inStem

CSCR
Centre for Stem Cell Research
(a unit of inStem, Bengaluru),
Christian Medical College Campus,
Bagayam, Vellore – 632002,
Tamil Nadu, India.

ABSTRACT BOOK

1ST TO 3RD AUGUST, 2024

CELL AND GENE THERAPY SYMPOSIUM

THEMATIC AREAS

- Orthobiologics in Cartilage Repair
- Application of iPSCs Technology
- Gene therapy
- Technology advances
- Gene editing
- Challenges and Opportunities in Gene Therapy
- Manufacturing and regulatory aspects

2024

9TH ANNUAL

CELL AND GENE THERAPY SYMPOSIUM

A Hybrid Meeting - 2024

1st to 3rd August, 2024

Organized by:

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

Supported by:

Human Resource Development Group, Council of Scientific & Industrial Research (CSIR – HRDG), New Delhi

Science and Engineering Research Board, Department of Science and Technology (SERB-DST), Government of India, New Delhi

Dear Colleagues,

We take immense pleasure in welcoming you to the **9th Annual Symposium on Cell and Gene Therapy.** This platform continues to bring together scientists and physicians from both academia and industry as well as all others interested in and responsible for developing this field in the country. This field itself is advancing with amazing speed with new transformational products coming to clinical care for various unmet needs. Considerable progress has also been made in India with several clinical trials initiated with gene modified cellular products including a first in human study for gene therapy of haemophilia A. It is also remarkable to note significant industry engagement in the field. These advances could provide much needed cost-effective solutions for several unmet health care needs in India.

The program this year will include topics and presentations which will capture the advances in the field. These include applications of cell and gene therapy in hemoglobin disorders, immune cell therapy, applications of iPSC technology and challenges and opportunities in Gene Therapy. We will also cover industry updates on evolving products and manufacturing along with regulatory issues related to them.

We are again fortunate to have among our speakers some of the global leaders in the field. This meeting is always structured to facilitate discussion both during formal presentations at the scientific sessions and through informal discussions and interactions during the breaks for those present in person at the venue. We also hope that we can follow-up on these deliberations after the meeting with suitable actions to move this field forward in India.

We would like to thank all of you for joining us in this endeavour.

Team CSCR for CGTS-2024

Message

The Institute for Stem Cell Science and Regenerative Medicine (iBRIC-inStem) is an autonomous institute of the Department of Biotechnology (DBT), Ministry of Science and Technology, Govt. of India. DBT-inStem is India's first stem cell institute. inStem's mandate is to undertake world-class research in the area of stem cells and regenerative medicine for the benefit of humankind and society. Capitalizing on its diverse and cross-disciplinary expertise with an aim to address complex problems, beyond the scope of individual laboratories, DBT-inStem has established numerous collaborative research programs with translational emphasis. With a significant core strength in stem cell biology, inStem is focused on increased mechanistic understanding of disorders of the blood, brain and heart disease, as well as developing technologies that will aid improvement of human health. Additionally, institutional programs focus on establishing stem cell and organoid models for research, training and drug discovery platforms.

The Centre for Stem Cell Research (CSCR), is the clinical translation unit of iBRIC-inStem in partnership with CMC, Vellore. CSCR receives administrative support from inStem and is funded by the DBT through inStem. CSCR is carrying out research at the forefront of Cell and Gene Therapy and application of iPSC technology, two important and critical areas for clinical translation. It is indeed commendable that the CSCR has entered the critical clinical trial phase for the treatment of Haemophilia A. Research from CSCR scientists has gained momentum. Several technologies for improved gene editing and prime editing in hematopoietic cells have been devised to correct sickle cell disease and thalassemia. Some of the products and technologies are being transferred for production. While the major efforts are centred on inherited blood disorders, specifically hemoglobinopathies, significant contributions have been made in musculoskeletal regeneration. CSCR has had a long and fruitful association and I am sure inStem will continue to support and benefit from their excellent efforts.

CSCR has hosted many scientific and outreach activities to promote knowledge and awareness on Cell and Gene Therapy, under the leadership of Dr. Alok Srivastava, Head CSCR who also setup this Centre. We are very glad that CSCR is hosting its 9th Annual Cell and Gene Therapy Symposium. We commend Head, CSCR and team for successfully keeping this excellent series running. This event has always been of interest to the stem cell community and attracts some of the best scientists and clinicians in this field of research.

I wish CSCR all the very best for a successful symposium.

Maneesha S Inamdar
Director, iBRIC_inStem

Message

The Annual Cell and Gene Therapy Symposium," organized by the Centre for Stem Cell Research (CSCR, a unit of inStem, Bangalore) at Christian Medical College (CMC) Vellore, aims to foster collaboration, innovation, and the exchange of cutting-edge research in the rapidly advancing fields of cell and gene therapy. These fields have seen significant advancements and innovations in recent times, and I am pleased to say that CMC Vellore, in collaboration with the Department of Biotechnology, has been at the forefront of research in this area. Recently, CSCR reported the first gene therapy trial for Haemophilia A, marking a major advancement in the field.

This year, the 9th Annual Symposium is being conducted in a hybrid format. It promises to be an engaging mix of scientific presentations, clinical breakthroughs, industry insights, networking opportunities, and poster presentations where young researchers and students can showcase their work. This platform will allow the next generation of scientists to share their contributions and receive valuable feedback.

The 9th Annual Symposium will not only celebrate the remarkable progress in cell and gene therapy but also highlight the significant challenges that lie ahead. By bringing together multidisciplinary expertise, this symposium aims to address these challenges and accelerate the development of transformative therapies, ultimately improving patient outcomes worldwide.

I extend my congratulations to the organizing team for their efforts and wish them the best of success.

Dr. Vikram Mathews MD.DM. FASc
Director,
CMC Vellore

Message

I am delighted to note that the faculty, staff and students at the Centre for Stem Cell Research (CSCR) are organising the 9th Annual Cell and Gene Therapy Symposium. This demonstrates their consistent commitment to high quality research and translational therapy.

The current therapies for several disease conditions are only supportive and not curative. There are several therapies that are extremely expensive and not affordable to the common man. Cell and Gene therapy provides a promising option for the treatment of several diseases and also a cost-effective option when developed in India. The Scientists at CSCR, through their dedicated and excellent research, have reached the translational stage for several therapies. The ongoing clinical trial involving gene therapy for Haemophilia is an example of the cutting-edge translational research that is being carried out.

This symposium with illustrious speakers and presentations of current cell and gene therapy research, will definitely provide an excellent platform for valuable discussions and deliberations on the way forward. I thank all the speakers of the symposium for their willingness to share their expertise, and wish all the participants a great time of learning and networking.

I take this opportunity to congratulate the team at CSCR and their Head Dr. Alok Srivastava, for the excellent translational research work that is being done, thus fulfilling the mandate for which CSCR was set up. It is indeed a time for celebrating 20 years of CSCR and sharing the noteworthy achievements that will impact the healthcare of our country, and the world at large.

Dr. Solomon Sathishkumar
Principal
Christian Medical College, Vellore

MESSAGE

It is a great privilege for all of us at the Centre for Stem Cell Research to be able to organize the 9th edition of the Annual Cell and Gene Therapy Symposium. Apart from pursuing translational science in the field of cell and gene therapy, it has also been the mandate of CSCR to support and promote this field in India. To this end, we had started organizing this annual symposium in 2016 to provide a platform for all stake holders involved in this area of science in the country to come together. This has included scientists from different scientific institutions and industry as well as physicians from medical institutions. There has also been participation from noted international scientists presenting their latest innovations in this meeting.

Over the last few years, it has been possible to extend the reach of this meeting through a hybrid platform which also allows many more presentations by scientists and participation of delegates who may not be able to join in person. It is reassuring to see the expansion of this field in the country within the basic science and translational research as well as industry participation.

As has been discussed for several years, having initiated such a platform through institutional mechansisms, with expanding interest from both academia and industry as well as exponential growth of this science in the world, there is a need to establish a national scientific society to take this mission forward. This will then become an independent activity which much greater possibilities for wider engagement and activities for further establishing and promoting this field in the country.

On behalf of everyone at CSCR, I want to thank all supporting institutions and sponsors who have helped us in this endeavour over the years.

Alok Srivastava MD, FRACP, FRCPA, FRCP
Head, Centre for Stem Cell Research
Senior Professor, Christian Medical College Vellore

9TH ANNUAL SYMPOSIUM ON CELL AND GENE THERAPY

1st to 2nd August, 2024

PROGRAMME SCHEDULE

DAY-1: Thursday, 1st August, 2024

1:00 to 1:05 PM **1:05 to 1:20 PM** **1:20 to 1:25 PM** **1:25 to 1:30 PM**	• **Prayer by Chaplain** • Welcome remarks: **Director, CMC / Director, InStem / Principal, CMC** • Remarks by Secretary, Department of Biotechnology, Ministry of Science and Technology, Govt. of India • Closing remarks: **Head, CSCR**

Session-1: ORTHOBIOLOGICS IN CARTILAGE REPAIR
Chair: Samuel Chittaranjan

India Time	Title	Speaker Name
1.30 to 2:00 PM	Cell-Based Therapeutics for Arthritic Disease	**Frank Barry** University of Galway, Ireland
2:00 to 2:30 PM	PRP for OA Knee: Bench to bedside	**Sandeep Patel** The Postgraduate Institute of Medical Education and Research (PGIMER) Chandigarh, India
2:30 to 3:00 PM	The Role of Extracellular Vesicles and Orthobiologics Secretome in Joint Preservation	**Elizabeth Vinod** Centre for Stem Cell Research (A unit of inStem, Bengaluru) and CMC, Vellore, India
3:00 to 3:30 PM	**Poster presentation and Industry Exhibition**	

Session-2: MANUFACTURING AND REGULATORY ASPECTS IN CELL AND GENE THERAPY
Chair: Cartikeya Reddy

India Time	Title	Speaker Name
3:30 to 4:00 PM	Regulatory Paradigm of CAR-T therapies in India ***and*** Challenges and Promise of CAR-based therapies in India	**Akhil Kumar** Aurigene Oncology Limited, Bangalore, India ***and*** **Priyadarshini Chatterjee** Aurigene Oncology Limited, Bangalore, India
4:00 to 4:30 PM	From Innovation to Translation to Patients: The Future of Genetically Engineered T-Cells for Human Therapeutics	**Bruce L. Levine** The University of Pennsylvania, Philadelphia, US
4:30 to 5:00 PM	Mesenchymal stromal cells for clinical applications: CMC Challenges and Paths Forward	**Sowmya Viswanathan** Schroeder Arthritis Institute, University Health Network and the University of Toronto, Ontario, Canada
5:00 to 5:15 PM	**Break**	

Session-3: TECHNOLOGY ADVANCES Chair: Soniya Nityanand		
India Time	**Title**	**Speaker Name**
5:15 to 5:45 PM	Development of targeted viral platform for selective gene transfer to human HSCs in vivo	**Dmitry M. Shayakhmetov** Emory University School of Medicine Atlanta, USA
5:45 to 6:15 PM	Genetic and Transcriptional Engineering of Primary Human Blood Cells	**Rasmus O. Bak** Aarhus University, Department of Biomedicine, Aarhus, Denmark
6:15 to 6:45 PM	Innovative Non-Genotoxic Cell and Gene Therapies for Fanconi Anemia	**Agnieszka Czechowicz** Stanford University School of Medicine, Dept of Pediatrics Div. of Stem Cell Transplantation and Regenerative Medicine, Stanford, US
KEYNOTE ADDRESS **Chair: Alok Srivastava**		
India Time	**Title**	**Speaker Name**
6:45 to 7:45 PM	The Concept of Innovation and Orchestration: Translating Engineered Cellular Therapies from Bench to Bedside	**Khalid Shah** Center for Stem Cell and Translational Immunotherapy, Brigham and Women's Hospital and Harvard Stem Cell Institute, Cambridge, USA
End of Day-1		

DAY-2: Friday, 2nd August, 2024

Session-4: INDUSTRY UPDATES Chair: Praveen Kumar Vemula		
India Time	**Title**	**Speaker Name**
12:00 to 12:20 PM	Non-Viral Chimeric Antigen Receptor (CAR) T Cells going Viral: Process Studies from Stanford Center for Cancer Cell	**Vimal Keerthi** Stanford University School of Medicine, California, US
12: 20 to 12:40 PM	Writing the Future of Biologics with an Integrated Offering of Immunization, Libraries, and Machine Learning	**Jay Yang** Twist Bioscience, San Francisco, CA, US
12:40 to 01:00 PM	Transforming Viral Vector Production: Enhancing Efficiency and Scale-Up with OmniBRx's Dynamic Bed Reactor Technology	**Ravindra Patel** OmniBRx Biotechnologies Pvt Ltd. Ahmedabad, Gujarat, India
01:00 to 02:00 PM	**Lunch Break**	

Session-5: APPLICATION OF IPSC TECHHNOLOGY Chair: Maneesha S. Inamdar Co-Chair: Dhandapani Perundurai		
India Time	**Title**	**Speaker Name**
2:00 to 2:30 PM	Challenges for the development of pluripotent stem cell based therapies and the role of international standards.	**Glyn Stacey** International Stem Cell Banking Initiative, Barley, UK
2:30 to 3:00 PM	Developing iPSC-derived RPE cell replacement therapy for the treatment of AMD	**Rajarshi Pal** Eyestem Research, Centre for Cellular and Molecular Platforms (C-CAMP), Bangalore, India
3:00 to 3:30 PM	Developing iPSC technologies to impact current challenges in the production of cell therapies	**Julia Neubauer** Fraunhofer Institute for Biomedical Engineering IBMT, Germany
3:30 to 3:45 PM	**Break**	

Session-6: GENE THERAPY Chair: Matthew Porteus		
India Time	**Title**	**Speaker Name**
3:45 to 4.15 PM	New insights into the use of gamma delta T cells to treat childhood cancers	**H. Trent Spencer** Emory University School of Medicine, Atlanta, USA
4:15 to 4:45 PM	Development of genome-modified generation ZZ (GenZZ) single-stranded AAV vectors with improved transgene expression	**Arun Srivastava** University of Florida, Florida, USA
4:45 to 5:15 PM	Preclinical development of gene therapy for Diamond-Blackfan anemia	**Senthil Bhoopalan** St. Jude Children's Research Hospital, Memphis, USA
5:15 to 6:00 PM	**Poster presentation and Industry Exhibition**	
Session-7: CHALLENGES AND OPPORTUNITIES IN GENE THERAPY Chair: RV Shaji		
India Time	**Title**	**Speaker Name**
6:00 to 6:30 PM	Development of pathophysiologicallyrelevant models of β-hemoglobinopahtues for therapeutic studies	**Sivaprakash Ramalingam** CSIR–Institute Of Genomics And Integrative Biology (CSIR–IGIB), New Delhi, India
6:30 to 7:00 PM	CAR T-cell induced T-cell malignancies	**Nirali Shah** Center for Cancer Research, National Cancer Institute, Maryland, US
7:00 to 7:30 PM	Genome Editing of HSCs to Develop Stem Cell Based Therapies	**Matthew Porteus** Stanford School of Medicine, California, USA

KEY NOTE ADDRESS Chair: Sanjay Singh		
India Time	**Title**	**Speaker Name**
7:30 to 8:30 PM	Gene therapy for haemophilia in India – The beginning of a New Era	**Alok Srivastava** Centre for Stem Cell Research, a unit of inStem, Bengaluru and Department of Haematology, Christian Medical College Vellore, India
End of Day-2		

DAY-3: Saturday, 3rd August, 2024

Session-8: INDUSTRY UPDATES Chair: Arvind Ramanathan		
India Time	**Title**	**Speaker Name**
12:20 to 12:40 PM	Autologous CAR-T Cell Therapy Manufacturing Solution	**Pankaj Salvi** Cytiva, Marlborough, US
12:40 to 01:00 PM	Thermo Fisher Scientific: Cell & Gene Therapy capabilities overview	**Uchenna Waturuocha** Thermo Fisher Scientific, Mumbai, India
01:00 to 02:00 PM	**Lunch Break**	

Session-8: NON-VIRAL VECTOR BASED GENE THERAPY Chair: N. Madhusudhana Rao		
India Time	**Title**	**Speaker Name**
2:00 to 2:30 PM	Enhancing Natural killer cells proliferation and cytotoxicity using Imidazole-based lipid nanoparticles encapsulating interleukin-2 mRNA	**Chantal Pichon** INSERM and University of Orléans, Orléans, France: Institut Universitaire de France, Paris
2:30 to 3:00 PM	MSC Extracellular Vesicles: Navigating Regenerative Medicine's Therapeutic Landscape	**Sujata Mohanty** All India Institute of Medical Sciences New Delhi, India
3:00 to 3:30 PM	Long-lasting mRNA enabled protein replacement therapy with liver-specific lipid nanoparticle system: Haemophilia B as a model disease	**Srujan Marepally** Centre for Stem Cell Research (a unit of inStem, Bengaluru), Vellore, India
3:30 to 3:45 PM	**Break**	
3:45 to 4:30 PM	**Poster presentation and Industry Exhibition**	

Session-9: GENE EDITING Chair: Sanjeev Galande		
India Time	**Title**	**Speaker Name**
4:30 to 5:00 PM	Genome editing strategies for treating sickle cell disease	**Thiyagaraj Mayuranathan** Centre for Stem Cell Research (A unit of inStem, Bengaluru), Vellore, India
5:00 to 5:30 PM	Epitope Editing for an Immunotherapy "Stealth" Hematopoiesis	**Pietro Genovese** Harvard Medical School, Boston, USA
5:30 to 6:00 PM	Next-generation gene editing of human hematopoietic stem cells: from research to clinical translation	**Samuele Ferrari** San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Milan, Italy
Session-10: IMMUNE CELL THERAPY **Chair: Amit Awasthi**		
India Time	**Title**	**Speaker Name**
6:00 to 6:30 PM	CART Therapy in Pediatric Acute Lymphoblastic Leukemia	**Sunil Bhat** Mazumdar Shaw Medical Centre, Narayana Health City, Bangalore, India
6:30 to 7:00 PM	Development of CAR-T cell products with non-viral vector system	**Yoshiyuki Takahashi** Nagoya University Graduate School of Medicine, Nagoya, Japan
7:00 to 7:30 PM	Genetic manipulation of NK cells for enhanced immunotherapy	**Rizwan Romee** Harvard Medical School, Dana Farber Cancer Institute, Boston, USA
CONCLUDING REMARK		
End of Day-3		

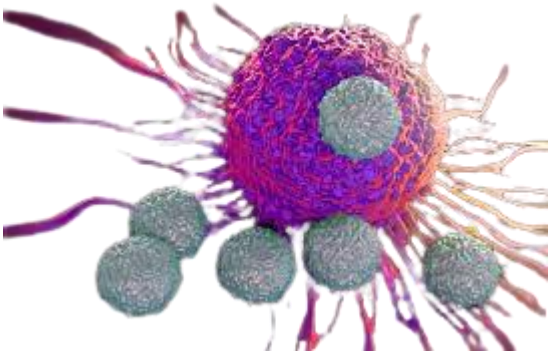
****

KHALID SHAH
Center for Stem Cell and Translational Immunotherapy, Brigham and Women's Hospital and Harvard Stem Cell Institute, Cambridge, USA

Plenary Lecture-1

The Concept of Innovation and Orchestration: Translating Engineered Cellular Therapies from Bench to Bedside

Biological therapies are emerging as a promising strategy for cancer. We have developed innovative cell and viral based immunotherapy approaches to tackle cancer. Our findings demonstrate the strength of using innovative approaches and clinically relevant preclinical models that pave a path for clinical translation. This presentation provides rationale and data of different biological therapies that we have developed to treat cancer in preclinical settings. Furthermore, this presentation will focus on how building experimental design and structure around innovative ideas allows us to translate the most promising studies into the clinic.



ALOK SRIVASTAVA
Centre for Stem Cell Research, a unit of inStem, Bengaluru and Department of Haematology, Christian Medical College, Vellore, India

Plenary Lecture -2

Gene therapy for haemophilia in India – The beginning of a New Era

In the constrained world of limited access to conventional treatment of haemophilia with regular replacement of the missing clotting factor concentrate (CFC), effective care of people with haemophilia (PWH) in low- and middle-income countries has been a challenge. As a leading centre for treatment of PWH in India, the Christian Medical College (CMC), Vellore has been the epicentre for management of haemostasis disorders in the country since the 1960s. Given the challenges of optimal replacement therapies, when gene therapy for haemophilia was attempted in the world in the early 2000s, it had become obvious that a one-time curative treatment could be the most effective way for PWH in LMICs could get the best care.

In 2004, CMC, Vellore was privileged to be selected as the only non-USA site for the first systemically infused intra hepatic administration of adeno associated virus (AAV) vector-based clinical trial of gene therapy of haemophilia. Unfortunately, that study closed while the proposal was still being evaluated by the regulatory authorities in India. However, by then collaboration was established with the University of Florida to develop our own AAV-based technology for gene therapy of haemophilia. While this was ongoing at a time when progress was slow in this field in the world, serendipity led to connections with the Emory University in 2011, a few months before the report of the first successful gene therapy for haemophilia.

This long standing unique tripartite collaboration between the Centre for Stem Cell Research (CSCR)-CMC, Vellore, the University of Florida, Gainesville and the Emory University, Atlanta for more than a decade has now become the basis of the most unusual model of developing gene therapy for haemophilia in the world. First this collaboration led to the development of a novel FIX transgene packaged in an AAV3 vector as an effective gene therapy product for haemophilia B. While this could have gone to clinical trials more than 5 years ago, challenges with scaled up GMP production prevented the translation of this technology. However, recognizing early sone of the limitations of AAV based gene therapy, we were also developing an alternate

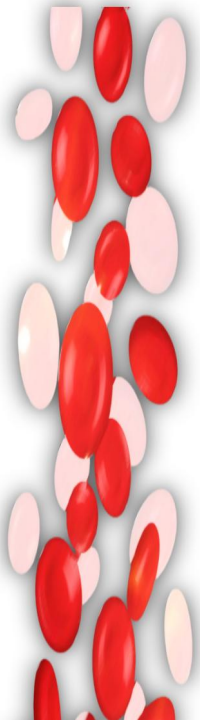
technology for expression of FVIII through a novel transgene transduced with a lentiviral vector into haematopoietic stem cells.

This led to a first in human clinical trial which has been recently completed with a most amazing outcome of all six participants showing clinically significant responses and half of them reaching high mild to near normal FVIII plasma levels. This achievement has only been possible due to the tremendous support from the Department of Biotechnology of the Government of India over these two decades since CSCR was established.

The next step is to transfer these technologies to industry for further product development. Unfortunately, this process has faced unexpected challenges in this unchartered space in the country affected by procedural delays leading to slowing down of the momentum established which is most unfortunate. Regardless, there is celebration of the first successful gene therapy in India with a first in human novel technology for a condition with huge unmet needs not only in India but also many LMICs – all achieved with grant funds and academic collaborations, a unique model in itself and indeed recognized by the Government of India.

https://pib.gov.in/PressReleaseIframePage.aspx?PRID=2009823#:~:text=Delhi%20on%20Wednesday.,India%20has%20conducted%20the%20first%20human%20clinical%20trial%20of%20gene,Minister%20(Independent%20Charge)%20Dr.

A new era of indigenously driven technology based solutions for unmet healthcare needs in India has begun.



Session-1: ORTHOBIOLOGICS IN CARTILAGE REPAIR

FRANK BARRY
University of Galway, Ireland

Cell-Based Therapeutics for Arthritic Disease

Cell transplantation strategies have found wide use in the treatment of acute cartilage injuries and also in osteoarthritis and degenerative disc disease. Much of this effort has focused on autologous chondrocyte transplantation and the use of mesenchymal stromal cells (MSCs). The latter have been used either as an expanded cell product or as a concentrate from bone marrow or the stromal vascular fraction of adipose tissue. There is an abundant preclinical database indicating that these approaches are effective. In the case of MSCs the mode of action appears to rely mainly on immunomodulation and anti-inflammatory effects associated with extracellular vesicles and paracrine factors released by the cells. Recent studies, carried out in our laboratory and others, provide some more precise information about the molecular events that occur following transplantation of cells to the arthritic joint and their response of the inflamed and degenerate environment. New insight has recently been obtained in large, well designed patient trials that raises significant questions about the true clinical utility of these approaches, with inconsistent outcomes being reported. One area of particular concern is the lack of fully standard manufacturing protocols and the persistent problem of heterogeneity in expanded MSC populations. A detailed analysis of the impact of different culture media shows extraordinary phenotypic variability, indicating that the critical biological attributes of the cells are highly dependent on the composition of the medium. The absence of a standardised processing model, coupled with the widespread adoption of quality release criteria tests that provide very limited phenotypic information about the cell dose, are likely to be major contributors to the variable clinical response. The solution lies in the adoption of stringent industry process standards and the development of disease-specific potency tests.

SANDEEP PATEL
The Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

PRP for Knee OA- bench to bedside

Background
PRP provides a cocktail of growth factors which modulates the internal milieu and through its anti-inflammatory properties and Chondral remodelling potential is effective for early OA Knee. The ideal type of PRP needs to be defined and there is need to standardize various variables concerning PRP.

Summary of our studies
Our RCT first established the superiority of PRP injection over normal saline for early OA knee where in better WOMAC scores and VAS scores were observed at 3 and 6 months compare to baseline in the PRP group. We also observed that single injection of PRP was as good as 2 injections of PRP in this RCT. We used 8 ml Leucocyte poor PRP along with exogenous activator CaCl2.
Simultaneously we carried on animal studies wherein we used Dunkin Hartley guinea pig which develops spontaneous Knee OA similar to human OA. We found better anti-inflammatory properties in terms of minimal synovial hyperplasia and inflammation with PRP injection compared to normal saline. We also observed better cartilage quality in terms of tide mark, minimal fissures and better proteoglycan profile in PRP group in PRP group.

Our next animal study focussed on comparing single PRP injection with multiple PRP doses and we observed better anti-inflamatory effect in multiple injections at 6 months and similar effect at 3 months. Better chondroprotective effects were seen with multiple PRP injections at 3 months. In our next studies we looked at caspase-3 as a marker for anti-apoptosis effects of PRP and aggrecan and Col-2 as marker of ECM. PRP group had anti-apoptosis effects and better ECM profile compared to normal saline.We also looked at improving PRP effects by combining PRP with injectable Chitosan as a scaffold. However, no added benefits were observed with the Chitosan-PRP injection.
Our constant efforts to improve PRP for clinical use resulted in better understanding of PRP and we developed superdose PRP which refers to 8 ml of PRP with absolute platelet number over 5 billion platelets. In our triple blind RCT, Superdose PRP was found to have better patient satisfaction as well as better clinically significant WOMAC, KOOS and VAS scores compared to conventional dose of PRP. In another Triple blind RCT, we also looked at the added advantage of using exogenous activator along with PRP and found no added benefit of using activator along with PRP.

Conclusion
The dose of platelets in PRP is crucial for better clinical results and atleast 5 billion platelets are required for knee OA. There is no role of additional activator. The effects of PRP are due to its anti-inflammatory effects and minimal chindral remodelling effect.

ELIZABETH VINOD
Centre for Stem Cell Research, a unit of inStem, Bengaluru and Christian Medical College, Vellore, India

The Role of Extracellular Vesicles and Orthobiologics Secretome in Joint Preservation

Diseases that impact the joints affect millions globally, presenting as a complex, chronic condition. They lead to changes in the hyaline articular cartilage and affect the entire joint structure, including subchondral bone, ligaments, capsule, and surrounding muscles. Currently, no therapies effectively halt or delay disease progression, making joint replacement the top option for improving patient quality of life. Consequently, research actively pursues treatments to reduce symptoms and prevent progression. Clinical trials mainly target enhancing cartilage regeneration and reducing inflammation through intra-articular injections of orthobiologics.

The paradigm of orthobiologics has recently garnered a lot of interest, utilizing biological substances to accelerate healing or alleviate the progression of articular cartilage-related pathologies. Notably, Mesenchymal stem cells (MSCs), bone marrow aspirate concentrate (BMAC), and stromal vascular fraction (SVF) are extensively researched for their immunomodulatory and regenerative capabilities. Their inherent potency stems from releasing bioactive molecules and extracellular vesicles(EVs), known collectively as the "secretome," which play crucial roles in joint preservation. Studies evaluating the potential cartilage resident-derived secretome, namely chondrocytes and their progenitors, are also currently being actively pursued. In-vitro analysis reports enhance growth kinetics, reduce inflammation, and promote healing. These positive findings were also observed in in-vivo studies, demonstrating beneficial effects on cartilage, subchondral bone, and synovial tissues in osteoarthritis (OA) and osteochondral models. The first clinical studies exploring clinical-grade MSC secretomes or EVs for treating OA are underway and have shown promising interim reports. Are we there yet with secretome-based therapy in the field of cartilage repair? Future research in this area holds significant promise as it will enhance our understanding of the therapeutic potential of MSCs and shed light on the molecules/miRNAs they release.

Session-2: MANUFACTURING AND REGULATORY ASPECTS IN CELL AND GENE THERAPY

AKHIL KUMAR
Aurigene Oncology Limited, Bangalore, India

Regulatory Paradigm of CAR-T therapies in India

Current CAR T-cell therapies harnesses the power of the patient's own immune system to combat cancer. These drugs have shown phenomenal success in treating certain types of cancers and have changed the natural history of these diseases. While the clinical value of CAR-T products are now well known, regulatory paradigm around the globe is still evolving. As an example, in our country, the current guidelines require application to multiple governmental agencies to start a clinical trial. Starting non-clinical work also needs permission from the government as genetic material is involved. Aurigene Oncology Limited is one of the few companies in India which has manufactured CAR-T in a GMP facility and has worked with haematologists across the country in administering the product to patients. While we extensively discuss the technical, scientific and clinical aspects of CAR-T therapies, it is important for all involved to understand the regulatory aspects of the treatment.

The ability to serve our patients is intertwined with our regulations, and we are witnessing an evolution and significant help from the government in this regard. The presentation will discuss the current paradigm and the desire of the community for way forward.

PRIYADARSHINI CHATTERJEE
Aurigene Oncology Limited, Bangalore, India

Challenges and Promise of CAR-based therapies in India

CAR T-cell therapy represents a groundbreaking approach in the field of cancer treatment, harnessing the power of the patient's own immune system to combat cancer. CAR T-cell therapy has shown remarkable success in treating certain types of hematologic malignancies, such as relapsed/refractory multiple myeloma (RRMM), demonstrating its potential as a transformative therapeutic option for patients who have not responded to conventional treatments. As an example, of the new modalities developed to target B-cell maturation antigen (BCMA), a member of tumor necrosis factor receptor (TNFR) superfamily, preferentially expressed by plasma cells and of interest in treatment RRMM, CAR-T (chimeric antigen receptor T) cells has shown striking overall response rates of 73% and 97% for the two US-FDA approved drugs, Abecma from Blue Bird/BMS and Carvykti from Janssen Pharmaceuticals respectively. As a discovery company that was among the earliest to initiate drug discovery in India, Aurigene Oncology Limited is one of the few players involved in CAR-T therapy in India and continues its endeavour at building innovation businesses based on intense and state-of-the-art science. Here, we describe development of a nanobody based CAR-T cell (DRL-1801) therapy directed against BCMA where in an initial clinical trial (wwww.clinicaltrials.gov NCT03661554) with 34 patients, an overall response rate of 88.2% was achieved with extremely promising safety profile. Like other autologous CAR-T products, DRL-1801 entails ex vivo transduction of patient's T cells with CAR gene construct that encodes the nanobody based antigen-binding domain fused with co-stimulatory and signalling domains, 4-1-BB and CD3ζ. Currently, a Phase I/II multi-centre trial (CMC, Vellore, St. John's, Bangalore, Apollo, Hyderabad, Fortis, Gurgaon and others) is ongoing in India. A single dose of DRL-1801 or Ribrecabtagene autoleucel (2.5–10.0 × 106 CAR+ T cells/Kg) administered by IV infusion to RRMM patients has shown desirable efficacy and safety.

With aging populations, rise in prevalence of cancers and low health expenditure driving market demand for lower-cost therapies, Aurigene stays committed to its motto of "Good Health Can't Wait" and of providing access and affordability to quality treatment. We therefore have endeavours underway like rapid CAR-T manufacturing, assessing automation versus "process-street" manufacturing, deciphering success of various bioreactors to reduce manufacturing failures in patient samples and developing allogenic routes of CAR-based cell therapies. Allogeneic CAR therapies we believe should be able to cut costs substantially, making a huge difference to patients, not just in India but across other emerging markets. Ability to develop affordable CAR cell therapy, coupled with India's growing medical infrastructure, can further position our country as a potential global hub for medical tourism in cancer treatment.

BRUCE L. LEVINE
The University of Pennsylvania, Philadelphia, US

From Innovation to Translation to Patients: The Future of Genetically Engineered T-Cells for Human Therapeutics

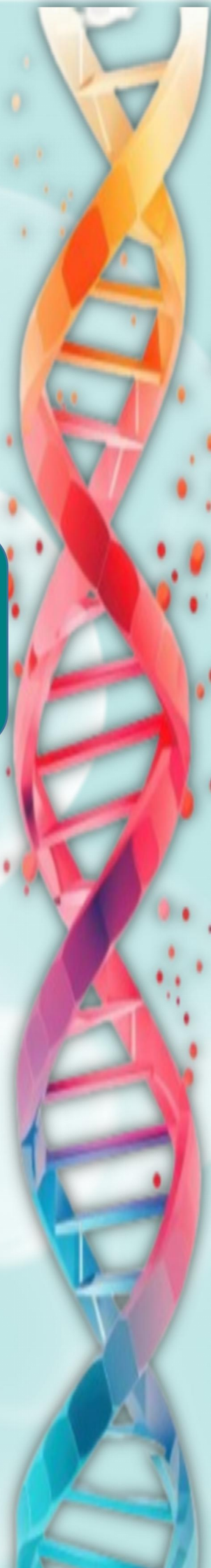
Since the 1990's, we have conducted clinical trials of gene modified T cells. Chimeric antigen receptor (CAR) T cells targeting CD19 on B cells leukemias and lymphomas which have induced durable complete responses in patients who are relapsed or refractory to all other available treatments. New designs for genetically modified T cells include switches and potency enhancements that will be required for targeting solid tumors. Determining the critical quality attributes, dose, potency, and anticipating pharmacokinetics of a living, dividing drug presents unique challenges. Improving patient access to advanced cell and gene therapies entails not only on scientific progress in targeting, gene modification and cellular manipulation, but also on meeting automation, engineering, clinical site onboarding, and health policy challenges.

SOWMYA VISWANATHAN
Schroeder Arthritis Institute, University Health Network and the University of Toronto,

Mesenchymal stromal cells for clinical applications: CMC Challenges and Paths Forward

Despite authorization of mesenchymal stromal cells (MSCs) in certain markets, there are no approved MSC products in Europe or North America. Heterogeneity in donors, lack of standardization in expansion processes and limited characterization of critical quality attributes (CQAs) combined with heterogeneity in recipient patient disease status has led to diminished efficacy, dampening investor and market enthusiasm. In this talk, I will speak to developing multivariate CQAs, and understanding the relationship between these CQAs and donor heterogeneity during MSC expansion processes. This will help address the underlying variability that has confounded clinical trial results. A roadmap for future clinical trials will also be outlined.

Session-3: TECHNOLOGY ADVANCES



DMITRY M. SHAYAKHMETOV
Emory University School of Medicine, Atlanta, USA

Development of targeted viral platform for selective gene transfer to human HSCs in vivo

Numerous genetic diseases of the blood and immune system could be cured or ameliorated if targeted and efficient in vivo delivery of gene editing technologies to hematopoietic stem cells (HSCs) is achieved. We explored a multiplexed targeting and de-targeting approach utilizing modification of the proteinaceous capsid of a non-replicating vector based on human adenovirus type 5, for in vivo gene delivery to human HSCs. To enable on-target specificity, we utilized a dual-moiety targeting approach, whereby the vector was engineered to attach to HSCs via DSG2 and to internalize into cells via a6-integrin receptors, both of which are expressed in, and present on the surface of, human HSCs. In vitro evaluation of human CD34+ transduction by a dually targeted vector demonstrated that transfer of the GFP reporter gene into the most primitive LT-HSC cell populations with a CD34+CD38-CD45RA-CD90+ phenotype was nearly 100% efficient. Intravenous administration of engineered vector to mice, even at extremely high doses, did not trigger cytokine storm, thrombocytopenia, or hepatotoxicity, suggesting significantly reduced vector sequestration in off-target cells and tissue types. In humanized NSG mice grafted with human CD34+ cells, intravenous administration of HSC-targeted vector led to gene transfer to the HSC-rich bone marrow compartment with ~1900-fold higher selectivity compared to CD34-negative lineage committed bone marrow cells. We anticipate that the multiplexed targeting/de-targeting strategy can be applicable to other viral and non-viral delivery platforms to achieve highly selective and efficient transduction of target cells for a variety of in vivo therapies.

RASMUS O. BAK
Aarhus University, Department of Biomedicine, Aarhus, Denmark

Genetic and Transcriptional Engineering of Primary Human Blood Cells

Hundreds of different monogenic diseases of the blood pose great unmet medical needs. Here, I will present our efforts to develop CRISPR-based gene therapies to cure Inborn Errors of Immunity (IEIs). We have developed Base Editors to correct STAT3 Hyper IgE Syndrome (HIES) and Chronic Granulomatous Disease (CGD) in CD34+ hematopoietic stem and progenitor cells (HSPCs). More recently, we have also developed CRISPR-based transcriptional modulation tools delivered as all-RNA reagents for transcriptional engineering of primary blood cells. This technology can impose transient transcriptional states into cells, which we hope can be implemented to improve different types of cell therapies.

AGNIESZKA CZECHOWICZ
Stanford University School of Medicine; Dept of Pediatrics Div. of Stem Cell Transplantation and Regenerative Medicine, Stanford, US

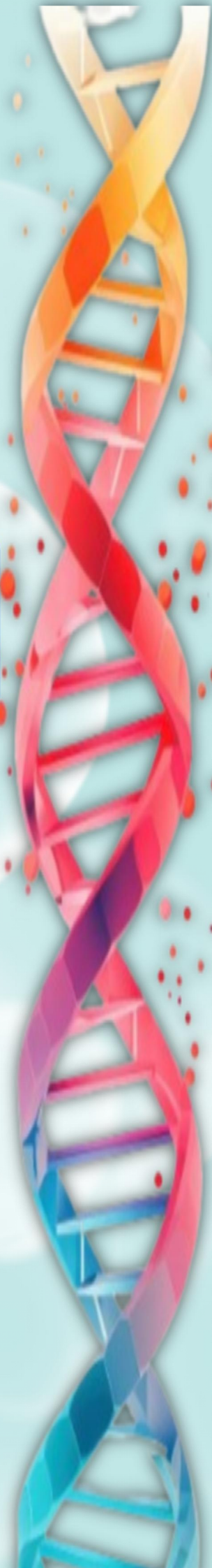
Innovative Non-Genotoxic Cell and Gene Therapies for Fanconi Anemia

Hematopoietic stem cell transplantation (HSCT) can be used to cure many different genetic, malignant and autoimmune blood and immune diseases. However, historic HSCT protocols have required the use of genotoxic chemotherapy and/or irradiation conditioning which cause toxicity and limit use of these curative cell therapies. Hence, we have focused our efforts on understanding the barriers of HSC engraftment and developing new protocols using HSC-targeted approaches.

We have specifically shown that host HSCs limit donor HSC engraftment and that this can be overcome by depleting host HSCs using anti-CD117 mAb-based conditioning. In addition, we have focused on development of new therapies for the DNA-repair deficiency Fanconi Anemia (FA). Given that FA patients experience especially pronounced toxicities from current genotoxic HSCT regimens, we have worked with various collaborators to develop innovative allogeneic transplant protocols and novel gene therapy approaches for these patients. Specifically, we have initiated an antibody-based conditioning regimen comprising briquilimab (anti-CD117 mAb) and a graft manipulation technique that depletes TCRαβ+ T-cells and CD19+ B-cells. This approach aims to reduce toxicity and enhance donor engraftment. This protocol is a TBI- and busulfan-free method that enables use of haploidentical allogeneic HSCT in these exquisitely sensitive immunocompetent patients, thereby restoring hematopoiesis while minimizing toxicity (Clinicaltrials.gov NCT#04784052). Further, in collaboration with Rocket Pharma we have been developing an autologous gene therapy for patients with FA-type A. Specifically, we have been involved in the global RP-L102 clinical trials using ex vivo lentiviral FANCA gene-modified CD34+ enriched hematopoietic stem and progenitor cells (HSPCs). These studies rely upon the proliferative advantage of gene-corrected FA HSPCs, enabling stabilization of hematopoiesis without any conditioning (Clinicaltrials.gov NCT#03814408/04248439).

Through these efforts we aspire to improve treatment of FA patients, while simultaneously better understanding HSPC dynamics to enable development of improved treatments for patients with many different blood and immune diseases.

Session-4: INDUSTRY UPDATES



VIMAL KEERTHI
Stanford University School of Medicine,California,US

Non-Viral Chimeric Antigen Receptor (CAR) T Cells Going Viral: Process Studies from Stanford Center for Cancer Cell Therapy

Non-viral delivery platforms present a promising alternative to address the limitations associated with viral delivery in CAR-T (Chimeric Antigen Receptor T-cell) cell therapies. Viral vectors exhibit other notable drawbacks such as elevated immunogenic potential, restricted insert size, theoretical possibility of insertional mutagenicity, and substantial production costs. In contrast, non-viral approaches alleviate these limitations through decreased manufacturing expenses, increased cargo capacity, greater design flexibility, and diminished immunogenic profiles. At Stanford's Center for Cancer Cell Therapy, we have designed robust processes for clinical scale manufacturing non-viral TRAC (T-cell receptor alpha constant) targeted CAR-T cells. Our platform leverages either the (HDR) Homology Directed Repair approach or (NHEJ) Non-Homologous End Joining based HITI (Homology Independent Targeted Insertion) for manufacturing CAR-T cells. Furthermore, we discuss CEMENT (CRISPR Enrichment) for enriching CAR-T cells and parameters for the electroporation-based delivery of Cas 9 RNP and nanoplasmid donor templates.

RAVINDRA PATEL
OmniBRx Biotechnologies Pvt Ltd. Ahmedabad, Gujarat, India

Transforming Viral Vector Production: Enhancing Efficiency and Scale-Up with OmniBRx's Dynamic Bed Reactor Technology

Today, process efficiency and scale-up are the most common bottlenecks for viral vector manufacturers. The CellBRx-IST (Integrated Seed Trail) platform is a series of closed-loop end-to-end fully integrated and automated variable scale bioreactors ranging from 1L to 200L (from 1 m2 to 1500 m2 surface area for cell attachment and growth) serving as a perfect platform for vaccine and viral vector manufacturing. The CellBRx® Single-use Bioreactors are developed upon the concept of Dynamic-bed reactor technology, which ensures nutritional & gaseous homogeneity across the cell carrier bed resulting in ultra-high-density cell culture and multifold increase in the harvest titres compared to the traditional technologies. The CellBRx bioreactors are specifically developed to provide linear scalability, and ultimate process efficiency and to reduce the batch to batch variability to minimizing process failures at large-scale operations.

PANKAJ SALVI
Cytiva, Marlborough,US

Autologous CAR-T Cell Therapy Manufacturing Solution

The development of CAR T cell therapies has been a decades-long journey from when the technology was first proposed in the late 1980s, to the Food and Drug Administration (FDA) approval of Novartis' Kymriah in 2017. Since then, 10 CAR T therapies have been approved worldwide and more than 20 000 patients have benefited from this revolutionary medicine. With over 1000 of such therapies in the clinical pipeline, the need for technologies that improve manufacturing productivity, automation, and scale-out capacity are even more critical to make these therapies less costly and widely accessible. We shall introduce a new platform that addresses the key challenges to manufacture autologous CAR T therapies at commercial scale. Discover how you will deliver what's next.

JAY YANG
Twist Bioscience, San Francisco, CA, US

Writing the Future of Biologics with an Integrated Offering of Immunization, Libraries, and Machine Learning

Utilizing its proprietary DNA technology to write synthetic libraries, Twist Bioscience provides end-to-end antibody discovery libraries including both (1) highly diverse synthetic naïve antibody phage display libraries, (2) target class-specific antibody phage display libraries against difficult-to-drug targets, and (3) in vivo animal immunization workflows (proprietary mouse, single B-cell cloning, llama VHH discovery). In this talk, Jay will present how Twist leverages in vitro, in vivo, and in silico approaches to accelerate the discovery and optimization of antibodies and other biological molecules.

UCHENNA WATURUOCHA
Thermo Fisher Scientific, Mumbai, India

Thermo Fisher Scientific: Cell & Gene Therapy capabilities overview

In the dynamic landscape of biomedical research, cell and gene therapies have emerged as transformative approaches, offering unprecedented potential to treat and even cure a wide range of diseases. One of which is the CAR-T cell therapy, that is used in treating various cancers. Our company is at the forefront of this scientific revolution, delivering cutting-edge cell and gene therapy solutions that push the boundaries and enhance precision medicine.

This talk will delve into the capabilities and applications of our advanced cell and gene therapy products, focusing on their critical roles in CAR-T production and lentiviral vector systems. We will explore our optimized protocols and reagents for efficient T cell isolation and expansion, precise gene editing and scalable manufacturing solutions. This session will provide valuable insights for researchers, clinicians, and industry professionals seeking to harness the full potential of CAR-T production and lentiviral vector systems to drive innovative treatments and improve patient care.

Session-5: APPLICATION OF IPSC TECHHNOLOGY

GLYN STACEY
IInternational Stem Cell Biobanking Initiative, Barley, UK

Challenges for the development of pluripotent stem cell based therapies and the role of international standards

Human pluripotent stem cells (hPSCs) are now demonstrating their significant potential as research tools to enhance our understanding of human development and the responses of human tissues to drugs and disease. They have been applied in a wide range of research and in the development of novel stem cell based therapies and diagnostic applications. However, their utility remains hampered by certain factors which will be explored in this presentation. The culture and differentiation of hPSCs to provide sufficient quantities and reproducible cultures of cells for use, is complex and prone to issues such as their colonial growth mode, the heterogeneity of stem cell populations, plus, lengthy and complex cell differentiation protocols, Furthermore, on passaging, hPSC cultures may give rise to genetic variants that may have altered characteristics and also raise concerns about the potential tumorigenicity of derivative cell therapies.
An important element in assuring reliable and reproducible cultures and research data from hPSC systems is the use of well qualified stem cell lines, reagents and methodologies. Some of the issues are common to all cell-based systems, but there are certain features of hPSCs where there are special considerations to address. The translation of research driven protocols and materials into final application requires careful development and qualification. In particular, moving to the manufacture of an hPSC based medicine requires considerable effort to assure product safety and quality. Such issues include the selection of suitable culture media, production bioreactors and analytical methods. In addition, it is vital to develop sufficient understanding of each hPSC-based product in question. Such aspects have been a keen focus for efforts to standardise some of these factors, but the development of such standards requires careful forethought to deliver valuable standards, whilst also avoiding redundancy and potential conflict between the output of different standardisation agencies.
This presentation will explore some of the crucial scientific issues for the use of hPSCs in research and development and the establishment of a path towards their standardisation

RAJARSHI PAL
Eyestem Research, Centre for Cellular and Molecular Platforms (C-CAMP), Bangalore, India

Developing iPSC-derived RPE cell replacement therapy for the treatment of AMD

Age-related macular degeneration (AMD) is one of the leading causes of vision loss among the elderly population worldwide. This condition occurs due to the degeneration of the retinal pigment epithelium (RPE) monolayer that supports photoreceptors in the eye. To address this, we have developed an allogeneic cell replacement therapy using induced pluripotent stem cells (iPSC) for treating dry AMD patients. The process involves differentiating iPSCs into RPE cells using a protocol developed in our lab. The RPE cultures are then enriched to promote complete maturation and grown for six to eight weeks to achieve scale during GMP manufacturing. Quality control assays are performed to confirm the identity, purity, sterility, functionality and stability of the cryopreserved iPSC-RPE. iPSC-RPE transplanted into immune suppressed RCS rats showed integration, neuroprotection and rescue of visual function. Preclinical studies in rats, mice, rabbits and non-human primates have demonstrated the safety profile of the iPSC-RPE cells. Transplanted immune-compromised rats with escalating doses of iPSC-RPE showed no signs of tumor formation after nine months, confirming the safety profile. We started a Phase I/IIa trial using the iPSC-RPE cells to treat AMD. The ongoing trial will assess the safety and efficacy of Eyecyte-RPE (drug product) after a single-dose of subretinal injection in 9 dry AMD patients (secondary to geographical atrophy) across three centers in India.

JULIA NEUBAUER
Fraunhofer Institute for Biomedical Engineering IBMT, Germany

Developing iPSC technologies to impact current challenges in the production of cell therapies

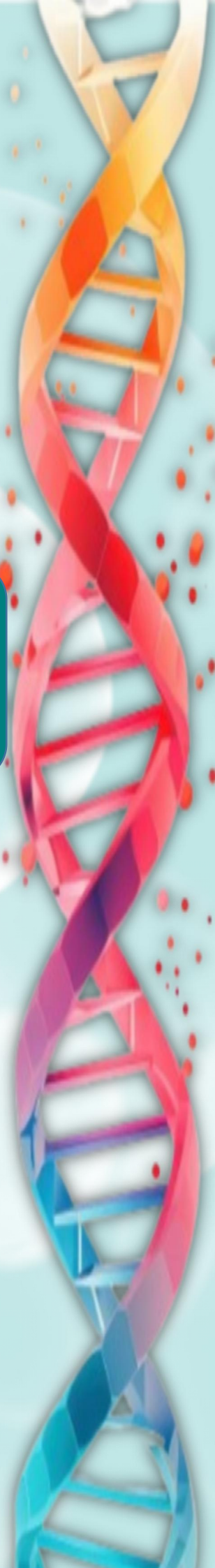
The advent of human induced pluripotent stem cells (iPSCs) has revolutionized the landscape of regenerative medicine, offering unprecedented opportunities for cell therapies. However, the translation of iPSCs from research to clinical applications necessitates efficient and reliable methodologies. Automation and cryopreservation are pivotal strategies in this endeavor. Automation addresses the challenges of scalability and reproducibility in iPSC production. It streamlines the processes of cell reprogramming, maintenance, differentiation, and quality control, minimizing human error and variability. Automated systems enable high-throughput generation and screening of iPSCs, which is essential for meeting the clinical demand. Additionally, they facilitate the consistent production of iPSCs with defined characteristics, ensuring the robustness and safety of cell-based therapies.

Cryopreservation is equally crucial, providing a means to store iPSCs long-term without compromising their viability and pluripotency. Effective cryopreservation techniques allow for the creation of cell banks, which are indispensable for standardized and on-demand cell supply. This ensures that iPSCs retain their therapeutic potential post-thaw, which is critical for timely and effective patient treatments. Moreover, cryopreservation supports the logistics of iPSC distribution across different geographical locations, enhancing the accessibility of cell therapies globally.

The integration of automation with advanced cryopreservation methods forms a cohesive framework that supports the scalability, standardization, and logistical viability of iPSC-based therapies. By ensuring consistent cell quality and availability, these strategies mitigate the risks associated with manual handling and storage, thus enhancing the clinical translation and therapeutic success of iPSCs. Consequently, the development and refinement of these technologies are essential to harness the full potential of iPSCs in regenerative medicine, promising more efficient and equitable delivery of novel treatments for a range of diseases.

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

Session-6: GENE THERAPY



H. TRENT SPENCER
Emory University School of Medicine, Atlanta, USA

New insights into the use of gamma delta T cells to treat childhood cancers

Gamma delta T cells are a unique immunocompetent cell source that can be expanded and genetically modified ex vivo for use as anti-cancer therapeutics. We have initiated a pediatric clinical trial using non-modified cells to treat neuroblastoma, which to date has shown a favorable safety profile. We recently tested and developed numerous expansion and engineering strategies that have resulted in more potent cell product candidates. These efforts will be summarized as well as efforts to advance beyond the treatment of neuroblastoma.

ARUN SRIVASTAVA
University of Florida, Florida, USA

Development of genome-modified generation ZZ (GenZZ) single-stranded AAV vectors with improved transgene expression

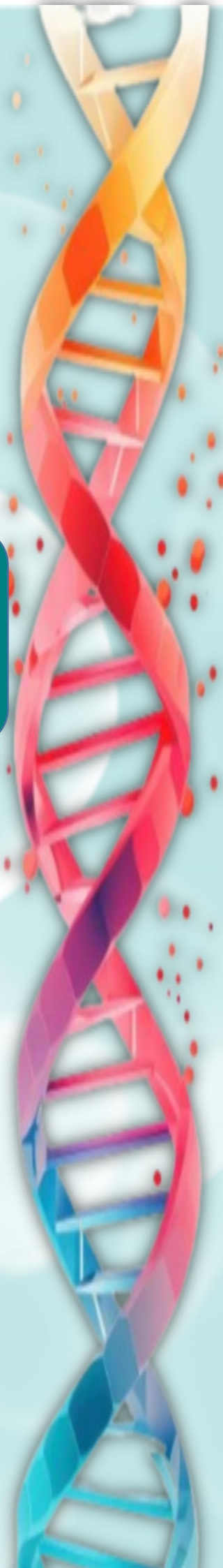
We previously reported the development of genome-modified generation X (GenX) AAV vectors in which deletion of one of the D-sequences in the AAV inverted terminal repeats (ITRs) mediates up to ~8-fold higher transgene expression from single-stranded AAV (ssAAV) vectors (J. Virol., 89: 952-961, 2015). We next reported the development of genome-modified generation Y (GenY) AAV vectors in which the replacement of the distal 10-nucleotides in the D-sequence with the glucocorticoid receptor-binding element (GRE) leads to up to ~10-fold higher transgene expression from ssAAV vectors (Mol. Ther.-Nucl. Acids, 35: 1-13, 2024). Recently, we reported the development of generation Z (GenZ) AAV vectors in which replacement of the proximal 10 nucleotides in the D-sequence with a random N10-sequence from a library leads to ~20-fold increase transgene expression from ssAAV vectors (Mol. Ther., 32: 141-142, 2024). In the current study, we combined the features of GenY and GenZ ITRs to develop a novel AAV vector, designated generation ZZ (GenZZ) vector. WT and Gen ZZ vectors containing a muscle cell-specific promoter (CDK8)-driven hrGFP expression cassettes were packaged into AAVrh74 capsids. The extent of the transgene expression from GenZZ AAVrh74 vectors was ~34-fold higher than that from WT AAVrh74 vectors in differentiated primary human skeletal muscle cells in vitro. The transduction efficiencies of WT and GenZZ AAVrh74 vectors were also evaluated in a mouse model in vivo. 1x109 vgs of WT or GenZZ-CK8-hrGFP AAVrh74 vectors were administered into gastrocnemius (GA) muscles in C57/BL6 mice (n=6 each). Cyro-sectioning and imaging of muscle tissues were performed 2-weeks post-injections. These results documented that GenZZ AAVrh74 vectors averaged ~24-fold increased transgene expression than that from WT AAVrh74 vectors. Packaging of GenZZ ssAAV DNA genomes in capsid-modified NextGen AAV serotype vectors should further enhance the performance of OptZ ssAAV serotype vectors at significantly reduced doses for gene therapy in humans.

SENTHIL BHOOPALAN
St. Jude Children's Research Hospital, Memphis, USA

Preclinical development of gene therapy for Diamond-Blackfan anemia

Diamond–Blackfan anemia (DBA) is a rare congenital bone marrow failure disorder that typically presents in infancy as macrocytic anemia with erythroid hypoplasia. The disease is most commonly caused by heterozygous germline loss-of-function mutations in one of 24 ribosomal protein (RP) genes. Current treatments, including red blood cell transfusions, glucocorticoids, and allogeneic hematopoietic stem cell transplantation (HSCT), are suboptimal. Despite recent scientific advances in defining the genetics and biology of DBA, no new therapies have been released for several decades. The only curative option, allogeneic HSCT, is hampered by the lack of suitable donors for many patients and immune toxicities such as graft rejection and graft-versus-host disease. Autologous gene therapy whereby patient hematopoietic stem and progenitor cells (HSPCs) are isolated, transduced with RP-encoding lentiviral vector (LV), then reintroduced following bone marrow conditioning to enhance engraftment of gene-corrected cells can be potentially therapeutic for hematopoietic defects associated with DBA. However, limited availability of DBA patient HSPCs has slowed development of gene therapy. Utilizing a novel Cas9-based approach to model DBA phenotype in HSPCs from healthy donor (Bhoopalan et al, JCI Insight, 2023), we have developed a LV approach to treat DBA caused by mutations in RPS19, which accounts for 25-35% of DBA patients. We have performed preclinical safety and efficacy studies of this RPS19-encoding LV towards an upcoming Phase I/II clinical study for DBA.

Session-7: CHALLENGES AND OPPORTUNITIES IN GENE THERAPY



SIVAPRAKASH RAMALINGAM
CSIR–Institute Of Genomics And Integrative Biology (CSIR–IGIB), New Delhi, India

Development of pathophysiologicallyrelevant models of β-hemoglobinopahtues for therapeutic studies

β-hemoglobin disorders, such as sickle cell disease (SCD) and β-thalassemia (BT), are the most common inherited monogenic blood disorders globally. They impose a significant burden on patients, their families, and the healthcare system due to high morbidity and mortality rates. Hence, ex vivo cellular system that accurately replicates the BT and SCD characteristics is a highly sought-after goal in the field of erythroid biology to study the disease mechanisms and for testing novel oral drugs as well as for developing cellular therapies . In this study, we present the generation of erythroid progenitor systems using CRISPR/Cas9. The disease cellular models exhibit similar differentiation profiles, globin expression and proteome dynamics as patient- derived HSPCs. Additionally, these cellular systems recapitulate pathological conditions associated with both the diseases. Hydroxyurea and pomalidomide treatment enhanced HbF levels. In summary, we present a major step towards the development of pathophysiologically relevant cellular systems for SCD and BT for identifying and investigating host-receptors for malaria invasion, novel regulators of γ-globin expression and a wider range of applications. Here, we further demonstrate for the first time the proof of concept of their ex vivo research potential by creating HPFH3 genotype and HBG1/2 promoter motif editing to reactivate HbF for the treatment of β-hemoglobin disorders.

NIRALI SHAH
University of Washington, Seattle, WA

CAR T-cell induced T-cell malignancies

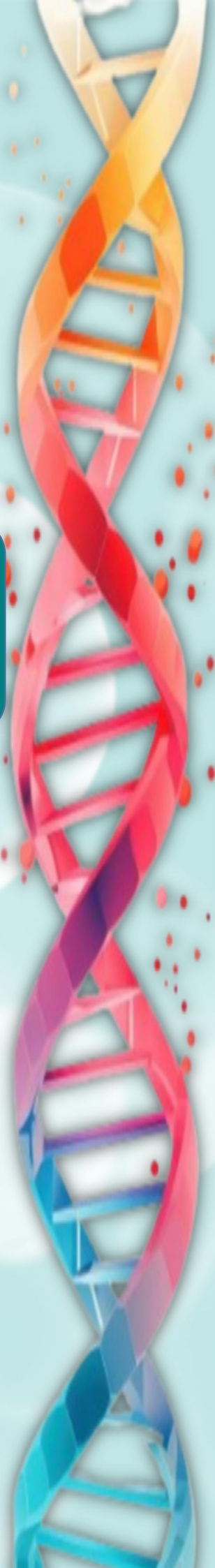
Since the first reports of T-cell malignancies arising in children receiving retrovirally transduced hematopoietic stem cells for the treatment of inherited immunodeficiencies, the risk of insertional mutagenesis has been of particular concern in the field of pediatric gene therapy. A goal for any potentially curative gene therapy is minimization of late effects particularly in a pediatric context where the time dependent burden may be greatest. Beyond the pediatric population, risk of secondary malignancies remains a concern in all patients with malignancies. The risk of CAR T-cell induced malignancies remains a concern—although initial experience suggests relatively safety in this regard. Based on updated guidance from the United States Food and Drug Administration, there is increased awareness of the risk of CAR T-cell induced malignancies. In the context of this discussion, risk of secondary malignancies and experience with CAR T-cell induced malignancies will be discussed.

MATTHEW PORTEUS
Stanford School of Medicine, California, USA

Genome Editing of HSCs to Develop Stem Cell Based Therapies

ematopoietic stem cells (HSCs) are rare cells but have the tremendous biologic potency of generating the blood system for the entirety of a person's life. Transplantation of HSCs (HSCT) has been successfully performed for over 50 years. Precision genetic engineering of HSCs has the potential to make HSCT safer and more efficacious across a broad range of diseases. In this presentation, I will present the latest updates on technology advancements in using homology directed repair to engineer HSCs by genome editing. HDR-editing has the unparalleled versatility to create single nucleotide changes in the genome as well as to precisely insert large gene cassettes into the genome with high efficiencies. I will discuss how we have applied HDR-editing to HSCs as a method to treat hemoglobinopathies, immunodeficiencies and HIV. I will also discuss some of the barriers to being able to use autologous genome edited HSCT more broadly as well as potential solutions to those problems.

Session-8: NON-VIRAL VECTOR BASED GENE THERAPY



CHANTAL PICHON
INSERM and University of Orléans, Orléans,
Institut Universitaire de France, France, Paris

Enhancing Natural killer cells proliferation and cytotoxicity using Imidazole-based lipid nanoparticles encapsulating interleukin-2 mRNA

Messenger RNA applications have undergone unprecedented applications - from vaccination to cell therapy. Natural Killer cells are recognized to have a significant potential in immunotherapy. NK -based cell therapy has drawn attention as allogenic graft with a minimal graft-versus-host risk leading to easier "off-the-shelf" production. NK cells can be engineered with either viral vectors or electroporation, involving high costs, risks, and toxicity, emphasizing the need for alternative way as mRNA technology. We successfully developed, screened, and optimized novel lipid-based platforms based on imidazole lipids. Formulations are produced by microfluidic mixing and exhibit a size around 100 nm with a polydispersity index less than 0.2. They are able to transfect NK-92 cells, KHYG-1 cells and primary NK cells with high efficiency without cytotoxicity while Lipofectamine Messenger Max and D-Lin-MC3 LNP based formulations do not. Moreover, the translation of non-modified mRNA was higher and more stable in time compared to modified one. Remarkably, the delivery of therapeutically relevant IL2-mRNA resulted in extended viability together with preserved activation markers and cytotoxic ability of both NK cell lines and primary NK cells. Altogether, our platforms feature all prerequisites needed for the successful deployment of an NK-based therapeutic strategies.

SUJATA MOHANTY
All India Institute of Medical Sciences, New Delhi, India

MSC Extracellular Vesicles: Navigating Regenerative Medicine's Therapeutic Landscape

Mesenchymal stem cells (MSCs) have garnered significant attention in regenerative medicine due to their potent therapeutic properties. MSC-derived extracellular vesicles (MSC-EVs) play a pivotal role in cell-to-cell communication, delivering bioactive molecules such as proteins, lipids, and nucleic acids, thereby influencing various cellular processes critical for tissue repair and regeneration

MSC-EVs have demonstrated remarkable therapeutic potential across various preclinical disease models, including Parkinson's disease, chronic liver disease, and bone defect. Their small size and natural origin offer advantages over cell-based therapies, such as reduced risk of immune rejection and the ability to cross biological barriers, including the blood-brain barrier.

MSC-EVs have been demonstrated to promote cell proliferation, differentiation, and angiogenesis and modulate immune responses, essential for effective tissue integration and repair. MSC-EVs can be integrated into biomaterial scaffolds or used with other cell types to create advanced tissue constructs that mimic the native extracellular matrix and support tissue regeneration.

However, the transition of MSC-EVs from bench to bedside is challenged by several hurdles. Standardization of isolation and characterization techniques is essential to ensure consistency and reproducibility in clinical applications. Additionally, understanding the biodistribution, and long-term safety of MSC-EVs is crucial for their successful translation into clinical therapies.

Despite these challenges, the therapeutic landscape of MSC-EVs is rapidly evolving, with numerous clinical trials underway to evaluate their efficacy in treating a variety of conditions.

In conclusion, MSC-EVs represent a promising frontier in regenerative medicine, offering innovative solutions for tissue repair and disease modulation. Ongoing research and clinical trials will be pivotal in unlocking their full potential, potentially revolutionizing the field of regenerative medicine and paving the way for innovative treatments for a wide range of tissue-related disorders.

SRUJAN MAREPALLY
Centre for Stem Cell Research (a unit of inStem, Bengaluru), Vellore, India

Long-lasting mRNA enabled protein replacement therapy with liver-specific lipid nanoparticle system: Haemophilia B as a model disease

The current standard of care for Hemophilia B necessitates frequent intravenous administrations of recombinant factor IX (FIX), which induces immune tolerance and imposes substantial financial burdens, leaving many patients without adequate treatment options. Messenger RNA (mRNA) delivery via lipid nanoparticles (LNPs) has emerged as a promising alternative to conventional protein replacement therapies in recent years. However, critical clinical challenges remain, including the need for liver-targeted specificity, prolonged mRNA half-life, and efficient translation to achieve therapeutically relevant protein levels. To address these unmet needs, we developed a novel Galactosylated Lipid Nucleic Acid system (GaLiNAs) designed to deliver chemically modified FIX mRNAs effectively. This biodegradable nanocarrier system incorporates an ionizable galactosylated lipid to facilitate receptor-mediated endocytosis, a transfection-ionized lipid for optimal mRNA complexation, and a helper lipid to maintain the structural integrity of the carrier. Additionally, we utilized truncated albumin UTRs to enhance mRNA translation in hepatic cells. In preclinical evaluations using a Hemophilia B mouse model, GaLiNAs demonstrated sustained FIX protein expression for one month following a single administration. Furthermore, *in vivo* safety assessments indicated that multiple doses of GaLiNAs could be administered without eliciting toxicity in major organs. These findings suggest that long-lasting mRNA therapy holds significant promise as a viable protein replacement treatment for Hemophilia B and inspires hope for the future development of mRNA therapeutics targeting other hepatic monogenic disorders.

Session-9: GENE EDITING

THIYAGARAJ MAYURANATHAN
Centre for Stem Cell Research (A unit of inStem, Bengaluru), Vellore, India

Genome editing strategies for treating sickle cell disease

Sickle cell disease (SCD) and β-thalassemia are caused by mutations in the HBB gene, which encodes the β-globin subunit of adult hemoglobin (HbA, α2β2). These disorders become symptomatic after birth, following the switch in expression from fetal γ-globin genes (HBG1/HBG2) to mutated β-globin (HBB). Reversing this developmental switch through genetic modification of autologous hematopoietic stem cells (HSCs) represents a promising therapeutic approach. Early clinical studies have demonstrated that suppressing the γ-globin repressor gene BCL11A by Cas9 nuclease-mediated disruption of its erythroid enhancer or RNA interference induces fetal hemoglobin (HbF, α2γ2) and reduces morbidities of SCD or β-thalassemia. New approaches to induce HbF and to correct the pathogenic variants for treating β-hemoglobinopathies are fueled by the development of genome engineering technologies and insights into globin gene regulation. The field is evolving rapidly, and the best strategy is not known. We explored multiple gene editing approaches to ameliorate the SCD phenotype. First, we compared the use of adenine base editors (ABEs) to generate HbF inducing mutations at γ-globin promoter and identified the best approach for potent HbF induction. Second, we used a custom adenine base editor (ABE8e-NRCH) to convert the SCD allele (HBBS) into Makassar β-globin (HBBG), a non-pathogenic variant that exhibit anti-sickling property. Third, we used a prime editor that replaces a target segment of DNA with a specified new sequence and our result supports the feasibility of a one-time prime editing SCD treatment that corrects HBBS to HBBA, does not require any viral or non-viral DNA template and minimizes undesired consequences of DNA double-strand breaks.

PIETRO GENOVESE
Harvard Medical School, Boston, USA

Epitope Editing for an Immunotherapy "Stealth" Hematopoiesis

Despite the considerable efficacy observed when targeting a dispensable lineage antigen, such as CD19, in B-cell acute lymphoblastic leukemia, the broader applicability of adoptive immunotherapies is hampered by the absence of tumor-restricted antigens3–5. Acute myeloid leukemia immunotherapies target genes expressed by hematopoietic stem/progenitor cells (HSPCs) or differentiated myeloid cells, resulting in intolerable on-target/off-tumor toxicity. Here, we show that epitope engineering of donor HSPCs used for bone marrow transplantation endows hematopoietic lineages with selective resistance to chimeric antigen receptor (CAR) T cells or monoclonal antibodies without affecting protein function or regulation. This strategy enables targeting genes essential for leukemia survival regardless of shared expression on HSPCs, reducing the risk of tumor immune escape. By performing epitope mapping and library screenings, we identified amino acid changes that abolish the binding of therapeutic monoclonal antibodies targeting FLT3, CD123, and KIT and optimized a base-editing approach to introduce them into CD34+ HSPCs, which retain long-term engraftment and multilineage differentiation ability. After CAR T cell treatment, we confirmed resistance of epitope-edited hematopoiesis and concomitant eradication of patient-derived acute myeloid leukemia xenografts. Furthermore, we show that multiplex epitope engineering of HSPCs is feasible and enables more effective immunotherapies against multiple targets without incurring overlapping off-tumor toxicities. We envision that this approach will provide opportunities to treat relapsed/refractory acute myeloid leukemia and enable safer non-genotoxic conditioning.

SAMUELE FERRARI
San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Milan, Italy

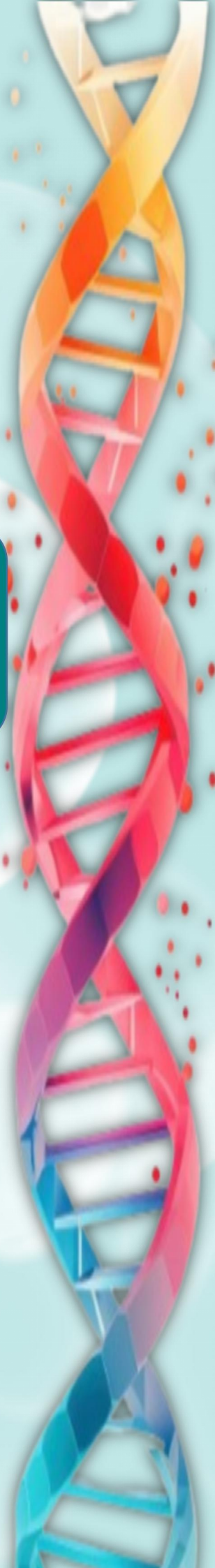
Next-generation gene editing of human hematopoietic stem cells: from research to clinical translation

Hematopoietic stem/progenitor cell (HSPC) gene editing presents an opportunity to treat a wide range of diseases through precise DNA modification using chimeric enzymes. Programmable nucleases, in particular, promise targeted integration of therapeutic transgenes or gene correction via homology-directed repair (HDR). Despite this potential, several challenges impede clinical application: i) low HDR efficiency in long-term repopulating HSPCs; ii) toxicity and loss of stemness during component delivery and ex-vivo culture; iii) safety issues from unintended, potentially harmful repair outcomes at nuclease target sites. To address these issues, we developed two enrichment strategies for HDR-edited HSPCs and combined them with clinically compliant culture conditions and optimized lipid nanoparticle (LNP)-mediated RNA delivery. The first strategy, Selection by Means of Artificial Transactivators (SMArT), uses HDR donor templates with a selector gene that activates upon precise on-target integration. This selector gene's expression is induced by transient mRNA delivery of an artificial transactivator (ArT) that binds near the homology region. This method enriched HSPC products to over 80% HDR-edited alleles. PCR assays confirmed that over 95% of clonogenic, enriched HSPCs had the intended HDR edit. SMArT also eliminated HSPC clones with on-target long-range deletions, addressing safety concerns. Xenotransplantation of SMArT-enriched HSPCs resulted in fully HDR-edited human grafts, with selector expression no longer detectable post-transplantation. Improved culture protocols and LNP-mediated delivery further enhanced HSPC preservation and HDR efficiency, increasing the yield of selectable HSPCs. SMArT is compatible with both in situ gene correction and targeted integration strategies.

The second approach is a platform for targeted transgene cassette integration, using negative selection of cells with unintended on-target edits. We screened gRNAs to knockout haploinsufficient genes in HSPCs. Effective gRNAs led to counterselection of indel-bearing cells, reducing their presence in vitro and in xenotransplantations. Integration of an HDR template restoring the target gene and incorporating a gene-of-interest (GOI) cassette rescued clonogenic capacity, enriching GOI-expressing cells up to 90% without needing sorting before infusion.

These combined enrichment strategies, optimized culture conditions, and safe delivery methods can overcome current HDR editing limitations, paving the way for clinical translation.

Session-10: IMMUNE CELL THERAPY



SUNIL BHAT
Mazumdar Shaw Medical Centre, Narayana Health City, Bangalore, India

CART Therapy in Pediatric Acute Lymphoblastic Leukemia

CART (Chimeric Antigen Receptor T-cell) therapy has emerged as a groundbreaking treatment for pediatric Acute Lymphoblastic Leukemia (ALL), especially in cases that are refractory (do not respond to standard treatments) or have relapsed (return after initial treatment).

Understanding CART T-cell Therapy

1. **Mechanism**: CART T-cell therapy involves genetically modifying a patient's own T-cells to express chimeric antigen receptors (CARs) on their surface. These CARs are designed to recognize specific proteins (antigens) present on cancer cells.
2. **Target in ALL**: In pediatric ALL, CART therapies are typically designed to target CD19, or CD22, proteins found on the surface of most B-cell malignancies, including B-cell ALL.

Treatment Process

1. **T-cell Collection**: T-cells are extracted from the patient through a process called leukapheresis.
2. **Genetic Modification**: The collected T-cells are then genetically engineered in the laboratory to express the CARs specific to CD19 or CD22 or combinations.
3. **Expansion and Infusion**: Once modified, these CAR T-cells are grown in large numbers and infused back into the patient's bloodstream. The CAR T-cells then multiply in the body and target cells with CD19 or CD22 on their surface, including ALL cells.

Efficacy and Challenges

1. **Efficacy**: CART T-cell therapy has shown remarkable efficacy in pediatric ALL, especially in patients who have exhausted conventional treatment options. High remission rates (often above 80%) have been reported in clinical trials.
2. **Challenges**: Despite its success, CART therapy can cause severe side effects, including cytokine release syndrome (CRS) and neurotoxicity, which need careful management. Long-term effects and durability of response are also areas of ongoing research.

Clinical Trials and Approval

1. **Clinical Trials**: Numerous clinical trials have demonstrated the efficacy and safety of CART T-cell therapy in pediatric ALL. These trials continue to refine protocols and expand indications.
2. **FDA Approval**: In 2017, the FDA approved the first CART therapy for pediatric and young adult patients with relapsed or refractory B-cell ALL.

Future Directions

1. **Improving Safety**: Research is ongoing to improve the safety profile of CART therapies, including strategies to mitigate severe side effects.
2. **Broadening Indications**: CART therapies are being explored in other types of leukemia and lymphomas, as well as solid tumors, to expand treatment options.

In conclusion, CART T-cell therapy represents a transformative treatment option for pediatric ALL, offering hope for patients who previously had limited treatment options. Ongoing research aims to further optimize its efficacy, safety, and accessibility, potentially leading to broader use in the future.

YOSHIYUKI TAKAHASHI
Nagoya University Graduate School of Medicine,
Nagoya, Japan

Development of CAR-T cell products with non-viral vector system

Chimeric antigen receptor-modified T cells targeting CD19 (CD19.CAR-T cells) have shown clinical success in patients with hematological malignancies. Despite the encouraging results obtained with this novel therapy, a major concern to its global spread, particularly in developing countries, is its high cost. We developed a method of non-viral gene transfer using piggyBac transposon to reduce the cost of CAR-T cell production. We started a human clinical trial to define feasibility, toxicity, maximum tolerated dose and clinical response of CD19.CAR-T cells in patients with relapsed or refractory B-ALL (jRCTa040190099).

We engineered autologous T cells via the piggyBac transposon system with CD19.CAR-expression transposon vector and piggyBac transposase-expression vector to express CD19.CAR incorporating CD28 costimulatory domain. In this study, patients in cohorts 1 (16-60 years old) and 2 (1-15 years old) receive 1 × 105 CAR-transduced T cells per kg. Patients in cohorts 3 and 4 (both 1-60 years old) receive 3 × 105 and 1 × 106 CAR-transduced T cells per kg, respectively. As no patients had DLT, we are enrolling the patients in the last cohort. Expansion of piggyBac CAR-T cells was observed in the peripheral blood of all patients treated with the drug.

Nagoya University School of Medicine signed a material transfer agreement to support the use of CAR-T cell therapy with the Faculty of Medicine at Chulalongkorn University. Since then, researchers from Nagoya University have been supporting CAR-T cell therapy clinical research in Thailand. PiggyBac CAR-T cell product was administered to five patients with relapsed and chemotherapy resistant malignant lymphoma in Thailand and PET scan evaluation showed that all five patients responded. PiggyBac transposon, a non-viral vector system, could be used for the production of CAR-T cells. Especially in Asian developing countries, it is important to reduce the manufacturing cost of CAR-T cells.

RIZWAN ROMEE
Harvard Medical School, Dana Farber Cancer Institute, Boston, USA

Genetic manipulation of NK cells for enhanced immunotherapy

Natural Killer (NK) cells offer several advantages over T cells for immunotherapy. IL-12 is one of the potent cytokines however it has been associated with systemic toxicity in the context of T cells. We have successfully engineered a novel tumor matrix directed IL-12 and incorporated it into several CAR constructs targeting both liquid and solid tumors. CAR-NK cells armed with this novel IL-12 demonstrate very potent anti-tumor responses against multiple cancer cell lines, PDX samples in vitro and can control tumors and prolong survival in vivo in xenograft mouse models. In parallel, we engineered nonpathogenic E.coli to express activating cytokines that mediate strong T and NK cell-mediated anti-tumor responses in colorectal cancer and melanoma mouse models. Furthermore, These bacteria enhance tumor trafficking of CAR NK cells to tumors mediating enhanced tumor control in NSG mouse models. These data make a strong case for the evaluation of these approaches in early-phase clinical trials.

Corporate Sponsors

Center for Stem Cell Research, Vellore would like to acknowledge the support from the following companies

MaxCyte®

cytiva

OmniBRx® BIOTECHNOLOGIES

ThermoFisher SCIENTIFIC

TWIST BIOSCIENCE

HIMEDIA CELLBIO™ LIFE FOR CELLS

32 YEARS Labmate (Asia) Pvt. Ltd. Sewing Science

INTELSIUS™ A DGP COMPANY

Cytocare TECHNOLOGIES PVT LTD Cellular Excellence

GENE TECHNOLOGIES

eppendorf

ProteoGen® >>> SUPPORTING PROGRESSIVE RESEARCH

Invitro Sciences

VG Life® VG Life Sciences Pvt. Ltd. EST.2021

inStem

CSCR
Centre for Stem Cell Research
(a unit of inStem, Bengaluru),
Christian Medical College Campus,
Bagayam, Vellore – 632002,
Tamil Nadu, India.

CELL AND GENE THERAPY SYMPOSIUM (CGTS)-2024

Contact Us

https://www.cscr.res.in/symposium/

+91 416 228 5108

cscrmeet@cmcvellore.ac.in