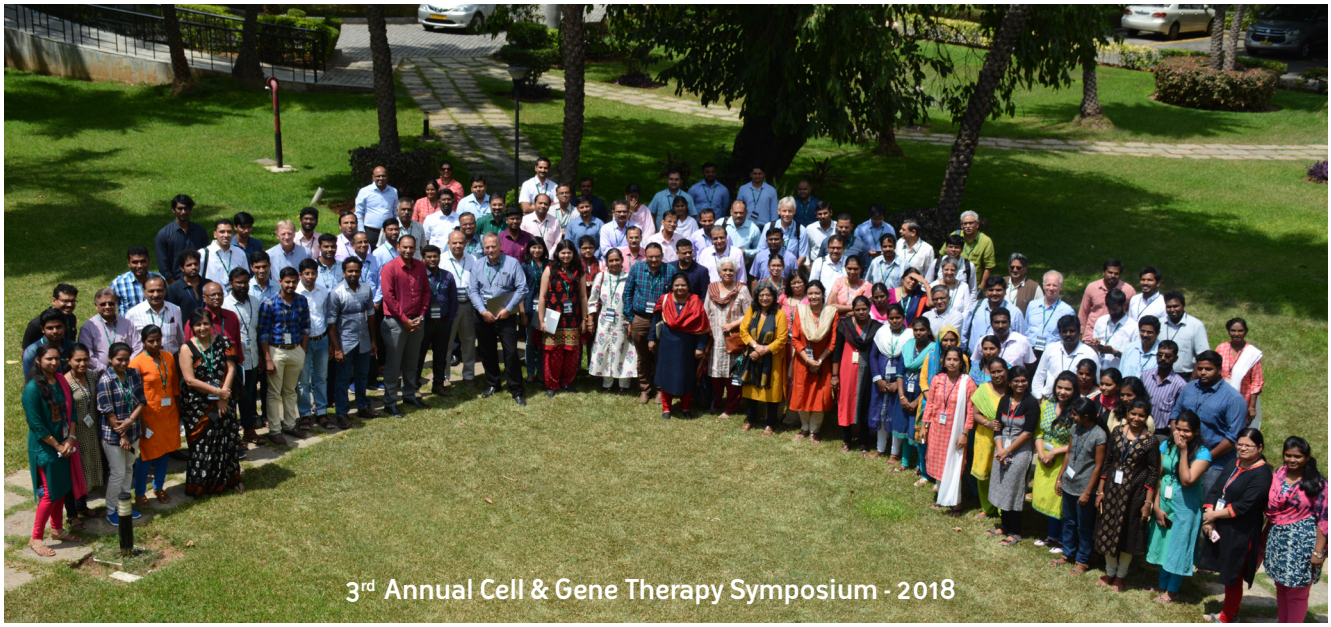
CSCR Core Committee *(appointed by Principal, CMC)*

»»Dr. Nihal Thomas, Department of Endocrinology	Chair
»»Dr. Prasad Mathews, Medical Superintendent (Ex-Officio)	Member
»»Dr. Molly Jacob, Department of Biochemistry	Member
»»Dr. Asha Mary Abraham, Department of Virology	Member
»»Dr. R V Shaji, Adjunct Scientist, CSCR	Member
»»Dr. Alok Srivastava, Head, CSCR	Member Secretary

CSCR Scientist Review Committee *(appointed by Principal, CMC)*

»»Dr. Prasad Mathews, Medical Superintendent z	Chair
»»Dr. B S Ramakrishna, SRM University	Member (External)
»»Dr. Molly Jacob, Department of Biochemistry	Member
»»Dr. Nihal Thomas, Department of Endocrinology	Member
»»Dr. Asha Mary Abraham, Department of Virology	Member
»»Dr. Alok Srivastava, Head, CSCR	Member Secretary

SAC 2018



3rd Annual Cell & Gene Therapy Symposium - 2018

Centre for Stem Cell Research
(a unit of inStem, Bengaluru)
Christian Medical College Campus
Bagayam, Vellore - 632002
Tamil Nadu, India
Phone: 91-416-2285101, 2285107, 2285120
Fax: +91- 416-2285103
Email: cscr@cmcvellore.ac.in | Website: www.cscr.in