CENTRE FOR STEM CELL RESEARCH

(a unit of inStem, Bengaluru)

Christian Medical College Campus, Bagayam, Vellore

ANNUAL REPORT

2017-18

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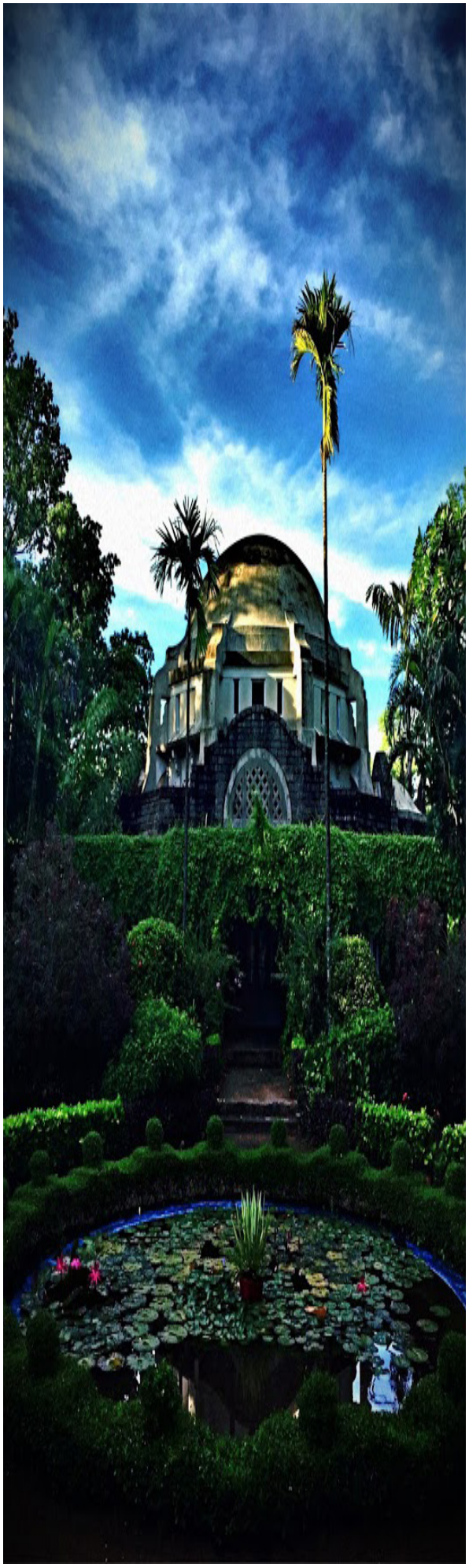


Centre for Stem Cell Research (CSCR)
(a unit of inStem, Bengaluru)
Christian Medical College Campus, Bagayam, Vellore

The Beginnings: 2005 - 2010

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

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

Mandate

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

Governance: 2005 - 2010

Even though it was initiated as a project by the DBT, CSCR was governed by a Governing Body, chaired by the Secretary DBT and also had a Finance Committee. There also was a DBT designated Scientific Advisory Committee that reviewed the work done at CSCR every year. In addition, there were two committees appointed by the CMC, Vellore to help with the management of CSCR on a regular basis, both from the administrative as well as the scientific aspects. These included a Core Committee of faculty from CMC and CSCR who meet regularly to resolve all matters at CSCR that require discussion and a Steering Committee, chaired by the Director, CMC, Vellore along with other administrative officers to provide policy guidance for CSCR in the early stages of its establishment.

CSCR – A unit of the Institute for Stem Cell Biology and Regenerative Medicine (inStem), Bengaluru from 2011

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

CORE SCIENTIFIC ACTIVITIES AND INITIATIVES

THEMATIC RESEARCH PROGRAMS

1. Musculoskeletal regeneration program

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

2. Gene therapy program

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

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

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

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

NOVEL APPROACHES TO HEMATOLOGICAL DISEASES (NAHD) PROGRAM

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

In addition, the ASHD program collaborates with the Centre for iPS Cell Research and Application (CiRA), Kyoto University, Japan, under the leadership of Prof. Shinya Yamanaka, a pioneer and Nobel Prize winner in stem cell technology. The program at NCBS, inStem, and NIMHANS - The Accelerator program for Discovery in Brain disorders using Stem cells (ADBS) – encompasses research to unravel complex problems in brain disorders / mental illnesses by exploiting the advances in modern human genetics, stem cell technology and clinical investigations. The program at CSCR / CMC - Novel Approaches to Hematological Disorders (NAHD) aims to enhance current methods / technologies including gene

therapy for hereditary blood disorders such as haemophilia, thalassemia and sickle cell disease, all of which are causes of significant morbidity and mortality in India. To ensure maximum impact on hereditary hemoglobin diseases in the population at risk in India, this collaborative initiative blends these efforts with a community outreach program for the control of major haemoglobin disorders.

The major components of this program are:

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

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

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

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

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

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

RESEARCH PROJECTS

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

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

Alok Srivastava
Head, CSCR

Scientific Research Profile

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

LABORATORY HIGHLIGHTS OF YEAR 2017-18

Musculoskeletal regeneration lab focuses on regenerative strategies using cell based therapy for musculoskeletal disorders. A number of clinical and preclinical studies relating to bone cartilage and tissue regeneration have been completed and are ongoing. Scaffolds from various partnering institutions are utilized to create new tissue engineered strategies.

1. Bone regeneration:

Patient recruitment and two year follwup for phase 1 trial for the treatment of large bone defects (gap non-union) in ten children using hydroxyapatite scaffold loaded with mesenchymal stem cells has been completed. While the strategiy was found useful for bone regeneration based on the outcome we have planned for making changes in our tissue engineered construct.

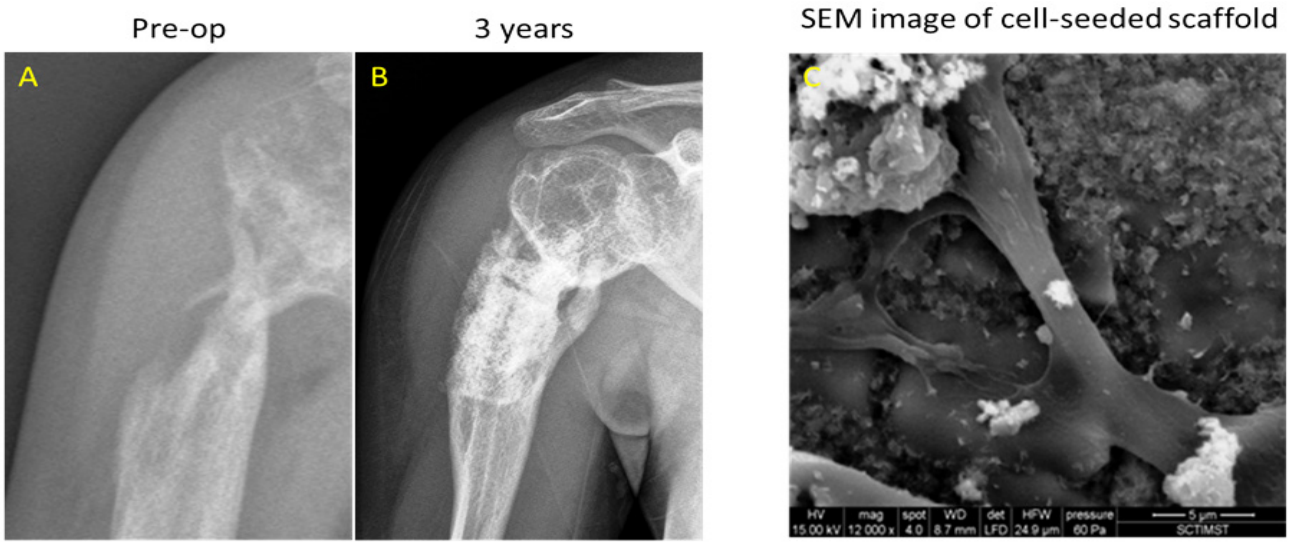


Figure 1 shows the outcome of tissue engineered bone transplant in proximal humerus at three years follow up. A. Preoperative radiographs of a 12 year old with chronic osteomyelitis and gap non-union B. Postoperative radiograph after 3 years shows union and well incorporated scaffold. C. Shows the scanning electron microscope analysis of cell seeded scaffold showing good cell to scaffold and cell to cell interaction at 8 days.

2. Physeal regeneration:

After the successful completion of a pilot study for the treatment of physeal bars in five children using autologous chondrocyte transplantation a phase I/II clinical trial for a total of 15 children has been sanctioned by department of health research. Regulatory clearance from the Drug Controller General of India has been obtained and CTRI registered.

3. Differentiation of mesenchymal stem cells (MSCs) into chondrocytes by sustained delivery of miRNAs using chitosan hydrogel:

The current protocol for the differentiation of MSCs into chondrocytes results in hypertrophic cartilage instead of hyaline like cartilage. We have shown that the quality of differentiated chondrocytes could be enhanced using biomolecules.

4. Musculoskeletal stem cell targeting:

This is a collaborative project with four Indian partners and two international partners from Denmark. The focus is on

1. Muscle-derived stem cells in the treatment of anal sphincter injury in a rat model:

2. Treatment of osteochondral defects using functionalised scaffold with or without mesenchymal stem cells:

3. Treatment of segmental bone defects using functionalised scaffold with or without mesenchymal stem cells:

5. Effect of shock wave treatment on growth plate cartilage:

This study aims to evaluate the effects of shock wave therapy on longitudinal bone growth.

Our results from ex-vivo cultured fetal rat metatarsal organ culture suggest that shock waves at higher energy level and frequency can significantly a. increase the final bone length accompanied by marked cellular proliferation, chondrocyte hypertrophy; b. Upregulate Gli-1 expression, a downstream target of Ihh on the shock wave treated bones.
In vivo studies in rabbits suggest that both high and low energy radial shock wave treatment can increase the thickness of growth plate cartilage.

6. Other translational research with surgical team within CMC:

Tissue regeneration using muscle derived stem cells in the treatment of ventral hernia in a rat model - Drs. Inian Samarasam and Joshua Frankyln

This study tested the combination of culture expanded autologous muscle derived stem cells over an electrospun poly-caprolactone scaffold in a ventral hernia model and found satisfactory outcome at 6 and 10 weeks.

Publications since last report

- Madhuri V., Rajagopal K., Ramesh S. (2017) Physeal Regeneration: From Bench to Bedside. In: Mukhopadhyay A. (eds) Regenerative Medicine: Laboratory to Clinic. Springer, Singapore
- Lise K. Hansen, Henrik D. Schrøder, Lars Lund, Karthikeyan Rajagopal, Vrisha Maduri, Jeeva Sellathurai. The effect of low intensity shockwave treatment (Li-SWT) on human myoblasts and mouse skeletal muscle. BMC Musculoskeletal Disorders BMC series 2017.18:557
- David Livingstone, Albert A Kota, Sanjay K Chilbule, Karthikeyan Rajagopal, Sukria Nayak, Vrisha Madhuri Isolation, in-vitro expansion, and characterization of human muscle satellite cells from the rectus abdominis muscle. Paediatric orthopaedics and related sciences. Volume 3. February 2017.

Invited Talks

1. Mr. Karthikeyan R gave an invited talk titled 'Autologous chondrocyte transplantation for the treatment of physeal defect in children' at the 46th Annual Conference of Orthopedic Surgeons Society of Andhra Pradesh, Nellore – February 24-26th, 2018

Patents:

Patent in Process- Invention Facilitation and Screening meeting – January 22, 2018 - Fabrication of a wound healing material using umbilical cord Wharton's jelly stem cells

Poster/ conference

1. Sowmya Ramesh. 'Modulation of longitudinal bone growth using radial shock wave treatment' Poster presented at the Nordic Doctoral Summit August 21-22, 2017, Karolinska Institutet, Sweden.
2.Sowmya Ramesh"Evaluation of safety and feasibility of novel tissue engineered bone for large segmental bone defects using autologous mesenchymal stem cells" and "Suitability of human muscle-derived stem cells on electrospun PCL scaffolds for skeletal muscle tissue engineering" posters presented at the European Chapter Meeting of Tissue Engineering and Regenerative Medicine Tissue Engineering and Regenerative Medicine International Society26 - 30 June, 201 , Davos, Switzerland.

3. Mr. Karthikeyan Rajagopal osters titled “Chitosan-gelatin nanocomposite scaffold for articular cartilage regeneration” and “Articular cartilage regeneration in osteoarthritis rat model” at the European Chapter Meeting of Tissue Engineering and Regenerative Medicine Tissue Engineering and Regenerative Medicine International Society (26 - 30 June, 2017) in Davos, Switzerland. He was selected for a travel grant from SERB, DST to attend the conference.

4. Sowmya Ramesh and.Karthikeyan Rajagopal was shortlisted for a scientific debate at the Tissue Engineering and Regenerative Medicine Tissue Engineering and Regenerative Medicine International Society 2017 on the topic ““Will 3D-Printing or Decellularised organs rescue the donor-based organ scarcity for transplantation?”

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. Dolly Daniel, CSCR / Department of Transfusion medicine & Immunohematology, CMC, Vellore
12. Antonisamy, Department of Biostatistics, CMC, Vellore

P.R.J.V.C. BOOPALAN, MS

Professor, Department of Orthopedics Unit-3, CMC, Vellore
Adjunct Scientist, CSCR

PROJECT-1

Project title: In vitro characterization and immunogenic profiling of human articular chondroprogenitor cells from normal and osteoarthritic knee joints.

Funding source: AO Trauma Asia Pacific Research Grant 2016

Duration: Ongoing March 2017 - October 2018

Brief description of the project:

PART I

Although there are established protocols for isolation of pure populations of chondroprogenitors and chondrocytes the need for a classical differentiating marker between the two cell populations still exist. Our primary objective was to compare both cell types and evaluate differences in their biological characteristics using flow cytometric analysis (MSC, chondrocyte and chondroprogenitor CD markers), RT-PCR studies for chondrogenic and hypertrophic markers and differentiation studies. Our second objective was to assess if osteoarthritis differentially affects the cell populations under consideration, therefore cell samples isolated from normal and osteoarthritic human cartilage were compared. Our final objective was to look at the effects of prolonged time (upto passage 10) in culture on the said populations.

PART II

Another objective was to conduct immunogenic profiling of human articular chondrocytes and chondroprogenitors from normal and osteoarthritic knee joints using flow cytometry analyses, T cell response in invitro stimulation assays, RT-PCR and ELISA assays.

Work done

The aforementioned objectives (Part I) are complete and has been submitted to Cartilage .

Support from CSCR: Lab space and core lab facilities.

Publications:

Elizabeth Vinod, P. R. J. V. C. Boopalan, Solomon Sathishkumar, 2018 'Reserve or Resident Progenitors in Cartilage? Comparative Analysis of Chondrocytes versus Chondroprogenitors and Their Role in Cartilage Repair'. http://journals.sagepub.com/doi/abs/10.1177/1947603517736108.

Collaborations:

External:

1. Ozlem Ozbey, Department of Histology and Embryology Campus, School of Medicine, Akdeniz University, Antalya, Turkey
2. Sabareeswaran Arumugam, Division of Experimental Pathology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram
3. George Thomas, Chief Orthopaedic Surgeon and Head of Emergency Services, St. Isabel's Hospital, Mylapore, Chennai

Internal:

1. Elizabeth Vinod, CSCR / Department of Physiology, CMC, Vellore
2. Solomon Sathishkumar, Department of Physiology, CMC, Vellore

PROJECT-2

Part I: Comparison of electrophysiological properties and gene expression between human chondrocytes and chondroprogenitors derived from normal and osteoarthritic cartilage.

Part II: Evaluation of whole genome transcriptome between human chondrocytes and chondroprogenitors derived from normal and osteoarthritic cartilage

Funding source: AO Trauma Asia Pacific Research Grant 2017

Duration: Ongoing March 2017 - February 2019

Brief description of the project:

PART I

Since chondrocytes and chondroprogenitors co-exist in cartilage and chondrocytes have been shown to acquire stemness in cultures, the ability to define and provide a clear-cut differentiation between the two-cell populations has been obscured. Since chondrocytes have been profiled extensively, our first attempt was to differentiate this cell population from chondroprogenitors based on electrophysiology by performing patch clamp analysis.

Furthermore, the growing interest in CPs is due to their MSC like properties and therefore our second objective was to look at mRNA expression of ionic channels already reported in MSCs as per literature. RT-PCR analysis used for assessing molecular evidence of channels was also extended to chondrocytes for comparison. Another dimension studied was whether cells obtained from normal and osteoarthritic (OA) knee joints showed any difference when said analysis was performed. Our interest was to see whether the disease altered cell biology enough so as to render the OA cartilage as a less than suitable cell source.

PART II

Another objective was to conduct whole transcriptome analysis of human articular chondrocytes and chondroprogenitors from normal and osteoarthritic knee joints to uncover novel biomarkers that may exist, thus help us establish ideal cell population with enhanced chondrogenic potential for cartilage repair.

Support from CSCR : Lab space and core lab facilities.

Collaborations:

External:

1. George Thomas, Chief Orthopaedic Surgeon and Head of Emergency Services, St. Isabel's Hospital, Mylapore, Chennai

Internal:

1. Abel Livingston, Department of Orthopaedics, CMC, Vellore
2. Upasana Kachroo, Department of Physiology, CMC, Vellore
3. Elizabeth Vinod, CSCR / Department of Physiology, CMC, Vellore
4. Solomon Sathishkumar, Department of Physiology, CMC, Vellore

ELIZABETH VINOD, MD

Assistant Professor, Department of Physiology, CMC, Vellore

Adjunct Scientist, CSCR

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 - February 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.

Publication:

Elizabeth Vinod, P. R. J. V. C. Boopalan, Solomon Sathishkumar, 2018 'Reserve or Resident Progenitors in Cartilage? Comparative Analysis of Chondrocytes versus Chondroprogenitors and Their Role in Cartilage Repair'. http://journals.sagepub.com/doi/abs/10.1177/1947603517736108.

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

Collaborations:

1. Boopalan Ramasamy, CSCR / Department of Orthopaedics, CMC, Vellore
2. Solomon Sathishkumar, Department of Physiology, CMC, Vellore

INIAN SAMARASAM, MS, FRCS, FRACS
Professor, Upper GI Surgery Unit, Department of Surgery, CMC, Vellore
Adjunct Scientist, CSCR

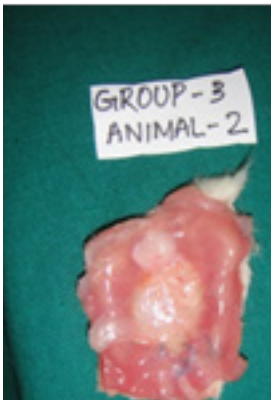
Project title: Tissue regeneration using muscle derived stem cells in the treatment of ventral hernia in a rat model

Funding source: CSCR

Duration: September 2017 – July 2018.

Brief description of the project:

This study attempts to test the combination of culture expanded autologous muscle derived stem cells over an electrospun poly-caprolactone scaffold in a ventral hernia model. The cell seeded construct along with a polypropylene (PP) mesh was transplanted into the abdominal wall of the rat (n=12) after the creation of a ventral hernia. The animals that received PP mesh alone and PP + PCL served as a control (n=12 each). The outcome at six and ten weeks post transplantation was assessed by adhesions, tensile strength and histology. Our preliminary results suggest gross healing (Fig.1), minimal adhesion and evidence of regenerated muscle towards the peritoneal layer histologically in the cell seeded group. While the PP and PP+PCL group had higher adhesion scores with no significant muscle regeneration. Thus our study establishes safety and feasibility of using tissue engineered construct for the treatment of ventral hernia.



Any specific highlights of the project: We observed minimal adhesions with and regenerated skeletal muscle bundles in the cell seeded group.

Support from CSCR: Funding, usage of laboratory animal facility, core lab facility

Publications:

- Manuscript submitted to Journal – Hernia and is being peer reviewed.
- Paper presented in the European Surgical Society conference in Poland and was awarded the best paper in the basic science category and overall conference 2nd best paper among all categories.

Collaborations:

Internal:

1. Vrisha Madhuri, CSCR / CMC, Vellore
2. Geeta Chacko, CSCR / CMC, Vellore

External:

1. Amit Kumar, VIT, Vellore

ALOK SRIVASTAVA, MD, FRACP, FRCPA, FRCP

Professor, Department of Haematology, CMC, Vellore

Adjunct Scientist / Head, CSCR

LABORATORY HIGHLIGHTS OF YEAR 2017-18

Highlights from Different Research Programs

A. The gene therapy program

The gene therapy program at CSCR has several components:

I. Within NAHD program (Novel Approaches to Hematological Diseases) program

1. CLINICAL TRIAL FOR GENE THERAPY OF HEMOPHILIA B

A novel transgene has been evaluated in two in vivo modelsthe First by packaging into AAV8 vector and comparing it with the LP1 transgene in the mouse model establishing comparable expression; Second, NOG mice with a 'humanized' liver (human hepatocytes) were transduced with this vector and transgene. This experiment also confirmed significant FIX expression. (Figure 1 a & b)). This has given us adequate data to be convinced of the functionality of this transgene and proceed towards the IND enabling non-human primate (NHP) studies with a GMP like product.

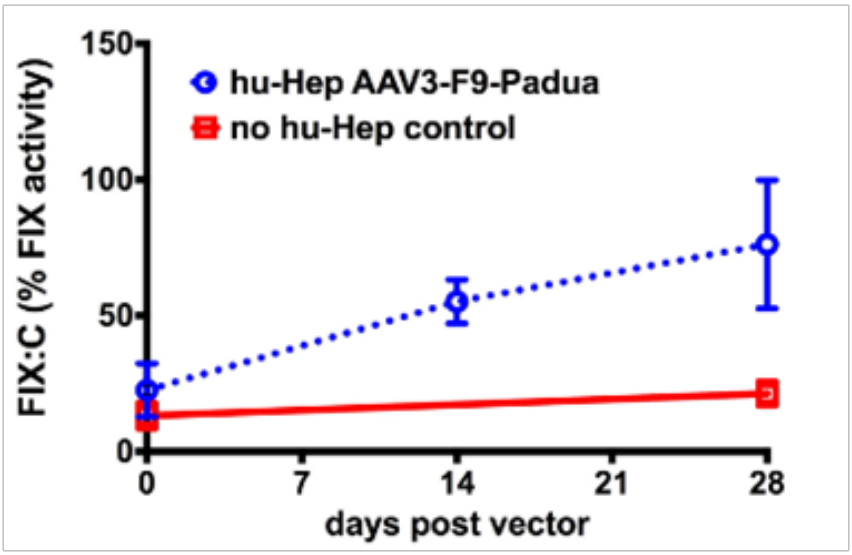


Figure 1: TK-NOG mice with humanized liver & AAV3-F9 Padua – hFIX activity 14 and 28 days post 5e11 vg vector infusion.

2.GENE THERAPY FOR THE MAJOR HAEMOGLOBIN DISORDERS

i. Lentiviral based – See report from R V Shaji
ii. Gene editing based approach – See reports from Saravanabhavan Thangavel and Mohankumar Murugesan

II. Other Gene Therapy Projects

1. LENTI VIRUS VECTOR GENE THERAPY FOR HEMOPHILIA A

Apart from this AAV3 based gene therapy for haemophilia B, we have filed a proposal for a clinical trial of a lentiviral vector (LVV) based gene therapy for haemophilia A. In the mouse model, it has been shown that very high levels of FVIII are secreted from hematopoietic stem cells transduced with a myeloid/monocytic cell specific promoter (CD68) (Figure 2). Further studies in human HSCs have also confirmed expression in myeloid/monocytic cells.

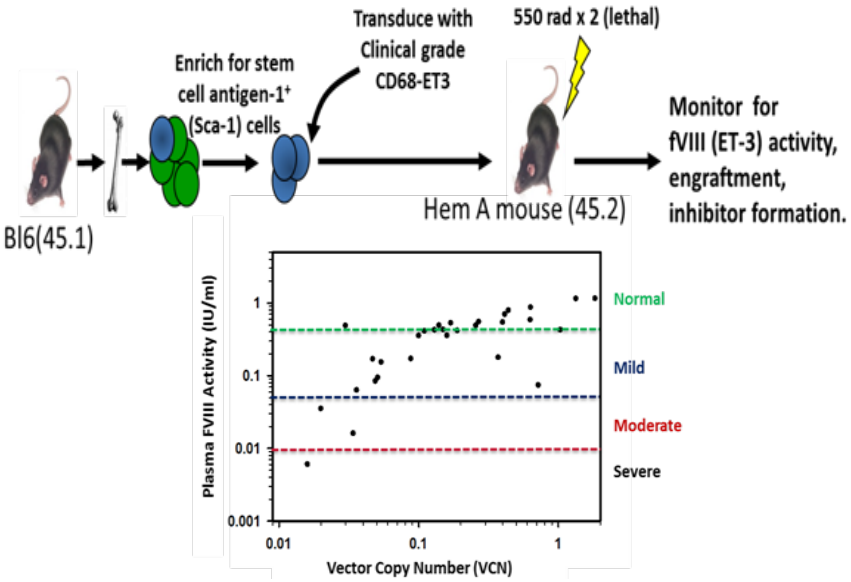


Figure 2. HSCT gene therapy using LV-CD68-ET3 in murine hemophilia A

We are also keen on this approach as nearly 50% of the patients with haemophilia may be ineligible for AAV based gene therapy due to pre-existing AAV antibodies to different serotypes. The concept here is similar to what is being done widely now in LVV based gene therapy for the major haemoglobin disorders which are in advanced stage clinical trials in the world. In brief, HSCs are mobilized in the patient and transduced ex-vivo with the LVV and then transplanted after suitable conditioning.

Collaboration with industry

Another AAV based gene therapy program for haemophilia B is being pursued with industry collaboration (INTAS Pharmaceuticals) through a formal MoU, this work is focused on both AAV8 and AAV 5 vector with the FIX Padua transgene for gene therapy. Please see the report of Dr. Sanjay Kumar for further details.

Collaboration with institutions/scientists

B. Haplobanking

This novel and a 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/. Please see the report of Dr. Dolly Daniel and Dr. R.V. Shaji.

C. Developing newer areas of research

An area of cell therapy that can paradigms of management of resistant cancer are the T cell therapies, particularly CAR T cells. We are trying intensively to establish this technology at CSCR. We have established a group consisting of basic scientists, scientific staff from cGMP as well as clinical faculty to work together to take this forward. While Dr. Sunil Martin from CSCR is working on gene modification of T cells to produce the CAR T cells, Dr. Aby Abraham from the Department of Haematology, CMC, Vellore is coordinating the establishment of protocols for NK and gamma delta T cell expansion. Please see their reports.

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

This program is led by Dr. Kuryan George and Dr. Shantidani Minz along with several other colleagues from the departments of Community Health, Haematology and Transfusion Medicine and Immunohaematology at CMC, Vellore, A collaboration was established with the Department of Health and Family Welfare and the National Health Mission of the Govt. of Odisha for developing a model for control of thalassemia and sickle cell disease in Odisha. In July 2017, the team from CSCR / CMC, Vellore had a meeting with the Commissioner-cum-Secretary of Department of Health and Family Welfare and the Mission Director, National Health Mission of the Government of Odisha along with other senior officials of the health department to discuss the project plans. A MoU was signed with the Department of Health and Family Welfare, Govt. of Odisha on 5th December 2017. For this project, a situational analysis in Odisha has been completed with the help of Indian Institute of Public Health Bhubaneswar (IIPHB). A Steering Committee and two Core Working Groups have been established.

The revised detailed implementation proposal is in its final stages will be submitted to the DBT for approval.

Selected publications (2017-18):

1. Manian KV, Bharathan SP, Maddali M, Srivastava VM, Srivastava A, Velayudhan SR. Stem Cell Res. 2018 May;29:148-151.
2. Mohanan E, Panetta JC, Lakshmi KM, Edison ES, Korula A, Na F, Abraham A, Viswabandya A, George B, Mathews V, Srivastava A, Balasubramanian P. Clin Pharmacol Ther. 2017 Dec 16. doi: 10.1002/cpt.988.
3. Fouzia NA, Edison ES, Lakshmi KM, Korula A, Velayudhan SR, Balasubramanian P, Abraham A, Viswabandya A, George B, Mathews V, Srivastava A. Bone Marrow Transplant. 2018 Feb;53(2):169-174.
4. George B, Pn N, Devasia AJ, Kulkarni U, Korula A, Lakshmi KM, Abraham A, Srivastava A, Mathews V. Biol Blood Marrow Transplant. 2018 Mar;24(3):494-500.
5. Korula A, Pn N, Devasia A, Lakshmi KM, Abraham A, Sindhuvi E, George B, Srivastava A, Mathews V. Biol Blood Marrow Transplant. 2018 Jan;24(1):103-108.
6. Abbas S, Kini A, Srivastava VM, M MT, Nair SC, Abraham A, Mathews V, George B, Kumar S, Venkatraman A, Srivastava A. Blood Cells Mol Dis. 2017 Jul;66:37-46.
7. Hashmi SK, Srivastava A, Rasheed W, Adil S, Wu T, Jagasia M, Nassar A, Hwang WYK, Hamidieh AA, Greinix HT, Pasquini MC, Apperley JF, Aljurf M. Hematol Oncol Stem Cell Ther. 2017 Dec;10(4):167-172.
8. Mohanan E, Panetta JC, Lakshmi KM, Edison ES, Korula A, Fouzia NA, Abraham A, Viswabandya A, Mathews V, George B, Srivastava A, Balasubramanian P. Bone Marrow Transplant. 2017 Jul;52(7):977-983.
9. Bharathan SP, Nandy K, Palani D, Janet A NB, Natarajan K, George B, Srivastava A, Velayudhan SR. Stem Cell Res. 2017 Apr;20:54-57.
10. Bharathan SP, Manian KV, Aalam SM, Palani D, Deshpande PA, Pratheesh MD, Srivastava A, Velayudhan SR. Biol Open. 2017 Jan 15;6(1):100-108.
11. Srivastava A, Shaji RV. Haematologica. 2017 Feb;102(2):214-223.

Collaborations:

Internal Collaborators:

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 Collaborators:

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

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

LABORATORY HIGHLIGHTS OF YEAR 2017-18

Molecular mechanisms of human erythropoiesis

We are trying to understand the transcriptional regulation of human erythropoiesis. We use a variety of cell and molecular biology methods in this research area. These include ex-vivo erythropoiesis to generate erythroid cells in the laboratory, immortalization of erythroid progenitors, small RNA Sequencing and ChIP-Sequencing, RNAi using lentiviral shRNA libraries and gene editing of target genes using CRISPR-Cas9. The following are the translational research projects that we are carrying out in this area:

II. Pre-clinical lentiviral gene therapy for haemoglobinopathies

In collaboration with Dr. Trent Spencer, Emory University, we have initiated a pre – clinical lentiviral gene therapy project for hemoglobinopathies. The vectors have shown upto 16% globin expression in the ex – vivo erythropoiesis model. Three vectors (CSCREU-V1, V3 and V4) were further tested in a mouse model for sickle cell disease to check their efficacy. In brief, Sca-1+ cells from Townes' sickle cell mouse were isolated, purified and transduced with the lentiviruses and these transduced cells were transplanted into irradiated C57Bl6 transgenic mice. Cellulose acetate electrophoresis analysis showed significant level of exogeneous haemoglobin expression.

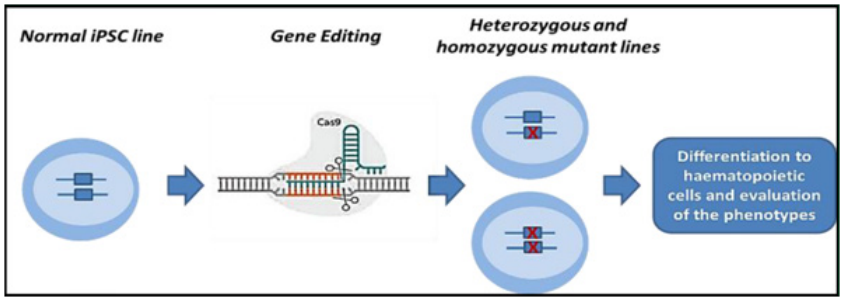
We are currently generating additional vectors for higher expression of transgene β – globin and γ – globin expression. We designed novel lentiviral vectors expressing the BCL11a shRNAs from the intron sequences of β – globin transgene for the simultaneous expression of β – globin and re – activation of the γ – globin genes.

For rapid analysis of lentiviral vectors, we will use immortalized erythroid cells from patients with Sickle cell disease and β – thalassemia. We have generated immortalized erythroid lines from normal CD34+ cells and PBMNCs and we will use the same strategy for the generation of immortalized erythroid lines for the patients.

EARLY PRE-CLINICAL RESEARCH – GENE THERAPY FOR ERYTHROID DISORDERS:

In this project, the aim is to create disease models for monogenic erythroid disorders. Two diseases have been chosen: Diamond Blackfan Anemia (DBA) and Congenital Dyserythropoietic Anemia (CDA). Both diseases manifest anemia secondary to ineffective erythropoiesis. DBA is caused by mutations in ribosomal genes and CDA is caused by mutations in CDAN1 and SEC23B genes.

We are currently creating disease causing mutations in a normal iPSC line by CRISPR/Cas9 mediated gene editing. We have established the methods for creating mutations in iPSCs and to differentiate these iPSCs to haematopoietic progenitor cells and erythroid cells (Figure 1).



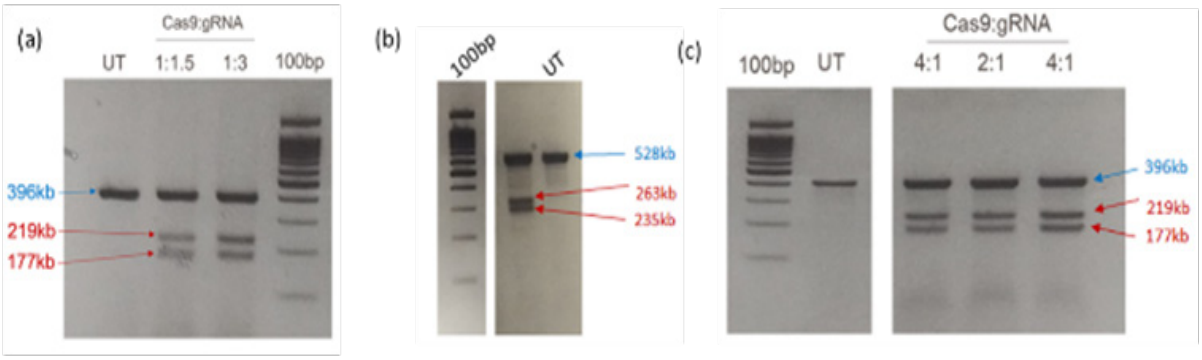


Figure: CRISPR/Cas9 targeting of CDAN1 by Nucleofection and Lipofection: Disruption of CDAN1 in iPSCs by: (a) RNP delivered by Nucleofection (b) Plasmid delivered by Lipofection and (c) RNP delivered by Lipofection

HAPLOBANKING – ESTABLISHING A BANK OF IPS CELLS FROM INDIVIDUALS WITH HOMOZYGOUS HLA HAPLOTYPES IN INDIA:

So far, we have collected blood samples from 186 donors who are homozygous for HLA haplotypes, and the PBMNCs were isolated and cryopreserved inside the GMP facility. For the expansion of erythroid cell progenitors from PBMNC culture, robust xeno-free erythroid expansion culture conditions have been standardized. Using also have standardized a xeno-free protocol to generate iPSCs from 8 donors. To generate transgene-free iPSCs from these erythroid progenitors, nucleofection using the episomal plasmids to express the reprogramming factors was performed. Two clones were isolated from each donor and they were expanded on feeder-free condition for 15-20 passages.

At passage 15, all the expanded clones maintained the ESC-like morphology and showed no signs of differentiation. Quantitative real-time PCR analysis showed high expression of pluripotency markers (NANOG, OCT4, SOX2, GDF3, STELLA, DNMT3B and TGFD1). The expression of pluripotency markers (OCT4, SOX2, NANOG, SSEA4, TRA-1-60, TRA-1-81) was also detected and confirmed by immunofluorescence staining. Embryoid body and tri-lineage differentiation potential were assessed which showed efficient differentiation into all three germ lineages. At passage 15, these clones were cryopreserved and the stability of the iPSC clones post freezing and thawing were also tested during this period.

Karyotype analysis confirmed the genomic stability of the expanded iPSC clones at passage 15. STR analysis was performed for the genetic identify of the clones and PCR analysis showed that was no vector integration in the clones. PCR analysis performed on the samples confirmed the absence of mycoplasma contamination. Whole genome sequencing of 4 iPSC samples were also performed during this period to evaluate the genomic stability of the established clones.

In the following years, the expansion of erythroid cell progenitors from PBMNCs and reprogramming will be peformed in our GMP facility. Analysis of the whole genome sequencing will be performed in depth to evaluate the safety of the established clones using this protocol.

Publications:

1. Generation of an integration-free iPSC line (CSCRi005-A) from erythroid progenitor cells of a healthy Indian male individual. Manian KV, Bharathan SP, Maddali M, Srivastava VM, Srivastava A, Velayudhan SR. Stem Cell Res. 2018 May;29:148-151.
2. Long-term outcome of mixed chimerism after stem cell transplantation for thalassemia major conditioned with busulfan and cyclophosphamide. Bone Marrow Transplant. 2018 Feb;53(2):169-174.
3. Fouzia NA, Edison ES, Lakshmi KM, Korula A, Velayudhan SR, Balasubramanian P, Abraham A, Viswabandya A, George B, Mathews V, Srivastava A. Bone Marrow Transplant. 2018 Feb;53(2):169-174.

SARAVANABHAVAN THANGAVEL, PhD
Assistant Investigator, CSCR

LABORATORY HIGHLIGHTS OF YEAR 2017-18

The prime focus of our lab is to develop gene-modified haematopoietic stem cells for the autologous stem cell transplantation. Using CRISPR/Cas9 gene editing tools we aim to correct Beta hemoglobinopathies, a common genetic disorder and Wiskott-Aldrich Syndrome (WAS), a rare disease. We also work on various technologies to improve the efficiency of generation of gene-modified haematopoietic stem cells.

Gene editing the haematopoietic stem cells for the gene therapy of β- haemoglobinopathies

β-thalassemia and Sickle Cell Anemia, the common genetic diseases in India, is caused by mutations in the human hemoglobin beta (HBB) gene. The mutations lead to reduced/absent synthesis of the beta globin chains of the hemoglobin tetramer. We are developing two different strategies towards the permanent correction of the disease phenotype.

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 achieved the genetic correction of disease-causing mutation in the primary hematopoietic stem cells of the patients and currently observing the functional consequences on the hemoglobin chain production.

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. Two targets for the HBF reactivation have been identified and gene edited in the erythroid progenitor cell lines. Preliminary experiments have shown the HbF reactivation in the erythroid cell lines. Our future plan is to test the same reagents in the HSPCs for the reactivation of fetal gamma globin.

Gene editing the haematopoietic stem cells for the gene therapy for Wiskott-Aldrich Syndrome (WAS)

Wiskott-Aldrich syndrome is one of the very first disease to be tested for viral vector -mediated gene therapy. The fitness advantage of gene- corrected WAS-HSCs 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.

WAS, a life-threatening X-linked recessive disorder is characterized by thrombocytopenia, eczema and immunodeficiency. Mutations in the WAS gene leads to compromised expression of the Wiskott - Aldrich syndrome Protein (WASP). 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 in 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 cell lines and currently being tested in the HSPCs from the healthy donors.

Technology development for the enhanced production of gene modified HSPCS

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 in the cell lines. Currently, we are testing these reagents for their efficiency in delivering the genome editing cargo into HSPCs.

ii) We are developing a novel platform for the ex vivo expansion of hematopoietic stem cells. The flow cytometric 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.

Project	stage						
	Target selection	Optimized editing reagents	Validation in cell lines	Validation in primary HSPCs	Validation in patient HSPCs	Engraftment studies in mice	Late pre-clinical studies
Gene editing for B-hemobinopathies i) Mutation correction							
Gene editing for B-hemobinopathies ii) HbF reactivation							
Gene editing for WAS							
HSPC expansion							
HSPC transfection							

Fig: Table summarizing the projects and the progress

Honors and awards:

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

Grants:

- CSCR start-up grant
- "Pre-clinical studies for gene therapy of Wiskott-Aldrich Syndrome (WAS)" SERB-Early Carrier Research award funded by-DST, India.
- Novel Approaches to Haematological Disease (Gene editing approach for major haemoglobin disorders)

Publications:

- Rangasami V, Lohchania B, Rachamalla R, Banerjee R, Dhayani A, Thangavel S, Vemula P, Marepally S. Exploring Membrane Permeability of Tomatidine to Enhance Lipid Mediated Nucleic acid Transfections: BBA – Biomembranes, 2018.
- Dharmalingam P, Reddy R H, Bhanuprakash B, Thangavel S, Murugesan M, Banerjee R, Chaudhuri A, Voshavar C and Mareaplly S. Green transfection: Cationic lipid pool derived from vegetable fat palm stearin enhances nucleic acid transfections. ACS Omega, 2017, 2 (11), 7892–7903

Invited talks:

Introduction and application of genome editing technology: Guest Lecture, September, 2017, VIT, Vellore

Academic Activities:

- Organizing committee member of 2nd annual symposium on Cell and Gene therapy, Vellore
- In-Charge: Imaging facility, Students presentation, Stem cell gene therapy class to JRFs

Collaborations:

Internal:

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:

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

Lab members:

1. Santhosh Chander Maddila: Post-doctoral fellow
2. Abisha Crystal: SRF
3. Vigneshwaran Venkatesan: JRF
4. Sarubala Malaiappan: JRF
5. Saranya Srinivasan: JRF
6. Porkizhi arjunan: Short-term project trainee
7. Muthu Ganesh: Short-term project trainee

MOHANKUMAR MURUGESAN, PhD
Assistant Investigator, CSCR

LABORATORY HIGHLIGHTS OF YEAR 2017-18

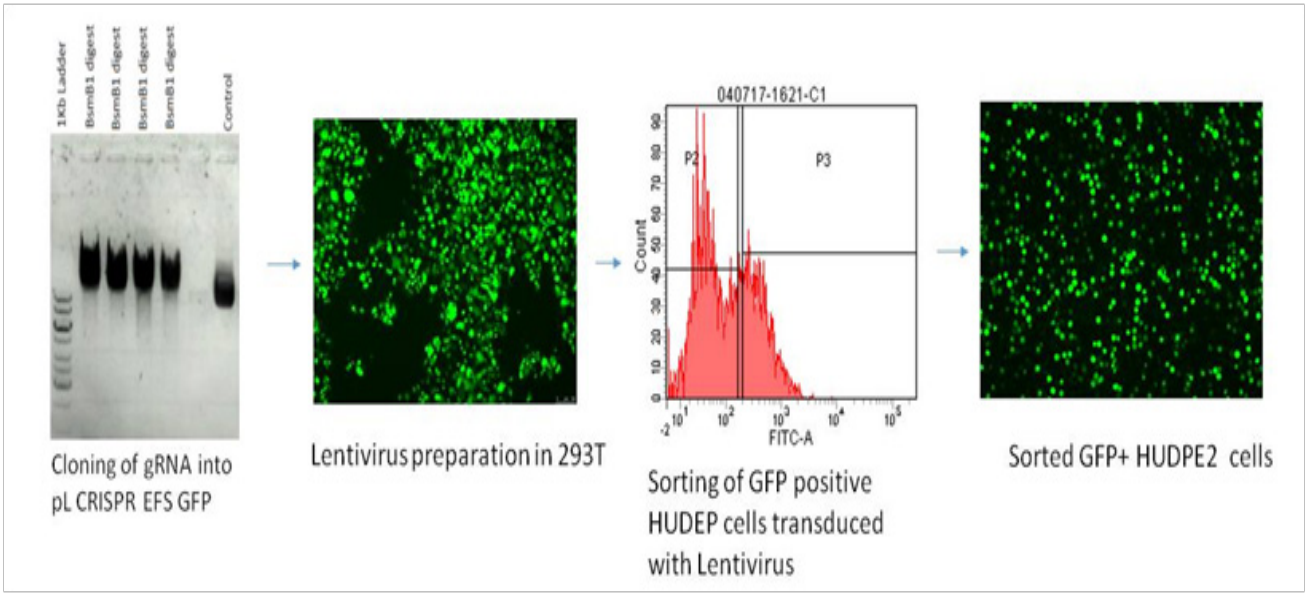
The current focus of my lab is to develop a novel genome editing approach for the treatment of β - hemoglobinopathies and Haemophilia-A.

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

Reversing the fetal to adult hemoglobin switch is substantial therapeutic interest, since persistence high levels of HbF ameliorates clinical symptoms of β – hemoglobinopathies. 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.

BCL11A

BCL11A as a key transcriptional factor that 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. To delete the critical sequence in the human BCL11A erythroid specific enhancer, we designed guide RNAs targeting the BCL11A intron-2, erythroid specific BCL11A enhancer and cloned these guide sequences into lentiviral vector. We transfected these guides into human embryonic kidney 293T cells and sorted for GFP. The validation of these guide RNAs was achieved through T7- endonuclease based in vitro screening in human embryonic kidney cells. We have successfully edited HUDEP2 cells with these guide RNAs by using lentivirus and validated the cleavage of target locus. We established the culture conditions and ex vivo erythropoiesis for HUDEP 2 cells. We determined the effect of targeted deletion of BCL11A enhancer on induction of fetal globin level in HUDEP2 cells.



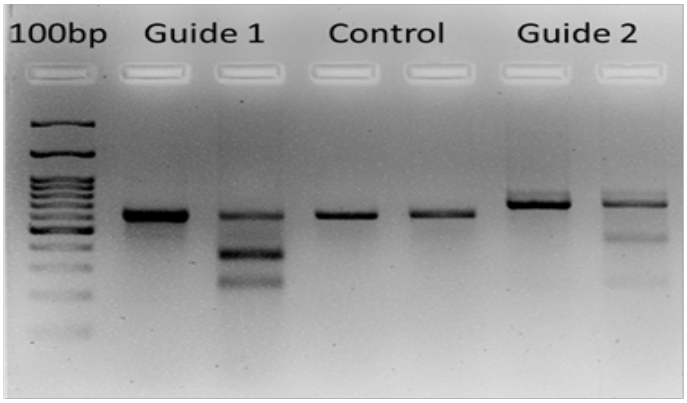
Lenti viral delivery of CRISPR Cas9 and gRNAs into HUDEP cells

Non deletional HPFH

Non deletional HPFH mutations are caused by point mutations in the Aγ-globin or Gγ-globin gene promoters. These point mutations greatly increase the production of Aγ-globin or Gγ-globin chain. 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.

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.



T7 endonuclease assay showing the cleavage 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 hematological malignancies, we are assessing the cytolytic activity of selectively expanded γδ2 T lymphocyte under different conditions and from different sources in invitro by rapid flow cytometry-based assay

Current lab members:

1. Nithin Sam, Junior Research Fellow
2. Vignesh R, Junior Research Fellow
3. Nazar M, Senior Research Fellow
4. Kartik lakhotiya, Short-term project trainee

Honors and Awards:

- Indo-U.S. Genome Engineering / Editing Technology Initiative (GETin) Overseas Fellowship program 2017 supported by the Department of Biotechnology (DBT), Govt. of India

Grants:

- Preclinical genome editing approach for the treatment of beta-globin disorders: SERB-DST, India
- Accelerating the application of stem cell technology in human diseases: CSCR core grant
- C11orf95-RELA fusions in supratentorial ependymomas: Relevance in prognostication: SERB-DST, India (co-PI)
- CSCR start-up grant

Academic activities:

- Organizing committee member of 2nd annual symposium on Cell and Gene therapy, CSCR, Vellore
- In-Charge: JRF review process
- Stem cell gene therapy class to JRFs

Publications:

- Dharmalingam P, Reddy R H, Bhanuprakash B, Thangavel S, Murugesan M, Banerjee R, Chaudhuri A, Voshavar C and Mareaplly S. Green transfection: Cationic lipid pool derived from vegetable fat palm stearin enhances nucleic acid transfections. ACS Omega, 2017, 2 (11), 7892–7903

Invited talks:

Therapeutic genome editing for beta hemoglobinopathies: BioMET, July 2018, VIT, Vellore

Collaborations:

Internal:

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. Sunil Martin, CSCR
6. Geeta Chacko, CSCR / Department of Neuropathology, CMC, Vellore
7. Premila Abraham, Department of Biochemistry, CMC, Vellore
8. Muthuraman N, CSCR / Department of Biochemistry, CMC, Vellore
9. Christhunesa Soundararajan, CSCR / Department of Neurochemistry, CMC, Vellore
10. Aby Abraham, CSCR / Department of Haematology, CMC, Vellore

External:

1. Jacob Corn, Innovative Genomics Institute, UC Berkeley, USA
2. Mark Dewitt, Innovative Genomics Institute, UC Berkeley, USA

SRUJAN K MAREPALLY, PhD

Scientist, CSCR

LABORATORY HIGHLIGHTS OF YEAR 2017-18

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.

PROJECT - 1

Understanding the mechanisms involved in lipid mediated nucleic acid transfections.

Cationic lipid guided nucleic acid delivery offers several advantages in gene therapy and genome editing applications owing to their biocompatibility and flexibility in delivering the genetic cargo. However, major challenge lies in achieving the therapeutically relevant efficiency. Prior attempts, including our own demonstrated that transfection properties of lipoplexes critically depend upon overcoming membrane barrier, early escape from the endosome and for pDNA nuclear localization.

Towards overcoming the membrane barrier and endosomal escape, we took two intriguing approaches

A. Exploring membrane permeability of liposomes with spirosolanes to enhance lipid enabled CRSIPR/Cas9 mediated genome editing in hematopoietic cells

Intracellular delivery of nucleic acids is one of the critical steps in the transfections. Prior findings demonstrated various strategies including membrane fusion, endosomal escape for the efficient cytoplasmic delivery. In our continuing efforts to improve the nucleic acids transfections, we harnessed cell permeable properties of solasodine (S), a steroidal alkaloid abundantly found in potatoes for maximizing intracellular delivery of lipoplexes. Firstly, we evaluated nucleic acid delivery efficacies in immortalized Human Erythroid Progenitor cells (HUDEP cells) using GFP mRNA. Nanoassemblies doped with spirosolanes (ECS) showed 3 fold superior transfection efficiencies when compared with control nanoassemblies (EC) and exhibited 50% more efficiency when compared with commercial control CRISPR Max. (Fig. 1)

Cas9 mRNA and sgRNA against BCL11A carrying lipid nanoassemblies for efficient genome editing in human erythroid progenitor Cells to elevate fetal globin levels Elevating fetal globin (HbF) is a promising approach for treating β-globinopathies. Prior findings demonstrated that BCL11A is a key regulator of γ-globin expression and functions as a switch of fetal-to-adult haemoglobin and a prime target for inducing fetal globin levels. Genome editing using CRISPR/Cas nuclease system offers great advantages in knock-in or knock-out of target genes. We have used our system to deliver CAs9 mRNA and sgRNA against to BCL-11A locus into hematopoietic cells. T-7 endonuclease assay demonstrated that ECS showed 2 fold superior percentage indels when compared to CRISPR Max. (Fig. 1C).

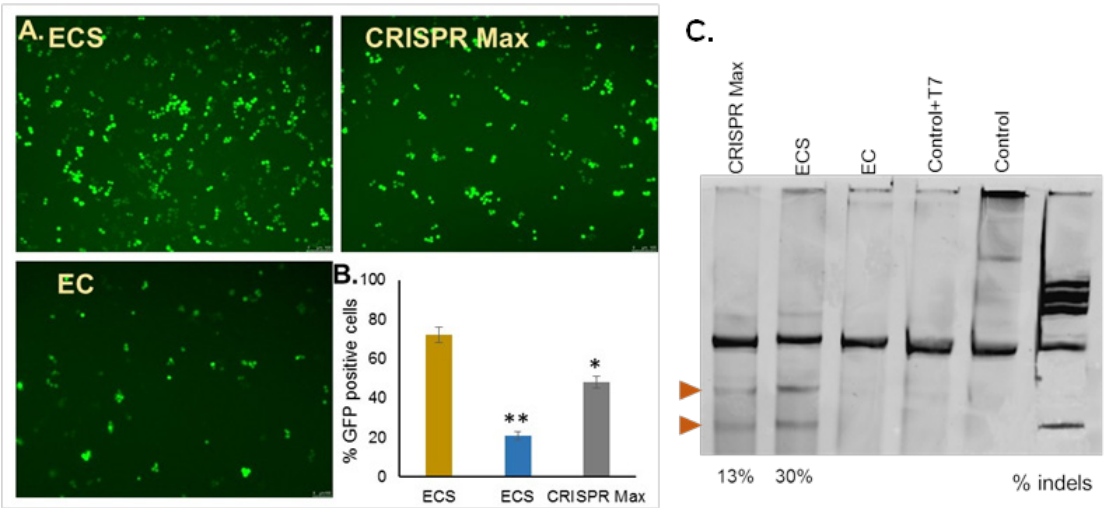


Fig. 1: A&B) mRNA Transfection studies with ECS and EC. Microscopic images of in vitro mRNA delivery with lipid nanoassemblies in HUDEP cells C) Lipid enabled genome editing with Cas9 mRNA and sgRNA against BCL11A in HUDEP cells.

Our ongoing experiments are directed towards quantifying the elevation in fetal globin levels after differentiating HUDEP cells to erythorcytes and quantifying BCL11A mRNA levels with real time PCR.

Taking cues from our findings, we would like to further explore this technology to edit Hematopoietic Stem and Progenitor Cells (HSPCs) under both in vitro and in vivo conditions.

B. An endocytosis switch significantly improves cationic lipid mediated nucleic acid transfections in endothelial cells

Delivering nucleic acids to vasculature has a great promise in treating vascular diseases. However, endothelial cells, which line the vasculature are considered as sensitive and hard-to-transfect cells in nature. Transfecting such endothelial cells with good efficiency is a challenging task. Towards, improving the transfection efficiency, we made an effort to understand the internalization of lipoplexes into cells, which is the first and most critical step in the nucleic acid transfections. In this study, we demonstrated that inhibition of the caveolae pathway with the use of chemical inhibitors, small interfering RNAs has significantly increased the transfection properties of cationic lipoplexes in SK-HEP-1 cells. Intriguingly, cholesterol sequestrating agents, Nystatin and Filipin III, have enhanced cationic lipid mediated transfections. In particular, Nystatin is more effective in increasing the transfections to 2-3 folds through switching lipoplex internalization predominantly through clathrin mediated endocytosis (CME) and macropinocytosis.

PROJECT - 2

Lipid enabled gene/mRNA therapy for hemophilia B

Gene therapy holds a promising alternative to protein replacement therapy for hemophilia. It would ideally involve 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. Since, the clotting factor (FIX) is secreted in hepatocytes, which are predominant cell type of the liver, we aim to deliver correct copy of gene encoding FIX/chemically modified mRNA with our proposed liver specific galactosylated nucleic acid delivery system (GALINAs) to produce functional FIX protein at therapeutically relevant level. We anticipate that our GALINAs deliver nucleic acids specifically and efficiently to liver without provoking immune responses.
To this end, we designed and synthesized Galactosyl head group lipids. Galactose is a ligand for ASGPRs, which are abundantly expressed in hepatocytes.

We fabricated liposomes with galactosylated lipids varying co-lipids in different concentrations and evaluated transfection efficiencies in HepG2. Transfection results demonstrated that one of our formulation MeOH 16DC found to be as efficient as commercial reagent LT-1. We are currently evaluating transfection efficiencies in primary human hepatocytes.

We would like to evaluate GALINAs efficiency in hemophilia B mouse model.

Current lab members:

1. Brijesh Lochania, Senior Research Fellow
2. Rashmi Prakash Chowath Junior Research Fellow
3. Aishwarya Prasannan, Junior Research Fellow

Honors and Awards: Nil

Publications and Patents:

1. Vignesh K. Rangasami#, Brijesh Lochania#, Chandrashekhar Voshavar, Harikrishna R. Rachamalla, Rajkumar Banerjee, Ashish Dayani, Saravanabhavan Thangavel, Praveen K. Vemula*, and Srujan Marepally*. Tomatidine, a steroidal alkaloid improves liposomal transfections BBA Biomembranes 2018 (Just accepted) (Corresponding author)
2. Venkanna MVN, Brijesh Lohchania, Harikrishnareddy Rachamalla, Rajkumar Banerjee, Srujan Marepally, Srilakshmi PV. Tocopherol-ascorbic acid hybrid antioxidant based cationic amphiphile for gene delivery: Design, Synthesis and transfection. Bioorganic Chemistry 2018, (Just accepted)
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

4. Priya Dharmalingam, Hari Krishna Reddy R, Bhanuprakash B, Saravanabhavan Thangavel, Mohan Kumar KM, Rajkumar Banerjee, Arabinda Chaudhuri, Chandrashekhar Voshavar* and Srujan Mareaplly*, Green transfection: Cationic lipid pool derived from vegetable fat palm stearin enhances nucleic acid transfections. ACS Omega, 2017, 2 (11), 7892–7903 (Corresponding author)
5. Omprakash Sunnapu, Niranjan G Kotla, Balaji Maddiboyina, Duraisamy Chellappa, Srujan Marepally*, Jayabalan Shanmugapriya Subramanian Singaravadivel*, Gandhi Sivaraman*. Rhodamine-Based Fluorescent Turn-On Probe for Facile Sensing and Imaging of ATP in Mitochondria. ChemistrySelect, 2017, 2 (25) 7654-7658. (Corresponding author)
6. Sasidharan Vidyanand#, Srujan Marepally#, Sarah Elliot, Vairavan Laxman, Dhiru Bansal, Alejandro Alvarado Sanchez, Praveen Kumar Vemula*, Dasaradhi Palakodeti* MicroRNA, miR 124c regulate axon guidance cues and planar cell polarity pathway essential for cephalic ganglion and photoreceptor organization during anterior regeneration in planarian Schmidtea mediterranea. Development, 2017, dev.144758. (# equally contribution)

Academic activities:

- Organizing committee member of 2nd annual symposium on Cell and Gene therapy, CSCR, Vellore
- Taught nanotechnology in stem cells applications for doctoral students

Collaborations:

Internal:

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:

1. Arun Shastry, DART, Bengaluru
2. Rajkumar Banerjee, CSIR-IICT, Hyderabad
3. Srilakshmi V. Patri, NIT, Warangal
4. V. G. M. Naidu, NIPER, Guwahati

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:

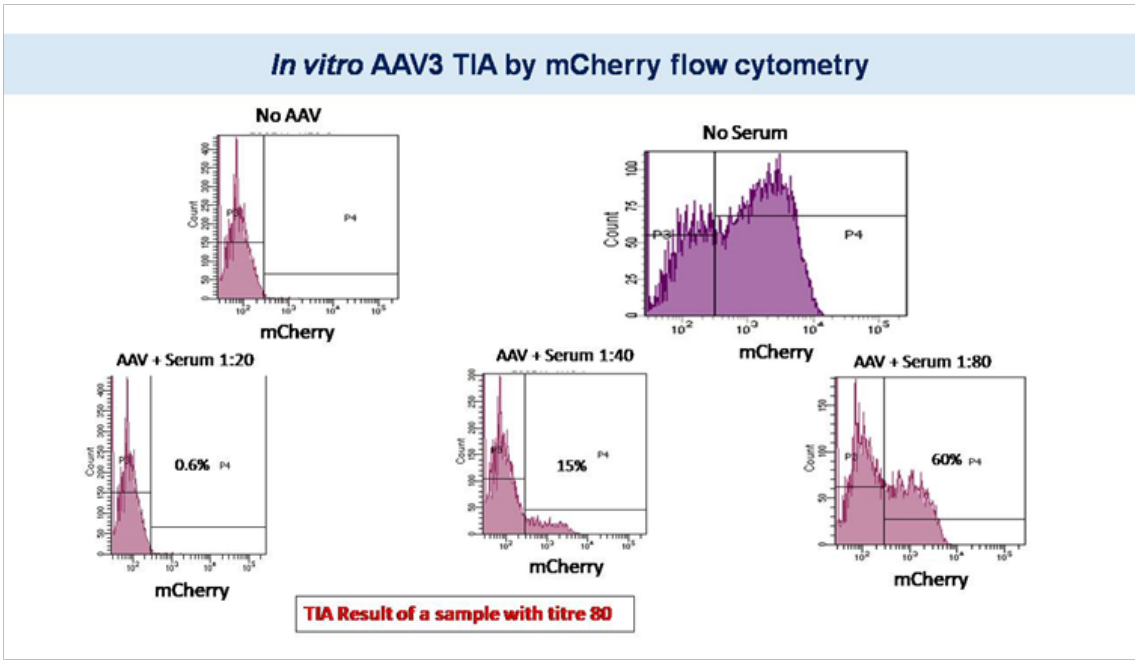
The overall objective is to establish an efficient method of screening for AAV antibodies to different serotypes in individuals for potential gene therapy.

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:

- Processes for virus packaging and AAV transduction for in vitro transduction inhibition assay have been established and optimized for AAV serotypes 3 and 5.
- Iin-house peptide ELISAs have been established for the detection of AAV serotypes 3, 5 and 8.
- In-house whole capsid ELISAs 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 flow cytometry.
- Transduction inhibition assay (TIA) for AAV serotypes 3 and 5 has been standardized using 96 well format luminometer detection method.
- One hundred serum samples were screened for AAV serotypes 3, 5 and 8 using whole capsid ELISA and in-house peptide ELISA.
- 15% of the samples were also screened using AAV3 TIA.



Specific highlights of the project:

- Designed peptides for the serotype specific detection of AAV.
- Established in-house peptide ELISAs for the serotype specific detection of antibodies against AAV 3,5 and 8.
- Establishing transduction inhibition assay (TIA) for quantitation of antibodies against AAV 3,5 and 8 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 prohibitive in terms of cost and labour. 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. However, 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 four stages:

- Identifying the most common 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 followed by;
- Banking of peripheral blood mononuclear cells (PBMNC) for generation of iPSC lines from the cultured donor cells in the laboratory, through Good Manufacturing Practice (GMP).

Peripheral blood samples collected from donors after consent are screened for infectious diseases at the CMC, Vellore blood bank, in a manner identical to blood donors. An efficient protocol has been established at CSCR to generate iPSC lines from cultured erythroid cells derived from PBMNCs. So far 222 samples have been collected through DATRI and PBMCs were isolated and cryopreserved. The donors are from Tamil Nadu covering Chennai, Thiruvannamalai, Coimbatore, and Thiruppur. This year, we expanded the sample collection to Bangalore, Karnataka. Generation and banking of iPSCs are shown in the report of R V Shaji and eight (8) iPSC lines were generated and extensively characterized for the expression of pluripotency markers and their ability to differentiate into tri-lineages. Karyotype analysis and VNTR analysis were also performed. All the details of the samples are maintained in a biobanking software for traceability and access.For the haplobanking program, the Centre for Stem Cell Research has been part of the International consortium through the Department of Biotechnology, Government of India aiming to establish harmonized approaches to generate iPSCs for regenerative medicine applications.

Collaborations:

Internal:

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

External:

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

1. 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.

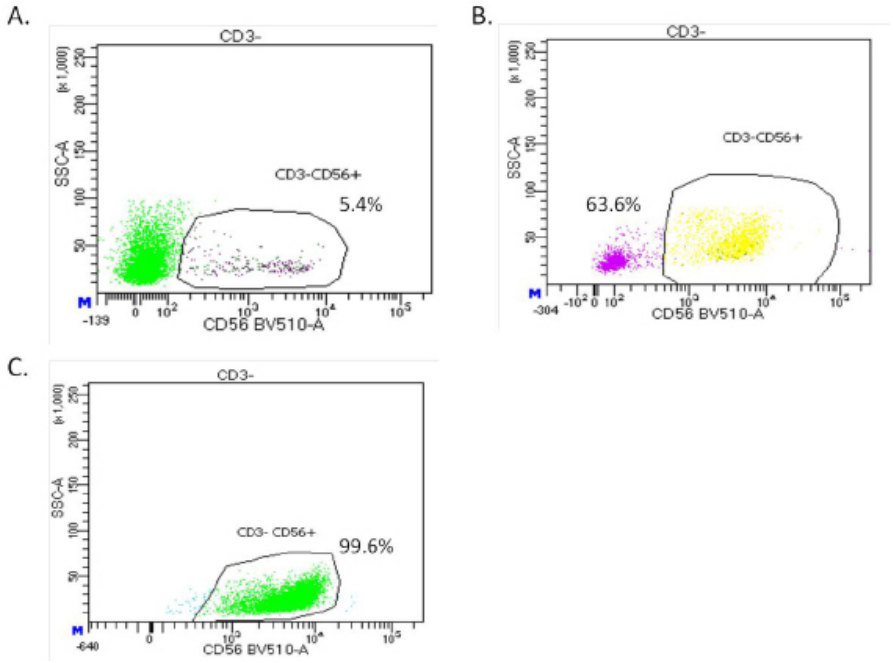


Figure: (A) In day 0 of culture, the PBMNC population had 5.4% of CD3-CD56+ NK cells. (B) On day 7 the CD3-CD56+ NK cells increased to 63.6% and (C) on day 14 it was 99.6%. This clearly shows the selective growth of only the CD3-CD56+ NK cells in culture.

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.

Support from CSCR: Funding and lab space

Collaborations:

1. Aniket Kumar, CSCR / CMC, Vellore
2. Sunil Martin, CSCR
3. Mohankumar Murugesan, CSCR
4. Augustine Thambaiah, CSCR
5. 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 9 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 cell 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 purity and fold expansion.

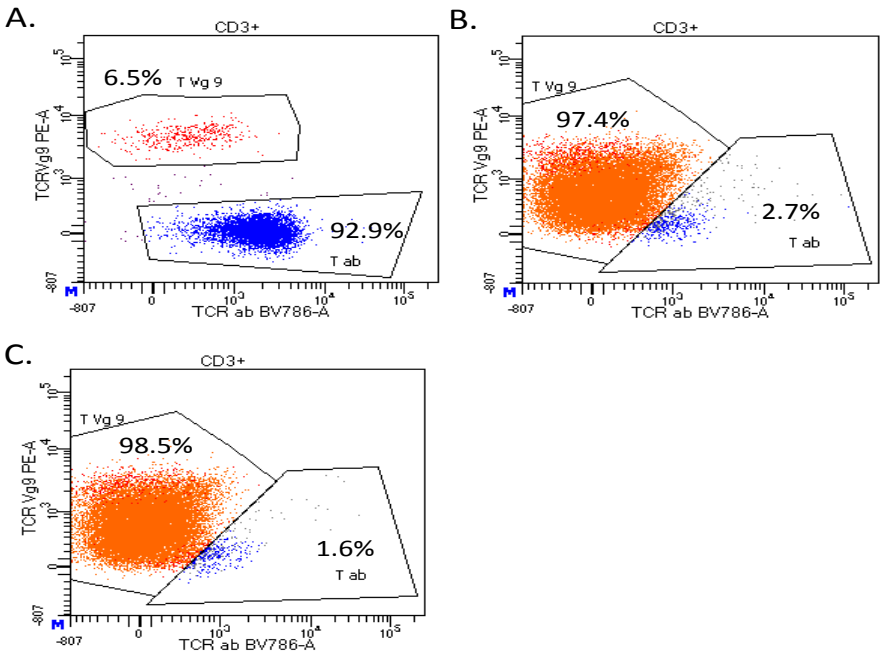


Figure: Typical surface phenotype of γδ T cells expanded from PBMNC using zoledronic acid and IL-2. PBMC were stimulated with zoledronate and IL-2 for 14 days. (A) Day 0, MNC's stained with PE-labelled anti-TCR Vγ9 and BV786 labelled anti-TCR αβ to monitor the expansion of γδ T cells and the reduction of αβ T cells. (B) The phenotype was compared between γδ T cells in PBMNC (day 0) and IL-2 and zoledronate-activated γδ T cells (day 14). (C) Further reduction in the level of αβ T cells was noticed in the same sample cultured in parallel with IL-15 along with IL-2 and zoledronate.

Support from CSCR: Funding and lab space

Collaborations:

1. Aniket Kumar, CSCR / CMC, Vellore
2. Sunil Martin, CSCR
3. Mohankumar Murugesan, CSCR
4. Augustine Thambaiah, CSCR
5. 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: 2015-2018

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:

AML cell line MV4-11 made resistant to cytarabine is developed in the laboratory and IC50 of developed resistant cell line was significantly higher than the parental line. Other markers for resistance need to be probed by RT-PCR. Representation and knockdown efficiency of the pooled shRNAs in Epigenetics Library was confirmed followed by preparation and concentration of the pooled virus incorporating the entire Library. To confer the best promoter for the AML cell lines, transduction efficiency of five different promoters was monitored in different cell lines at various time points and it was decided that hEF1a is the best promoter for all the AML cell line.

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

Brief description of the project:

As part of this project, the objectives were to develop mouse model of AML and CML for testing combination therapy to overcome chemoresistance in these myeloid leukemia

a. Development of FLT3 mutated AML mice model for in-vivo investigation.

Leukemic splenocytes (FLT3/DNMT3a-cMYC-eGFP splenocytes, a kind gift from Dr. David E. Muench, Professor, Division of Immunobiology, Cincinnati Children's Hospital Medical Center, Ohio, USA) were revived and injected (viable 4*106cells) via tail vein into 4 weeks old sub-lethally irradiated C57Bl/6 mice. Retro-orbital bleeding was performed to observe cell count from the 4th week of transplant to monitor the development of leukemia. Spleen and liver and femur bones of morbid mice were collected to confirm the development of AML. Morbid mice had classical splenomegaly also blast infiltration in the liver leading to hepatomegaly. Flow cytometric analysis of bone marrow cells showed greater than 20% (eGFP positive cells in the CD117+ fraction) confirming the development of AML

Development of CML mouse model by retro-viral transduction method (additionally funded by CMC Fluid grant, 2018-2020)

BCR-ABL onco-transcript plasmid MIG-BCR-ABL-IRES-GFP was a kind gift from Dr. Warren Peer's lab (Professor, University of Pennsylvania School of Medicine, Philadelphia, USA). The authenticity of plasmid was confirmed by bacterial transformation followed by clone picking. Plasmids were isolated from the selected individual clones and subjected to restriction digestion. The digestion pattern of the selected clone that matched the parental digestion pattern was selected and BCR-ABL fusion transcript was validated in these clones using nested PCR.
BCR-ABL plasmids transfected into the plat-E cells and the virus collected at 3 time points 48hrs, 60hrs and 72hrs, pooled and stored at -80°C until use.

C57Bl/6 - 9 weeks old mouse was euthanized and bone marrow cells was collected from both femur and tibia (9*106 cells). 1*106 cells were seeded in the 6 well plate with cytokines – Stem cell factor (SCF), IL-3 and IL-6 for 24 hours. The cells were transduced with BCR-ABL containing virus by spinfection and the GFP expression was checked by Flowcytometry at 48 hours post transduction.

Around 30% of the bone marrow cells were transduced with BCR-ABL. The transplantation of these BCR-ABL transduced cells into lethally irradiated recipient mice will develop CML disease within 2-3 weeks which will be confirmed by the spleen size, CBC profile, Morphology and Flowcytometry (GFP). Splenic cells positive for GFP will be harvested and stored for further experiment.

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

Collaborations:

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

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

Project title: C11orf95-RELA fusions in supratentorial ependymomas: Relevance in prognostication. (EMR/2016/002251)

Funding source: External agency : Department of Science and Technology

Duration: 20 th June 2017- 19th June 2020

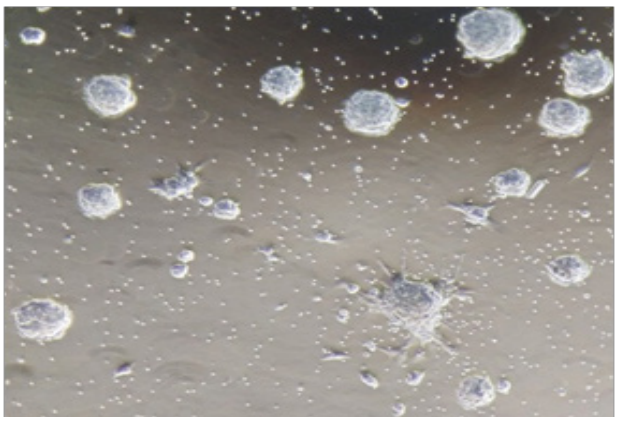
Brief description of the project:

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?

We have observed C11orf95-RELAtype 1 and type 2 translocations in a subset of supratentorial ependymomas. Cases are still being recruited. Tumor from cases suspected to be glioma are being cultured and neurospheres from SEs are being investigated for the presence of C11orf95-RELA translocation.



Neurospheres in a case of Anaplastic ependymoma

Collaborations:

Internal :

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 S. CHRISTUDASS, PhD

Associate Professor – Neurochemistry, Department of Neurological Sciences, CMC, Vellore

Adjunct Scientist, CSCR

Project title: Isolation of Cancer Stem Cells from primary and secondary high grade gliomas - their response to microenvironmental cues and Notch signaling blockade.

Funding source: Department of Biotechnology, Government of India

Duration: August 2016 to August 2019

Brief description of the project:

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

Specific highlights of the project:

Developed a FACS- acquisition protocol for GBM derived CSCs using A2B5 and Nestin markers. We also have found that inhibition of NF-κB pathway down regulates CSC population and disrupts tumor micro-environment.

Support from CSCR: Regularly using CSCR culture facility, and core facility for FACS analysis, nanodrop and real-time PCR.

Collaborations:

1. Ari George Chacko, Department of Neurosurgery Unit-I, CMC, Vellore
2. Geeta Chacko, Department of General Pathology, CMC, Vellore

ANIKET KUMAR, PhD

Senior Lecturer, Department of Pharmacology & Clinical Pharmacology, CMC Vellore

Adjunct Scientist, CSCR

Project title: Study of human keloid fibroblasts in culture and effects of novel drugs

Funding source: Fluid grant / CSCR

Duration: April 2016 – March 2018 (2 years)

Brief description of the project:

Samples of normal skin and keloid were taken from the patients, after getting their consent in the department of plastic surgery. One portion of the obtained specimen of keloid and normal skin was utilized for morphological studies, by histopathology and immunohistochemistry for identification of keloid specific structures and proteins. From the other part of normal skin and keloid sample, fibroblasts were isolated and cultured. Cultured cells were further utilized for understanding their in-vitro characteristics by different techniques. After the characterization studies, cultured fibroblasts (normal and keloid) was treated with log doses for 24 and 48 hours, with novel drugs of interests, for dose optimization, and its effect on the proliferation and expression of various biomarkers in cultured fibroblasts was analysed.

Objectives:

1. To isolate and culture keloid fibroblasts obtained from keloids of patients.
2. To identify the expression of specific biomarkers for keloid in the cultured keloid fibroblasts.
3. To evaluate the effect of various pharmacological therapies on proliferation and differentiation of keloid fibroblasts in-vitro.

Work Done:

All the samples have been collected from department of plastic surgery and the protocol for the culture, expansion and cryopreservation of keloid as well as normal fibroblasts have been established. Master cell bank and working cell banks are also prepared. To differentiate keloid fibroblasts and normal fibroblast morphologically, we have already done histopathological analysis with the help of a trained pathologist. The base line expression of biomarkers, in normal and keloid fibroblasts, such as TGFß, PAI-2, COL-I and III has already been done using qRTPCR. Also the effect of Thalidomide, Verteporfin and Salirasib in log doses has been tested on keloid and normal fibroblasts to evaluate its role in down regulation of above mentioned biomarkers.

The effect of Thalidomide and Salirasib is evident on TGF beta and Col-I, where the drug at higher concentration has significantly reduced the levels of TGF beta and Col-I in keloid fibroblasts. Verteporfin has showed effect only on TGF beta at high concentration where as it failed to show any significant effect on other biomarkers in keloid sample.

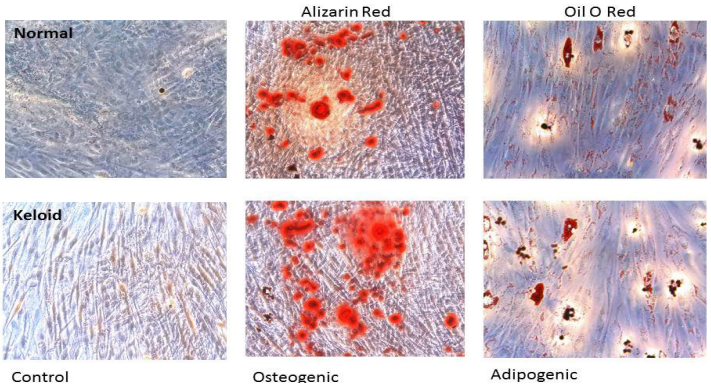
Support from CSCR: Infrastructure/Funding/Scientific

Collaborations:

1. Alok Srivastava, CSCR / Department of Haematology, CMC, Vellore
2. Sanjay Kumar, CSCR
3. Ashish Kumar Gupta, Department of Plastic Surgery, CMC, Vellore
4. Meera Thomas, Department of Pathology, CMC, Vellore
5. Margaret Shanthi, Department of Pharmacology & Clinical Pharmacology, CMC, Vellore

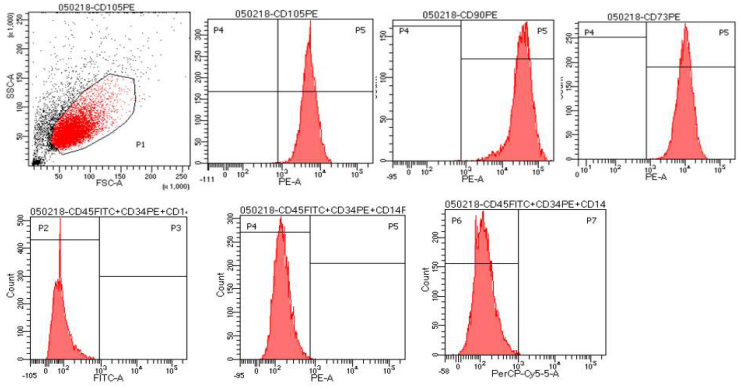
Characterization study: Differentiation

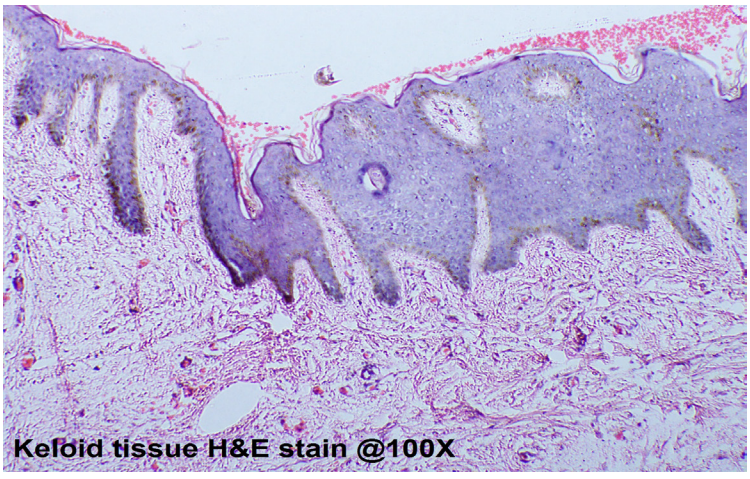
In vitro differentiation experiments:



Characterization study: Flow cytometry

Keloid Fibroblasts:





SANJAY KUMAR, PhD
Scientist, CSCR

LABORATORY HIGHLIGHTS OF YEAR 2017-18

PROJECT - 1

Relevant AAV-gene therapy work contribution related to AAV Gene therapy program:

My scientific contributions in AAV gene therapy program is mostly related to pre-clinical AAV based gene therapy studies. 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 project work. 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 cell lines and primary cells as well as in vivo transduction testing in suitable transgenic mice models. c) Troubleshooting in the AAV vector assays based on AAV based scientific facts and AAV specific knowledge.

Contribution towards Affordable Excellence in AAV gene therapy program:

Co-ordinating an AAV8-FIX-Padua vector based in vivo study in Hemophilia B transgenic mice model at CSCR in collaboration with Intas Pharmaceuticals. 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.

1. Safety and efficacy study of (adeno associated virus vector based) AAV8-hFIX vector in hemophilia B mice model.

Results: Negative stain TEM analysis of rAAV8-hFIX-Padua shows reasonably pure genome containing AAV particles.

To assess overall purity qualitatively and to know the amount of genome-containing (full) versus empty particles of the rAAV8-hFIX-Padua preparation, we imaged rAAV8 particles using negative stain on TEM. It is well known that empty particles are generated when producing rAAV8 in any of the current production technologies. Discovering purification procedures that will effectively remove the empty particles from full particles are needed.

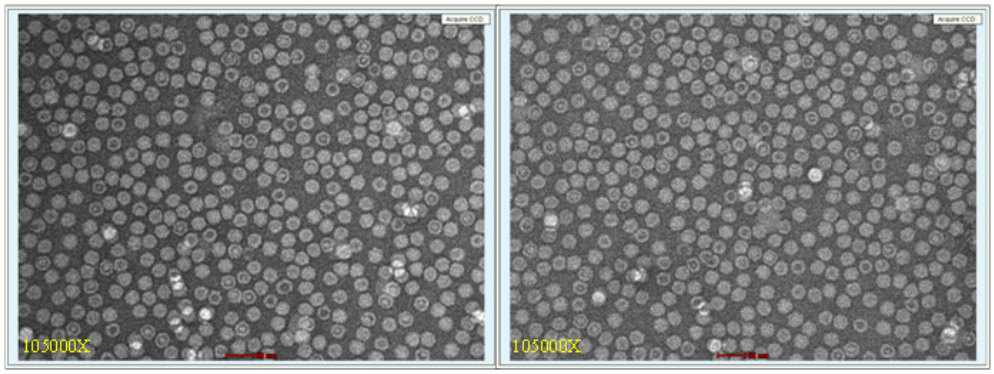


Figure 1: Ultrastructural characterization of rAAV8-hFIX-Padua particles: rAAV8 particles are observed as 25-nm spheres. Genome-containing AAV particles are impermeable to uranyl acetate, while empty AAV particles TEM images show differentially negatively stained empty particles, which can be distinguished based on the electron dense center within the particle.

scAAV8-hFIX treatment significantly reduced the bleeding-time in Hemophilia B mice:

In our experimental setting (Figure 2), untreated hemophilic mice had an average bleeding time of more than 20 minutes. None of the hemophilia B control mice stopped bleeding within the 20 minutes time frame thus we sacrificed the mice after 20 minutes of the bleed episode. However, low and high dose treatment cohorts with scAAV8-hFIX showed significant improvement in the bleeding-time.

ELISA assay shows 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

Mice were injected intravenously with scAAV8-hFIX human secreted factor IX or PBS. The injections were well tolerated by all of the animals. The amount of human FIX antigen in hemophilia B mice plasma was assessed by the analysis of the presence of hFIX proteins in the plasma of the scAAV8-hFIX administered hemophilia B mice and non-injected mice. In general, figure 3 shows the level of hFIX protein in plasma of transgenic hemophilia mice injected with scAAV8-hFIX was significantly higher than in plasma of non-injected transgenic hemophilia mice correlating very well with the tail bleed assay.

2: To test, if targeted therapy by transient-niche modulation using PTEN gene alteration in injured spinal cord may help augment functional recovery & management of spinal cord injury (SCI) in preclinical mice models.

3. Preclinical experiments towards development of standardized in vitro culture system for in vitro expansion of human mesenchymal stem cells (hMSCs).

The stem cell, as a product, falls under the stringent quality control requirements imposed on a therapeutic product by industry regulators. These include validated measurements of purity, potency, efficacy, and stability. Problematically, there is no current measurement system that can completely define a cell using either an individual or a set of assays. Each cell type has different properties, and mechanism for their therapeutic influence on tissue homeostasis in vivo is frequently unclear and mostly attributed to paracrine factors and other MSCs derived factors influencing the homed tissue microenvironment. Thus, working on standardizing all the variables in the in vitro culture conditions, where the only variable will be the tissue of origin.

4. Testing whether human Mesenchymal Stem Cells (hMSCs)-derived Exosomes/ Exosome Mimetics can be utilized as a potential novel therapeutic tool for regenerative purposes? & "How we can arm hMSCs with a cargo of our choice and deliver them for specific therapeutic purposes?"

Project Model: Human Wharton-jelly-derived mesenchymal stem cells (hWJ-MSCs) / genetically engineered hWJ-MSCs-derived exosomes as therapeutic delivery vehicles for tumor-targeted therapy in mouse models or maintaining tissue homeostasis in preclinical mice model.

Collaborations:

Internal

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 OF YEAR 2017-18

Immune Cell Engineering and Therapy (iCET) Laboratory

The laboratory of immune Cell Engineering and Therapy (iCET) thematically focuses to advance the antitumor capabilities of the adoptively transferred therapeutic immune effector cells by the genetic modification. We are particularly interested in engineered expression of Chimeric Antigen Receptors (CARs) to redirect the antitumor functions of NK cells, γδ T cell and αβ T cells against haematological malignancies.

The immune cells receive and respond to the biochemical cues from its microenvironment by the surface receptor gateway. Dysfunctional immune cells in the tumour microenvironment have suboptimal reception and processing of these signals with consequent failure to sensitize them against the tumour cells. CARs are artificial receptors synthesised by genetically fusing canonical signalling components of the extracellular antigen binding and intracellular signalling domains of costimulatory molecules. CAR constitute HLA independent, durable and versatile platform that can be engineered in variety of immune cells against multitudes of antigens in several formats. CAR therapy has emerged as the treatment of choice against relapsed or chemotherapy resistant haematological malignancies.

Of late, Anti CD19 CAR T cells exhibited outstanding antitumor functions in the preclinical and clinical studies against haematological malignancies. However CAR therapy is not entirely free from the effector cell dysfunction and antigenic escape associated with targeted therapies. Clinical side effects such as tumour lysis syndrome, cytotoxic syndrome and neurotoxicity still limit its wider application in global cancer medicine, apart from the expenses involved.

In this framework iCET joins formulating strategies to make CAR therapy safer without losing its effectiveness and affordability window. We envision achieving this goal through two different endeavours.

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

NK cells/ γδ T cells detect the tumour using a series of germline encoded activating and inhibitory receptors and directly lyse the tumour cells without prior sensitisation in an HLA independent manner. Moreover they express high affinity Fc receptors to facilitate ADCC which might very well sync with antibody therapeutics. All the more, γδ T cells can present tumour antigens to the αβ T cells. Both NK cells/ γδ T cells invokes remarkably lower GvHD (Graft versus host Disease) and remarkable GvL (Graft versus Leukemia). The role of innate lymphoid cells as host cells of CARs is only beginning to be addressed.

Refractory or relapsed B cell leukemia (r/r B-ALL) treatment is clinically challenging; in spite of the advances in mainstay chemotherapy and allogeneic hematopoietic stem cell transplantation (allo-HSCT). Remarkable clinical results with up to 90% complete remission (CR) rates have been reported in patients with B cell lymphoblastic Leukemia, receiving T cells genetically redirected with CAR which lyse the tumor cells antigen specifically. Inspired by the foregoing clinical success and preclinical assays; we propose to generate and expand NK cells and γδ T cells engineered to express CAR targeting CD19 which is over expressed in neoplastic B cell progenitor cells. We will also introduce an inducible caspase 9 switch which can trigger apoptosis upon pharmacological induction at will, should there be any adverse events.

The current project has three objectives.

- To generate and validate the plasmid constructs encoding gene cassettes for chimeric antigen receptor (CAR) targeted against CD19; a leukemic antigen.
- To optimize the protocols for genetic engineering of cord blood NK cells (CBNK) and γδ T cells (CB γδ) with antiCD19CAR.
- To assess the fold expansion, phenotype and tumor lysis function of CBNK19/ CBγδ19 against established leukemic cell lines.

On-going work:

Aim1: We have two sets of CAR constructs. The first set is purchased from Creative Bio labs (Shirley, New York, USA). The antigen binding extracellular domain of this CAR is derived from scFv of the mouse anti CD19 hybridoma, FMC63. A linker and transmembrane domain from CD8 alpha will connect the extracellular domain to the intracellular domain. The intracellular cellular domain consists of costimulatory moieties from CD28, CD137 and signalling domains from CD3ζ. An induced caspase9 also known as iCas9 to trigger apoptosis upon pharmacological induction with chemical inductor of dimerization (CID) CID (AP1903 or AP20187). This induced dimerization of caspase 9 activates molecules inducing apoptosis in the immune cells expressing the construct).

The second set of constructs is from Gene and Cell Therapy lab at Emory University School of Medicine. This is a second generation CAR with the same anti CD19 scFv extracellular domain from FMC63, CD8 transmembrane domain. However this construct has CD28 and CD3ζ signalling domain, but lacks the CD137 signalling domain. Moreover GFP in this construct would allow us to precisely monitor the phenotype and functions of CAR transduced cells. We have set up the protocols to generate and titer lentiviral vectors in our lab. We could generate K562 cells overexpressing GFP tobe used as a target in tumor toxicity assays. FACS based assays to detect Tumor toxicity of engineered immune cells are also in progress.

Future works:

1. Generation of high titer lentiviral particles containing CAR: We program to accomplish the aim 1 by generating endotoxin free and transduction quality plasmid before sequence confirm it against the construct map. We will package the plasmid in lentivirus and estimate the titer of the preparation. We plan to titer the pseudo virus by p24 ELISA (Origene) and quantitative PCR of RRE (Rev Response Element).

2. Transduction efficiency: Lentiviral vectors with superior transduction efficiency is a prerequisite for generating effective CAR transduced immune cells. We will estimate the surface expression of CARs by staining with anti Fab antibody which would bind the scFv region of the CAR.

3. Expansion of the engineered immune cells: Our goal is to generate high quality functional GD-T cells and NK cells expressing CARs. GD-T cells will be generated from the stimulation of the cord blood derived monocytes with Zoledronic acid which is a bisphosphonate drug approved by FDA. On day 07, the GDT cells will be lentivirally spinfected in presence of Retronectin before expanding them. We will assay the proliferation, phenotype and tumour toxicity of the transduced cells.

   NK cells will be generated by the Ionomycin treatment of Cord Blood derive Mononuclear Cells followed by the expansion of NK cells with rIL-2. We will collect the NK cells from Day 02, Day 04 and Day 06 to empiricise the CAR transduction and expansion protocol. We will then gauge the fold expansion; phenotype and cytotoxic functions of the Cord blood Derived NK cells transduced with CAR (NK-CAR).

II. Targeting CD19 CAR to TCRα locus of T cells with CRISPR/Cas9 to augment efficacy

Generating universal engineered immune cell system resolves multiple caveats of CAR therapy such as toxicity due to GvHD, batch to batch variability and cost. Advances in gene editing technologies such as CRISPR/cas9 would allow the cell engineer to delete endogenous TCRs and HLA molecules that triggers Graft versus Host Disease. Cord Blood derived immune effector cells have lower GvHD but significant GvL due to its naiveté; allowing lesser disparities on HLA restriction.

The proposed project has 3 objectives

- To generate and validate the guide RNAs and donor templates for anti CD19 chimeric antigen receptor (CAR) targeted to T Cell Receptor Alpha Constant (TRAC) locus.
- To optimize the protocols for CRISPR/cas9 mediated targeting of anti CD19 CAR to TRAC locus.
- To assess the fold expansion, phenotype and tumor lysis function of CBT19-TRAC against established CD19 positive leukemic cell lines.

Ongoing work:

We have designed series of guide RNAs against exon1 of the TRAC to shut down the expression of TCRα and the donor vector containing the antiCD19 CAR flanked by left and right homology arms

Honors and awards:

- Ramalingaswamy Re-entry Fellowship 2016-17, Department of Biotechnology, Government of India.

Academic Activities:

- JRF review process
- Co-organiser: 3rd Annual Cell and Gene Therapy Symposium, Vellore

Lab Members

1. Ms. G. Thamizhselvi, JRF

Collaborations:

Internal:

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

External

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

MUTHURAMAN N, MD

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

Project title: 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:

The incidence of endometrial cancer is increasing worldwide. 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 the present project we are interested in studying the properties of cancer stem like cells obtained from these two different types of cancer. This study will help in understanding whether a difference in cancer stem like cells between these two types of endometrial cancer can be a cause of treatment failure and high rate of relapse in type II endometrial cancer. We will isolate cancer stem like cells from type I and type II endometrial cancer tissue by establishing primary endometrial cell culture. We will characterize the stem cells in terms of the expression of surface markers, their proliferative potential, migratory potential and invasive capability. We will analyze the similarities and differences of stem like cells obtained from these two different types of endometrial cancer.

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

RAVIKAR RALPH , MD

Associate Professor, Department of Medicine Unit-1, CMC, Vellore

Adjunct Scientist, CSCR

Project title: β-chemokine expression and HIV-1 infection in CD34+ haematopoetic stem cells – A pilot study

Funding source: Fluid research grant

Duration: 2016-2018

Brief description of the project:

Despite the advent of highly active anti-retroviral therapy, a functional cure for HIV has been impossible to achieve due to the existence of cellular reservoirs with latent infection. These latently infected cells are all derived from CD34+ haematopoetic progenitor cells. Engineered CD34+ cells could potentially result in mature cells resistant to HIV infection thereby resulting in a functional cure. In this context, CD34+ cellular susceptibility to HIV-1 infection is an important question. CD34+ cells are largely resistant to HIV-1 infection in-vivo despite a subset expressing the CD4 and CCR5 co-receptors. Autocrine binding of cellular β chemokines to the CCR5 co-receptor has been suggested as a putative hypothesis to explain this phenomenon.

This study aims to compare mRNA expression levels of the β chemokines MIP-1α, MIP-1 β and RANTES in CD34+ cells with CD4 and CCR5 co-expression, derived from HIV-1 infected patients with expression levels in cells from healthy HIV negative consenting donors using reverse transcriptase polymerase chain reaction (RT-PCR) techniques, to determine whether or not an upregulation of β chemokines genes in these CD34+ cell subsets occurs in response to an in-vivo HIV-1 infection. Transcriptional upregulation if present would strengthen the above hypothesis and suggest an autocrine effect of β chemokines in blocking an HIV-1 infection of CD34+ cell subsets in-vivo.

Aim : To compare mRNA expression levels of the β chemokines MIP-1α, MIP-1 β and RANTES in CD34+ CD4+ cells with CCR5 co-expression, from HIV-1 infected patients with expression levels in CD34+ CD4+ CCR5+ cells from HIV negative donors using reverse transcriptase polymerase chain reaction (RT-PCR) techniques.

Objectives:

1. Relative quantification of β chemokine (MIP-1α, MIP-1 β and RANTES) mRNA in CD34+ CD4+ cells with CCR5 co-expression, from HIV-1 infected patients compared to HIV negative healthy controls using qRT-PCR
2. HIV-1 DNA quantification in CD34+ CD4+ cells from HIV-1 infected patients using qPCR

Work done: Indian guidelines for antiretroviral therapy (ART) initiation have been amended by the national AIDS control organisation (NACO) in May 2017, based on recommendations of the ART technical resource group and the 2016 WHO guidelines. The revised national guidelines recommend that all PLHIV be initiated on ART regardless of CD4 count; clinical stage, age or population.

Consequentially, all PLHIV being referred to CMC since 2017 have already been initiated on ART, at centers where the diagnosis was initially made. They are therefore unable to fulfil the inclusion criteria of this study which require HIV patients to be ART naive. Of individuals diagnosed with HIV for the first time at CMC, none of those screened (n =10) gave consent for receiving G-CSF injections prior to being initiated on ART. The study has therefore been unable to recruit any participants fulfilling inclusion criteria for the year 2017-2018.

Support from CSCR: Lab space, flow cytometry

Collaborations:

1. George Varghese, CMC, Vellore
2. Alok Srivastava, CSCR / CMC, Vellore
3. Anand Zachariah, CMC, Vellore
4. Rajesh Kannangai, CMC, Vellore
5. John Fletcher, CMC, Vellore
6. Jaiprasath, CMC, Vellore

JEYANTH ROSE, MS

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

PROJECT-1

Project Title: Bioengineered Corneal Substitute using decellularized human donor cornea rejected for corneal transplant.

Funding Source: Fluid Research Grant, CSCR core fund

Duration: November 2016 to November 2018

Objectives: The objectives of this study are to standardize a biomimetic stromal scaffold using decellularized human corneal stroma and to measure the transparency, biomechanical strength and structural integrity of the decellularized scaffold.

In this study, we aim to standardize a stromal scaffold using human corneal stroma. The corneo-scleral button of 16 mm will be excised and the epithelium and endothelium will be removed by treating in Dispase II. Then NaCl based decellularization will be performed and then treated with DNAse and RNAse. Then the cornea will be dehydrated using 10%, 20% and 30% sucrose consecutively. Then decellularized scaffold will be characterized.

Work Done:

- Decellularization protocol was adjusted with Hepes-buffer solution and standardised.
- Extensometer was constructed to analyse the tensile strength of the decellularized cornea.
- Thirteen corneas have been decellularized using standardised protocol.
- Four samples have been used to standardise the Extensometer and one sample was analysed using DAPI staining. The laser quantification and Font recognition are done in remaining samples and now being subjected to Extensometer and DAPI & Haematoxylin and Eosin staining.

Support from CSCR: Lab infrastructure and funding

Collaborators:

1. Sanita Korah, Department of Ophthalmology, CMC, Vellore
2. Thomas Kuriakose, Department of Ophthalmology, CMC, Vellore
3. Deepthi Kurien, Department of Ophthalmology, CMC, Vellore
4. SharmiliLalgudi, Department of Ophthalmology, CMC, Vellore
5. Syrpailyne Wankhar, Department of Bioengineering, CMC, Vellore

PROJECT-2

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: Major research grant

Duration: November 2016 to April 2018

Objectives:

1. To evaluate the viability and distribution of placenta derived Mesenchymal stem cells in the corneal stroma in an ex-vivo organ culture model of human corneal scarring over a 30 days period.
2. To compare the influence of intrastromal MSC's on corneal transparency in a ex-vivo organ culture model of evolving corneal scarring.
3. To compare the influence of MSC's on the basic histopathology of the cornea.
4. To compare the influence of MSC's on markers of Fibrotic corneal scarring.
5. To investigate the mechanism of action of MSC's in corneal scarring.

The aim of this project is to use intrastromal injection of Placenta derived Mesenchymal stem cell as a treatment for corneal scar. Briefly the Placenta derived MSC's 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 mesenchymal stem cells. 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 that were cryopreserved were revived for flowcytometry analysis. Live-Dead assay was done using 7AAD to check the viability of the revived cells.
- Flowcytometry was done for MSC characterisation using CD73, CD90, CD105 anti-body and negative control using CD 45, CD 34, CD 14 anti-body.
- Differentiation of MSC into Adipocyte and Osteocyte in a six well plate to demonstrate the differentiation capacity of MSC's is done.
- All five pairs of cornea have been cultured for 28 days and transparency measurement, digital photographs were done. Currently the Immunohistochemistry analysis are being done.

Support from CSCR: Lab infrastructure and funding

Collaborators:

Internal:

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. Augustine Thambaiah, CSCR
8. Alok Srivastava, CSCR / Department of Haematology, CMC, Vellore
9. Aarwin Joshua R, CSCR
10. Suresh Devasahayam, Department of Bioengineering, CMC, Vellore
11. Syrpailyne Wankhar, Department of Bioengineering, CMC, Vellore
12. Geeta Chacko, Department of Pathology, CMC, Vellore
13. Tripti Jacob, Department of Anatomy, CMC, Vellore
14. Joe Varghese, Department of Biochemistry, CMC, Vellore
15. Vinay Oomen, Department of Physiology, CMC, Vellore

External:

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

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: J. Saranya
- 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: J. Saranya & T. Abdul Muthallib
- 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
- Technical Staff: J. Saranya
- 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

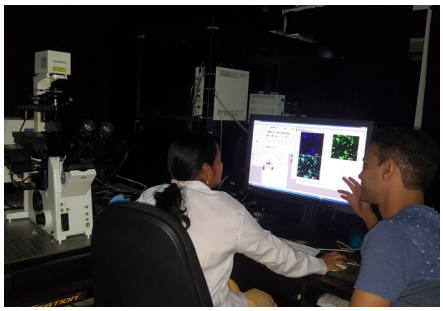
Leica DMIL (upright) and Leica DMI1000 (inverted) microscopes are available for users to perform routine light microscopy imaging. Both microscopes are provided with an interchangeable Leica DFC290 camera for high resolution bright-field imaging. The Leica DMI1000 is also installed in the tissue culture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

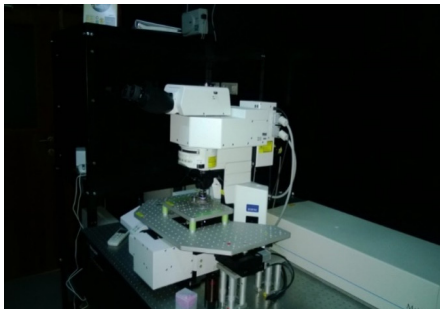
The EVOS® FL Auto Imaging System is a fully-automated, digital, inverted multi-channel fluorescence and transmitted light imaging system with outstanding workflow efficiency. Designed to meet demanding requirements over a broad range of applications, the EVOS® FL Auto system supports high-resolution 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 CO_2 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
- CO_2 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

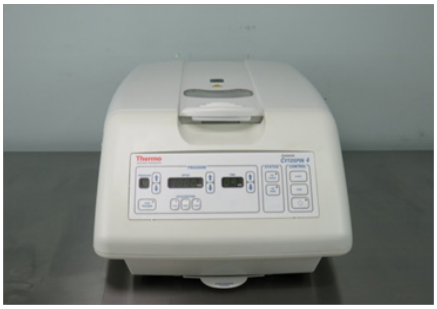
The RM2245 is a semi-motorized rotary microtome, designed for routine in histopathology.

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.

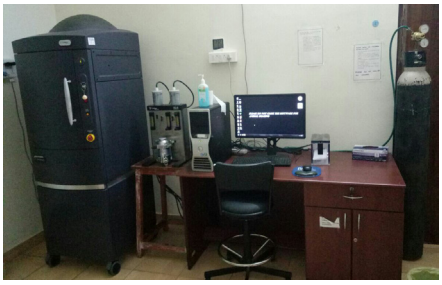
The Veterinary and technical staffs of the CSCR-LAF are also supporting and facilitating all animal facility users (including PhD students and Project Assistants) on mouse and rat bio-methodologies, principles of three R's, ethics, IAEC laws and guidelines on the regulation of scientific experiments 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	Dr. Poonkuzhali
2	Identification of novel nuclear receptor drug targets in myeloid leukemia (To identify the effect of selected NHR ligands in modulating drug resistance in AML mouse model)	Dr. Poonkuzhali
3	Pre-clinical studies for the gene therapy for Wiskott-Aldrich Syndrome (WAS)	Dr.Saravanabhavan
4	To study the effect of shock wave on rat metatarsal organ culture	Dr. Vrisha Madhuri
5	Muscle derived stem cells in the treatment of anal sphincter injury in rat model – an interventional study	Dr. Vrisha Madhuri
6	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	Dr. Sanjay Kumar
7	Identification of novel nuclear receptor drug targets in myeloid leukemia (To identify the effect of selected NHR ligands in modulating drug resistance in CML mouse model)	Dr. Poonkuzhali
8	Safety and efficacy study of AAV based FIX gene therapy productin Hemophilia B mice	Dr. Priyanka Priyadarsini
9	Pre-clinical model for gene therapy for Thalassemia and Sickle Cell disease	Dr. Mohan kumar

Protocols established

- Rodent enrichment program was established to improve the behaviour of animals.
- SOPs for semen collection and analysis in rat and mice were established.
- Irradiation and transplantation of hematopoietic stem cells (HSC) in NSG mice has been optimized.

Training sessions

The Lab Animal Facility conducts training and 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.

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: Aniket Kumar & Alok Srivastava

Clinical grade cells manufactured

- Bone Marrow derived Mesenchymal Stromal Cells (MSC)
  No. of samples processed: 58
  Total Cell yield: 6663.29 x 10^6 MSC
- Placenta derived MSC
  No. of samples processed: 7
  Total cell yield: 2458 x 10^6 MSC

Description

The facility is designed to develop and manufacture cellular and tissue engineered products for clinical applications. It provides the infrastructure to conduct Phase I/II clinical trials by supporting translational medicine in the fields of cell therapy and regenerative medicine. The trained staff, directly interact with investigators and help in process development and manufacture of clinical grade products for use in early phase clinical trials.

Facility Layout

Approximately 1200 square feet, the clean room area is divided into four independent manufacturing suites and one common staging room which are all ISO Class 7 (Class 10,000). The manufacturing rooms have positive pressure to adjacent areas. Each suite is equipped with biological safety cabinet, CO2 Incubators, refrigerated high speed centrifuge and inverted phase contrast microscope. Also a one pass-through both sides is fitted to each room. The facility maintains unidirectional traffic flow for personnel and materials.

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.

Facility Resources

- Manufacturing Suites cleaned twice weekly (includes ceiling, wall, floor)
- Change over cleaning between each manufacturing step
- Environmental Monitoring Program for both viable & non-viable contaminates- monthly
- Daily QC checks for door pressure, temperature, etc.

Current scientific activities

The cGMP facility is currently involved in the banking of clinical grade iPSC. Peripheral blood sample will be collected from HLA haplotyped donors. Mononuclear cells will be isolated and used as a starting material for the derivation of iPSC. The iPSC will be characterized and cryopreserved for future use.

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

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

Access

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. For any service related queries please contact Augustine Thambaiah at +91-416-307-5168 or e-mail cscrcpf@cmcvellore.ac.in

SECOND ANNUAL SYMPOSIUM ON CELL AND GENE THERAPY
7 & 8 SEPTEMBER, 2017

The Centre for Stem Cell Research (CSCR), (a unit of inStem, Bengaluru) organized the 2nd Annual Symposium on Cell and Gene Therapy on 7th & 8th September, 2017. 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. VijayRaghavan, Secretary, DBT and Dr. Soumya Swaminathan, Secretary, Department of Health Research (DHR) and Director General, ICMR 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.

Number of participants:

International speakers: 05
National Speakers: 13
Delegates: 110
Total number: 128

Participating institutes:

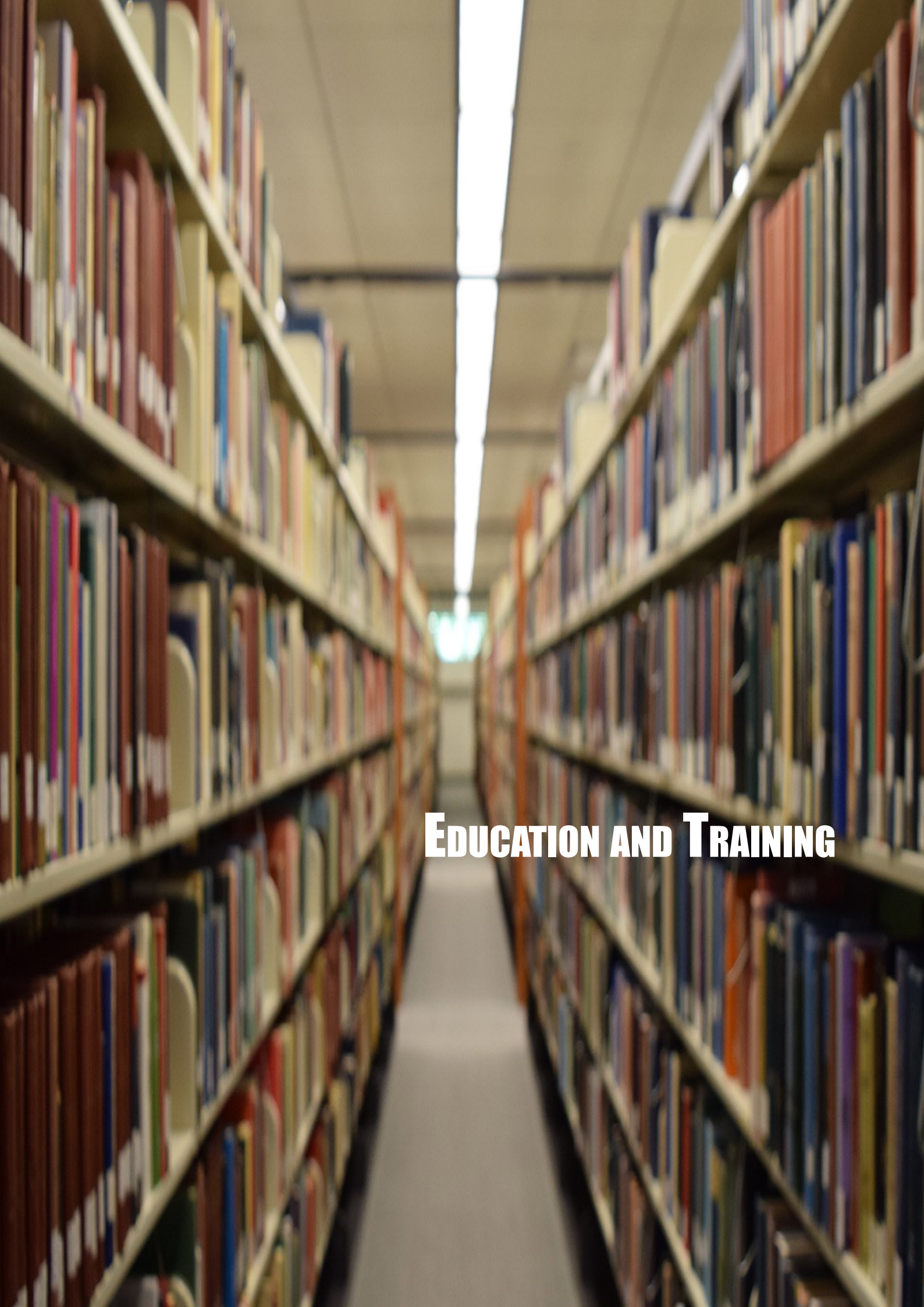
International:

1. University of Florida College of Medicine, Florida, USA
2. Emory University School of Medicine, Atlanta, USA
3. UCSF Benioff Children's Hospital, Oakland, USA
4. Children's Hospital Oakland Research Institute, USA
5. Cell and Gene Therapy Lab, Necker Hospital, France

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 Council of Medical Research, New Delhi
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. Vellore Institute of Technology, Vellore

The 3rd Annual Symposium on Cell and Gene Therapy is scheduled on 6 & 7 September, 2018.



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, CSCR or Thiruvalluvar University. One student has registered for PhD in 20172018.

Thesis Submitted

»»Mr. Salar Abbas
»»Mr. Janakiraman R
»»Ms. Ezhil Pavai M
»»Ms. Sreeja K

PhD Completed

»»Ms. Kannan VM
»»Ms. Savitha V
»»Ms. Divya M

II. Other training programs:

Short term student projects (Bi-annual)

S. No	Name	Duration	Qualification	University	Project title	PI /Lab
1	Ms. Dhakshanya Predheepan	Jan - Jun 2018	B.Tech - Biotech	Anna University	Construction of Lentiviral Vectors for Gene Knockdown	Dr. Shaji / Lab-2
2	Ms. Sumitha. B	Jan - Jun 2018	M.Sc Biotech	VIT University	Construction of Lentiviral Vectors for Gene Knockdown	Dr. Shaji / Lab-2
3	Ms. Janitri Venkatachala Babu	Jan - Jun 2018	M.Sc Regenerative Medicine	Manipal University	Investigating human Wharton's Jelly Mesenchymal Stem Cells (h-WJ-MSCs) /derived extracellular vesicles as a cell free therapeutic product in mice model	Dr. Sanjay / Lab-3
4	Ms. Antra Pant	Jan - Jun 2018	M. Pharm.	NIPER, Guwahati	Targeting stress induced alteration in psoriatic plaque with antimiR HSP90 loaded lipid nanoparticles	Dr. Srujan / Lab-6
5	Ms. Swapna L	Jan - Jun 2018	M. Pharm.	NIPER, Guwahati	Evaluating the topical gene therapy for chronic wound healing: delivering pdgf-b plasmid with nanocarrier system	Dr. Srujan / Lab-6
6	Mr. Muthuganesh	Jan - Jun 2018	M.Sc Biotech	Bharathidasan University	Screening novel solutions for hematopoietic cell nuclefection	Dr. Saravana / Lab-7
7	Ms. Porkizhi	Jan - Jun 2018	M.Sc Biotech	Bharathidasan University	Generation and characterization of Cas9 expressing HEK293T, K562 & HUDEP cell lines for genome editing applications	Dr. Saravana / Lab-7
8	Mr. Subhajit Hazra	Jan - Jun 2018	M. Pharm.	Gupta College of Technological Sciences	Synthesis and Characterization of Chitosan based Nanogels for Stem Cell Delivery	Dr. Murugan/ Lab- 8

9	Ms. Gowri P	Jan - Jun 2018	M.Sc Biotech	Bharathidasan University	CRISPR aided Ex-vivo gene therapy for β- hemoglobinopathies	Dr. Mohan / Lab-9
10	Ms. Shanmathi. D	Jan - Jun 2018	M.Sc Biotech	Bharathidasan University	CRISPR aided ex-vivo gene therapy for β- hemoglobinopathies	Dr. Mohan / Lab-9
11	Mr. Arup kumar Bishoyi	Jul - Dec 2018	M. Pharm.	NIPER, Guwahati	Fabrication of galactosylated liposomes for liver specifc nucleic acid delivery	Dr. Srujan / Lab-6
12	Mr. Lakhan Sahu	Jul - Dec 2018	M. Pharm.	NIPER, Guwahati	Factor IX protein replacement therapy with lipid mediated nucleic acid delivery	Dr. Srujan / Lab-6
13	Mr. Vedha Viyas T	Jul - Dec 2018	M.Tech Biomed	Karunya University	Anti-tumor Functions of CAR Engineered Immune Cells	Dr. Sunil / Lab-6
14	Ms. Salini Kumari	Jul - Dec 2018	M.Sc Biotech	Kalinga University	Lentiviral Delivery of CAR Genes to Immune Cells	Dr. Sunil / Lab-6
15	Ms. Sahana Sadhasivam	Jul - Dec 2018	M.Tech Biotech	Bannari Amman Institute of Technology, Sathyamangalam	Characterization of gene edited Hematopoietic stem cells	Dr. Saravana / Lab-7
16	Mr. Kartik Lakhotiya	Jul - Dec 2018	B.Tech	VIT University	CRISPR aided Ex-vivo gene therapy for β- hemoglobinopathies	Dr. Mohan / Lab-9

PERSONNEL AT CSCR

Scientific / Technical Staff

»»Dr. Alok Srivastava	Head / Adjunct Scientist
»»Dr. R. V. Shaji	Adjunct Scientist
»»Dr. Vrisha Madhuri	Adjunct Scientist
»»Dr. Sanjay Kumar	Scientist
»»Dr. Poonkuzhali Balasubramanian	Adjunct Scientist
»»Dr. Murugan Ramalingam	Associate Professor *(up to May 2018)*
»»Dr. Saravanabhavan Thangavel	Assistant Investigator
»»Dr. Srujan Kumar Marepally	Scientist
»»Dr. Jeyanth Rose	Adjunct Scientist
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»»Dr.Geeta Chacko	Adjunct Scientist
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»»Dr. Md. Manzoor Akheel	Scientist, Research Development Office
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»»Mr. Rajesh A.	Technical Officer
»»Dr.Vasanth Thomodaran	Post Doctoral Fellow
»»Dr Santosh Chandar Maddila	Post Doctoral Fellow
»»Mr. Kannan Thoppil	Senior Research Fellow *(up to Jun 2018)*
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»»Ms. Aneesha Nath	Senior Research Fellow
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»»Mr. Abhirup Bagchi	Senior Research Fellow
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»»Mr. Thulaj M	Junior Research Fellow *(up to Jun 2018)*
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»»Ms. Nimmi Vincy	Junior Research Fellow
»»Mr. Bandlamudi Bhanu Prasad	Junior Research Fellow *(up to Jan 2018)*
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