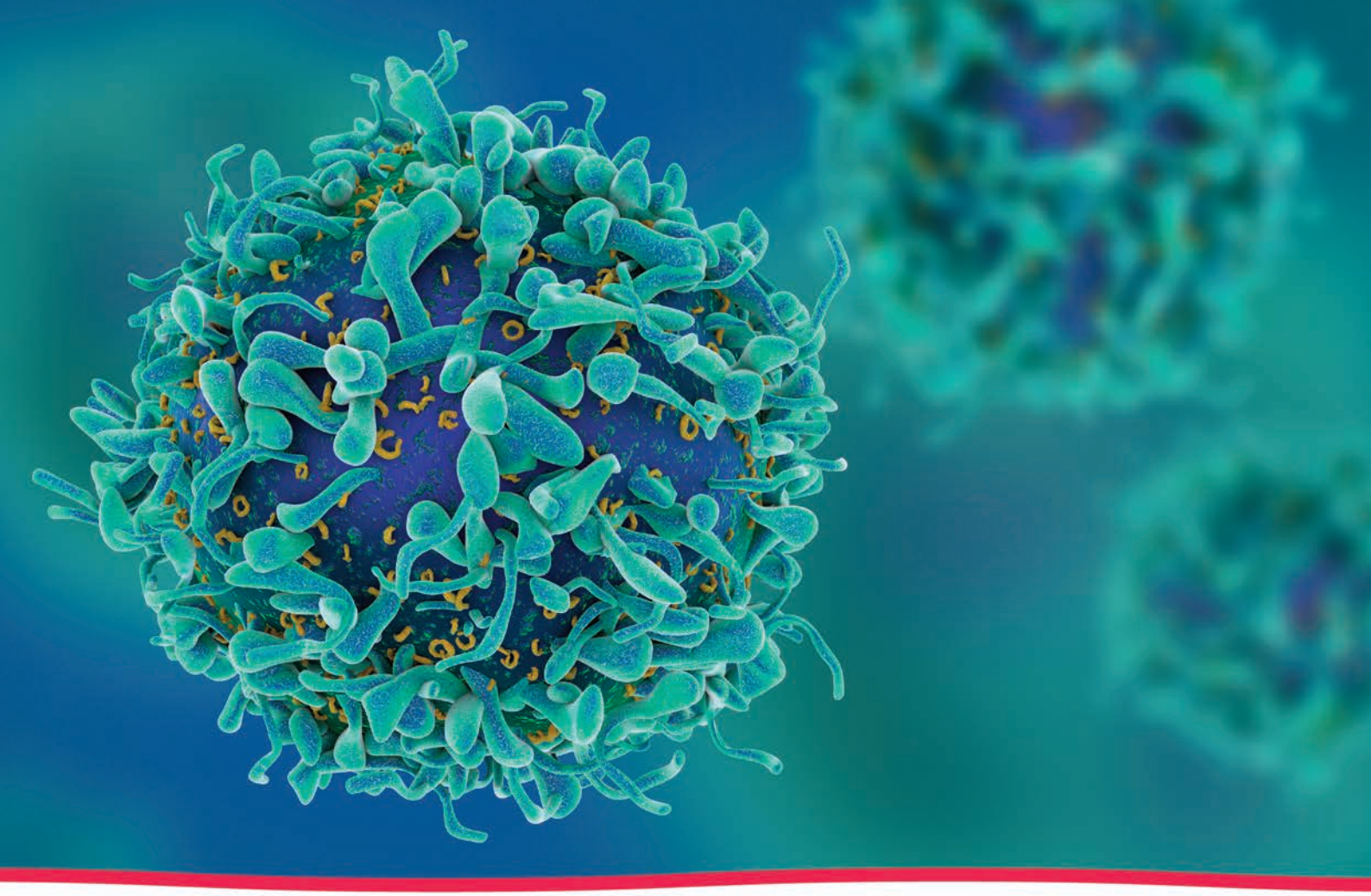


RESTORE

Health by Advanced Therapies

1st ADVANCED THERAPIES SCIENCE MEETING

25th-26th November 2019 - Berlin, Germany

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 820292.

Table of content

Health by Advanced Therapies

1st ADVANCED THERAPIES SCIENCE MEETING

25th-26th November 2019 - Berlin, Germany

Shifting from treating symptoms to curing chronic diseases by making the transformative promise of Advanced Therapies a reality for the benefit of patients, this is the vision of the international project RESTORE Health by Advanced Therapies.

For certain rare genetic and immune diseases as well as selected cancers and tissue degenerative conditions Advanced Therapies already exist. Some of these products have already entered the market, mostly for rare diseases. However, so far only a few thousand patients worldwide have benefitted from Advanced Therapies.

What are the research challenges slowing development of curative treatments and what are the roadblocks for the wider adoption of Advanced Therapies in clinical routine?

"Living drugs" are a disruptive innovation that challenge the tried and tested paradigms of modern drug development, clinical implementation and collaboration across disciplines.

At the start of such a trailblazing change scientific, industrial and regulatory challenges are plentiful. All recently approved Advanced Therapy products required a long (>20 years) and costly added-value chain and the complexities of manufacturing and clinical development result in high product prices. Addressing these challenges and making Advanced Therapies more quickly available and accessible as the "standard of care" for all patients provided the motivation to found RESTORE.

The packed two-day **1st ATSM** programme will include talks from Nobel Prize winner Ada Yonath (Director of Weizmann Institute of Science, Israel), Michele De Luca (University of Modena, Italy), Timothy O'Brien (National University of Ireland Galway, Ireland), Maksim Mamonkin (Baylor College of Medicine, United States), Manuela Gomes (University of Minho, Portugal) and many many more!

RESTORE welcomes you to the **1st ATSM** in Berlin to explore the latest trends in the field of regenerative Therapies and to discuss the future of Advanced Therapies in Europe.

RESTORE Consortium

Health by Advanced Therapies

CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN
BIH Regeneration

University of Zurich[UZH]

CATAPULT Cell and Gene Therapy

TISSUSE Emulating Human Biology

Pluristem Therapeutics Inc.

MACS Miltenyi Biotec

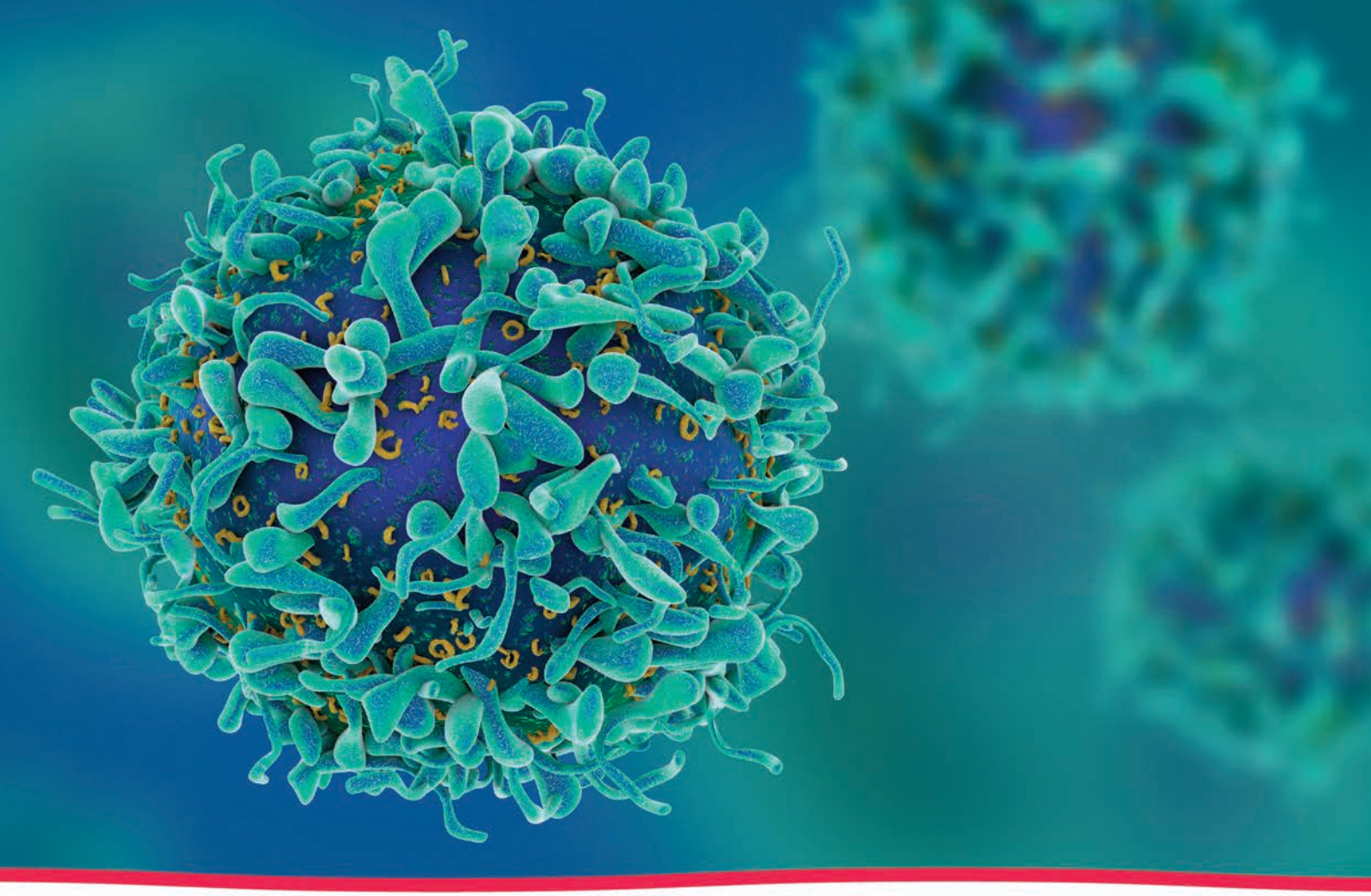
Inserm

Inn·Acta

FONDAZIONE Telethon

Universidade do Minho

RESTORE is supported by the Center for Alternative to Animal Testing in Europe (CAAT-Europe)



Agenda

RESTORE

Health by Advanced Therapies

Monday, 25th November 2019 - Day I

08.00-09.00 Registration

Welcome

Hall Maritim

09.00-09.05 **Welcome**
Hans-Dieter Volk - Charité Universitätsmedizin Berlin, Germany
Petra Reinke - BeCAT - Charité Universitätsmedizin Berlin, Germany

09.05-09.40 **Introduction**
RESTORE - Health by Advanced Therapies - Opportunities and Challenges
Hans-Dieter Volk - Charité Universitätsmedizin Berlin, Germany

Plenary Session Topic 1 - **Foundational Research (New Targets and New Indications)**

Hall Maritim

Chairs: Annelise Bennaceur - Institute National de la Santé et de la Recherche, France
Georg Duda - BCRT, Charité Universitätsmedizin Berlin, Germany

09.40-10.05 **On the way to improved therapies for skin diseases and disorders**
Alexandra Marques - University of Minho, Portugal

10.05-10.20 **Targeting the tumour vasculature with CAR-T cells for treatment of solid tumours**
Andrea Keogh - Cell and Gene Therapy Catapult, United Kingdom

10.20-10.45 *Coffee break* & **Posters set up**

10.45-11.00 **Characterizing immune cells by Single-Cell-Sequencing - Identification of novel therapeutic targets for precision cell therapy in chronic inflammation**
Mir-Farzin Mashreghi - Deutsches Rheuma-Forschungszentrum, Germany

11.00-11.15 **Bioengineered implants to repair the heart**
Maximilian Emmert - University of Zurich, Switzerland

11.15-11.40 **Engineering T-cells to target malignant and pathogenic T-cells**
Maksim Mamonkin - Baylor College of Medicine, United States

Plenary Session Topic 2 - **Preclinical Models and Technologies (Focus on Human-on-a-chip)**

Hall Maritim

Chairs: Ali Turhan - Institute National de la Santé et de la Recherche, France
Uwe Marx - TissUse GmbH, Germany

11.45-12.10 **Engineered polymeric matrices as instructive cells' artificial micro-environment**
Antonella Motta - University of Trento, Italy

12.10-12.35 **Stimuli-responsive and multifunctional scaffold-cells systems for tendon tissue regeneration**
Manuela Gomes - University of Minho , Portugal

12.35-12.50 **From Organ-on-a-Chip Tools Towards Patients on Chips - Enforcing a Paradigm Shift in Drug Development**
Uwe Marx - TissUse GmbH, Germany

12.50-13.05 **Monitoring CAR T cell efficacy in fresh human solid tumor slices**
Emmanuel Donnadieu - Institute National de la Santé et de la Recherche, France

13.15-13.30 **Photo session**

13.30-14.30 *Lunch* & **Poster setup finalisation**

Plenary Session Topic 3 - **Manufacturing Technologies (Including Product Characterisation and Automation)**

Hall Maritim

Chairs: Rui Reis - University of Minho, Portugal

14.30-14.55 **Engineering personalized tissue implants: From 3D printing to bionic tissues**
Tal Dvir - Tel Aviv University, Israel

14.55-15.20 **Genome-wide CRISPR Screens in Primary Human T Cells Reveal Key Regulators of Immune Function**
Eric Shifrut - University of California San Francisco, United States

15.20-15.35 **Becoming cell therapy Makers - Opportunities and challenges in manufacturing of cell therapy products**
Lior Raviv - Pluristem LTD, Israel

15.35-15.50 **Pioneering Cell Processing of personalized ATMPs**
Andrew Kaiser - Miltenyi Biotec GmbH, Germany

15.50-16.05 **Generation of hematopoietic cells with lymphoid potential from induced pluripotent stem cells (iPSC) for adoptive immunotherapy of cancer and leukemia**
Annelise Bennaceur - Institute National de la Santé et de la Recherche, France

16.05-16.20 **Functional Surfaces and Scaffolds Combined with Stem Cells for Bone and Cartilage Tissue Engineering**
Nuno Neves - University of Minho, Portugal

Break out sessions in Berlin D Room, Hall Maritim and London Room for topics 1-3 (in parallel)

Break out Session Topic 1 - **Foundational Research (New Targets and New Indications)**

Berlin D Room

Chair: Michel Apel - Miltenyi Biotech GmbH, Germany

16.30-16.40 **Developing Novel siRNA Nanoparticle Therapies For Cystic Fibrosis by Silencing of the Epithelial Sodium Channel**
Stephen Hart - UCL Great Ormond Street Institute of Child Health, United Kingdom

16.40-16.50 **Epigenetic control of T cell differentiation: From genome-wide signatures to local regulators and their targeted manipulation.**
Julia K. Polansky-Biskup - BCRT, Charité Universitätsmedizin Berlin, Germany

16.50-17.00 **Subconjunctival injection of low-dose mesenchymal stem cells promotes corneal allograft survival in a mouse cornea transplantation model**
Thomas Ritter - National University of Ireland Galway, Ireland

17.00-17.10 **Immunomodulatory properties of amniotic membrane derivatives for tissue regeneration: a 20-year experience**
Ornella Parolini - Università Cattolica Santo Cuore, Italy

17.10-17.40 *Coffee break*

17.40-17.50 **Pre-activated mesenchymal stromal cells induce regulatory immune populations *in vivo* and prolong corneal allograft survival**
Oliver Treacy - National University of Ireland Galway, Ireland

17.50-18.00 **Treatment of osteochondral defects of the knee in rats using bilayered scaffold free cell-based constructs**
Luis Freitas Mendes - Katholieke Universiteit Leuven, Belgium

18.00-18.10 **Highly activated Natural Killer cells to treat pediatric cancer**
Cristina Ferreras - Hospital La Paz Institute for Health Research, Spain

18.10-18.20 **Neural precursor/stem cell-based therapy for Rett syndrome**
Nicoletta Landsberger - University of Milan, Italy

18.20-18.30 **Drive regeneration by employing the immune-structure interface**
Georg Duda - BCRT, Charité Universitätsmedizin Berlin, Germany

18.30-18.40 **Towards efficient strategies of HIV-1 cure: Analysis of HIV-1 latency-reverting agents by single cell RNA-seq**
Christine Laqmani-Goffinet - Charité Universitätsmedizin Berlin, Germany

Break out Session Topic 2 - **Preclinical Models and Technologies (Focus on Human-on-a-chip)**

Hall Maritim

Chair: Nuno Neves - University of Minho, Portugal

16.30-16.40 **Tissue engineered mimetic autografts for bone regeneration**
Froilán Granero-Moltó - Clínica Universidad de Navarra, Spain

16.40-16.50 **A millifluidic dynamic system to unveil the interaction among breast cancer cells, fibroblasts and mesenchymal stem cells**
Virginia Brancato - University of Minho, Portugal

16.50-17.00 **Aryl hydrocarbon receptor - driven immunometabolic checkpoints in type-1 dendritic cells restrains antitumor responses**
Francesca Fallarino - University of Perugia, Italy

17.00-17.10 **Amniotic mesenchymal stromal cell secretome: a potent pleiotropic strategy for acute brain injury**
Francesca Pischiutta - Istituto di Ricerche Farmacologiche "Mario Negri" IRCCS, Italy

17.10-17.40 *Coffee break*

17.40-17.50 **A preclinical non-human primate model to investigate the regenerative potential of human induced pluripotent stem cell-derived cardiomyocytes**
Ina Gruh - Hannover Medical School, Germany

17.50-18.00 **Adapter CAR T cells - A new versatile platform for controllable CAR T cell function**
Joerg Mittelstaet - Miltenyi Biotec GmbH, Germany

18.00-18.10 **Microvessels-on-Chip - Bridging the gap between blood and lymph**
Barbara Bachmann - Ludwig Boltzmann Institute for Experimental and Clinical Traumatology and Vienna University of Technology, Austria

18.10-18.20 **MSC improve skeletal muscle regeneration via locally damplen the pro-inflammatory capacity of CD8+ T cell subpopulations**
Sven Geissler - BCRT, Charité Universitätsmedizin Berlin, Germany

18.20-18.30 **Treatment of a naturally occurring dog model of Alport Syndrome by means of CRISPR/Cas9 gene therapy approach**
Sergio Daga - University of Siena, Italy

18.30-18.40 **Effectiveness and safety of adoptive cell therapy with regulatory type 1 (Tr1) cells in pancreatic islet transplantation**
Georgia Fousteri - San Raffaele Diabetes Research Institute, Italy

18.40-18.50 **Immunosuppressant-resistant T cells for advanced adoptive T cell therapy in immunosuppressed patients**
Leila Amini - BCRT, Charité Universitätsmedizin Berlin, Germany

Break out Session Topic 3 - **Manufacturing Technologies (Including Product Characterisation and Automation)**

London Room

Chair: Michael Schmück-Henneresse - BCRT, Charité Universitätsmedizin Berlin, Germany

16.30-16.40 **Tissue Manufacturing by Bioprinting: Challenges and Opportunities for Regenerative Medicine**
Fabien Guillemot - Poietis, France

16.40-16.50 **Self-assembly of stem/progenitor cells creates human neo-vascularized skin and skin organoids**
Patricia Peking - Paracelsus Medical University, Austria

16.50-17.00 **Using clinical data for manufacturing design and release criteria to improve the quality of a cell-based ATMP for cartilage repair**
Giulietta Roel - CO.DON AG, Germany

17.00-17.10 **Harmonisation of multicenter MSC production for a phase III clinical trial**
Melissa Van Pel - Leiden University Medical Center, Netherlands

17.10-17.40 *Coffee break*

17.40-17.50 **Manufacturing of stem cell derived *in-vitro* tissues using robot technology**
Marco Metzger - Fraunhofer Institute for Silicate Research, Germany

17.50-18.00 **Human induced pluripotent stem cells from universal donors as starting material for regenerative therapies**
Micha Drukker - Helmholtz Center Munich, Germany

18.00-18.10 **Gene therapy getting personal: mutation-specific editing and gene addition strategies**
Carsten Werner Lederer - The Cyprus Institute of Neurology and Genetics / TEDDY, Cyprus

18.10-18.20 **T cell immunity toward CRISPR-associated nucleases**
Dimitrios Laurin Wagner - BCRT, Charité Universitätsmedizin Berlin, Germany

18.20-18.30 **Tissue-Engineered ATMPs for cartilage healing**
Oliver Pullig - Fraunhofer Translational Center Regenerative Therapies, Germany

18.30-18.40 **Personalized therapy for TP53 mutated cancer patients based on CRISPR-Cpf1 and suicide gene delivery**
Flaminia C. Lorenzetti - University of Siena, Italy

18.40-18.50 **Generation, cultivation and characterization of stem cell-derived bioartificial cardiac tissue of clinically relevant dimensions**
Ina Gruh - Hannover Medical School, Germany

Poster session & Networking Get-Together

19.30-21.00 **Poster session & Discussion** *with Cheese & Wine buffet*

21.00-22.00 **Poster session continued**

Tuesday, 26th November 2019 - Day II

Introduction Day II

Hall Maritim

08.30-08.45 Registration

08.45-08.50 **Introduction**
Hans-Dieter Volk - Charité Universitätsmedizin Berlin, Germany

08.50-09.15 **Opening Remarks of Ada Yonath, Nobel Prize for Chemistry**
Ada Yonath - Director of Weizmann Institute of Science, Israel

Plenary Session Topic 4 - **Clinical Implementations (Including Reimbursement Models)**

Hall Maritim

Chairs: Zami Aberman - Pluristem LTD, Israel
Terri Gaskell - Cell and Gene Therapy Catapult, United Kingdom

09.15-09.40 **Combined cell and gene therapy for Epidermolysis Bullosa**
Michele De Luca - University of Modena, Italy

09.40-10.05 **Clinical implementation of cornea and skin artificial tissues generated with fibrin-agarose biomaterials**
Antonio Campos - University of Granada, Spain

10.05-10.30 **Car-T cell therapy - a clinical experience report**
Michal Besser - Ella Lemelbaum Institute of Immuno-Oncology Sheba Medical Center, Israel

10.30-10.45 **Reshape immune balance by next-generation regulatory T cells**
Petra Reinke - BeCAT - Charité Universitätsmedizin Berlin, Germany

10.45-11.00 **Cellular therapy: Boosting innovation in technology and clinical translation**
Mario Assenmacher - Miltenyi Biotech GmbH, Germany

11.00-11.15 **Development of allogeneic Placenta-derived (PLX) cell therapy - from bench - to bedside**
Racheli Ofir - Pluristem LTD, Israel

11.15-11.50 *Coffee break*

Plenary Session Topic 5 - **Regulatory Science and Clinical Trials**

Hall Maritim

Chairs: Petra Reinke - BeCAT - Charité Universitätsmedizin Berlin, Germany
Racheli Ofir - Pluristem LTD, Israel

11.50-12.15 **Role of the EMA Committee for Advance Therapies (CAT) in the assesment of ATMPs**
Paolo Gasparini - University of Trieste IRCCS, Italy

12.15-12.40 **MSCs as a therapy for diabetic complications: a translational journey in the EU**
Timothy O'Brien - National University of Ireland, Ireland

12.40-12.55 **An Integrated Eco-System of Research and Development for Orphan Medicinal Products: the IRDiRC Orphan Drug Development Guidebook**
Michela Gabaldo - Fondazione Telethon, Italy

13.00-14.15 *Lunch*

Plenary Session Topic 6 - Special Workshop - **Ethics, Economics, Big data and AI**

Hall Maritim

Chairs: Joana Namorado - IBMT Fraunhofer Institut, Germany
Michal Rosen-Zvi - IBM Research, Israel

14.15-14.30 **State and potential for data-driven strategies in the cell and gene therapy industry**
Marc-Olivier Baradez - Cell and Gene Therapy Catapult, United Kingdom

14.30-14.45 **Ethics management of science and the public**
Andreas Kurtz - BCRT, Charité Universitätsmedizin Berlin, Germany

14.45-15.00 **Ethics in scientific communication**
Pier Maria Fornasari - Regen Health Solutions, Italy

15.00-15.15 **A Consistent Mechanism for Designing a Clinical Trial based on AI Technologies Applied to Observational Cohort**
Michal Rosen-Zvi - IBM Research, Israel

15.15-15.25 **In silico regenerative medicine: from living implants to digital patients and back again**
Liesbeth Geris - Katholieke Universiteit Leuven, Belgium

15.25-15.35 **Stem cell data standards and data utility**
Andreas Kurtz - BCRT, Charité Universitätsmedizin Berlin, Germany

Break out sessions in Hall Maritim and Berlin D Room for topics 4-5 (in parallel)

Break out Session Topic 4 - **Clinical Implementations (Including Reimbursement Models)**

Hall Maritim

Chair: Mohamed Abou El-Enein - BCRT, Charité Universitätsmedizin Berlin, Germany

15.45-15.55 **Stem cells for treatment of Amyotrophic lateral sclerosis**
Eva Sykova - Scimed Biotechnologies, Czech Republic

15.55-16.05 **Chimeric antigen receptor (CAR) immunotherapy for patients with solid tumors**
Gabriele Pecher - Charité Universitätsmedizin Berlin, Germany

16.05-16.15 **SLAMF7 CAR-T cells for immunotherapy of multiple myeloma: 'real-world' experience of GMP-manufacturing using virus-free Sleeping Beauty gene-transfer**
Michael Hudecek - Universitätsklinikum Würzburg, Germany

16.15-16.25 **Cartilage regeneration with ATMP produced cells; past, present and future pertspectives**
Anders Lindahl - University of Gothenburg, Sweden

16.25-16.35 **Adoptive Transfer of Thymic Derived Regulatory T-Cells in Therapy-Refractory Chronic Graft versus Host Disease in Children**
Sybille Landwehr-Kenzel - BCRT, Charité Universitätsmedizin Berlin, Germany

Break out Session Topic 5 - **Regulatory Science and Clinical Trials**

Berlin D Room

Chair: Michela Gabaldo - Fondazione Telethon, Italy

15.45-15.55 **Translational support in tissue engineering by the Andalusian Network for Design and Translation of Advanced Therapies**
Rosario Sánchez Pernaute - Andalusian Network for Design and Translation of Advanced Therapies, Spain

15.55-16.05 T**ranslation of CAR-based innovation into clinics: how to overcome regulatory hurdles**
Bernd Schröder - Miltenyi Biotec GmbH, Germany

16.05-16.15 **Personalized tissue-engineered grafts**
Raimund Strehl - Verigraft AB, Sweden

16.15 -16.25 **Placental Cell Therapy for the Treatment of Muscle Trauma: From Preclinical Models to Clinical Phase III**
Tobias Winkler - BCRT, Charité Universitätsmedizin Berlin, Germany

16.40-17.40 **Poster session** *& Coffee break*

Final Remarks, Farewell

Hall Maritim

17.40-18.00 **Final Remarks, Farewell**
Hans-Dieter Volk - Charité Universitätsmedizin Berlin, Germany

Scientific Committee

BCRT and Charité Universitätsmedizin Berlin, Germany
Hans-Dieter Volk
Petra Reinke
Mohamed Abou El-Enein

Institut National de la Santé et de la Recherche, France
Annalise Bennaceur-Griscelli
Ali Turhan

Universität Zürich, IREM, Switzerland
Simon Hoerstrup
Maximilian Emmert

Cell and Gene Therapy Catapult, United Kingdom
Michaela Sharpe
Anna Williams
Terri Gaskell

TissUse GmbH, Germany
Uwe Marx
Silke Hoffmann

Pluristem LTD, Israel
Racheli Ofir
Zami Aberman

Miltenyi Biotec GmbH, Germany
Mario Assenmacher
Michael Apel

Fondazione Telethon - San Raffaele, Italy
Stefano Benvenuti
Manuela Battaglia

University of Minho, Portugal
Rui Reis
Nuno Neves

I-Stem, France
Marc Peschanski

IBMT Fraunhofer Institut, Germany
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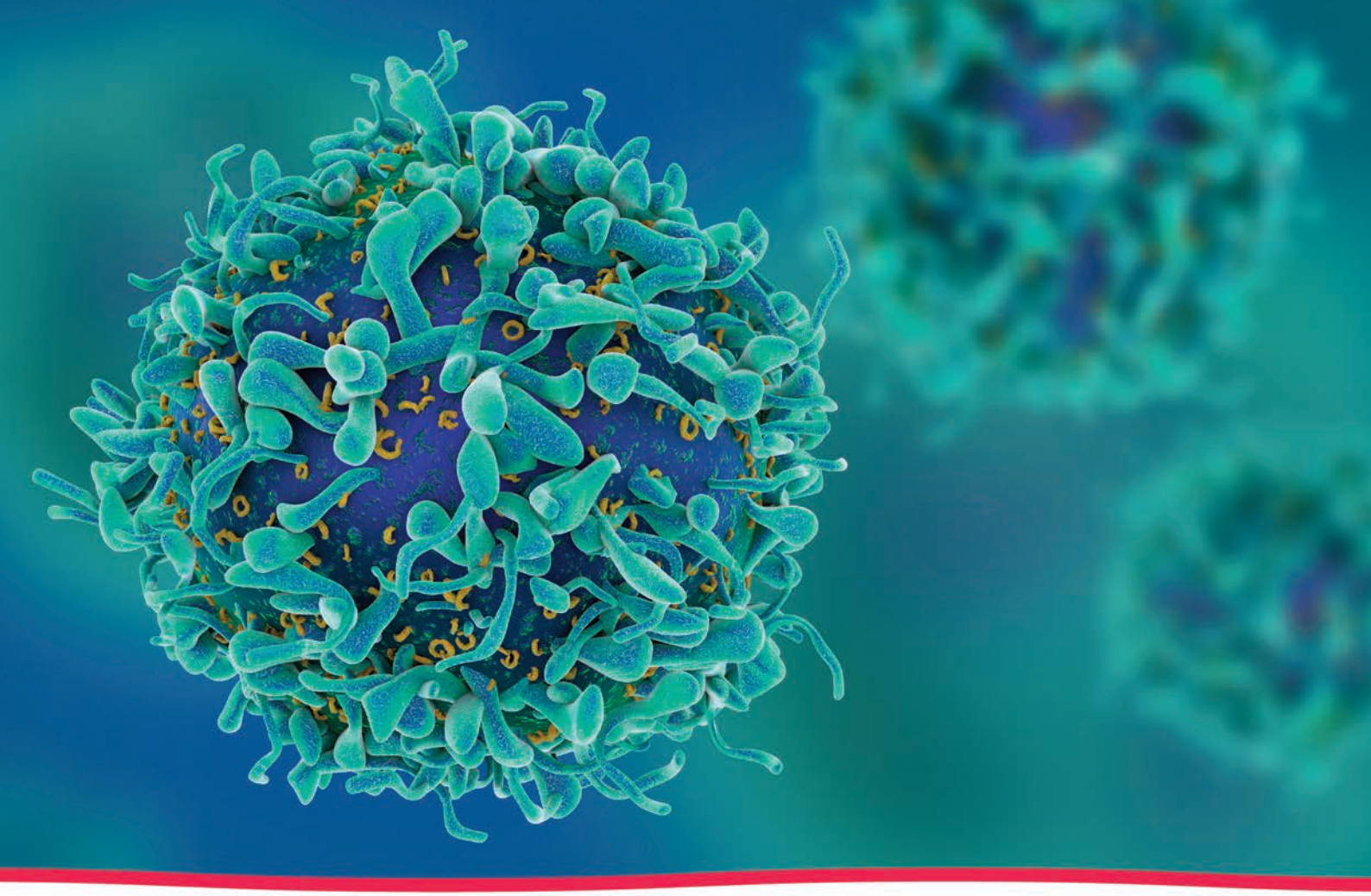


Biographies

RESTORE

Health by Advanced Therapies

Antonio Campos

University of Granada, Spain

Prof. Antonio Campos is a full-professor of the Histology Department in the University of Granada in Spain and the director of the Tissue Engineering Group of this University. He has held the following positions: President of the Spanish Society of Histology (1983-1993), Dean of the Faculty of Medicine of Granada (1992-2000), President of the Spanish Conference of Medical Schools (1996-2000), President of the European Association of Faculties of Medicine (2000-2001), Representative of Spain in the Consultative Committee of the European Union for the Training of Physicians (1998), Advisory Board Member of Science and Technology (2000-2004), Director of the National Institute of Health Carlos III (2000-2004), Member of Editorial Boards and Scientific Evaluator in journals and national and international institutions, Member of the Royal National Academy of Medicine (since 2004) and Honoris Causa degree for Cordoba National University in Argentina, Autonomous University of Santo Domingo, in Dominican Republic (first university founded in America) and the Aveiro University in Portugal. Moreover, he has published relevant books and papers in high impact factor journals related to tissue engineering and also Medical Education. His group has generated several artificial tissues by tissue engineering that are presently being applied or in clinical trial.

Michele De Luca

University of Modena, Italy

Michele De Luca, MD, is Director of the Centre for Regenerative Medicine "Stefano Ferrari" and the Interdepartmental Centre for Stem Cells and Regenerative Medicine at the University of Modena and Reggio Emilia and Scientific Director and co-founder of the university spin-off Holostem Terapie Avanzate.

De Luca was Scientific Director of Veneto Eye Bank Foundation in Venice (2000-2007) and Head of Laboratory of Tissue Engineering at Istituto Dermopatico dell'Immacolata in Rome (1996-2002), Deputy Head (1992-1995) and Senior Investigator (1986-1992) at Laboratory of Stem Cell Differentiation at National Institute for Cancer Research in Genova, Visiting scientist at the Department of Physiology and Biophysics of Harvard Medical School in Boston (1985) and Fogarty Fellow at the NIH (NIADDK) in Bethesda (1982-1985).

De Luca is author of over 120 peer reviewed international publications, has been invited speaker in approximately 200 international meetings worldwide. He is internationally recognized as a leading scientist in epithelial stem cell biology aimed at clinical application in regenerative medicine and played a pivotal role in epithelial stem cell-mediated cell and gene therapy.

Tal Dvir

Tel Aviv University, Israel

Tal Dvir is a Professor at Tel Aviv University, Israel. He obtained his B.Sc. (2003) and Ph.D (2008) degrees from the faculty of Engineering at Ben-Gurion University of the Negev in Israel. His Ph.D research focused on cardiac tissue engineering and regeneration. Tal continued his postdoctoral studies in the laboratory of Prof. Robert Langer in the Department of Chemical Engineering at MIT. His postdoc research focused on advanced materials for tissue engineering and regeneration.

On October 2011 Tal was recruited by the Department of Biotechnology and the Center for Nanotechnology at Tel Aviv University to establish the Laboratory for Tissue Engineering and Regenerative Medicine. On 2013, Tal has also joined the newly established Department of Materials Science and Engineering at Tel Aviv. Since 2017, Tal is the founding director of the Sagol Center for Regenerative Biotechnology. Currently, Tal's laboratory designs and develops smart bio and nanomaterials and technologies for engineering complex tissues, such as the heart, brain, spinal cord, intestine, eyes and more.

Tal has published many papers in top journals and received numerous awards, including the Rappaport prize and the Juludan prize.

Paolo Gasparini

University of Trieste IRCCS, Italy

Prof. Paolo Gasparini graduated in Medicine at the University of Turin in 1985, and specialized in Haematology and in Medical Genetics at the University of Verona in 1988 and 1992, respectively. He is full professor of Medical Genetics at the University of Trieste, Head of Medical Genetics and Head of the Department for Advanced Diagnostics and Clinical Trials at IRCCS Mother Child Hospital Burlo Garofolo in Trieste. He has contributed to the identification of several inherited disease genes as well as genes underlying complex and quantitative traits. He spent some time as visting professor at CHOP (Philadelphia, USA) and Sanger (Cambridge, UK). He is directly involved in a series of large studies on isolated populations in Italy, Caucasus and Central Asia in which samples from more than 5000 individuals were collected, accurately phenotyped and genotyped. He also established a series of collaborations in Qatar, where he spent two years at Sidra Medical and Research Center developing research on genetics diseases.

His research activities focus on the identification of genes related to hereditary illnesses and mitochondrial metabolic illnesses. Major fields of research include the understanding of the genetics bases of hearing function and loss, aging, and taste and food preferences and their implications on health status.

Manuela Gomes

University of Minho, Portugal

Manuela E. Gomes is Associate Professor and President of the Research Institute on Biomaterials, Biodegradables and Biomimetics (I3Bs) of the University of Minho, Portugal. She is a founder member of the 3B's Research Group.

Manuela has been part of numerous European and national/regional projects as PI/member; in 2018 she was awarded a Consolidator Grant from the European Research Council (ERC CoG) to develop magnetically assisted tissue engineering technologies for tendon regeneration and with a EC funded Twinning Project in collaboration with NUI Galway (Ireland), Regensburg University (Germany) and the Mayo Clinic (USA).

Manuela E. Gomes is editor of 2 books and Assistant Editor of the Tissue Engineering Encyclopaedia, published by Elsevier in June 2019 and author of 40 book chapters, 195 full papers published in international refereed journals, and more than 300 communications in international Conferences. She is also co-author of 8 patents (national/international).

Manuela E. Gomes is an active member of several International Scientific Organizations, but she has been particularly involved in the Tissue Engineering and Regenerative Medicine International Society (TERMIS), presently as member at large of TERMIS-Global and Member of the Governing Board and Chair of the Membership Comitttee. She has received several awards, including the 2013 Tissue Engineering and Regenerative Medicine International Society - European Chapter (TERMIS-EU) Young Investigator Award.

Alexandra Marques

University of Minho, Portugal

Alexandra P. Marques has a PhD on Materials Science and Technology - Biomaterials from the University of Minho, Portugal and in cooperation with the University of Liverpool, UK, and a BSc in Biochemistry from the University of Porto, Portugal. She is a Founder and Principal Investigator of the 3B's Research Group at the University of Minho in Portugal, a research unit of the I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics of which she is vice-president. She is also Member of the Governing Board of the PT Government Associate Laboratory ICVS/3B´s. From January 2017 she is Associate Editor of the Journal of Tissue Engineering and Regenerative Medicine as from 2016 was Assistant Editor. She has been actively participating in the activities (organising and scientific committees, and International Advisory Board of conferences) of different Societies including the Tissue Engineering and Regenerative Medicine International Society and the European Wound Management Association (Elected Council Member from July 2018). After receiving Starting and Development Career Grants from the Portuguese Science Foundation, in 2016 she was awarded a Consolidator Grant from the European Research Council, adding to the different National and International projects she has been coordinating or she was core member. She edited 2 books, is author of 99 peer-reviewed publications, 19 book chapters, 95 Indexed conference abstracts and proceedings, and is co-inventor of 13 patents (5 families).

Antonella Motta

University of Trento, Italy

Antonella Motta is Associate professor at Department of Industrial Engineering, University of Trento, Italy. Graduated in Natural Sciences at the University of Padova (Italy), with PhD in Biomaterials by University of Trento (Italy). The research topics include engineered polymer-based materials for regenerative medicine applications, chemical-physical and biological characterization of materials for biomedical use, interactions between implants materials, proteins and cells, protein based materials, immobilization and adhesion mechanisms of proteins and cells, blood-contacting materials, nanostructured materials for biomedical applications; in particular, a 20 years experience on silk-based matrices design for application in tissue engineering and 3D *in vitro* models.

Co-Editor-in-Chief of Journal of Biomaterials, Polymer Edition; Associate Editor of Journal of Bioactive and Compatible Polymers; Member of the Scientific Editorial Board of International Journals, and referee for international Journals in the Biomaterials and Biomedical Technologies field.

Visiting professor in several Universities, i.e. Tufts University (MA, USA), Chulalongkorn University (Bangkok, Thailand), University of Texas at Arlington (TX, USA), University of Colorado at Boulder (CO, USA).

About 160 publications in International Journals, Co-editor of a book on Tissue engineering, 9 book chapters, 8 International Patents. Invited Speaker at several International and National Conferences and Schools.

Eric Shifrut

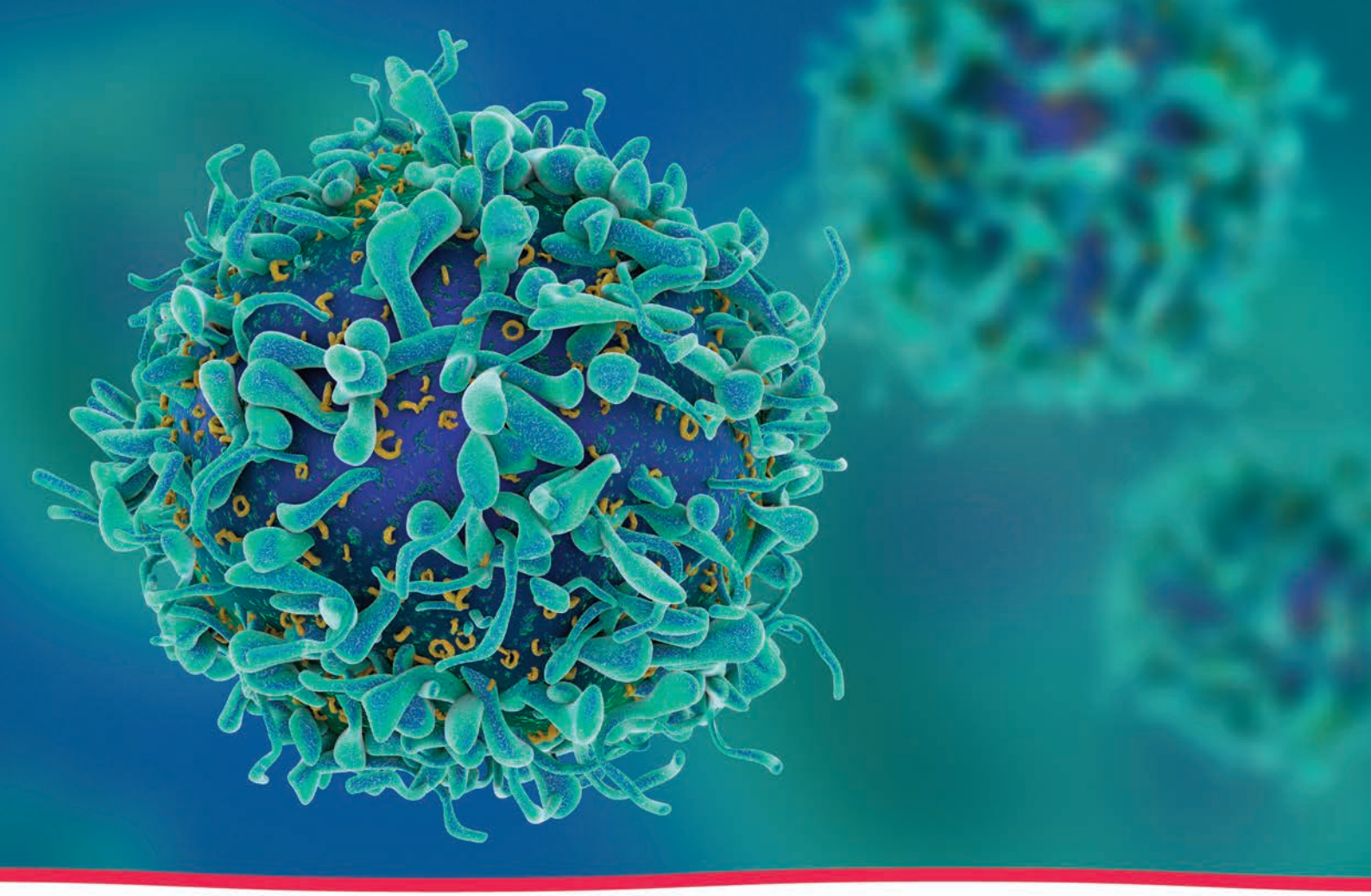
University of California San Francisco, United States

Eric Shifrut is currently a senior postdoctoral fellow at UCSF working with Dr. Alexander Marson. Eric completed his PhD at the Weizmann Institute of Science in Israel, under supervision of Prof. Nir Friedman. In his thesis work, Eric developed computational and experimental platforms to map the landscape of T cell receptor repertoires in health and disease. Since joining the Marson lab in 2016, Eric has been working to pioneer CRISPR applications in primary human T cells. These efforts aim to design discovery platforms to explore and exploit genetic circuits to boost T cell function in cancer immunotherapy. His research focuses on engineering human T cell therapies with an enhanced ability to survive in the suppressive tumor microenvironment. Eric is a co-author of multiple patents in T cell engineering and his work has generated more than 750 citations. He serves as a scientific consultant to cell therapy companies and has been an invited speaker at several international scientific meetings. He continues to develop new experimental platforms and computational frameworks to pursue key questions in adaptive immunity, immune tolerance and engineered cell therapies.

Ada Yonath

Weizmann Institute of Science, Israel

Ada Yonath focuses on genetic code translation by ribosomes, on antibiotics paralyzing this process, on designing novel antibiotics for resistant pathogenic bacteria and on origin of life. She graduated from Hebrew University, earned PhD from Weizmann Institute (WIS) and completed postdoctoral studies at CMU and MIT, USA. In 1971 she established the first biological-crystallography laboratory in Israel, which was the only lab of this kind in the country for almost a decade and became a WIS faculty member. Since 1989 she is the Director of Kimmelman Center for Biomolecular Structures at WIS. In 1978-9 she spent a Sabbatical in Chicago U, and during 1980-2004 in parallel to her WIS activities, she headed the Max-Planck-Research-Unit for Ribosome Structure in DESY, Hamburg. Among others, she is a member of US National Academy of Sciences (NAS); Israel Academy of Sciences & Humanities; German Academy for Sciences; European Molecular Biology Organization; Pontifical Academy of Sciences; International Academy of Astronautics; Accademia Nazionale dei Lincei, Rome; and Royal European Academy of Doctors (RAED). She holds honorary doctorates from over 20 universities worldwide, in Israel, USA, Latin America, Europe and the Far East. Her awards include the Israel Prize; Linus Pauling Gold Medal; Albert Einstein World Award for Excellence; UNESCO-L'Oréal Award; the Wolf Prize; the Golden DESY Pin; the Paul Ehrlich Medal; the Erice Peace Prize and the Nobel Prize for Chemistry.



Oral Presentations

RESTORE

Health by Advanced Therapies

On the way to improved therapies for skin diseases and disorders

Alexandra Marques - University of Minho, Portugal

Tissue dysfunction after skin wound healing still is a reality for most of the employed therapeutics. This is greatly because a reparative process rather than a regenerative one occurs. Improved therapeutic approaches are therefore a major demand in the field but are likely to be achieved with enhanced and sustained knowledge on skin pathologies and associated wound healing.

Tissue engineering will certainly play a key role in targeting this issue by developing skin substitutes with improved functionality capable of directing the healing mechanisms into the regenerative pathway. However, as one solution does not fit all wounds, future therapies must be adapted not only to the type of wound but also to the healing stages and for that we still need to better understand the pathophysiology of each wound as well its progression under specific conditions. Under this context we have been focusing on tailoring biomaterial properties to accurately recreate cell-cell and cell-extracellular matrix (ECM) interactions representative of each skin cell niche to be able to modulate cellular crosstalk and tissue (patho)physiology. A direct comparison with microenvironments, based on cell's own ECM, generated using cell sheet technology has allowed to identify for example specific mediators that were missing for the adequate progression of the healing. This is also in line with our 4D printing approach that integrates time as the fourth dimension when taking advantage of 3D printing technologies to develop dressings with improved functionality.

Ultimately, we envision taking advantage of the generated knowledge to contribute to advance the development of new or improved therapies for skin diseases and disorders.

Targeting the tumour vasculature with CAR-T cells for treatment of solid tumours

Andrea Keogh - Cell and Gene Therapy Catapult, United Kingdom

CAR-T cell therapies have proven to have significant efficacy in targeting haematological malignancies such as acute lymphoblastic leukaemia (ALL). Conversely, their use in treating solid tumours has provided fewer encouraging results, mainly due to the difficulty in identifying specific target antigens and the hostile microenvironment which presents itself as a barrier to CAR activity. An alternative therapeutic approach is to target the tumour vasculature rather than the malignant cells directly. We have demonstrated that CLEC14A, an endothelium-specific gene is highly upregulated on vessels associated with a variety of solid tumours when compared to normal tissue endothelium, highlighting its potential for tumour vasculature targeting. We hypothesised that a functional CLEC14A-targeted CAR-T cell therapy can disrupt the vasculature causing haemorrhagic necrosis and tumour bulk reduction, which may open further treatment strategies. In support of this hypothesis, CLEC14A- targeted CAR-T cells have demonstrated a reduction in tumour size by 50-60% in three different mouse tumour models with no evidence of toxicity.With a view towards a clinical trial to assess safety, tolerability and preliminary efficacy we have developed a robust, automated and closed manufacturing process to produce mRNA-based CLEC14A CAR-T cells. This process has reproducibly achieved 77-98% transfection efficiency with a cell viability > 80%. To quantify the amount of CAR mRNA and monitor its persistence post-transfection we have developed a droplet digital PCR assay. With this assay we have demonstrated absolute quantification without the requirement of a standard curve and achieved a LoD of 5.6 copies of CAR mRNA. The resultant cells demonstrate robust killing of target cells in an impedance-based assay with transient expression expected to minimise the risk of cytokine release syndrome often associated with viral-based products.

Characterizing immune cells by Single-Cell-Sequencing - Identification of novel therapeutic targets for precision cell therapy in chronic inflammation

Mir-Farzin Mashreghi - Deutsches Rheuma-Forschungszentrum, Germany

It is well known that immune cells, including T and B lymphocytes contribute to the pathogenesis of chronic inflammatory diseases (CID). Therapies completely ablating the immune system followed by its regeneration with autologous hematopoietic stem cells can lead to therapy-free remission. But the loss of all protective immunity and vulnerability to lethal opportunistic infections hinders the widespread application of such therapies. For the development of more selective therapies, there is a medical need for the identification of the molecular identity of those immune cells that drive and maintain chronic inflammation as opposed to immune cells which protect against pathogens or help to resolve chronic inflammation. Therefore, we aim to define the transcriptional and clonal identity of particularly T and B cells in inflamed tissues and peripheral blood of patients with CIDs using state-of-the-art single-cell transcriptomics. Our results demonstrate a considerable heterogeneity among e.g. T cells within the inflamed tissues. We identify both, T cell populations which express either pro- or anti-inflammatory genes showing that not all immune cell infiltrates contribute to the pathogenesis of CIDs. The selective ablation of CID driving immune cell populations can be achieved by targeting genes important for their lifestyle and tested in preclinical models of chronic inflammation. Thus, single cell transcriptomics is a powerful tool for the development of agents to selectively ablate even small populations of immune cells driving pathology or help to identify those cells, which qualify as living drugs for the therapy of CIDs.

Bioengineered implants to repair the heart

Maximilian Emmert - University of Zurich, Switzerland

Congenital heart defects and structural heart disease represent a major cause of death around the globe. Although current therapy strategies have rapidly developed over the last decades, the currently used artificial prostheses (i.e. vascular grafts, heart valves) are still considered to be suboptimal and complications. They do not promote regeneration, physiological remodeling or growth (particularly important aspects for children) as their native counterparts. This leads to the continuous degeneration and subsequent failure of such substitutes which is associated to an increased morbidity and the need for multiple re-interventions. To overcome this problem, the concept of regenerative medicine comprising of tissue-, bio-engineering and hybrid technologies has been suggested as a next generation approach to enable native-like cardiovascular replacements with regenerative and growth capacities, amendable to young adults and children. However, despite promising data from preclinical and first clinical pilot trials, the translation and clinical relevance of such technologies is still very limited. The reasons for that are multifaceted and comprise of scientific, logistical, infrastructural and regulatory challenges that need to be systematically addressed in order to facilitate clinical translation of such next generation cardiovascular substitutes. We have recently developed a novel human cell derived extracellular matrix (ECM) based technology to engineer next generation cardiovascular implants in the setting of heart valves, blood vessels and many other applications. Based on very encouraging preclinical data and the implementation of GMP-/ISO-compliant manufacturing methods, clinical translation into first-in-man studies is underway.

Engineering T-cells to target malignant and pathogenic T-cells

Maksim Mamonkin - Baylor College of Medicine, United States

Extending the success of chimeric antigen receptor (CAR) T-cells to treat T-cell malignancies or suppress deleterious T-cell responses has been difficult due to the shared expression of most targetable antigens between pathogenic and normal T-cells, possibly leading to self-elimination of CAR T-cells and ablation of the endogenous T-cell compartment post-infusion. To overcome these limitations, we have developed a CAR targeting CD5, a pan-T cell marker commonly expressed in T-ALL and T-cell lymphoma. The CD5 CAR enables T-cells to resist CAR-mediated fratricide while retaining high cytotoxicity against malignant T-cells. CD5 CAR T-cells can be easily manufactured from patients with T-ALL and lymphoma and produce high anti-tumor activity without eliminating normal circulating T-cells. Of interest, adapting the same approach to target CD7 - another pan-T cell marker widely expressed in T-cell malignancies - results in an overwhelming fratricide of CD7 CAR T-cells and requires prior disruption of the CD7 gene in T-cells using CRISPR/Cas9. The resulting CD7-edited CD7 CAR T-cells expand normally and produce robust cytotoxicity in preclinical models of human T-cell malignancies and AML.
Finally, T-cells can be engineered to recognize and eliminate non-malignant pathogenic T-cells using a 4-1BB-specific alloimmune defense receptor (ADR). We show that ADR-expressing T-cells can specifically target activated alloreactive T- and NK-cells and are protected from alloimmune rejection in preclinical models of allogeneic "off-the-shelf" CAR-T cell therapy. These studies demonstrate the feasibility of using engineered fratricide-resistant T-cells to selectively target malignant and non-malignant pathogenic T-cells.

Engineered polymeric matrices as instructive cells' artificial micro-environment

Antonella Motta - University of Trento, Italy

In vitro 3D models are becoming crucial for drug screening, therapeutic strategies, and to investigate mechanisms and pathways of tumor development. In this context, the artificial cellular microenvironment in combination with culture protocols, can influence cell behavior such as differentiation, proliferation, ECM synthesis, and 3D organization. Hydrogels are particularly attractive for the design of cellular artificial microenvironments, and can be used to fabricate temporary artificial extracellular matrix while providing extrinsic *in vitro* factors-stimuli. An ideal instructive matrix should possess mechanical, rheological, and biological properties that should be properly designed in order to ensure the correct interaction with cells, and promote and act as templates for the generation of functional tissue-like constructs. Cell-laden hydrogels can be used as building blocks of additive manufacturing techniques, i.e., for bioprinting. The lecture will focus on nature-derived polymers offering specific biomolecular recognition moieties, hence being particularly attractive for the design and fabrication of 3D tissue models. Among them, silks, hyaluronic acid, and alginate will be considered. Their functionalization with biologically active chemical groups, their processability and properties, and their use as matrices for the fabrication of 3D constructs with different techniques will be explored.

Stimuli-responsive and multifunctional scaffold-cells systems for tendon tissue regeneration

Manuela Gomes - University of Minho, Portugal

The common theme in tissue engineering is to supply cells with an environment that provides appropriate behavioural instructions to stimulate cells to synthesize new tissue. For that, the scaffold should direct cell behaviour via cues similar to those found in the native environment. Nevertheless, significant challenges remain to accomplishing the development of fully functional tissue substitutes that can lead to clinically effective and successful applications. In the specific case of tendon tissue, several scaffolding materials have been investigated. However, an optimal scaffold is yet to be engineered, meeting the demanding requirements for tendon substitutes, namely: i) mimic the hierarchical and anisotropically aligned structure of tendon tissues from the nano- up to the macroscale, ii) meet tendon mechanical requirements and non-linear biomechanical behavior, iii) provide the necessary biophysical/biochemical cues and mechanical responsiveness to induce the tenogenic differentiation of stem cells and potentiating the effects of biochemical supplementation. Herein we will present and discuss recent progresses in our lab research towards the design of multifunctional and stimuli-responsive biomaterials-cells constructs, considering the importance of biomechanical and structural cues in the regeneration of this tissue, but with potential to be transposed to other applications. Particularly, this work reports on recent studies concerning the development of specific scaffolds architectures based on various polymers using technologies that enable obtaining structures based on the arrangements of fibers from the nano to the micro scale. In particular cases, these scaffolds materials are doped with superparamagnetic nanoparticles (SPMNs) rendering magnetic responsive systems that can be *in vitro* and *in vivo* remotely actuated. Synergies of scaffolds designs and magnetic responsiveness can impact significantly cells behaviour as well as *in vivo* response and thus widen the therapeutically range of such cell-laden tissue engineered constructs.

From Organ-on-a-Chip Tools Towards Patients on Chips - Enforcing a Paradigm Shift in Drug Development

Uwe Marx - TissUse GmbH, Germany

Aim: Microfluidic microphysiological systems (MPS) have proven to be a powerful tool for recreating human tissue- and organ-like functions at research level. This provides the basis for the establishment of qualified preclinical assays with improved predictive power but industrial adoption is progressing slowly due to their complexity. Methods: A universal microfluidic Multi-Organ-Chip (MOC) platform of a size of a microscopic slide integrating an on-chip micro-pump and capable to interconnect different organ equivalents has been established. Sixteen different human single organ equivalents have been established on that platform and nine organ combinations have been tested for stable long-term crosstalk yet. The challenges to translate a MOC-based combination of four human organ equivalents (intestine, liver, kidney and neuronal tissue) into a useful tool for ADME profiling and toxicity testing of drug candidates will be highlighted. Furthermore, selected single- and multi-organ models bearing advanced therapy test potential, such as human bone marrow equivalents, chip based co-culture of tumour with a healthy organ equivalent and immune tissues on a chip will be introduced. Conclusion: The presentation concludes on status quo of pharmaceutical assay adoption for the presented MOC platform in particular, and the entire MPS field in general. It emphasis challenges towards MPS-based organismal homeostasis, which bear the potential of a paradigm shift in advanced therapy development.

Monitoring CAR T cell efficacy in fresh human solid tumor slices

Emmanuel Donnadieu - Institute National de la Santé et de la Recherche, France

Adoptive transfer of T cells expressing chimeric antigen receptors (CARs) has shown remarkable clinical efficacy against advanced B cell malignancies. This clinical success has generated urgent interest in the development of new CARs and the extension of CAR T cell therapy to solid tumors that are, up to now, refractory to this strategy. Prior to initiating clinical trials, model systems in which CAR T cells can be characterized and tested for their potency and safety should be in place. To date, few models perfectly recapitulate the human immune system and tumor microenvironment. We have pioneered an experimental approach of fresh tumor slices that retain the complexity of human tumor *in vivo*. This non-animal model, combined with extensive fluorescent imaging techniques, enabled us to monitor T cell activities, namely their migration, activation and cytotoxic abilities in a preserved human tumor microenvironment. The detrimental impact of cells and elements of the tumor environment - macrophages and the extracellular matrix - on T cell migration and ability to reach tumor cells have recently been demonstrated. Through the European consortium H2020 CARAT, we have transposed this approach to CAR T cells. Our results that will be presented reveal that engineered T cells infiltrate tumor cells regions in a two step-process dependent on IFN gamma and ICAM-1. The different optimizations of this innovative model as well as the possibility to combine it with the organ-on-chip approach will be discussed.

Engineering personalized tissue implants: From 3D printing to bionic tissues

Tal Dvir - Tel Aviv University, Israel

In this talk I will describe cutting-edge technologies for engineering functional tissues and organs, focusing on the design of new biomaterials mimicking the natural microenvironment, or releasing biofactors to promote stem cell recruitment and tissue protection. In addition, I will discuss the development of patient-specific materials and 3D-printing of personalized vascularized tissues and organs. Finally, I will show a new direction in tissue engineering, where, micro and nanoelectronics are integrated within engineered tissues to form cyborg tissues.

Genome-wide CRISPR Screens in Primary Human T Cells Reveal Key Regulators of Immune Function

Eric Shifrut - University of California San Francisco, United States

Human T cells are central effectors of immunity and cancer immunotherapy. CRISPR-based functional studies in T cells could prioritize novel targets for drug development and improve the design of genetically reprogrammed cell-based therapies. However, large-scale CRISPR screens have been challenging in primary human cells. Critical biology of human immune cells, including key signaling pathways and effector functions, may not be recapitulated in immortalized cell lines. We developed a new method, sgRNA lentiviral infection with Cas9 protein electroporation (SLICE), to identify regulators of stimulation responses in primary human T cells. Genome-wide loss-of-function screens identified essential T cell receptor signaling components and genes that negatively tune proliferation following stimulation. Targeted ablation of individual candidate genes characterized hits and identified perturbations that enhanced cancer cell killing. SLICE coupled with single-cell RNA-Seq revealed signature stimulation-response gene programs altered by key genetic perturbations. SLICE genome-wide screening was also adaptable to identify mediators of immunosuppression, revealing genes controlling responses to adenosine signaling. In summary, we have developed a novel pooled CRISPR screening technology with the potential to explore unmapped genetic circuits in primary human cells and to guide the design of engineered cell therapies.

Becoming cell therapy Makers - Opportunities and challenges in manufacturing of cell therapy products

Lior Raviv - Pluristem LTD, Israel

For the last few years, the cell therapy industry is evolving from a "research" state of mind to a commercialization focus. The last approvals for Kite, Novartis and Tigenix have boost up the evolutionary process and changed the focus from "how to bring the product to approval?" to "what will be in the day after?". Based on the proof that cell therapies do work and hold the potential to change the way medicine is being practiced, the cell therapy industry is now mature enough to start facing the gaps in translating the manufacturing lines into industrialized platforms.
Keeping the product under control during the process actually means keeping the process controlled from the isolation step and up to the patient's body. However, current technologies hold many gaps for every process step. Most of these gaps are around manual operation, poor process control, open manipulations and scale. In order to transform cell therapy to an industry and be able to supply the post-approval clinical demand, companies must have the following five aspects covered during their process development evolution:

1. Good and potent cell source
2. Closed manufacturing systems
3. Reproducible process
4. Ability to produce trillions of high-quality cells per year
5. Low cost of goods.

Advancing through the process development evolution requires initial investment in the form of time and money. However, the course of increasing the process knowledge and understanding will lead to improved yields, higher process robustness and reduced cost of goods. In this presentation case studies will be presented on the implementation of the five elements during the process development stages.

Pioneering Cell Processing of personalized ATMPs

Andrew Kaiser - Miltenyi Biotec GmbH, Germany

Background and aims: With the approval of two CAR-T cell therapies for B cell malignancies consisting in the genetic manipulation of patient's own immune cells, the field of immuno-oncology is truly entering a new era. The implementation of autologous gene engineered T cells towards standard-of-care is an incredible step towards personalized medicine. However, the potential associated with these new therapeutic approaches comes with new challenges. In a very timely fashion T cells from patients must be harvested, enriched, activated, gene engineered, expanded and formulated *ex vivo* prior to reinfusion. Analytics should be in place to ensure the cell product is according to specification, chain of identity must be maintained and many more aspects must be considered for successful clinical application of these "living drugs".
Methods and conclusion: Here we will present some of the solutions to integrate novel cell manufacturing tools and enabling technologies into a comprehensive platform that will facilitate the safe, automated, and cost-efficient preparation of highly effective gene-modified T cells and ultimately aid in the dissemination of the manufacturing process around the globe and thereby help patients get access to personalized cell based therapies in the future.
The fully-automated CliniMACS Prodigy T Cell Transduction process minimizes user interactions and enables the preparation of high numbers of functional CAR T cells in a serum free process. Thereby, classical process risks, such as viral contamination due to human components, use of different devices or unnecessary manipulations in a non-automated system are reduced to a minimum. Accordingly, these improvements are the next step towards a commercial fully-automated manufacturing of CAR T cells for the treatment of a large number of patients.

Generation of hematopoietic cells with lymphoid potential from induced pluripotent stem cells (iPSC) for adoptive immunotherapy of cancer and leukemia

Annelise Bennaceur - Institute National de la Santé et de la Recherche, France

Human pluripotent stem cells (hPSCs) hold a potential source of hematopoietic progenitor cells for producing mature T and NK cells and to achieve next generation immune cell therapy strategies using engineered CAR-immune cells targeting relevant antigens from leukemia and solid tumor. Previous data from our group have demonstrated that hematopoietic potential of hPSCs derived from PBMC, is highly variable from one cell line to another healthy donors, whatever the reprogramming process. We explore miRNA expression at pluripotent stage and during differentiation. Gene profiling of several iPSC and ESC with low and high hematopoietic generation ability allows us to select on the basis of their miRNome those cell lines with increased hematopoietic potential. Hematopoietic competent IPSC line were expanded in clinically applicable conditions by using CliniMACS Prodigy as close system culture (Myltenyi Biotech). The seeding of 1.106 IPSC allows a 1000-Fold expansion (109) by using IPISC Brew XF, with the preservation of their genomic integrity and pluripotency as assessed by the expression of Oct 4, Nanog, SSEA4 and Tra-1 60, trilineage differentiation culture kit (StemMACS) and teratoma assay. This IPSC line batch is now used to generate Functional T cells and NK cells using our established protocol were generated and robust protocols are underway to generate homogenous clinical grade NK cells with increased lysis ability and iPSC-derived NK-CAR cells.

Functional Surfaces and Scaffolds Combined with Stem Cells for Bone and Cartilage Tissue Engineering

Nuno Neves - University of Minho, Portugal

Many biomaterials have been proposed to produce scaffolds aiming the regeneration of many tissues. We have a particular interest in developing systems combining natural and synthetic biodegradable polymers. By proposing those systems for those demanding applications, we aim at obtaining biomaterial devices with enhanced properties namely mechanical properties, processability, cell-active and friendly surfaces and tunable biodegradability. Our biomaterials may be processed by melting or solvent routes into devices with wide applications such as biodegradable scaffolds, films or particles and adaptable to many biomedical applications. As an example of processing technologies, electrospinning has recently gained popularity as a simple and versatile technique to produce synthetic polymeric ultrafine fibers. This technique allows the production of non-woven meshes with fiber diameters in the nanometer range, which results in a high surface area-to-volume ratio and high porosity. Additionaly, these nanofiber meshes can mimic the extracellular matrix of human tissues and, therefore, can be used as scaffolds for Tissue Engineering (TE) applications. Many sources of cells may be considered for successful tissue engineering applications. Embryonic, iPS and adult stem cells are among the most promising to achieve the cell numbers required to have therapeutic relevance. We have been proposing adult stem cells from different sources for bone and cartilage tissue engineering applications. This talk will review our latest developments using natural-based biomaterials and nanofibre meshes in the context of bone and cartilage tissue engineering applications.

Developing Novel siRNA Nanoparticle Therapies For Cystic Fibrosis by Silencing of the Epithelial Sodium Channel

Stephen Hart - UCL Great Ormond Street Institute of Child Health, United Kingdom

Cystic Fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane regulator gene (CFTR) but also involves upregulated activity of the epithelial sodium channel, ENaC, which leads to excessive sodium and water absorption, depleting the watery, periciliary liquid (PCL) layer and mucus overlaying the ciliated airway epithelium. This markedly impairs mucociliary clearance leading to small airway obstruction and chronic bacterial infections leading to progressive lung damage. We are developing a genetic therapy for CF aimed at maintaining lung hydration by silencing of αENaC by RNA inhibition with siRNA, a natural method of gene regulation. We have developed a novel, proprietary, mucus-penetrating, epithelial-targeted, lipid/peptide/siRNA nanoparticle formulation for siRNA transfection of lung epithelium. These nanoparticles protect siRNA from shearing and RNase digestion and achieved silencing of αENaC in transfections of differentiated human CF cells to correct physiological factors including PCL depth, mucus hydration and membrane ion transport defects. A single dose of the siRNA nanoparticle in mouse lung targeting ENaC silenced its expression by approximately 30%, which persisted for at least 7 days. Three doses of siRNA increased silencing to approximately 50%. Thus the nanoparticle mixture achieved similar levels of silencing of αENaC to human CF air-liquid interface cultures, which persisted for at least one week after administration while repeat dosing led to cumulative levels of silencing. In this presentation we discuss the challenges of scale-up manufacture of the components and of the nanoparticle formulation itself using advanced microfluidic mixing protocols, producing active, monodisperse nanoparticles formulated with cryoprotectants that will be stable to long-term storage, frozen. We have also investigated the stability of the siRNA nanoparticle formulations to nebulisation since this will be an inhaled therapy. it is important that the aersol is the correct size and that the nanoparticles are stable to the shearing forces in the nebuliser. With these formulations we will progress to preclinical PD/PK and toxicology studies.

Epigenetic control of T cell differentiation: From genome-wide signatures to local regulators and their targeted manipulation

Julia K. Polansky-Biskup - BCRT, Charité Universitätsmedizin Berlin, Germany

Adoptive T cell therapy is a promising approach in various clinical settings, from target-specific immune reconstitution fighting cancer and chronic infections to combating undesired immune reactivity during auto-immunity and after organ transplantation. For such clinical applications, the expanded T cell products need to maintain functional stability and fitness during the production process and after transfusion into the patients. We address these T cell features from an epigenetic viewpoint and generated genome-wide epigenetic maps for several CD4+ T cell subpopulations. These rich data allowed the extraction of epigenetic elements which are essential for imprinting a functional T cell phenotype ('Epi-stabilizers'). Such elements are reliable biomarkers for functional T cell subsets, but might also be utilized as molecular switches for the targeted induction of advantageous T cell qualities during the production process. To this end, we established a powerful 'hit-and-run' CRISPR/Cas9-based epigenetic editing approach for the targeted demethylation of the Epi-stabilizer for immuno-suppressive regulatory T cells (Treg). Using this system, we were able to induce the Treg master transcription factor FOXP3 from the physiological chromatin context of primary human naïve, but also fully differentiated pro-inflammatory memory T cells. The induced demethylated state was stable over weeks in clonal T cell proliferation cultures even after expression of the editing complex has ceased. With this we show, that epigenetic editing is suited to change the molecular profile of T cells and suggest the presented technique as a tool for improving Treg products for advanced T cell therapies. The method might also be of value for many other types of cellular therapy as epigenetic regulation has been identified as the major driver of phenotype imprinting.

Subconjunctival injection of low-dose mesenchymal stem cells promotes corneal allograft survival in a mouse cornea transplantation model

Thomas Ritter - National University of Ireland Galway, Ireland

Background: Systemic administration of mesenchymal stem/stromal cells (MSC) has been shown to promote corneal allograft survival in rodents. Aim: Here we investigate the potential of low-dose subconjunctival injection of MSC to promote corneal allograft survival in a mouse corneal transplant model. Methods: MSC were isolated from 8 to 14-week-old female C57BL/6J (H-2k) or BALB/c (H-2d) mice or from healthy human donors and extensively characterised. A fully allogeneic mouse corneal transplant model was employed for these studies with BALB/c mice serving as recipients of female C57BL/6J grafts. A low-dose dual injection strategy was developed with mice receiving subconjunctival injections of 50.000 MSC on day -1 and day +1 before/after transplantation. Graft transparency was used as the primary indicator of rejection while neovascularisation was also recorded. To evaluate if MSC-injections modulate host immune cell populations, draining lymph node and spleen cells will be isolated from treated animals on day 2 and at the estimated time of rejection and characterised by flow cytometry. *In vitro*, MSC modulate key components of the corneal allograft rejection mechanisms by directly suppressing lymphocyte proliferation. *In vivo*, low-dose dual subconjunctival injection of 50.000 C57BL/6J MSC leads to 100% allograft survival in grafted mice (n=7). Interestingly, the same injection strategy using BALB/c (syngeneic) or human MSC (xenogeneic) only leads to 50% (n=8) or 30% graft survival (n=8), respectively, which is not significantly different from control transplanted mice (40%, n=6). With an aim to optimising administration strategies we also demonstrate that single administration of C57BL/6J MSC on either day -1 or day +1 promotes rejection free graft survival in 87% (n=8) and 85% (n=7) of grafted mice, respectively. Moreover, early time point *ex vivo* analysis suggests modulation of innate immune responses by local MSC administration. Conclusion: This work demonstrates that low-dose dual subconjunctival injection of allogeneic MSCs successfully promotes corneal allograft survival which can be optimised to a single administration strategy.

Immunomodulatory properties of amniotic membrane derivatives for tissue regeneration: a 20-year experience

Ornella Parolini - Università Cattolica Santo Cuore, Italy

Background: For almost 2 decades our research has afforded greater clarity about the therapeutic properties of stem/stromal cells from human term placenta. Term placenta is an appealing cell source because it is discarded after birth and considered biological waste, and its procurement does not require an invasive procedure nor pose ethical issues. Aim: Overall our studies have been aimed at understanding how mesenchymal stromal cells from the amniotic membrane (hAMSC) contribute to tissue regeneration. We have shown that amniotic cells contribute to the resolution of inflammation that limits pro-inflammatory pathways and boosts mediators/cells responsible for tissue repair. We demonstrate that MSC from fetal-derived placenta are most apt for inducing immunomodulation when compared to MSC from other tissues. Results: *In vitro* we have shown that hAMSC and their conditioned medium (CM) reduce T cell proliferation, enhance T regulatory and reduce Th1 and Th17 populations, inhibit the differentiation of monocyte-derived dendritic cells, and induce macrophage differentiation toward M2 macrophages. We are currently studying the effect of hAMSC and CM on B cells and our results demonstrate a block of proliferation of CpG-ODN-stimulated B cells and in the formation of antibody-secreting cells. We have also shown that hAMSC promote functional recovery when applied in preclinical models of inflammatory diseases such as lung and liver fibrosis, myocardial ischemia, autoimmune diseases, and traumatic brain injury. In addition, we show that macrophages generated in the presence of CM enhance wound healing in diabetic mice, and CM improves motor deficits, ameliorate brain pathology, and decrease microglia activation in mice with Huntington's disease. Conclusion: Collectively, these results help shape/guide the development of novel therapeutic strategies based on the ability of perinatal cells to contribute to tissue regeneration by resolving inflammation. As we move forward, an improved understanding of the correlation between inflammation and tissue regeneration (and degeneration) will be essential to better understand the mechanisms of action.

Pre-activated mesenchymal stromal cells induce regulatory immune populations *in vivo* and prolong corneal allograft survival

Oliver Treacy - National University of Ireland Galway, Ireland

Background: Corneal transplantation is the final treatment option for millions of people suffering from severe corneal disease. Mesenchymal stromal cells (MSC) are regarded as a promising therapeutic option for multiple immune diseases/disorders; however, efficacy of MSC treatments varies for currently unknown reasons. Aim: To investigate a novel licensing strategy to significantly improve the immunosuppressive capacity of MSCs *in vitro* and their therapeutic efficacy *in vivo* in a fully allogeneic murine corneal transplant model. Methods: Pre-conditioning of murine MSC with TGF-β1 (TGF-β MSC) significantly improved their ability to modulate innate and adaptive immune cells *in vitro* and *in vivo*. We found that TGF-β MSCs significantly suppressed the proliferation of stimulated CD3+CD4+ and CD3+CD8+ T lymphocytes while significantly increasing the numbers of CD3+CD4+FoxP3+ regulatory T lymphocytes (Tregs) following co-culture assays. We tested if TGF-β MSC had an enhanced ability to prolong rejection free survival in a fully allogeneic mouse cornea transplant model (C57BL/6 to Balb/c). TGF-β MSC treated mice presented with a rejection free survival rate of 70% (n=13) compared to 25% (n=14) in the non-activated MSC treatment group. Prolongation of graft survival was associated with; (i) increased Treg populations in the draining lymph nodes (dLNs) and lungs of TGF-β MSC treated mice, and (ii) decreases in antigen presenting cell populations in the dLNs, lungs and spleens of TGF-β MSC treated mice. Finally, we confirmed that (i) TGF-β MSC mediated their therapeutic effects via canonical Smad2/3 signalling, that (ii) the potent immunosuppressive effects mediated by TGF-β MSC were contact-dependent and (iii) prostaglandin E2 (PGE2) (via prostaglandin EP4 receptor) plays a vital role in TGF-β MSC mediated immunosuppression of T lymphocytes. Conclusion: We have shown for the first time that pre-treatment of MSC with TGF-β significantly enhances their immunosuppressive capacity *in vitro* and therapeutic efficacy in a murine corneal transplant model. These findings will contribute to the improvement of currently unsuccessful MSC therapies.

Treatment of osteochondral defects of the knee in rats using bilayered scaffold free cell-based constructs

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Background: Restoration of deep osteochondral defects represents a significant unmet clinical need. Moreover, untreated lesions lead to a high rate of osteoarthritis [1]. Current strategies to repair osteochondral defects such as osteochondral grafting or "sandwich" strategies (e.g. bone autografts plus ACI/MACI) fail to generate long-lasting osteochondral interfaces. Aim: This study aimed at providing proof-of-principle of the increased capacity of immature osteochondral grafts (OCGs) to repair deep osteochondral defects of the knee in rat model, and generating biomimetic TE constructs inspired by the immature OCGs.Methods: Dermal punches were used to transplant cylindrical OCGs from the knee of skeletally immature rats into osteochondral defects created in skeletally mature rats. To create bilayered TE constructs, micromasses of human periosteum-derived progenitor cells and human articular chondrocytes were produced *in vitro* using chemically defined medium formulations. These constructs were subsequently implanted in osteochondral defects created in the knees of nude rats. Results and Conclusions: Our results suggested that the immature OCGs hold the capacity to repair osteochondral defects in skeletally mature animals, i.e., allowed new subchondral bone and tidemark formation, and maintained a Safranin O-positive hyaline cartilage at 16 weeks after transplantation. The bilayered TE constructs could partially recapitulate this regenerative process, including the formation of new subchondral bone and generation of the typical joint surface architecture, including similar tissue structure and zonation. In summary, while immature OCGs provide an attractive model to investigate osteochondral maturation and repair, cell-based TE constructs displaying a hierarchically organized structure comprising of stable and transient cartilage intermediates seem an attractive strategy to repair osteochondral defects of the knee.

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Highly activated Natural Killer cells to treat pediatric cancer

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Background: Natural Killer (NK) cells are able to recognize and kill virally infected or transformed cells in a short time and without prior sensitization, representing a promising therapeutic option for patients with various malignancies. Methods: Our group is focused in the use of highly activated NK cell products from haploidentical donors for cell therapy in pediatric cancer: i) after haplo-identical stem cell transplantation for pediatric refractory solid tumors, NCT01337544; ii) after salvage chemotherapy in children with relapsed/refractory leukemia, NCT01944982 and NCT02074657; iii) as consolidation strategy in acute myeloid pediatric patients, NCT02763475. In collaboration with: TEDDY- European Network of Excellence for Paediatric Clinical Research.We have used two good manufacturing practice (GMP)-compliant methods for the production of high numbers of highly activated NK cells from peripheral blood mononuclear cells: 1) overnight culture with IL-2 cytokine; 2) 14-21 days co-culture with irradiated stimulatory cells, K562-mb15-41BBL or K562-mb21-41BBL cell lines. Conclusions:In our preclinical and clinical experience, the use of NK to treat pediatric cancer patients is safe and feasible. More than 75 NK cell products have been infused in 45 patients with different solid and hematological cancers inducing no GvHD or any other severe adverse effects related to the cell therapy. However, the anti cancer efficacy is limited in quality and time.Aims:We propose to overcome those limitations by NK cells engineering to: 1) improve their cytotoxic capacity (CAR-NK cells); 2) improve their delivery into the tumor (chemokine receptors expressing-NK cells); 3) minimize their exhaustion ("memory phenotype"-NK cells).

Neural precursor/stem cell-based therapy for Rett syndrome

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MECP2 mutations cause Rett syndrome (RTT), the first cause of severe intellectual disability in girls. No cure is available and the identification of therapies is hampered by the need of maintaining physiological protein levels in brain and the limited comprehension of RTT pathophysiology. Several studies proved that reduced levels of neurotrophic factors play a major role in RTT and their augmentation represents a valid therapeutic approach. Indeed, manipulation of their levels or activation of the downstream pathways represents one of the most promising therapeutic strategy that, however, is limited by the low blood brain barrier permeability. Neural Precursor/Stem Cell (NPC) transplantation was proved safe and efficacious in many neurological disorders, including autism. Willing to respond to the unmet need of a cure for RTT, we are investigating the therapeutic potential of NPCs in Mecp2 null mice, modelling RTT. Although the long-thought prime mechanism of NPC action is the replacement of damaged cells, it is now clear that transplanted cells often exert their benefits through bystander mechanisms. Indeed, by sensing the pathological environment, they promote immunomodulation, neuroprotection and brain plasticity through the secretion of a plethora of molecules, including neurotrophic factors. Our data demonstrate that intra-cisterna magna NPC transplantation can significantly prolong the lifespan of Mecp2 null mice and improve memory and motor functions.We report that NPCs localize along the meninges in the caudal zone of the brain up to 40 days after transplantation; grafted cells reveal an immature phenotype and generally a commitment towards the astrocytic lineage.To identify the molecular mechanisms responsible for the observed neurological benefits, we are analyzing typical molecular defects of RTT, reporting rescue effects that vary depending on the specific molecule and brain region. Importantly, NPCs modulate molecular pathways both proximally and distally to the grafted cells.Using an *in vitro* transwell-based co-culture system, we demonstrate the paracrine ability of NPCs to promote neuronal morphological and electrophysiological rescues.

Drive regeneration by employing the immune-structure interface

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Background: To steer regeneration in compromised patient settings, it is essential to understand how systemic challenges alter endogenous regenerative cascades. Aim of our work is to analyse the alterations in bone regeneration and adaptation with increasing age and how these are driven by the linkage of the immune and the bone systems. Material & Methods: The immune status of aged mice was determined by flow cytometry (FACS). Their bone structure analysed by µCT and biomechanical competence was assessed using torsional testing. The interaction of the bone and immune system was analysed *in vitro* using conditioned immune cell medium on MSCs. Bone regeneration was investigated in a "humanized" mouse model by injection of human donor immune cells, reflecting an in-patient situation. Finally, a mechano-therapeutic experiment was performed to rescue an experienced immune system's lack of bone adaptation. Results: Femurs of aged mice with a "naïve" immune system showed age effects in their bone structure. However, mice of the same age, with an experienced immune system, displaying a higher percentage of effector/memory T cells had stiffer (more brittle) bone, a changed microarchitecture and thus were clearly negatively influenced by the pro-inflammatory impact of the experienced, "aged" immune system. The same effects were observed in the "humanized" mouse model. *In vitro* assays revealed that the cytokine milieu of activated effector T cells negatively impacted the osteogenic differentiation of MSC. Under limb loading, a more naïve phenotype was induced and thus the immune system's negative effects were partially rescued. Discussion & Conclusions: In old age, there is a pro-inflammatory milieu in the bone which reduces the regenerative ability. The findings explain why not only bone structure but the immune systems inflammatory capacity and experience is a key aspect to be addressed if regeneration is to be reached. A better understanding on the interaction of the immune and skeletal system opens new treatment avenues.

Towards efficient strategies of HIV-1 cure: Analysis of HIV-1 latency-reverting agents by single cell RNA-seq

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Background. HIV-1 infection is not curable to date. The "shock-and-kill" approach represents the most promising principle towards HIV-1 cure: In the context of antiretroviral therapy, a "shock" event, triggered by latency-reverting agents (LRAs) shall re-activate HIV-1 gene expression in latently infected T-cells, rendering them immunologically visible and thus susceptible to the subsequent "kill" event, which may be effected by CD8+ T-cell-mediated killing or pharmacological destruction. *In vivo*, this approach suffers from incomplete reduction of the viral reservoir despite detectable reactivation. Specifically, the relative percentage of latently infected cells (defined as bearing a replication-competent HIV-1 integrant) which end up expressing HIV-1 proteins upon treatment with individual LRAs is unknown. It is suspected that a substantial proportion of HIV-1-positive T-cells fully or partially resists reactivation and that a combination of LRAs may be required to quantitatively reverse HIV-1 latency.
Aim and methods. I propose to analyze the true potential and efficiency of several LRAs by monitoring, at the single cell level, the extent and quality of HIV-1 reactivation following *ex vivo* treatment of PBMCs isolated from aviremic HIV-1 patients with LRAs.
Parameters analyzed will include the three different mRNA (fully spliced, partially spliced, unspliced) species generated upon HIV-1 reactivation, cell surface HIV-1 Env expression, and the cellular transcriptomic profile.
Conclusion. The recent breakthrough of single cell RNA-sequencing technology provides a new dimension for the analysis and quantification of latently infected cells in HIV-1 patients and will help to generate hypotheses for the improvement of thus far inefficient approaches of HIV-1 cure.

Tissue engineered mimetic autografts for bone regeneration

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Bone tissue has an intrinsic regenerative capacity. However, this regeneration can be compromised, leading to delayed fracture healing and nonunion. Due to the scarcity of bone tissue that can be used as autograft, tissue engineering strategies arise as a sound solution by using biocompatible materials functionalized with cells and morphogens. Our objective is to design engineered autografts capable of efficiently treat fracture nonunion. For this purpose, we designed polycaprolactone (PCL) based mimetic autografts composed of an inner cylindric scaffold, produced by PCL extrusion, providing mechanical stability and an osteoconductive environment. Additionally, we created a mimetic autograft (MA) by the addition of an exterior thin and highly porous fibrillar tube of PCL, synthesized by melt electrospinning writing (MEW), mimicking the periosteum. To evaluate their regenerative capacity, these scaffolds were placed in critical size femur defect model in rats and compared with rats were the defect was left untreated (Empty). Ten weeks after surgery μCT and histological studies were carried out. At the μCT level, structural mimetic PCL scaffolds, devoid of cells and morphogens, showed no significant differences in healing or bone formation (Empty group, 11.47±4.93 mm3; MA, 14.95±3.09 mm3, p=0.1711). Histological analysis demonstrates that MEW PCL mimicking periosteum enhances bone growth and present good implant integration, but insufficient for successful healing. In conclusion, acellular mimetic autografts need to be optimized by functionalization with morphogens (BMP-2, BMP-7) and/or mesenchymal progenitor cells.

A millifluidic dynamic system to unveil the interaction among breast cancer cells, fibroblasts and mesenchymal stem cells

Virginia Brancato - University of Minho, Portugal

Background: Cancer disease is the results of the interplay among cancer cells, fibroblasts, endothelial and immune system cells [1]. The role of mesenchymal stem cells (MSCs) in breast cancer progression is still under debate. In some cases, MSCs are able to trigger tumor progression by modulating the immune surveillance, promoting angiogenesis and metastasis and by interfering with the epithelial-mesenchymal transition (EMT) [2]. MSCs show a "double-edged sword" behavior since they are also able to inhibit cancer invasion [3]. The development of more realist 3D *in vitro* tumor models is an urgent need in order to understand the role of MSCs in cancer [4]. Aim: In this work, we investigate the paracrine interaction between bone marrow derived MSCs (bmMSCs) and a heterotypic breast cancer microenvironment composed of cancer cells and fibroblasts using a dynamic millifluidic system. Methods: Two chambers of a millifluidic dynamic reactor (LIVEBOX 1, IVTech, Italy) are serially connected to a peristaltic pump. The cell viability in static and dynamic condition are analyzed. Moreover, the differences in the exosome content of single (3D-bmMSCs and 3D-HMF/MDA-MB-231 in separate LIVEBOX 1) and co-culture (3D-bmMSCs and 3D-HMF/MDA-MB-231 in two connected LIVEBOX 1) are analyzed both in static and dynamic conditions. Finally, the expression of genes related to the extracellular matrix remodeling are investigated in static and dynamic conditions for both single and co-cultured systems. Conclusion: The result shows a modulation of exosome-related protein in the different conditions. This also modulates the expression of the genes related to the extracellular matrix remodeling. The dynamic *in vitro* model developed in this work unravel the role of MSCs in the tumor progression.

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Aryl hydrocarbon receptor - driven immunometabolic checkpoints in type-1 dendritic cells restrains antitumor responses

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Introduction: The immune system has a key role in controlling tumor initiation and growth. Conventional dendritic cells (DCs) are immune cells critical for innate and adaptive immune responses, and they include cDC1 and cDC2 subtypes. cDC1s are critical for CD8+ T-cell priming early in an antitumor response, and this function is affected by environmental signals. The transcription factor Aryl hydrocarbon Receptor (AhR) is an environmental "sensor" of specific metabolites, both endogenous and exogenous in nature, via association with other cell-intrinsic transcription factors. Emerging data have shed light on an unexpected role of AhR in fostering tumor escape mechanisms. Objectives: We assessed the impact of AhR deletion selectively in cDC1 on the immune response to tumors. Materials & methods: cDC1 were differentiated from C57BL/6J bone marrow cells after FLT3L treatment for 9 days. Cytokine production in cDC1 and in cDC1-CD8+ T-cell co-coltures was analyzed by intracellular staining. Ahrfl/fl XCR1-Cre and Ahrfl/fl control mice were inoculated subcutaneously with fibrosarcoma tumor cell line. Tumor growth was monitored daily for 30 days. Results: Whole genome analysis revealed that AhR is expressed in mature CD24+CCR7+ cDC1 to greater extent than in cDC2 and pDCs. In particular, in mature cDC1, AhR in combination with the cDC1-specific factor IRF8 drived the expression of immunosuppressive indoleamine 2,3-dioxygenase (IDO1). Accordingly, AhR deletion in cDC1 abrogated IDO1 expression. Surprisingly, we found that deficiency of either Ahr-/- or Ido1-/- in CCR7+ cDC1 strongly potentiated immunostimulatory IL-12 and TNF-α productions by cDC1. Therefore, in a cDC1-T CD8+ co-colture system, we demonstrate that Ahr deletion in cDC1 greatly increases IFN-γ, Granzyme B and Perforin production. Notably, in an *in vivo* mouse model, selective AhR deletion in XCRI expressing cDC1 accelerated spontaneous immune rejection of an otherwise progressive fibrosarcoma cell line. Conclusion: Overall, these data point to AhR as a new immune inhibitory target in cDC1, which can be targeted pharmacologically to overcome immune tolerance and resistance to immunotherapy.

Amniotic mesenchymal stromal cell secretome: a potent pleiotropic strategy for acute brain injury

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We have demonstrated that human amniotic mesenchymal stromal cells (hAMSC) protect the brain after traumatic brain injury (TBI) in mice. In an *in vitro* model, we demonstrated that hAMSC or their secretome exert similar protective effects after acute brain injury. Here we evaluate if hAMSC-secretome protects TBI mice and investigate possible mechanisms of protection in the *in vitro* model. *In vivo*: C57BL/6J male mice (8 weeks old) were subjected to sham or TBI followed by daily intraperitoneal injection of 150 µl of saline or hAMSC-secretome starting 3 h after injury. From 1 week on, TBI hAMSC-secretome treated mice show an improvement of sensorimotor function assessed by SNAP and neuroscore tests, compared to TBI untreated mice. *In vitro*: organotypic brain slices from prefrontal cortex of newborn mice were injured by oxygen and glucose deprivation (OGD) for two hours. One hour after OGD, slices were cultured in culture medium (control), or with 50% hAMSC-secretome. Gene expression analysis at 48h after injury revealed that, compared to control slices, treatment with hAMSC-secretome: 1) rescued OGD-induced MAP2 (a neuronal marker) downregulation; 2) reduced the OGD-induced oxidative stress (downregulation of HO-1 and NQO1 expression); 3) polarized microglia activation toward a protective phenotype (upregulation of Ym1 expression); 4) reverted OGD-induced astrocyte activation and toxic polarization (downregulation of GFAP and Serping 1 expression). In conclusion, hAMSC-secretome modulates glial activation toward beneficial phenotype possibly contributing to the protective effects observed *in vivo*. These data support hAMSC-secretome as a therapeutic strategy for acute brain injury.

A preclinical non-human primate model to investigate the regenerative potential of human induced pluripotent stem cell-derived cardiomyocytes

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Background - Induced pluripotent stem cells (iPSCs) and their progeny are promising sources for cell-based therapies. Human iPSC-derived cardiomyocytes (hiPSC-CMs) can be generated *in vitro* and have been proposed for myocardial repair *in vivo*, but so far, limited data is available from large animal models. We have previously established transplantation and tracking of human iPSCs in a pig model of myocardial infarction (Templin et al., Circulation 2012) as well as implantation of pulmonary, aortic and mitral valves in sheep (Tudorache et al., Eur J Cardiothorac Surg 2016 & Iablonskii et al., Eur J Cardiothorac Surg 2018).
Aim - In an ongoing study, it was our aim to establish a pre-clinical non-human primate model to investigate the regenerative potential of hiPSC-CMs after myocardial infarction.
Methods - Myocardial infarction (MI) was induced by coronary artery ligation in cynomolgus monkeys (Macaca fascicularis; n=6) under general anesthesia. Animal medication included analgesic, antibiotic, antiarrhythmic, and immunosuppressive drugs. Cardiac function was assessed via telemetric ECG recording, echocardiography and MRI at different time points. Human iPSC-CMs expressing a fluorescent reporter gene (Venus) were generated by targeted differentiation in large-scale suspension cultures. 5 - 7 x 10 7 hiPSC-CMs were injected directly into the myocardium 2 weeks after MI. After 2 weeks or 12 weeks, animals were sacrificed and graft survival was assessed histologically.
Conclusion - Both 2 weeks and 12 weeks after cell transplantation, large hiPSC-CM grafts (>1 mm2) were identified in myocardial tissue sections based on their reporter gene expression and after staining with a species-specific antibody targeting human cardiac troponin I. Human iPS-CMs expressed cardiac markers with visible cross striations reflecting sarcomeric structures and showing cellular alignment. Currently, the evaluation of functional cardiac data is ongoing. In conclusion, our pre-clinical non-human primate model allowed hiPSC-CMs to engraft and survive in the infarcted heart for up to 12 weeks and will be further used to investigate the regenerative potential of hiPSC-CMs.

Adapter CAR T cells - A new versatile platform for controllable CAR T cell function

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CAR T cells have shown tremendous clinical success in a wide range of B cell malignancies, but thus far have had limited efficacy in other forms of cancer. Some of the main challenges still associated with the technology are on-target/off-tumor toxicity, cytokine release syndrome, antigen escape, and generally a lack of control over CAR T cell function once infused into the patient. Integration of an adapter molecule that reversibly links the CAR T cell to the tumor cell, represents one strategy to overcome these challenges. Here we have investigated a novel adapter CAR system targeting a neo-epitope like structure, which can be easily linked to a wide range of therapeutic antibodies and antibody fragments to enable their use as adapter molecules. We have screened different adapter CAR sequences regarding their expression on primary T cells, absence of tonic signaling, specificity, and efficacy in adapter-mediated tumor cell lysis. Optimized CAR candidates showed target cell lysis, which was strictly dependent on the presence of the adapter molecule and could be finely tuned by adjusting the adapter concentration. Functionality of the optimized CARs was further studied in pre-clinical mouse models, in which adapter CAR T cells showed similar efficacy in tumor cell lysis compared to direct CAR T cells. Overall, our results suggest that the adapter CAR system enables precise pharmacological control of CAR T cell function, supports multitargeting strategies, and thereby can improve toxicity management and safety of CAR T cell therapy.

Microvessels-on-Chip - Bridging the gap between blood and lymph

Barbara Bachmann - Ludwig Boltzmann Institute for Experimental and Clinical Traumatology and Vienna University of Technology, Austria

Organ-on-a-chip technology has demonstrated the ability to recreate both physiologic and pathologic cellular microenvironments for a number of single tissue models. Building on these achievements, next generation organ-on-a-chip systems attempt to connect multiple organs into a single device, thus emulating tissue to tissue communication. A crucial aspect in developing so called multiple organ or body-on-a-chip devices is the incorporation of vascular systems that mimic functional blood vessels. (cooperation Uwe Marx group: Knezevic L, Front Bioeng Biotechnol. 2017 Apr 18;5:25.). Although a number of vasculature-on-a-chip devices system focusing on the cardiovascular system have been reported in recent years, the establishment of a biomimetic lymphatic system is still in its infancy. The lymphatic system has long been regarded as the body's sewage system, but recent studies suggest that the lymphatic system also plays a vital role in the immune system. This means that in addition to recirculating excess fluids, proteins and lipids back to the cardiovasculature, the lymphatic vasculature influences a wide range of diseases such as cancer, metabolic disease and inflammation. To gain a deeper understanding of lymphangiogenesis, we are combining the cardio- and lymphatic microvascular systems to re-engineer the interface between blood and lymphatic vasculature for creation of an *in vitro* model of vascularized interstitial tissue that can ultimately be connected to any organ model of the body. The microvasculature-on-chip device can mimic a physiological interstitial flow of 1 - 3 µm/s through the cell-laden (e.g. endothelial and adipose-derived stem cells) fibrin hydrogel matrix by employing a hydrostatic pressure-driven flow via fluid reservoirs. It is envisioned that the proposed organ-on-a-chip system can serve as a powerful tool to investigate lymphatic vasculature as a novel drug delivery target and as an improved lymphedema disease model.

MSC improve skeletal muscle regeneration via locally damplen the pro-inflammatory capacity of CD8+ T cell subpopulations

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Skeletal muscles have a significant potential to heal, but beyond a certain injury severity threshold, this endogenous process proves insufficient, leading to fatty degeneration and fibrotic scar tissue, which cause long-term deficits in muscle structure and strength. We have recently shown that the local transplantation of mesenchymal stromal cells (MSCs) improves the muscle repair in pre-clinical models and humans. Despite these promising results, the underlying mechanism, in particular the complex interaction between the cells and the injury environment is largely unknown. Since our previous studies suggest a close interaction between the immune and the musculoskeletal system, we here investigate the relevance of adaptive immunity for successful muscle repair and how it is altered by MSC transplantation. The longitudinal comparison of the immune cell compositions in severely injured muscle with the corresponding levels in the blood or uninjured muscles revealed that specifically T cells are important modulators of the healing process. We found that the accumulation of CD3+Tcells and CD8+ effector memory T-cells (CD8+ TEM) in the trauma area correlates with the injury severity and limits the regeneration potential. Conversely, local MSC transplantation improves the healing outcome and correlates with reduced levels of CD8+ TEM and enhanced levels of regulatory CD4+ T cells. *In vitro*, we found that conditioned media of CD3/CD28 activated PBMCs inhibited myogenic differentiation, whereas their co-culture with MSCs rescued this effect and led to improved myotube formation. Based on these observations, we showed *in vivo* that the systemic depletion of the whole CD8+ T cells or more specifically of the CD8+ TEM cells almost completely restores the function of severely damaged muscle, whereas the depletion of whole CD4+ T cell has no beneficial effect. Our study demonstrate that CD8+ effector T cells are not only found in persistently inflamed muscle, but also accumulate in traumatic injury area. The local reduction of CD8+ T cell levels and concurrent enrichment of regulatory CD4+ T cells is a promising therapeutic strategy to improve muscle repair.

Treatment of a naturally occurring dog model of Alport Syndrome by means of CRISPR/Cas9 gene therapy approach

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Alport syndrome (AS) is an inherited genetic disorder characterized by glomerular basement membrane abnormalities up to ESRD. Mutations in the COL4 genes are causative of both the ADAS and XLAS forms. No cure is available, and up to now only symptomatic treatments are accessible. We have proven how it is possible to isolate podocyte-lineage cells, the only kidney cells able to produce the COLIVα3-α4-α5 heterotrimer, from patients' urine. Thanks to the availability of these key disease-relevant cells, we have established the efficiency of AAV2-CRISPR/Cas9 to restore the wild-type genotype *in-vitro*. Employing a bi-plasmid system we have corrected the causative mutation in two stable podocyte-lineage cell lines, with a mutation in the X-linked gene COL4A5(p.(Gly624Asp)) and in the autosomal gene COL4A3(p.(Gly856Glu)), obtaining an HDR greater than 44% with in/dels rate lower than 12%. Gene editing experiments on podocytes-lineage cells from a naturally occurring dog model harboring a COL4A5 deletion confirmed a rate of correction greater than 50%. *In-vivo* experiments to correct the COL4A5 mutation on the dog model are currently ongoing. A single femoral artery catheterization, performed at 8 weeks of age, typically before the appearance of microalbuminuria, is used to deliver the AAV2-CRISPR correction system into both renal arteries on two distinct affected males, while a third one is used as negative control. This patent covered approach, providing the proof-of-principle for AS gene therapy strategy, can open up the possibility of *in-vivo* clinical trials through a new personalized medicine tailored to the pathogenic mechanism and acting on the key cells.

Effectiveness and safety of adoptive cell therapy with regulatory type 1 (Tr1) cells in pancreatic islet transplantation

Georgia Fousteri - San Raffaele Diabetes Research Institute, Italy

Adoptive cell therapy (ACT) with regulatory T cells is considered an emerging approach to improve transplant tolerance and enhance allogeneic graft survival. Among the different Treg subsets, regulatory type 1 (Tr1) cells stand out as one of the most promising approaches to promote tolerance in the context of allogeneic organ/tissue transplantation. However, it remains unclear how Tr1 cells promote transplant tolerance and whether they will be safe and stable in the face of an acute viral infection. By employing a mouse model of pancreatic islet transplantation, we report that Tr1 cell therapy promoted transplant tolerance via de novo induction of Tr1 cells in the recipients. Acute viral infection with lymphocytic choriomeningitis virus (LCMV) had no impact on Tr1 cell number and function, neither on the Tr1 cells infused nor on the ones induced, and that was reflected in the robust maintenance of the graft. Moreover, Tr1 cell immunotherapy had no detrimental effect on CD8 and CD4 anti-LCMV effector T-cell responses and viral control. Together, these data suggest that Tr1 cells did not convert to effector cells during acute infection with LCMV, maintained transplant tolerance and did not inhibit antiviral immunity. Thus, ACT with Tr1 cells is a highly promising approach to promote tolerance to allogeneic grafts.

Immunosuppressant-resistant T cells for advanced adoptive T cell therapy in immunosuppressed patients

Leila Amini - BCRT, Charité Universitätsmedizin Berlin, Germany

Solid organ transplant (SOT) patients require lifelong immunosuppressive medication to prevent rejection of the allogenic organ, which can cause morbidities and death due to viral infections. Recently, personalized T cell therapy approaches using regulatory T cells (Treg) to prevent organ rejection or antiviral T cell therapy to control chronic virus infections such as Epstein Barr or cytomegalovirus have become attractive living drug alternatives to conventional often toxic and unspecific medications. However, still baseline immunosuppression has to be maintained in SOT patients, which can negatively impact regulatory and antiviral T cell products (TCPs) resulting in limited efficacy or long-term performance. To improve performance and effectiveness of Treg as well as antiviral TCPs, we hypothesized that knock out (k.o.) of an adaptor protein required for function of a particular immunosuppressive drug, would desensitize T cell products to this immunosuppressant and allow sustained function even in the presence of immunosuppressants. Indeed, we generated Treg and CMV-specific TCPs, which are resistant to a commonly applied immunosuppressant by transfer of nucleoprotein complexes of the nuclease CRISPR-associated protein 9 (Cas9) with a site-specific guide RNA resulting in vector-free knock out (k.o.) of the cell-intrinsic target protein. We proofed the concept by assessment of the function of Treg and CMV-specific T cell products in the presence of distinct immunosuppressive drugs, in suppression assays or upon CMV-specific stimulation, respectively. Currently, we exclude off-target effects for safety assessment and as a prerequisite for ultimate GMP-compliant translation of T-k.o. -TCPs to first-in-human application. Once safety issues are addressed, this minimally manipulilative k.o. strategy may allow for imense increase of functionality of regulatory, antiviral and even anti-cancer TCPs in immunosuppressed patients and substantially reduce suffering, morbidities and deaths in this patient group.

Tissue Manufacturing by Bioprinting: Challenges and Opportunities for Regenerative Medicine

Fabien Guillemot - Poietis, France

Despite substantial investments to meet clinical and commercial expectations, and while scientific achievements at the preclinical research stage have sometimes been impressive, scaffold-based Tissue Engineering approaches are struggling to find the way to therapeutic and industrial success depriving patients to benefit from innovative treatments. Main challenges for the manufacturing of tissue engineered advanced therapy medicinal products (ATMPs) concern the improvement of the standardization of manufacturing processes, tissue functionality, and cost-effectiveness and profitability of related treatments. Producing advanced therapy medicinal products remains a cumbersome process with costs, reproducibility and scalability issues. Poietis develops biomanufacturing solutions based on bioprinting technologies for the design and the production of tissues, like the full thickness skin model Poieskin®, where automation of production processes is key to achieve quality controlled and repeatable bio-manufacturing. Based on our experience we will discuss how next-gen bioprinting technology - thanks to its characteristics resulting from the convergence of automation, biology and digital technology - should make it possible to overcome current tissue manufacturing bottlenecks and also provide new therapeutic opportunities.

Self-assembly of stem/progenitor cells creates human neo-vascularized skin and skin organoids

Patricia Peking - Paracelsus Medical University, Austria

Stem/progenitor cells bear the potential to self-assemble, creating organoids that resemble the organ functions *in vitro*. Here we established a humanized skin regeneration mouse model, based on self-assembly of adult as compared to iPSC-derived skin cell lineages forming neo-vascularized human skin. Adult endothelial cells (EC), skin fibroblasts (FB) and epidermal keratinocytes (KC) were propagated in 2D under xeno-free conditions. In addition, umbilical cord blood-derived iPSC were differentiated into iPS-EC,-FB, and-KC. Cell identity and purity were confirmed by flow cytometry and clonogenicity indicating their stem/progenitor potential. Skin organoid formation was performed to investigate cell self-organisation supported by human platelet-derived growth factors. Via life cell tracking sequential organoid assembly starting from stromal-vascular aggregation and followed by superficial anchorage of KC was revealed. Xeno-free human cell grafts, containing a mixture of KC, FB and EC in human platelet lysate (HPL) were transplanted onto full-thickness wounds of NSG mice using a transplant chamber to circumvent murine skin contraction. Two weeks after transplantation, histological analysis demonstrated appropriate cell organization into layered skin and a regular distribution of collagen fibers and ground substance. Immunohistochemistry confirmed the human origin of the grafts and a combination of murine and human neo-vasculature. Quantification showed significantly increased vessel numbers upon co-transplantation of EC compared to limited murine in-sprouting angiogenesis after transplantation of KC+FB only. The data show that self-assembly of human KC+FB combined with co-transplanted EC and HPL can create complex organoids *in vitro* and human neo-vascularized skin *in vivo*, building the basis for novel skin regeneration strategies.

Using clinical data for manufacturing design and release criteria to improve the quality of a cell-based ATMP for cartilage repair

Giulietta Roel - CO.DON AG, Germany

Background: Autologous cell-based ATMPs are characterized by a high biological variability between produced batches. This makes it very challenging to design a manufacturing process that performs consistently and delivers products that meet their intended function and the high quality criteria for cell-based ATMPs. Process validation and acceptance limits for in-process controls and release parameter require a different approach as used for other drug products. Aim: Apart from finding the right parameters, it is essential to addresses how the operational ranges of the manufacturing process can be optimized using clinical data. In addition, it is essential to use clinical data to define true decisive acceptance limits for release parameter to avoid release of suboptimal batches. Methods: Therefore, statistical correlation analyses were conducted between process parameters and clinical improvement data of 120 patients from Phase II and III treated with ACI (KOOS score, 1 year follow-up). This approach identified cell culture time as a critical process parameter that negatively correlates with the product's efficacy. In addition, responder versus non-responder analyses, were used to define clinical effective ranges of process parameters, as well as acceptance criteria for biological activity of the final product. Conclusion: These findings underscore the need to use clinical data to optimize the manufacturing process for autologous cell-based therapies. Moreover, clinical data are vital to understand process validation results as well as to define clinical relevant acceptance criteria for quality control parameter.

Harmonisation of multicenter MSC production for a phase III clinical trial

Melissa Van Pel - Leiden University Medical Center, Netherlands

The RETHRIM consortium conducts the first Europe-wide placebo controlled randomised phase III trial using bone marrow-derived mesenchymal stromal cells (MSC) for the treatment of steroid-resistant Graft versus-Host Disease. In this study, eligible patients receive second line immune suppression with i.v. mycophenolate mofetil and are randomised to receive additional treatment with either MSC (2 x10^6 per kg) or placebo i.v. at 1 day and 8 days following randomisation. In this study, centers from 6 different EU countries are participating and MSC are generated in 3 production centers across Europe. Therefore, comparability studies are required to demonstrate product equivalence. Following harmonisation of the complete production process at the level of an Investigational Medicinal Product Dossier (IMPD), a comparability study was designed to compare MSC product characteristics, including identity, purity and potency as well as in-process monitoring tests and in-process controls. The starting material for these comparability tests were obtained in a single center and distributed to the other manufacturing sites. MSC production was started at the same time in all centers and cells were produced under real-time conditions using local standard operating procedures (SOPs). Initially, a two-fold difference in ficoll yield and the amount of MSC harvested were observed, while viability and phenotype of the cells (including expression of CD73, CD90, CD105 and CD271) were similar. After a thorough comparison and revision of local SOPs, manufacturing procedures, such as the ficoll procedure, were further harmonised. In subsequent comparability tests, product characteristics and in process controls were similar, indicating the importance of further protocol harmonisation. However, differences in cell yield remained, which may be explained by differences in T flask manufacturers.In conclusion, harmonisation of cell therapy product generation at multiple production sites not only requires harmonisation of the IMPD, but also of SOPs at a level far beyond the details addressed in an IMPD.

Manufacturing of stem cell derived *in-vitro* tissues using robot technology

Marco Metzger - Fraunhofer Institute for Silicate Research, Germany

Background: One of the biggest challenges in the *in-vitro* generation of functional tissues for applications in regenerative medicine is the provision of scalable and highly reproducible manufacturing procedures. Using a flexible robot production platform according to the concept "build-2-order" (BTO) and an automised documentation system could provide a high quality and flexibility in the generation of a variety of different tissues. We have experiences in the automised generation of nanoparticles, organoids (intestine) and transwell-like cultures (skin). Aim: We are aiming to apply an existing two-arm robot system in the different RESTORE subprojects. The robot can work autonomously and interact with the relevant periphery equipment. By working under a GMP-like environment this allows the production of individual tissues in the most standardized way needed for clinical applications. Methods: We establish protocols for standardized 24/7 production of a variety of stem cell derived tissues using an existing robot station. The platform includes a state-of-the art cell culture lab environment and analytical equipment e.g. automated 3D microscopy, which can interact with the robot. Conclusion: The combination of automisation strategies and 3D cell culture techniques will allow patient-specific manufacturing of tissues according to the BTO concept.

Human induced pluripotent stem cells from universal donors as starting material for regenerative therapies

Micha Drukker - Helmholtz Center Munich, Germany

The use of human induced pluripotent stem cell (iPSC) derivatives such as pancreatic islet beta cells for clinical applications necessitates the creation of human iPSC lines manufactured in compliance with governmental directives and regulations. Defining the processes of iPSC manufacturing will furthermore create the basis for production of patient-derived iPSCs for autologous cell therapies.

The goal of our project is to create standardized processes and a well-qualified collection of GMP-grade human iPSC lines derived from healthy donors that harbor a unique repertoire of immunoregulatory molecules. Based on more than 70.000 donors from the German Red Cross Blutspendedienst Nord-Ost, we calculated that iPSC lines from only 3 donors with most prevalent homozygous HLA-class-I haplotypes, termed "universal donors", will be sufficient to provide transplantation tolerance in more than 30% of the German population and around 50-75 millions of individuals of Western Europe decent. We developed a process that begins with the derivation of primary fibroblasts from skin punch biopsies of the most suitable, healthy "universal donors". The reprogramming process is based on transient transfection of synthetic RNA transcripts (mmRNA) that encode for pluripotency factors, which we believe represents the safest current methodology to derive iPSC lines. We aim to produce human iPSC lines in compliance with current regulations of medicinal products and GMP standards, in order to use them as starting material for the production of ATMPs in a variety of medical indications.

Gene therapy getting personal: mutation-specific editing and gene addition strategies

Carsten Werner Lederer - The Cyprus Institute of Neurology and Genetics and TEDDY, Cyprus

Background: Thalassemia is caused by deficient production of α- or β-globin. The latter form, β-thalassemia, has particular clinical relevance and is common in many Southern regions and in recent immigrant populations in the EU. Beta-thalassemia as an early-onset severe monogenic blood disorder is an ideal target for advanced therapy medicinal product (ATMP) development based on gene addition or gene correction. It is also a frequent testbed for more widely applicable ATMP technology, including the establishment of efficient mutation-specific therapies. Aim: With focus on the common β-thalassemia mutation, HBBIVS1-110(G>A), this study demonstrates the development of highly efficient mutation-specific gene addition and gene disruption strategies applicable in primary cells. It further extrapolates the applicability of one of our approaches, the disruption of aberrant regulatory elements by designer nucleases, to a large number of mutations causing human disease. Methods: NHEJ-mediated disruption was performed in erythroid cell lines and patient-derived CD34+ cells, using plasmid transfection, lentiviral vectors or nucleofection of *in vitro* synthesized TALEN mRNAs and CRISPR/Cas9 RNA-guided nuclease (RGN) ribonucleoprotein complexes. Post-disruption analyses were performed at the level of DNA, RNA, protein and cell-morphology. Additional mutations, subjected to in silico disruption and splice site analyses, were retrieved from molecular databases and systematic literature searches. Conclusion: Gene addition based on small lentiviral transgenes and functional repair by TALEN and RGN based on target disruption showed exceptional functional correction for the targeted β-thalassemia mutation. Application of the latter with directly translatable (virus- and DNA-free) delivery showed high bulk disruption efficiencies, minimal toxicity and minimal off-target activity on the HBB paralog HBD in primary patient-derived cells, and is suitable for a large number of human diseases. Application of the current approaches therefore has great scope for personalised ATMP development.

T cell immunity toward CRISPR-associated nucleases

Dimitrios Laurin Wagner - BCRT, Charité Universitätsmedizin Berlin, Germany

CRISPR-Cas9 gene editing is a promising technology for the treatment of inherited diseases. Previous gene therapy trials suggest that pre-existing T cell immunity towards the delivery vector leads to decreased efficacy (e.g. gene therapy with AdV or AAV). We investigated the prevalence of T cell responses towards different CRISPR-associated nucleases (Cas) (Streptococcus pyogenes SpCas9, Acidominococcus Sp. AsCpf1) in the peripheral blood of healthy human donors and detected a ubiquitious effector/memory T cell response with multi-functional cytokine profile and cytolytic activity towards Cas9-transduced target cells. Cas-specific effector T cells could potentially migrate to CRISPR/Cas9-edited tissues and inflict damage to the targeted organ. In depth analysis of the activated T cells revealed a high frequency of Cas-reactive regulatory T cells. Cas-reactive regulatory T cells were able mitigate Cas-specific effector T cell proliferation and function *in vitro*. In conclusion, immunosuppressive treatment should be considered in first clinical trials using the current CRISPR-Cas9 technology *in vivo*. Our results emphasize that immune monitoring before, during and after application of CRISPR/Cas9 must be performed to identify high risk patients (potentially with low Treg/Teff ratio) and stratify immunosuppressive treatment accordingly. Further studies should investigate how pre-existing immune responses to Cas can be adequately prevented or delayed. We propose that adoptive transfer of Cas-specific regulatory T cells may circumvent the need for global immunosuppression in patients with pronounced pre-existing T cell immune reactivity to Cas.

Tissue-Engineered ATMPs for cartilage healing

Oliver Pullig - Fraunhofer Translational Center Regenerative Therapies, Germany

Background & Aim: Translational Center Regenerative Therapies (TLC-RT) in Würzburg, Germany is focusing on innovative strategies for fast-track translation of ATMPs into the clinic. Recently, we received manufacturing authorization for two combined ATMPs covering cartilage defects. Beside GMP conform production, our innovative manufacturing comprises

- Bio-reactor technology for 3D ATMPs
- Non-invasive quality control and advanced potency assays under regulatory validation
- Process automation under GMP

Method & Clinical trial: The phase II clinical trial "Nose-to-Knee2", granted by the EU (BIO-CHIP project) is addressing two innovations in the ATMP field.

1) the use of autologous nasal chondrocytes as a cell source superior compared to articular chondrocytes
2) the clinical application of a mature graft as opposed to an immature graft

The goal of this trial is the comparison of the clinical efficacy of a mature graft (Nasal chondrocyte tissue engineered cartilage, N-TEC) with that of an immature graft (Nasal chondrocyte cell activated matrix, N-CAM) for the treatment of cartilage lesions in the knee.

Results: More than 70 grafts have been produced and implanted successfully. No SAE related to the product have been observed. Mid-term analysis of clinical data revealed a beneficial effect in patient outcome. This result has been seen in both trial arms for the long-time and short time maturation.

Conclusion: GMP conform ATMP production can be realized in an academic environment. Next stage of development and reaching market readiness level requires industrial mindset plus innovative solutions for an efficient process workflow and cost reduction (Automation, non-invasive QC). These infrastructural requirements plus participating hospitals are present at TLC-RT as an Academic Translational Hub.

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Personalized therapy for TP53 mutated cancer patients based on CRISPR-Cpf1 and suicide gene delivery

Flaminia C. Lorenzetti - University of Siena, Italy

Mutations in the TP53 gene are one of the leading causes of both solid and hematological tumors. In a cohort of 96 advanced cancer patients with 13 different solid tumors, using NGS-liquid biopsy, we demonstrated that relapse is led by a clone with TP53 point mutation in more than 50% of patients. Unfortunately, drugs targeting TP53 mutations are not yet available and patients with either advanced solid cancer or leukemia can't be treated. We present here an example of transformative medicine based on gene editing of a recurrent TP53 mutation (c.548C>G; p.Ser183*) using CRISPR/Cpf1 and suicide-gene approach. We successfully treated engineered HEK293 holding the target mutation and experiments on a mouse model of Chronic Lymphocytic Leukemia (CLL) are ongoing. The treatment is based on locus specific integration of thymidine kinase (TK) suicide gene and *in vivo* delivery of a lentiviral (LV) vector bearing both the CRISPR/Cpf1 system and the TK sequence. Once the suicide TK gene is properly integrated, the treatment with the antiviral drug ganciclovir (GCV) induces death of cancer cells. We successfully detected 40% of cell death *in vitro* in the samples carrying the TK sequence and treated with GCV compared to controls. Xenograft NOD/SCID IL2Rgnull mice are obtained by intravenous injection of peripheral blood mononuclear cells (PBMC) isolated from CLL patients. Animals are treated with busulfan a day before cells injection for the correct growth of neoplastic B cells. CLL is obtained after 4 weeks from PBMC injection in control mice. Treated mice are intravenously injected with CRISPR/Cpf1 viral particles and the day after GCV is delivered by intraperitoneal injection. Animals are monitored weekly to detect a decrease in neoplastic B-CLL cells count. This study can open the way for an efficient and specific approach which might move to the clinic in the near future. Our system has been patented in 2018.

Generation, cultivation and characterization of stem cell-derived bioartificial cardiac tissue of clinically relevant dimensions

Ina Gruh - Hannover Medical School, Germany

Background - Human induced pluripotent stem cells can be differentiated efficiently into functional cardiomyocytes (iPSC-CMs). Combined with tissue engineering strategies, iPSC-CMs offer the unique opportunity to generate myocardial tissue *in vitro* as a potential therapeutic option to replace damaged myocardium after myocardial infarction.
Aim - We aimed at the generation of a large-scale stem cell derived bioartificial cardiac tissue (BCT) and an investigation of tissue maturation and functionality *in vitro*.
Methods - Large-scale BCT was generated from iPSC-CMs mixed with human fibroblasts and a hydrogel solution in a newly designed tissue chamber with a diameter of 43 mm. Tissue development was recorded microscopically. Maturation of CMs within the tissue was confirmed by immunofluorescence staining for cardiac markers (α-SA, cTnT) as well as cell junction proteins (Cx43, N-Cadherin). The integration of the tissue chamber in the novel custom-made bioreactor allows investigating the initial parameters for electromechanical stimulation (such as pressure, stress amplitude, frequency) necessary for proper tissue maturation in a more physiological manner. To investigate the contraction-associated calcium oscillations through the tissue a new transgenic hiPSC line containing a genetically encoded calcium indicator (GCaMP6f) was developed.
Conclusion - Microscopic evaluation revealed a progressive reduction of nearly ~27% of the initial volume after 15 days as a result of matrix remodeling. After 48h, the tissue exhibited first spontaneous contractions, which were coordinated and rhythmic within the whole tissue after 6 days. Video-optical analysis showed an estimated beating rate of 102 beats per minute and GCaMP6f fluorescence confirming periodic calcium increase in BCTs. Immunofluorescence staining of BCT sections performed at day 15 showed highly organised cross-striations of sarcomeric proteins. Thus, our results confirm that functional large-scale BCTs can be generated from human iPSC-CMs. Electromechanical stimulation and characterization in our bioreactor system is ongoing, and will be followed by *in vivo* testing in small and large animal models.

Combined cell and gene therapy for Epidermolysis Bullosa

Michele De Luca - University of Modena, Italy

LAMB3-dependent generalized Junctional Epidermolysis Bullosa (JEB) was targeted by transplantation of epidermal cultures originated from transgenic epidermal stem cells. We report life-saving regeneration of the entire epidermis on a seven-year-old JEB child suffering from a devastating form of JEB. The regenerated transgenic epidermis remained stable throughout the entire follow-up period and did not form blisters, even upon shear force. The proviral integration pattern was maintained *in vivo* and epidermal renewal did not cause any clonal selection. Clonal tracing showed that the human epidermis is sustained by a limited number of long-lived stem cells, detected as holoclones, that can extensively self-renew and produce short-lived progenitors that replenish terminally differentiated keratinocytes.
In studying the different behaviour of JEB and COL7A1-dependent generalized Dystrophic EB (RDEB) cultures we discovered a pivotal role of YAP in sustaining human epidermal stem cells, which explains the progressive stem cell loss observed in JEB. Epidermal stem cell depletion of primary JEB keratinocytes is due to perturbation of the YAP/TAZ pathway. YAP/TAZ expression is significantly decreased in JEB keratinocytes, which do not contain nuclear YAP but only phosphorylated, inactive YAP. The JEB phenotype is recapitulaled by Laminin 5 ablation and consequent YAP/TAZ down-regulation in normal cells. Restoration of adhesion properties by Laminin 5-gene therapy rescues normal nuclear levels of YAP/TAZ and clonogenic potential. Enforced YAP recapitulates Laminin 5-gene therapy in JEB cells, thus uncoupling adhesion from proliferation in epidermal stem cells. This work has important clinical implication for an efficient *ex vivo* gene therapy of JEB.

Clinical implementation of cornea and skin artificial tissues generated with fibrin-agarose biomaterials

Antonio Campos - University of Granada, Spain

Development of novel tissues by tissue engineering for clinical use is one of the main objectives of present-day medical histology. The tissue engineering group of the Histology Department of the University of Granada (Spain) has designed new models of human cornea and skin that have been translated to the clinical setting following the EU rules for advanced therapies. Clinical implementation of these tissues was the result of a cooperation program between the University and the public health system. This cooperation was promoted by the specific agency supporting clinical translation of advanced therapies in the autonomous region of Andalusia (Andalusian Initiative for Advanced Therapies - IATA).
In the first place, the Department of Histology was responsible for the design of the novel tissues, including development of novel fibrin-agarose biomaterials and biofabrication methods and all required preclinical quality controls (genetic, histology, histochemistry, immunohistochemistry, biomechanical, optic and *in vivo* analyses), and histological analysis of samples implanted in patients.
In the second place, IATA elaborates the necessary documentation and forms for the authorization of the clinical use of the different tissues by the National Medicines Agency under the EU regulation, and the subsequent fabrication of the tissues as pharmacy-degree medical products in GMP facilities.
Finally, professionals of the health system are the responsible for the clinical use of these novel therapies in the context of a clinical trial or as compassionate use.
Preliminary results of the clinical implementation of the new models of cornea and skin advanced therapies medical products are positive, and the cooperation designed for the clinical translation of these products resulted to be very effective.

Car-T cell therapy - a clinical experience report

Michal Besser - Ella Lemelbaum Institute of Immuno-Oncology Sheba Medical Center, Israel

Autologous CD19 chimeric antigen receptor (CAR) T cells demonstrate outstanding remission rates in pediatric and adult patients with relapsed and refractory acute lymphoblastic leukemia (ALL) and Non-Hodgkin lymphoma (NHL). Two CD19 CAR T cell products received recently FDA approval.
Patients and method: In October 2017, the Sheba Medical Center initiated the first phase 1b/2 study with in-house produced CD19 CAR T cells in Israel. Gamma-retrovirus encoding for a CD19 CAR based on an FMC63 derived scFv, CD28 costimulatory domain and CD3-zeta signaling domain was used for the study. CAR-T cells were produced from patients' PBMC within 9-10 days.
Results and discussion: Until June 2019, 95 patients (half ALL and half NHL) were enrolled to the trial and 90 (95%) patients were treated. CAR T cells were successfully produced for 94 of 95 (99%) patients. Four patients dropped out of the study due to clinical deterioration. The median age of patients was 34 years (range 22 months – 71 years). Patients had a median of 3 prior regimens, including other CD19-based therapies and stem-cell transplantations. The overall response rate of evaluated patients was 70% (81% in ALL and 56% in NHL), including complete remission in 53%. The 1-year overall survival was 61%.
Conclusion: The clinical response rate and overall survival in refractory patients treated with in-house produced CAR T is remarkable and comparable with commercial CAR T products. The extremely fast turn-around time from PBMC collection to CAR T infusion of only 9-10 days, in contrast to 30-60 days with commercial CAR T products, and the high production success (99%) enabled the treatment of 95% of enrolled patients. We are currently aiming to transfer the production process into an automated system and make the therapy available to even more patients.

Reshape immune balance by next-generation regulatory T cells

Petra Reinke - BeCAT - Charité Universitätsmedizin Berlin, Germany

Adoptive transfer of regulatory T cells (Treg) is a promising new therapeutic option to reshape undesired intra-tissue immune imbalance in immune-related disease entities. It supports long-term function of allografts and use of Advanced Therapy Medicinal Products (ATMP) by overcoming the challenge of unwanted immune reaction by the recipient of the ATMP. Therefore, adoptive Treg therapy is a potential game changer in health care, particularly in immune diseases, organ & hematopoietic stem cell (HSC) transplantation, and regenerative medicine, including gene therapy.
Based on the Triple-T concept - Transdisciplinarity, Technology, Translation - the major goal of RESHAPE is to transform the treatment of patients suffering from undesired immunity/inflammation, who presently have limited curative treatment options, by applying novel Treg approaches that overcome the limitations of 1st generation Treg product developments.
Members of the consortium, with academic and biotech backgrounds, are pioneers in the development of Treg therapy from basic science to very recent encouraging First-In-Human (FIH) clinical trials of the 1st generation Treg products. They have a longtrack record of collaboration, including in EC-funded projects. The first clinical trials were performed to combat organ transplant rejection and Graft-versus-Host-Disease. However, promising preclinical studies offer a broad application field of Treg therapy beyond allotransplantation.
Based on our preclinical & clinical data, we have identified several opportunities for improving Treg therapy, such as enhanced antigen specificity & functional stability, and recipient conditioning, that will be addressed by RESHAPE. The next-generation Treg products, developed by advanced technologies including CRISPR/Cas9, will be tested on platforms applying new methods for cell characteristics in both *in vivo* / *in vitro* models, and finally proven in FIH-clinical trials accompanied by biomarker and health economic studies.

Cellular therapy: Boosting innovation in technology and clinical translation

Mario Assenmacher - Miltenyi Biotech GmbH, Germany

Background and aims: Presently, an exponentially growing number of CAR T cell trials is driving the field. This is reflected by the recent increase in CART trials - from 35 worldwide in 2013 to more than 500 in 2019. However, ATMPs as "living drugs" are different from conventional therapeutics in their requirements for implementation into clinical routine. This lies in the complexity of the manufacturing, logistics and supply chain processes with multiple steps and high technical demands. In addition, treatment of the patients is extremely complex and requires an experienced multidisciplinary team (clinicians and nurses, geneticists, biologists, regulators, quality experts, pharmacists, etc) that is able to handle the specific requirements of cellular therapies as well as their possible complications (e.g. cytokine release syndrome or neurotoxicity in case of CAR T therapies).
Methods and conclusion: Here we will present some of our clinical experiences: Miltenyi Biotec is not only a recognized provider of advanced technologies and innovative tools but also is a leading European driver to advance translation of clinical cell therapy studies. For instance, we have recently received approval for three company-initiated CAR trials (CIT) in Germany and are recruiting patients since 2018. In addition, we were the first in Germany to receive manufacturing authorization for CAR T cells for our GMP facility and have been actively contributing to more than 150 investigator-initiated cell therapy studies (IIT) worldwide during the last decade. Our experiences in manufacturing and regulatory matters will be transferred to other researchers in order to support them in setting up their own cell manufacturing sites. For instance, we are developing "blueprints" for regulatory documents that will help other researchers to deal with their specific regulatory issues. Also, we are working on innovative approaches to improve efficacy and safety of ATMPs to overcome their current clinical limitations especially for treatment of solid tumors.

Development of allogeneic Placenta-derived (PLX) cell therapy - from bench - to bedside

Racheli Ofir - Pluristem LTD, Israel

The placenta provides a noncontroversial source of young, healthy cells of both maternal and fetal origin from which cell therapy products can be obtained. The two advantages of using live cells as therapeutic entities are their environmental-responsive, multifactorial secretion profile and their activity as a "slow-release drug delivery system". PLX cells are placenta-derived, mesenchymal-like stromal cells for allogeneic use. PLX are manufactured using a 3D bioreactor which provides tightly controlled, completely automated, efficient and scalable cell expansion system. Data from multiple *in vitro* and *in vivo* experiments indicate that PLX act via a paracrine/endocrine manner to facilitate healing of damaged tissue. Two lead cell products- PLX-PAD and PLX-R18, are in clinical development for several indications. PLX-PAD secretes proteins known to support angiogenesis, muscle regeneration and to modulate the immune system. Phase I trials of PLX-PAD in Critical Limb Ischemia (CLI) showed positive results, and a randomized, double-blind Phase II study demonstrated safety and efficacy of PLX-PAD in treating muscle injury in the context of hip replacement surgery. Positive results were also seen in a completed Phase II trial in Intermittent Claudication. PLX-PAD is currently studied in advanced clinical trials in CLI and in supporting muscle recovery following Femoral Neck Fracture. PLX-R18 secretes factors that enhance regeneration of damaged Hematopoietic system and is in advanced development stages for Acute Radiation Syndrome and in a Phase I study for support of incomplete engraftment following BM transplantation. Presentation will focus on the MoA of the cells and data from animal and clinical studies.

Role of the EMA Committee for Advance Therapies (CAT) in the assesment of ATMPs

Paolo Gasparini - University of Trieste IRCCS, Italy

Advanced Therapeutic Medicinal Products (ATMPs) are complex medicinal products that offer great promise to address some of today's unmet medical needs. ATMPs include: gene therapy medicinal products (GTMP); somatic cell therapy medicinal products; and tissue engineered products. The development of ATMPs requires levels of high-specialization biomedicine and technical know-how.

In the EU, ATMPs are governed by Regulation 1394/2007 and receive their marketing authorization (MA) on the basis of this legislation. Although hugely promising, AMTPs can pose risks, including immunogenicity/rejection, possible development of tumours, and dedifferentiation or loss of cell function. Therefore, ATMPs undergo a thorough review to ensure that products are safe and effective by European Medicines Agency (EMA) (i.e. Committee for Advanced Therapies (CAT) for draft opinion preparation followed by final adoption by Committee for Medicinal Products for Human Use (CHMP)).

Taking into consideration the intrinsic characteristics and variability of ATMPs as distinct treatments, a regulatory flexibility can be applied such as the adoption of a specific Good Manufacturing Practice (GMP) framework and the possibility to relies on the Risk Based Approach (RBA) when providing an opinion on MA. This determines the extent of quality, non-clinical and clinical data that needs to be included in MA applications.

Of the currently-authorised products, most have been approved in the past five years. As new technologies emerge (i.e. genome editing) and more ATMPs undergo clinical trials, it is expected that the number of applications for marketing authorisation will significantly increase. Due to the inherent differences between the types of ATMPs and difference in how the products work, creative thinking is required.

MSCs as a therapy for diabetic complications: a translational journey in the EU

Timothy O'Brien - National University of Ireland Galway, Ireland

Mesenchymal stromal cells (MSCs) have substantial therapeutic potential by virtue of paracrine and immunomodulatory properties. Our laboratory has been interested in the use of MSCs for the treatment of diabetic microvascular and macrovascular complications. Diabetes mellitus is assuming pandemic proportions and approximately 600 million people will have this condition by 2030. We have used bone marrow derived MSCs in a phase 1 clinical trial of no option critical limb ischemia and currently are recruiting patients to a phase 1b/2a clinical trial in patients with diabetic kidney disease. We also have translational programmes in diabetic retinopathy and neuropathy. In addition, we have developed a consortium of GMP facilities in the EU for production of MSCs for clinical trials. Data from the pre-clinical pathways for the use of MSCs for the treatment of diabetic complications will be presented. In addition the results of the CLI trial showing the challenges associated with autologous MSC approaches to this condition will be discussed. Finally, issues of cell manufacturing of MSCs for clinical trials in EU countries included experience with the Voluntary Harmonized Procedure will be described.

An Integrated Eco-System of Research and Development for Orphan Medicinal Products: the IRDiRC Orphan Drug Development Guidebook

Michela Gabaldo - Fondazione Telethon, Italy

There are about 400 million people worldwide suffering from 6000-8000 rare diseases. However, therapies are available for only a subset of patients, and the development of new therapies for rare diseases remains challenging. It is therefore why the International Consortium for Rare Diseases (IRDiRC) was set up, with as goal the approval of 1000 new therapies for rare diseases. However, in order to enable the eco-system of research and drug development in rare diseases to achieve this goal, a quantum change is needed in the way orphan medicinal products are developed. IRDiRC set up the Orphan Drug Development Guidebook Task Force which aims at creating a simple guidebook describing the available tools and initiatives specific for rare disease development and how to best use them. Integration of such elements within a defined drug development pipeline is set out to generate better data quality, shorter development timelines, and better R&D efficiency. To address these challenges, the IRDiRC Task Force gathered multi-stakeholders drug development experts, to create the guidebook, annotating the available tools, resources, and initiatives specific to rare disease drug development. Different associated cases (scenarios implying development of a traditional small molecule/biologic or an advanced therapy medicinal product) were discussed on how best to use them. Furthermore, an additional "impossible" case was studied (low disease prevalence, high paediatric population, slowly progressing disease), which in fact presents the majority of drug developments for rare diseases, all while focusing on the patient's needs as the focal point. The Guidebook includes fact-sheet descriptions of each drug development tool or resource (covering a large number of initiatives that are available worldwide), a series of standard use cases defining how and when to use them, and a series of practical checklists of items to consider at each step. The aim of the Guidebook is to benefit the various stakeholders working in orphan drug development, thereby creating a new research and drug development ecosystem, by highlighting and clarifying already existing tools in the rare disease space, so that we can jointly progress towards treatment for all rare diseases.

State and potential for data-driven strategies in the cell and gene therapy industry

Marc-Olivier Baradez - Cell and Gene Therapy Catapult, United Kingdom

With the accelerating pace of cell and gene therapy products being developed and reaching the clinic, there is an awareness that opportunities around data utilisation are yet to be fully realised. The field is transitioning from the use of conventional analytics such as flow cytometry and RT-qPCR to characterise and evaluate the products, to more advanced analytics such as spectroscopies, NGS, metabolomics, as well as outputs from integrated sensors. This ever-increasing mass of data should open up improvements in product design, process optimisation, adaptive manufacture, as well as reduce product life cycle. Current limitations to exploiting this wealth of data lies with the fragmented nature of the datasets, the complexity of merging structurally different datasets, the discrete nature of the data acquired over different phases of product development with breaks or steps in the data continuum (e.g. when new technologies are introduced), and more often than not the lack of depth in the available data. This talk aims to describe the current state of the art for data utilisation in this field and the opportunities arising from agile data capture, seamless data flow and automated processing. Cloud-based infrastructures and the emerging potential of artificial intelligence to complement the portfolio of statistical and modelling tools will be discussed in relation to product development, optimisation, manufacture and the wider healthcare implications.

Ethics management of science and the public

Andreas Kurtz - BCRT, Charité Universitätsmedizin Berlin, Germany

The lack of a unified strategy to deal with ethical and project risks can slow progress and isolate the group from highly rewarding areas of research. The strategy to minimize risk and liability can, however, be transformed from a defensive posture into a publicly visible advantage. This can occur if the effort to identify and minimise risk provides a comprehensive set of general ethics and regulatory documents and support. Centralized Ethics (Risk) assessment and management should push this comprehensiveness and moreover provide a way to optimize use of resources, adequate start-up support and oversight. An effective ethics tool for example based on consent categorization and automated gap identification may streamline this process and make it integrative. Moreover, proactive risk management will promote confidence in public discourse and shaping public and regulatory opinion. This requires the integration of the concrete ethics issues into a future oriented framework that is able to anticipate ethics dilemmas and support answering these scientifically and ethically. The outlined ethics approach will be described using examples from the area of gene editing, genetic data and stem cell research and application.

Ethics in scientific communication

Pier-Maria Fornasari - Regen Health Solutions, Italy

Background: The primary societal mission of basic biomedical research and its clinical translation is to alleviate and prevent human suffering caused by illness and injury. Stem cell research receives a great deal of attention from policy makers, the popular press and popular culture, including social media. Popular coverage and reporting in the medical literature are frequently far from ideal. Potential benefits are sometimes exaggerated and the challenges to clinical application and risks are often understated. Inaccurate or incomplete representations of this sort can have tangible impacts on the expectations of the general public, patient communities, physicians and on the setting of health and science policies. Aim: Misinformation or information that is false or misleading can quickly reach thousands or millions of readers, helped by inattentive or malicious sharers and algorithms optimized for engagement. In this presentation the ethics in scientic communication will be approached in a double direction: research publishing and media information. 1) The pubblication process is based on credibility, truth, authenticity and scientific honesty. The researchers, the academies and the editors should follow the European Code of Conduct for Research Integrity published il 2017 by ALLEA. 2) Media Ethics concerns itself with ethical practice in journalism and information dissemination, and includes issues as diverse as conflicts of interest, source transparency, fairness, fake news, and information accuracy, as set in Associated Press Managing Editors. Code of Ethics. 1995 Methods: Misinformation or false information in communication of stem cells research and results has exponentially increased in the last years. The pervasive presence of misinformation related to stem cells can influence the wide range of perceptions that individuals have. An exaggeration of benefits or risks, especially when accompanied by highly inaccurate information, can only serve to confuse or mislead the public on issues related to stem cells, including the readiness of novel stem cell interventions for clinical application. In the presentation, a review of the most recent studies on stem cells research communication will be evaluated. Conclusion: The stem cell research community needs to strive to make sure there are realistic and scientifically accurate portrayals of the research in popular culture and if an inaccurate misrepresentation is seen, steps to correct should be taken. At last the scientific community needs to take care not to hype their work as hyped representations can be leveraged by the creators of fake news. RESTORE community can help European people to have an accurate information on Advanced Therapies field.

A Consistent Mechanism for Designing a Clinical Trial based on AI Technologies Applied to Observational Cohort

Michal Rosen-Zvi - IBM Research, Israel

Often the endpoints of innovative clinical trials of advanced therapies are events or outcomes that are recorded in a standard electronical medical record of a person who is diagnosed with the condition that is at the center of the clinical trial. In these cases, the design of a clinical trial might benefit from a study of an observational cohort that contains EMRs with longitudinal patients records of medical tests, medical interventions and other related medical information which have a large overlap with the information planned to be collected in a two arms case-control randomised clinical trial. That is, the patients at the control arm and the patients in the observational cohort are similar and insights drawn from analysing the observational cohort regarding a subgroup that has better prognosis than other, for instance, can serve in designing the selection criteria for the clinical trial.

Machine Learning is a common methodology for deriving insights from observational cohorts. Particularly, prediction models are often used as a mechanism to make a personalized assessment of disease prognosis. There are many established ways to assess the performance of such models e.g. evaluating the ability to predict a binary outcome through area under the ROC curve (Ferri et al. An experimental comparison of performance measures for classification 2009). However, a well corroborated association between certain patient characteristics and a binary outcome is not sufficient for the design of a clinical trial where the questions of interest are what would have happened to a patient had she was exposed to different interventions and the answer can serve for selecting patients more prone to successful outcome. Casual inference provides a mechanism for automatically and consistently analysing causal relations and results of such analysis might affect clinical trial, it is important to evaluate the analysis performance. A framework to evaluate causal inference studies was recently offered (Shimoni et al. Benchmarking framework for performance-evaluation of causal inference 2018); it provides a set of automatic tools to assess the quality of the inferred casual relations.

In silico regenerative medicine: from living implants to digital patients and back again

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As basic science advances, one of the major challenges in Tissue Engineering (TE) is the translation of the increasing biological knowledge on complex cell and tissue behavior into predictive and robust engineering processes. Mastering this complexity is an essential step towards clinical translation of TE applications. Computational (in silico) modeling allows to study the biological complexity in a more integrative and quantitative way. We have developed computational models related to all aspects of the TE product development cycle. Depending on the specific questions that need to be answered and on the available information, model systems can be purely data-driven (machine learning/AI) or more hypothesis-driven (mechanistic) in nature. At cell level we study cellular regulation through knowledge-based and multi-omics approaches (transcriptomics & metabolomics). For scaffolds, we use models that combine effects of geometry, composition and degradation on tissue growth to optimize scaffolds printed in a variety of materials. With respect to culture strategies, we combine the development of *in vitro* set-ups (e.g. stand-alone perfusion bioreactor) with their digital twins. For a prediction of *in vivo* processes, we look at normal and pathophysiological healing cases and design possible treatment strategies that we test in in silico clinical trials for pediatric orphan indications. To bring these models to the clinics and/or the market, various collaborations have been set up with clinicians and companies, in Belgium and Europe. An important prerequisite to this translation is the acceptance of digital evidence generated by in silico tools in biomedical R&D activities and in regulatory submissions to EMA and USA-FDA (the latter is a driver of the use of in silico tools). Through our involvement in the Virtual Physiological Human institute and the Avicenna Alliance, we are involved in interactions with a variety of stakeholders, including policy makers and regulators across the world, to establish proper policies, regulations and harmonized guidelines related to the use of in silico tools in advanced medicinal therapeutic product development & translation.

Stem cell data standards and data utility

Andreas Kurtz - BCRT, Charité Universitätsmedizin Berlin, Germany

Stem cell registries provide information and transparency for available cell lines. Their utility for assessing stem cell line quality and supporting a specific application depend on the type, quality and quantity of data associated with each registered stem cell line. Ideally, data on stem cells should also be annotated with data on clinical, preclinical or organ modeling applications. Such an enriched registry will only become a cell - focused Information hub if relevant data entities and data standards are used, which allow interoperability between research, manufacturing and clinical fields. The requirement for data standardization is often in conflict with current state of the art and fast technological developments, which affect data quantity, types and formats, making them a moving target for application of advanced machine learning algorithms. The example of the human pluripotent stem cell registry (http://hpscreg.eu) will be used to discuss obstacles and possible solutions to make cell - related data applicable for regenerative medicine and machine learning in the framework of other data sources, international initiatives and stem cell banks. Registries can furthermore serve as a community - monitored quality assessment tool for cell lines and data, eventualy be able to provide quality certificates to regulatory and funding agencies as well as publishers.

Stem cells for treatment of Amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder leading to the death of upper and lower motoneurons (MN). In our preclinical study human mesenchymal stem cells (hMSCs), were delivered intrathecally into SOD1 G93A transgenic rats. Survival in the hMSC-treated group was prolonged and rats showed better motility and grip strength. Quantitative analyses of wisteria floribunda (WFA) fluorescence intensity measured in the spinal cord, revealed greater numbers of perineuronal nets (PNNs) in the hMSCs-treated animals and MSCs have antiapoptotic and immunomodulatory effects. The clinical study was designed as a prospective, non-randomized, open-label study (phase I/IIa, EudraCT No. 2011-000362-35) to assess the safety and efficacy of autologous BM-MSC in the treatment of ALS. BM-MSC were applied via lumbar puncture into the cerebrospinal fluid. During the 18 month follow-up period potential adverse reactions. The clinical outcome was evaluated by a ALS functional rating scale (ALSFRS), Norris spinal and bulbar scale (NSS and NSB), forced vital capacity (FVC) and weakness scale (WS). To date, 26 patients were enrolled in the study. No suspected serious adverse reactions or new cerebrospinal pathology on MR examinations were observed. In almost 80% of patients FVC values remained above 60% for a time period of 12 months. A group of 14 patients, with remarkable pretreatment decline in functional scales (ALSFRS + NSS), had significant reduction/stabilization in their total functional score decline at 3 months after application ($p<0.001$ in ALSFRS, $p<0.05$ in NSS), which was less pronounced at 6 months ($p<0.01$ in ALSFRS) and 9 months ($p<0.05$ in ALSFRS + NSS). In this group we also observed stable WS values for a time period of 3 months after application. Our results demonstrate that the intrathecal application of BM-MSC in ALS patients is a safe procedure and that it can, at least temporarily, slow down progression of the disease.

Chimeric antigen receptor (CAR) immunotherapy for patients with solid tumors

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Chimeric antigen receptor (CAR)-T cell therapies have achieved revolutionary success in the treatment of CD19+ hematological malignancies, but their applications in solid tumors require further exploration. Results from a clinical phase I trial targeting carcinoembryonic antigen (CEA)-positive metastatic colorectal cancers will be reported. Through escalating dose procedure, the tolerable and effective dose of CEA-CAR-T cell therapy, together with lymphodepletion, was determined. CEA-CAR-T cell therapy was well tolerated. Of the ten patients in the trial, two patients who experienced progressive disease (PD) in previous treatments remained with stable disease for more than 30 weeks following CAR-T cell therapy. Nevertheless, to make CAR-T cell therapy successful in solid tumors, this therapy has to address two main challenges; these include overcoming tumor microenvironment-related immunosuppression, and improving the persistence of immune cells at the tumor site. These challenges can be overcome by using next generation CAR vectors and by optimizing the potency of the immune cells used.

SLAMF7 CAR-T cells for immunotherapy of multiple myeloma: 'real-world' experience of GMP-manufacturing using virus-free Sleeping Beauty gene-transfer

Michael Hudecek - Universitätsklinikum Würzburg, Germany

Introduction: We are developing immunotherapy for multiple myeloma (MM) with chimeric antigen receptor-T cells (CAR-T) specific for the SLAMF7 antigen, and are preparing a phase I/IIa clinical trial (CARAMBA project). We have shown that SLAMF7 CAR-T cure MM in pre-clinical models, and have validated GMP-compliant manufacturing for SLAMF7 CAR-T using Sleeping Beauty (SB) gene-transfer.
Methods: CAR gene-transfer into CD8 and CD4 T cells was performed by nucleofecting mRNA encoding hyperactive SB100X transposase and a minicircle DNA transposon encoding the SLAMF7 CAR. Training, scale-up and validation runs were performed with T cells from >20 healthy donors and MM patients.
Results: Training and scale-up runs were completed and optimal conditions for CD3/CD28-activation, subsequent nucleofection and expansion in gas-permeable culture flasks established. Three validation runs were passed and allowed obtaining therapeutic doses of SLAMF7 CAR-T cells. The average gene transfer rates were 51.9% in CD4 and 71.4% in CD8 T cells. SLAMF7 CAR-T exhibited an effector phenotype and were SLAMF7-/low, consistent with deletion of SLAMF7+/high T cells due to fratricide. SLAMF7 CAR-T products were formulated at 1:1 CD8:CD4 ratio and conferred complete elimination of MM1.S myeloma xenografts in mice. In a subset of mice, we observed myeloma relapse in extramedullary lesions, which was controlled by (memory) SLAMF7 CAR-T that re-expanded and re-induced remission. Genome analyses revealed average transposon copy numbers between 6 and 12, and showed that transpositions had occurred with an overall random insertion profile, characteristic for SB. The absence pf SB transposase in the infusion product was confirmed by Western blotting.
Conclusion: We present 'real-world' data on the manufacture of clinical-grade SLAMF7 CAR-T prepared by SB gene-transfer. This approach provides CAR-T products with excellent characteristics in safety and potency, and has favourable practical and socioeconomic attributes. A phase I/IIa clinical trial with SLAMF7 CAR-T in MM is being initiated and is the first in the EU to take advantage of SB as virus-free tool in T-cell engineering.

Cartilage regeneration with ATMP produced cells; past, present and future pertspectives

Anders Lindahl - University of Gothenburg, Sweden

Cartilage regeneration in patients are challenging due to the low tissue regeneration potential. Cell therapy for cartilage injuries was pioneered in the late 1980s by our group and after the published pilot study using autologous chondrocyte transplantation (ACT or ACI) in New England Journal of Medicine in 1994, we have subsequently treated over 2000 patients with cell from our ATMP approved laboratory. Worldwide the technology has been adopted with over 40 000 treated patients. In long-term follow up after 15 to 20 years we have demonstrated a restored knee joint function in over 80 % of treated patients. The clinical success has not been followed by a commercial success due to reimbursement issue with the exception in the US. Today there are few companies remaining on the market although the implantation technology has been improved with chondrocytes cultured on scaffolds thus enabling an arthroscopic procedure with shorter rehabilitation for the patient. The scientific view of cartilage as a non-regenerating tissue has been challenged and there is scientific evidence that the physiological homeostasis as well as repair are potentially emanating from stem cell niches in the joint. Patients with osteoarthritis (OA) suffer from several other systemic diseased e.g. arteriosclerosis or diabetes. The future treatment for the OA disease will reside on early diagnosis by biochemical and imaging monitoring enabling early intervention as well as treatment with ATMP products based on universal donor cell lines derived from induced pluripotent stem cells or allogeneic donors.

Adoptive Transfer of Thymic Derived Regulatory T-Cells in Therapy-Refractory Chronic Graft versus Host Disease in Children

Sybille Landwehr-Kenzel - BCRT, Charité Universitätsmedizin Berlin, Germany

Background: The pharmacological control of T-cell-mediated immune responses continues to be one of the greatest challenges in (transplant) medicine. Modern immunosuppressants remained insufficient to adequately improve long-term morbidity and mortality after solid organ and hematopoietic transplantation. Therefore, CD4+CD25+FoxP3+ thymus-derived regulatory T-cells (tTregs), the physiological mediators of immune homeostasis, have extensively been studied in recent years and their therapeutic potential appears promising in various allo- and autoreactive indications.
Aim: The aim of this project is the clinical implementation of *ex vivo* expanded tTregs as adjunctive treatment option for patients suffering from graft-versus-host disease (GvHD) after hematopoietic stem cell transplantation.
Methods: Over the last decade, we have overcome the technical and regulatory hurdles to manufacture tTregs for clinical applications. Due to lack of alternative treatment options we have treated 3 children suffering from severe chronic, therapy-refractory GvHD. At the same time, the pharmacological immunosuppression was substantially reduced. Regular follow-up included a broad spectrum of clinical, biochemical and immunological parameters.
Results: Initially, the clinical status of all patients stabilized. In the long-term follow-up, 2/3 patients showed a remarkable reduction in cGvHD activity at all manifestation sites and improvement in quality of life. Both infection frequency and immunosuppressive side effects were substantially reduced. The immunological follow-up revealed an improved hematopoietic engraftment while the number of circulating lymphocytes and the innate immune defense system remained stable.
However, due to acute, unexpected pulmonary bleeding 2 patients died 6 weeks and 19 months after tTreg therapy.
Conclusion: Adoptive transfer of *ex vivo* expanded tTregs is a promising therapeutic approach to modulate undesired T-cell mediated immune responses. However, further data are necessary to evaluate safety and efficacy. We have thus developed a clinical Phase I/IIa trial protocol which will include 25 patients with therapy-refractory or steroid-dependent cGvHD.

Translational support in tissue engineering by the Andalusian Network for Design and Translation of Advanced Therapies

Rosario Sánchez Pernaute - Andalusian Network for Design and Translation of Advanced Therapies, Spain

Together with multiple stakeholders, the Andalusian Network for Design and Translation of Advanced Therapies (AND&TAT) has developed a Research & Development program around tissue engineering. As a technology maturation platform fostering the clinical use of cell & gene based therapies, AND&TAT has provided its experience in activities such as advising researchers in regulatory aspects, setting up the manufacturing of ATMPs, designing and monitoring Clinical Trials, identifying industrial partners and generating new consortia, among others. This R&D program has generated a pipeline with two products in clinics and other ten in animal studies. Clinical studies include a Phase I/II clinical trial for the treatment of corneal ulcers using a nanostructured artificial human cornea and the clinical use of an allogeneic temporary substitute and an autologous artificial skin for the treatment of severely burned patient through compassionate use. On the other hand, the most relevant preclinical studies include a hydrogel as a novel hemostatic agent in liver resection and a scaffold with human iPSC-derived retinal pigment epithelium cells for the treatment of macular degeneration. AND&TAT is committed to establish strategic collaborations and public-private partnerships in order to foster the bench to bedside translational development. We have undertaken various maturation and process development activities including the generation of two strong consortia which combine regenerative medicine and nanotechnology. On one hand, the European "NanoGSkin" project involves six partners from four different countries and aims to develop a skin substitute using growth factor- and antibiotic-loaded nanoparticles towards an improved chronic wound therapy; on the other hand, "NanoGrow" consortium involves 3 industrial partners and aims to manufacture a bioengineered corneal substitute using growth factor-loaded nanoparticles towards an innovative ophthalmological treatment. Last but not least, the collaboration with the Swedish biotech company Verigraft in tissue engineered veins for chronic venous insufficiency needs to be remarked.

Translation of CAR-based innovation into clinics: how to overcome regulatory hurdles

Bernd Schröder - Miltenyi Biotec GmbH, Germany

Typical regulatory issues will be discussed that come up in the course of practical CAR T cell manufacturing from the experience of an industrial supplier of ATMPs. In general, those problems can be attributed to three categories: 1. Raw materials: There is an urgent need for reliable products that allow seamless transition from research into clinical applications, for qualified suppliers and secured supply chains, for regulatory support for interaction with regulatory bodies and last-but-not-least for support with the huge amount of paperwork to apply for manufacturing license, Investigational Medicinal Product Dossier (IMPD), Environmental Risk Assessment (ERA), Investigator's Brochure (IB) and Clinical Trial Protocol. 2. Starting material: In Europe, lentiviral vectors (LV) are regulated as a starting material that has to be produced following GMP requirements. However, in Germany LV are both, starting materials and Active Pharmaceutical Ingredients (API) which implies that you need a manufacturing license for the API or an import license e.g. if the LV is produced in the US or somewhere else outside Germany. 3. Clean room requirements: For the decision of the authorities which clean room class is required for the manufacturing process, the question is critical whether the manufacturing system is closed or not. For manual steps a class B clean room is required with laminar air flow as well as multiple devices that each require stringent qualifications (IQ/OQ). For a closed system, it is possible to use class C or D clean rooms (depending on the decision or the responsible authorities). In this case, several cellular products can be processed in parallel. Miltenyi has established such a Class C clean room concept with multiple prodigy systems in parallel in Bergisch Gladbach, Germany and received a generic manufacturing authorization for CAR T cell products in 2018. For spreading of CAR T cell manufacturing, Miltenyi aims for a decentralized manufacturing model that is presently investigated in three clinical trials in Germany.

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Personalized tissue-engineered grafts

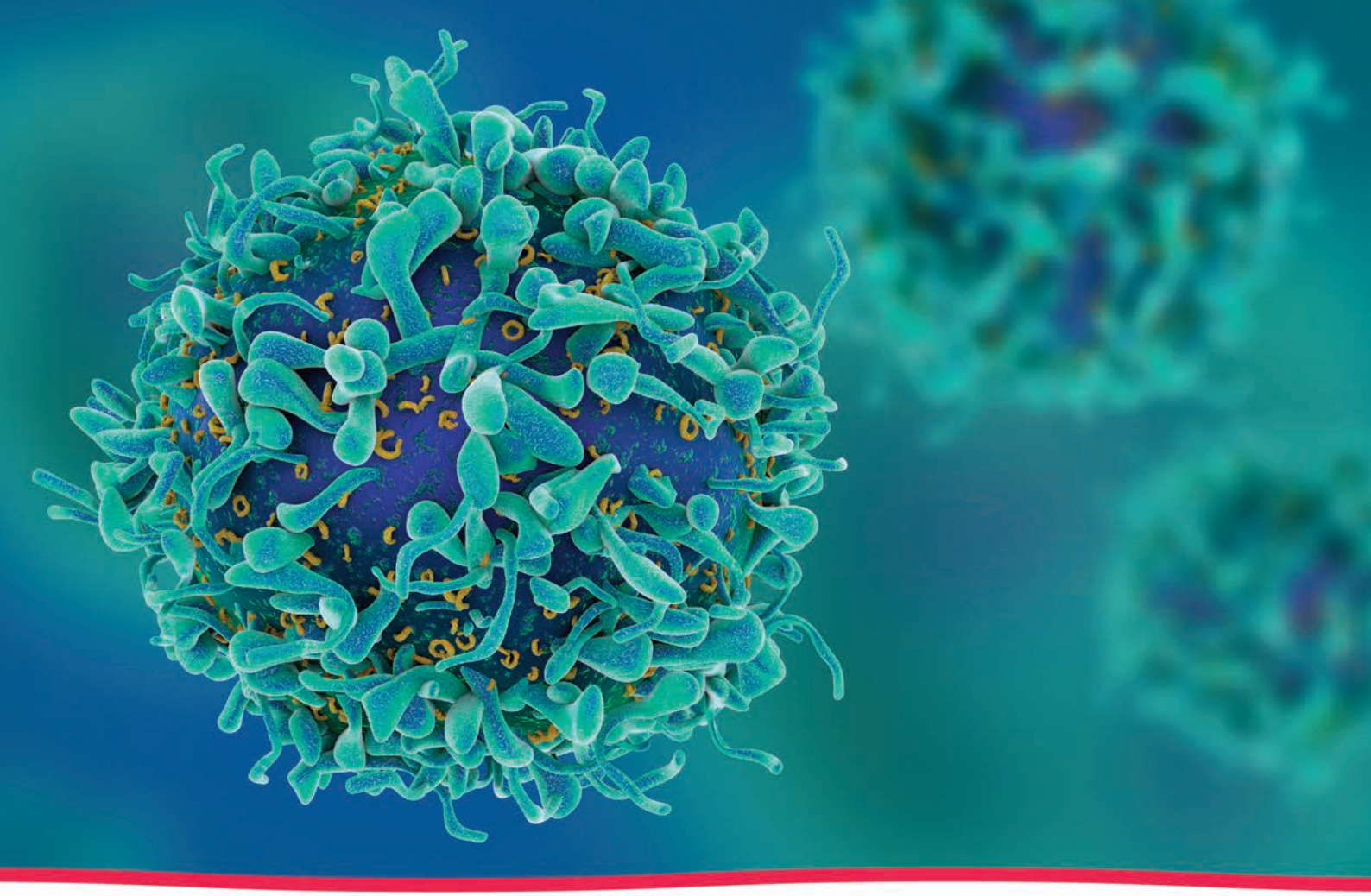
Raimund Strehl - Verigraft AB, Sweden

VERIGRAFT generates personalized tissue-engineered transplants for use in regenerative medicine. The company's unique technology platform turns donated allogeneic ("foreign") tissue into an autologous-like ("personalized") tissue and thus avoids transplant rejection and the need for life-long immunosuppression. Donated, allogenic tissue is patient-individualized by a combination of DC (Decellularization) and RC (Reconditioning/Recellularization) processes. VERIGRAFT's development pipeline comprises products in the areas of vascular regeneration as well as peripheral nerve repair. Donated blood vessels that serve as a starting point are obtained from deceased organ donors. The DC process uses specifically developed reagents to gently remove donor cells and DNA. This results in a clean extracellular matrix scaffold which has been depleted of immunogenic cellular material. At the same time the important three-dimensional structural properties as well as the mechanical strength and functionality of the tissue have been preserved.
The RC process is based on the patient's own peripheral blood which is supplemented with growth factors, nutrients and balancing agents. The resulting solution is used for the perfusion culture of the cell free scaffold which leads to reconditioning and repopulation of the tissue by cells and other biological components from the patient's blood. In this way the engineered blood vessel resembles the patient's own, and the risk for thrombosis and infection is greatly reduced compared to synthetic grafts. It has also been shown that such tissue engineered products do not trigger the recipient's immune system. The fact that the patient's blood is used directly, without artificially selecting or expanding cells in the lab, contributes favorably to the product's safety profile.
VERIGRAFT's personalized tissue-engineered grafts have gone through preclinical development. Safety data from large animals has been generated in pigs, minipigs and sheep. The first clinical trial has received approval.

Placental Cell Therapy for the Treatment of Muscle Trauma: From Preclinical Models to Clinical Phase III

Tobias Winkler - BCRT, Charité Universitätsmedizin Berlin, Germany

To date, there is no effective therapy to address skeletal muscle injuries. Our studies have explored therapy with mesenchymal stromal cells (MSC) for skeletal muscle injuries and transferred this therapy from preclinical tests to the patient.
We achieved PoC with autologous MSC transplantations. Following this, we successfully tested the efficacy of an allogeneic approach, using human placental stromal cells (PLX-PAD). We translated this therapy into clinics using acute iatrogenic muscle damage after hip arthroplasty as a model and conducted a phase I/II study. 20 patients were included, received a transplantation of 300x10^6 (300M), 150x10^6 (150M) PLX-PAD or placebo into the injured gluteus medius muscles (GM). Preclinical experiments showed improved muscle healing with increased force. In the phase I/II study no relevant AEs have been observed. The primary efficacy endpoint, change of GM strength after 6 months, showed a significant increase in the 150M group (p=0.0067) compared to placebo, which was accompanied by an increase in muscle volume (p = 0.004). Histology indicated faster healing and we found a reduction of the postoperative immunological stress. Based on these results we designed a phase III study (HIPGEN study) treating hip fracture arthroplasty patients (N=240) with an intramuscular injection of 150M PLX cells to improve muscle healing, mobility and mortality. Our consortium received funding from EU Horizon 2020 and is currently enrolling patients in 19 sites in Germany, England, Denmark, Israel and the US. The patients are followed for function, biomechanics, QoL, muscle volume and biomarkers. We further look into the mechanism of action by additional *in vitro* experiments on the effect of PLX cells on muscle cells of healthy donors and patients.
In summary, our data showed consistent positive results of MSC therapy for skeletal muscle regeneration in different preclinical application modes and finally in patients, where we are currently conducting a phase III study. Treatment with allogeneic cells could be a game changer not only in the treatment of the analyzed injuries but also for other skeletal muscle injuries.



Poster Presentations

REBROTS

Health by Advanced Therapies

1 - Foundational Research (New Targets and New Indications)

Mutually exclusive expression of ENTPD1 and EOMES among human PDCD1+ T memory cells differentiates pathogenic and suppressive autoreactive cell subsets in chronic inflammation

Mir-Farzin Mashreghi - Deutsches Rheuma-Forschungszentrum Berlin, Germany

Background/Aim: Memory T cells are critical perpetuators of chronic inflammation in autoimmunity. However, the transcriptional phenotype of autoreactive pathogenic memory T cells driving chronic inflammation still remains elusive. Thus, we aimed to define the transcriptional and clonal identity of autoreactive memory T cells in chronic inflammation of autoimmune patients. Methods: We isolated paired samples of memory T helper, memory regulatory T and memory cytotoxic T cells by FACS from the synovial fluid (SF) and the blood of seven patients with juvenile idiopathic arthritis - a chronic immune-mediated disease in children. Subsequently, we performed single-cell sequencing combined with T cell receptor (TCR) sequencing on these cells to dissect their cell heterogeneity due to their transcriptional profiles and clonal repertoire. Results: Our data reveal transcriptional heterogeneity among both CD4+ and CD8+ T memory cells. Clonal expansion and gene expression of TCR signaling-induced genes enabled us to distinguish autoreactive from bystander memory T cells. Clonally expanded, autoreactive T helper memory cells expressed PDCD1 and could be further divided into a pro-inflammatory subset expressing ENTPD1 as well as into a subset expressing EOMES, *ex vivo*. Conclusion: Taken together, these results might offer a basis for developing diagnostic and therapeutic strategies for patients with autoimmunity i), by developing biomarkers on the basis of recirculating autoreactive memory T cells and ii), by treating patients with agents to selectively deplete memory T cells driving pathology in chronic inflammation.

2 - Foundational Research (New Targets and New Indications)

Generation of Directly induced Neural STEM Cells from People with Progressive Multiple Sclerosis for Autologous Treatment (iSTEM)

Alexandra Nicaise - University of Cambridge, United Kingdom

Advances in stem cell medicine have suggested that diseases and injuries of the central nervous system (CNS) may be ameliorated through non-hematopoietic stem cell therapies. The transplantation of neural stem cells (NSCs) exerts trophic effects on endogenous brain cells, and provides beneficial modulatory actions on immune responses, thus promoting healing of the injured CNS. Multiple sclerosis (MS) is an immune-mediated disease of the CNS, where most patients develop a progressive form of the disease. There are currently very little to no therapies which modify progressive multiple sclerosis (PMS). Promoting endogenous brain repair is viewed as a potential strategy. Here we aim to explore the generation and scale up of clinical-grade, GMP-compliant, patient-specific induced NSCs (iNSCs) from people with PMS. Representing an advanced stem cell-based therapy that is a safer alternative compared to allogenic stem cells, avoiding many of the previous concerns associated with allogenic cells. Standard operating procedures and quality and safety criteria will be developed in order to facilitate the translation of stem cell therapies from academia towards a therapeutic opportunity for patients, through clinical and regulatory pathways. We will establish methods to generate safe, clinical-grade iNSCs that will then be characterized *in vitro*, including, but not limited to, a thorough analysis of transgene expression, mycoplasma and microbial contamination, expandability, protein marker expression, karyotype, differentiation potential, function, and genomic stability using whole genome bisulphite sequencing. Safety and efficacy will be tested using *in vitro* and *in vivo* function and potency assays. iSTEM will clarify potential issues in the generation and scale up of patient iNSCs that can then be mitigated if need be. This will lead to a better understanding of the potential therapeutic use of iNSCs in PMS and the strategy that needs to be taken to forward stem cell based therapies into clinical trials. The methods used to develop iNSCs will not only be applicable to treatment of PMS but can also be used in other chronic neurological diseases.

3 - Foundational Research (New Targets and New Indications)

Modeling Cell Senescence in Primary Progressive Multiple Sclerosis with iNSCs and Animal Disease Models

Alexandra Nicaise - University of Cambridge, United Kingdom

Primary progressive multiple sclerosis (PPMS) is a chronic demyelinating disease of the central nervous system (CNS) currently lacking any effective therapies that provide regeneration. Transplantation of induced neural stem cells (iNSCs) has been shown to promote healing of the injured CNS in animal disease models, by exerting trophic effects on endogenous brain cells which could be used as a strategy to foster repair and restore neurologic function. Previous work has demonstrated that NSCs generated from iPSCs of patients with PPMS have a senescent phenotype, associated with an increased secretion of HMGB1, which in turn impairs oligodendrocyte differentiation *in vitro*. Cellular senescence causes a pro-inflammatory phenotype that impairs tissue regeneration, linked to stress, and is implicated in several human neurodegenerative diseases. Increased HMGB1 is also associated with senescent progenitor cells within white matter lesions of PMS autopsy brain tissues. We have now generated iNSCs from patients with progressive MS and aim to understand the role of HMGB1 in PPMS NSC senescence using CRISPR-Cas9 knockout technology and high-throughput proteomics to better characterize the senescence phenotype in patient cells, perform functional assays, and discover new therapeutic targets. Mouse modeling using genetic and pharmacological targeting of HMGB1, in aged mice with delayed remyelination, will allow us to determine if inhibition of HMGB1 enhances repair after a demyelinating event. Overall this work will advance the understanding of the role of HMGB1 and cellular senescence in NSCs derived from patients with PPMS, in the aims of the development of personalized NSC therapies for patients with PPMS.

4 - Foundational Research (New Targets and New Indications)

Direct reprogramming of LGR5+ endodermal progenitor using defined signaling pathways

Frank Griscelli - Institute National de la Santé et de la Recherche UMR S935, France

Leucine-rich repeat (LRR)-containing G-protein-coupled receptor 5 (LGR5) is a marker of mouse liver stem cell population that appears *in vivo* near bile ducts exclusively upon liver damage. Here we succeeded in converting adult mouse hepatocytes into expandable and stable LGR5+ endodermal progenitor cells (EndoPCs) *in vitro* after transient exposure of 4 transcriptional factors (OCT3/4, SOX2, KLF4 and cMYC) and STAT3 activators. EndoPCs are shown to be dependent on three interrelated signaling pathways including gp130/JAK/STAT3, LGR5/Rspondin and WNT/βcatenin controlling their proliferation and self-renewing capacities. EndoPC can differentiate into liver restricted lineages in two- and three-dimensional long term culture systems including hepatocytes-like cells and bill duct-like structures *in vitro* and to repopulate liver tissue *in vivo*. After intra-muscular injection EndoPCs have generated well vascularized liver-like buds containing liver Alb+ parenchyma-like structures and KRT7/KRT19+ substantial bile duct-like structures. Clonal long-term expansion of LGR5+ adult endodermal stem cells opens up experimental avenues for disease modeling, toxicology studies and regenerative medicine.

5 - Foundational Research (New Targets and New Indications)

Amniotic mesenchymal stromal cells as drug carriers in ovarian carcinoma

Antonietta Rosa Silini - Centro di Ricerca "E. Menni" Fondazione Poliambulanza-Istituto Ospedaliero, Italy

Background: Mesenchymal stromal cells (MSC) are intensely studied for their potential in controlling tumor growth. Among their most interesting properties are their ability to migrate to inflammatory microenvironments and tumors. MSC isolated from the amniotic membrane of human term placenta (hAMSC) have attracted particular interest due to unique, tissue-related characteristics, and virtually absent expression of human leukocyte antigens and co-stimulatory molecules, making them very attractive for transplantation in allogeneic settings. hAMSC have been shown to modulate the functions of a wide variety of immune cells both *in vitro* and *in vivo*. These properties have been mostly attributed to their paracrine actions. Our study is focused on understanding the potential use of hAMSC as an anti-tumor strategy. Our previous data have shown that hAMSC per se inhibit the proliferation of a variety of tumor cell lines. Aim: The aim of our current study is to understand if hAMSC uploaded with the chemotherapeutic agent paclitaxel (PTX) can have significant anti-tumor effects in a three-dimensional spheroid model of human ovarian carcinoma. Methods: hAMSC were treated with 2µg/mL PTX and then collected 48 hours after priming. Primed cells were trypsinized and evaluated for their potential anti-proliferative potential on tumor spheroids. Tumor spheroids were developed from immortalized ovarian tumor cell lines (HEY, PEO-1, COV318) and from ascites of patients with epithelial ovarian carcinoma. Cellular spheroids were characterized based on their proliferation and size, and a comparative screening with 2D cultures was performed in order to determine the effects of naïve and PTX-loaded hAMSC on spheroid growth. Results: hAMSC are able to upload the chemotherapeutic agent paclitaxel and can release it in a sufficient amount to inhibit the proliferation of tumor cells *in vitro*. Preliminary results demonstrate an anti-proliferative effect of hAMSC uploaded with PTX on 2D and 3D spheroids obtained from ovarian carcinoma cells. Conclusion: Our data provide preliminary evidence suggesting hAMSC as interesting candidates for drug delivery vehicles.

6 - Foundational Research (New Targets and New Indications)

B lymphocytes as targets of the immunomodulatory properties of human mesenchymal stromal cells

Marta Magatti - Centro di Ricerca "E. Menni" Fondazione Poliambulanza-Istituto Ospedaliero, Italy

Background. Human amniotic mesenchymal stromal cells (hAMSC) from term placenta, and their conditioned medium (CM-hAMSC), have been proposed for the treatment of inflammatory-based diseases, favoring tissue repair and regeneration mainly due to their immunomodulatory action on inflammatory mediators. Indeed, hAMSC and CM-hAMSC have been shown to affect cells of the innate and adaptive immune systems (Th1, Th17, dendritic cells, monocytes and macrophages), supporting the expansion of regulatory T cells and anti-inflammatory macrophages (M2). However, the effect on B lymphocytes remains poorly addressed. Aim. Herein we investigated the *in vitro* properties of hAMSC and CM-hAMSC on B-cell proliferation and differentiation. Since we have previously shown that prostanoids are partially responsible for the hAMSC-induced inhibition of T-cell proliferation, we investigated whether they could be involved in any effects observed on B cells. Methods. Peripheral blood mononuclear cells were stimulated *in vitro* by CpG-ODN and cultured with hAMSC, or in presence of CM-hAMSC or CM without prostaglandins. The effect of hAMSC and CM-hAMSC on B-cell proliferation and differentiation was analyzed by flow cytometry.Results. CpG efficiently triggered B-cell (CD19+) proliferation and the generation of antibody-secreting cells (ASC, CD27highCD38high), which are largely terminally differentiated plasma cells (CD27highCD38highCD138+). Our results showed that both hAMSC and CM-hAMSC strongly suppressed B-cell proliferation and reduced ASC differentiation. Resulting ASC were mainly plasmablasts (CD27highCD38high CD138-), while plasma cell formation was abrogated. When CM depleted of prostaglandins was used, we observed only a slight reduction of B-cell proliferation and ASC formation, indicating that prostanoids interfere with these processes. However, the resulting ASC were mainly plasmablasts, suggesting that prostanoids are not involved in the terminal maturation into plasma cells. Conclusion. Our data indicate that secreted factor(s) released by hAMSC exert a suppressive effect on B cell proliferation and plasma cell formation; these effects are partially mediated by prostanoids.

7 - Foundational Research (New Targets and New Indications)

Mesenchymal Stromal Cells Modulate the Immune Response to Sepsis

Jack Brady - The Regenerative Medicine Institute and CURAM, Ireland

Sepsis is a syndrome defined as a dysregulated immune response to infection. It is characterised by an early hyper-inflammatory phase which can develop into a second phase of prolonged immune suppression, with vast immune cell death and dysfunction. This loss of immune homeostasis in sepsis remains to be poorly understood and to date, research efforts have paid little attention to the later phase of sepsis despite the fact that most patients die in this phase. Mesenchymal stromal cells (MSCs) are a potent immune-modulating therapy with proven pre-clinical efficacy in early phase sepsis. There is limited knowledge however, on their effects in the later phase due to a lack of appropriate pre-clinical models. Nonetheless, it is has been shown that the effect of MSCs is dependent upon environmental cues and that they have the ability to both support or suppress the immune system under different conditions. Thus, MSCs may have therapeutic potential for late phase sepsis. The aims of this study are a) to develop a relevant pre-clinical model of late phase sepsis, b) to characterise the innate and adaptive immune response in this model and c) to examine the potential of MSCs to restore immune homeostasis and stop the progression to late phase sepsis.Male Sprague Dawley rats received an intratracheal dose of Klebsiella Pneumoniae followed by intravenous vehicle or MSC administration two days later. Blood samples were collected at day five. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by Ficoll separation. PBMCs were counted, washed and stained with a multi-colour panel of antibodies and the cell profile was assessed by flow cytometry.There was a significant increase in the frequency of monocyte, natural killer and regulatory T cells in the septic rats that was significantly reduced by MSC treatment. This shows that MSCs can indeed modulate both the inflammatory and suppressive signals as induced by sepsis. Future functional assays will determine the exact mechanisms of MSC action on the immune response to sepsis and further pre-clinical studies are warranted to prove the therapeutic potential of MSCs for this severe syndrome.

8 - Foundational Research (New Targets and New Indications)

Imprecise lineage definition associates with functional dissimilarity observed between iPSC-derived MSCs and primary MSCs

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Background and Aim: Induced pluripotent stem cells (iPSCs) have been considered as a potential alternative source for generating mesenchymal stem cells (MSCs) to meet the increased cell demands for both research and therapeutic applications. Based on the minimal criteria for defining MSCs published by the International Society for Cellular Therapy (ISCT standard), several different protocols have claimed success in differentiating MSCs from iPSCs (iMSCs). However, questions have arisen relating to the phenotypic fidelity of iMSCs because, irrespective of the derivation methods used, the cells generally have less capacity for adipo- and chondrog-enesis when compared with primary MSCs. The objective of this study was to understand the cellular mechanism behind these discrepancies. Methods: iMSCs were derived using the embryoid body-based outgrowth method. We compared the differentiation ability, immunophenotype and gene expression profiles (GEPs) between multiple iMSC and BM-MSC lines. In addition, the impact of culture expansion on cell state was evaluated. Conclusion: The results showed that iMSCs met the ISCT standard but displayed significantly negligible adipogenic and chondrogenic capacity compared to BM-MSCs. GEPs analysis revealed that iMSCs expressed very high levels of vascular progenitor cell (VPC) genes (KDR and MSX2), whereas BM-MSCs had significantly greater levels of paraxial mesodermal genes (PDGFRα and MESP2). These distinct GEPs were maintained during culture expansion, indicating that, unlike BM-MSCs, iMSCs were more closely related to VPCs. Interestingly, the revealed characteristics of iMSCs corresponded to the known cell property of VPCs, where the latter one displays partially overlapped immunophenotype as primary MSCs. However, although VPCs have osteo-, adipo- and chondro-genic potential, which can not be fully activated in primary MSCs preferred differentiation condition. In summary, our results suggest a lineage misidentification of iMSCs, indicate the inadequacy of using the ISCT standard to distinguish different mesodermal progenitors and emphasize a necessity to validate the iMSCs identity with various lineage markers.

9 - Foundational Research (New Targets and New Indications)

In vivo assessment of glomerular filtration within transplanted human induced pluripotent stem cell-derived kidney organoids

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Induced pluripotent stem cell (iPSC)-derived kidney organoids are 3-dimentional structures grown *in vitro* that exhibit nephrons consisting of glomerular, proximal and distal tubular structures. Their generation provides opportunities for studying kidney disease, development and toxicity, but potentially also clinical use as auxiliary tissue therapy for patients with kidney failure. However, these kidney organoids are not yet applicable in regenerative medicine and several hurdles need to be overcome. For example, kidney organoids *in vitro* lack a functional vascular network, are still immature and lack a glomerular filtration barrier. Upon transplantation of these kidney organoids under the renal capsule we observed perfused host- and organoid-derived vasculature as well as vascularized glomerular structures. These glomerular structures showed advanced morphologic maturation, as did the tubular structures, raising the question whether under these conditions the glomeruli also become functional. We applied high-resolution intravital imaging that allowed the visualization and functional assessment of glomerular filtration and glomerular barrier function in the transplanted kidney organoids by live *in vivo i*maging of dextran sieving. After vascularization, organoid glomeruli displayed dextran size selectivity across their glomerular filtration barrier. Visualization of proximal tubular dextran re-uptake was also evident. Together this shows clear evidence of functional filtration. This approach to visualizing glomerular filtration function will be instrumental for translation of organoid technology towards clinical development.

10 - Foundational Research (New Targets and New Indications)

Transplantation of human PSC-derived kidney organoids induces significant glomerular and tubular maturation *in vivo*

Cathelijne W. van den Berg - Leiden University Medical Center, Netherlands

Human pluripotent stem cells (PSCs) have the unique ability to differentiate into all lineages that may facilitate approaches for regenerative medicine, such as the generation of tissues for renal replacement. Long term application, however, will require transferability between PSC lines and significant improvements in organ maturation. A key question is whether time or a patent vasculature is required for ongoing morphogenesis. We generated kidney organoids by applying the temporospatial mechanisms that regulate the induction of renal structures during development. The 3-dimensional structures, contain structures derived from both ureteric epithelium and metanephric mesenchyme progenitor populations. These kidney organoids remain disorganized and immature upon prolonged culture. We therefore transplanted the kidney organoids under the renal capsule in immunodeficient mice and observed host-derived vascularisation of the organoid. We employed the combinatorial approach of organoid transplantation and an abdominal imaging window to serially image the transplanted organoids *in vivo*. Imaging of organoids under the renal capsule confirms functional glomerular perfusion as well as connection to pre-existing vascular networks in the organoids. Wide-field electron microscopy demonstrates that transplantation results in formation of a glomerular basement membrane, fenestrated endothelial cells and podocyte foot processes. Furthermore, compared to non-transplanted organoids, polarisation and segmental specialisation of tubular epithelium is observed. These data demonstrate that functional vascularisation is required for progressive morphogenesis of human kidney organoids. The matured mini-kidneys are an important step forward for future applications in the development of a bioengineered kidney.

11 - Foundational Research (New Targets and New Indications)

Impact of Type 2 Diabetes Mellitus on the Functional Capacity of Human Bone Marrow-Derived Mesenchymal Stem Cells

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An increase in bone fragility, abnormal bone mineral density and delayed bone fracture repair are complications of diabetes mellitus (DM). This study proposes that alterations in bone marrow MSC number and capacity in humans living with DM contributes to the pathology underlying DM-associated osteopathy through MSC inability to support organ homeostasis. Bone marrow samples were donated by individuals undergoing elective hip arthroplasty or trauma intervention. Donations were categorized as age/gender-matched controls or from individuals living with type 2 diabetes (T2DM). After quantifying the mononuclear cells (MNC) per ml, neat marrow was plated for CFU-F and CFU-O quantification. The MSCs were then isolated through direct plating methods and culture expanded. Their proliferation, differentiation and angiogenic capacities were evaluated and their secretome and transcriptome profiled. No significant difference was identified between MSC cohorts with respect to the number of MNCs, CFU-Fs normalized to MNCs or CFU-Os normalized to CFU-Fs. However, there were gender-related differences in that bone marrow donations from males contained significantly more CFU-Fs than those from females, a phenomenon that was decreased in the presence of DM. All MSCs exhibited comparable proliferative, osteogenic and adipogenic differentiation capacity, information that could be utilized in designing a cellular therapy for individuals living with DM. However, analysis of the transcriptome and characterization of the secreteome do indicate subtle molecular differences between the donor cohorts. Ongoing experiments investigate the nuanced impact of DM on intra- and extra-cellular signalling and *in vivo* osteogenic capacity, thereby further investigating the relationship between MSCs, DM and DM-induced osteopathy

12 - Foundational Research (New Targets and New Indications)

The potential of biophysical therapies to enhance angiogenesis and wound healing

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Biophysical therapies, like extracorporeal shockwave therapy (ESWT) and photobiomodulation (PBM), have (and sometimes still are) been considered as voodoo treatment, but represent promising advanced therapies and get increased interest in various therapeutic settings. Typical indications for these therapies are the improvement of wound healing and tissue regeneration, scarring, and perfusion as well as pain therapy. Tissue perfusion is mandatory for successful wound healing. However, the mechanisms underlying the beneficial effects of biophysical therapies have not yet been fully revealed.
Because cell proliferation is a major requirement in the wound healing cascade, we used *in vitro* studies and an *in vivo* wound healing model to study whether ESWT influences proliferation by altering major extracellular factors and signaling pathways involved in cell proliferation. We identified extracellular ATP, released in an energy- and pulse number-dependent manner, as a trigger of the biological effects of shock wave treatment. Purinergic signaling-induced Erk1/2 activation was found to be essential for this proliferative effect, which was further confirmed by *in vivo* studies in a rat wound healing model where shock wave treatment induced proliferation and increased wound healing in an Erk1/2-dependent fashion.
In a similar fashion, PBM enhances cell proliferation and angiogenesis. Endothelial cells and stem cells are key factors in angiogenic processes. Pulsed blue (475 nm), green (516 nm) and red light (635 nm) from LED were applied and confirmed the stimulatory effects on regenerative potential. In a subsequent study the angiogenic stimulation was confirmed in a more complex CAM assay. Finally, PBM was tested in various *in vivo* models of impaired wound healing.
Our studies confirmed significant beneficial effects of biophysical therapies on vascularization potential and proliferation capacity both in various *in vitro* cell culture models as well as in the CAM model and *in vivo* models. Further studies have to focus on intracellular mechanisms induced by these therapies in order to optimize this promising applications in tissue regeneration.

13 - Foundational Research (New Targets and New Indications)

Effects of Cryopreservation Temperature and Storage Period on Lipoaspirate Cell Viability

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Background: There has been an increasing demand in recent years to use autologous fat from lipoaspirate in different clinical applications such as cosmetic, reconstructive and regenerative medicine. However, potential of lipoaspirate is somehow restrained due to the need of harvesting fresh adipose tissue within 24 hours. Cryopreservation of adipose tissue could be a simple solution to overcome this issue. However, survival rate of the thawed adipocytes is very important as cryopreservation may have great impact on adipose tissue engraftment following injection. Aims: This study aims to investigate the effect of storage temperature and storage period on the quality of the adipose tissue derived cells. Methods: Patients undergoing voluntary liposuction (n=4) will be recruited with signed informed consent. Lipoaspirate with temperature monitors provided by the collaborator company (Biostór) will be shipped to the Centre for Cell Manufacturing in Ireland (CCMI). Tissues will be divided into three portions. One portion will be processed immediately to separate stromal derived fractions (SVF) followed by cryopreservation at -800C and -1500C for 2 and 4 weeks using CCMI's standard operating procedure (SOP). Second portion will be cryopreserved immediately using the CCMI's SOP and labelled as "Crude-tissue". The third portion will be washed with PBS ("Washed-Tissue") prior to cryopreservation. Freshly prepared SVF will be cultured to grow adipose-derived-stem-cells (ADSCs) for multiple passages and colony formation assay. After 2 and 4 weeks, cryopreserved tissues will be thawed, SVF will be isolated and cultured to grow ADSCs. ADSCs from frozen tissues will be compared with ADSCs from freshly prepared SVF. ADSCs will be assessed for cell viability and CD90, CD73, CD105, CD31, CD36, CD13, CD45, CD14 and CD34 expressions using SOP. Conclusion: So far, from one lipoaspirate sample that we tested to validate our hypothesis, we found that the viability of the SVF from the cryopreserved "crude-tissue" and "washed-tissue" were 77% and 70% respectively. This data provide us an indication about the great therapeutic potential of cryopreserved lipoaspirate.

14 - Foundational Research (New Targets and New Indications)

Harnessing NK cells for cancer immunotherapy. Impact of NK cell immunosenescence in cancer patients

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The incidence of some types of tumours is increasing due in part to the aging of the population. Aging is associated with changes in the immune system involving both adaptive as well as innate immunity. Immunosenescence refers to the deterioration of the immune system function associated to aging. The failure of tumour immunosurveillance may be partly responsible for the age-associated increase in cancer incidence. Natural killer (NK) cells are innate lymphoid cells specialized in killing tumour cells as well as virus infected cells without the requirement of prior sensitization. NK cells are also involved in regulating immune function as they produce several cytokines and chemokines. NK cell immunosenescence affects the frequency, phenotype, and subset distribution of human NK cells. A decreased expression of activating receptors is observed in the elderly that may contribute to the decline of NK cell function. NK cells in cancer patients frequently show phenotypic and functional alterations, such as a reduced expression of several activating receptors, that impair NK cell function and may result from the cell interaction between NK effectors and cancer cells. The design of new therapies based on NK cells opens new possibilities especially for the treatment of elderly patients who are particularly susceptible to the toxicity of conventional chemotherapy treatments. Targeting NK cells by the use of cytokines (such as IL-15), agonist monoclonal antibodies (mAbs) or bi- or tri-specific engagers to activate NK cells, or the using anti-checkpoint mAbs such as TIGIT, TIM3, LAG3 and PD1, represent novel strategies to increase NK function in cancer patients. These advances have allowed the emergence of NK cell-based immunotherapy procedures based on the adaptive transfer of activated-expanded NK cells as novel treatments for cancer patients including acute leukaemia patients.

15 - Foundational Research (New Targets and New Indications)

Use of CRISPR/Cas9 to elucidate mechanisms of action of novel biomarkers in Osteoarthritis

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Osteoarthritis (OA) is a debilitating joint disease severely affecting elderly populations. At present the mechanisms of disease progression are poorly understood and there are no effective treatments. Currently, cell therapy is a promising candidate to help slow the progression and promote regeneration of the cartilage in the joint. The best cell phenotype has yet to be determined which can aid this process. The CRISPR/Cas9 system is an effective molecular biology tool that can be used to elucidate mechanisms of disease by investigating potential novel meditators of OA progression and also create a cell phenotype that aids chondrocyte repair in the joint. Previous work has identified that neuronal Interleukin-16 (nIL-16) as a novel biomarker that is significantly up-regulated in cartilage during the later stages of OA. Preliminary investigations identified co-localisation of nIL-16 with the Transient Receptor Potential cation ion channel sub-Family-V-member-4 (TRPV4) in the primary cilium and in the pericellular matrix of human OA chondrocytes. Perturbation of both elements are strongly associated with OA. We propose a strategy in which CRISPR/Cas9 can be used to investigate our hypothesis that nIL-16 and TRPV4 work in tandem in a pathway that leads to chondrocyte hypertrophy and calcification seen in late OA. CRISPR technology will be used to knock out nIl-16 PDZ domains to investigate whether this is the mechanism in which nIL-16 functions to anchor TRPV4 to the membrane of chondrocytes. In the future this strategy could be used to develop a potently chondrogenic cell that would improve prognosis for patients with OA.

16 - Foundational Research (New Targets and New Indications)

Human tolerogenic dendritic cells regulate immune responses through lactate synthesis

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Cell therapy is a promising strategy to treat patients suffering from autoimmune or inflammatory diseases, or receiving a transplant. Our preclinical studies revealed that treatment with Autologous Tolerogenic Dendritic Cells (ATDCs) is safe in non-human primates and promotes allograft tolerance in rodents. We have now established a robust procedure for manufacturing human ATDCs, which are currently being tested in a first-in-man phase I/II clinical trial as an adjunct immunosuppressive therapy in patients undergoing kidney transplantation. In the present study, we report human ATDCs properties and their mechanisms of action on T cells. The defining characteristics of ATDCs are their suppression of T cell proliferation and their expansion of regulatory T cells through secreted factors alone. We found that ATDCs produce high levels of lactate which shape T cell responses towards tolerance. Our results showed that T cells take up ATDC-secreted lactate leading to a decreased of their glycolytic metabolism. In a humanized mouse model, ATDCs delay graft-versus-host-disease development by decreasing the ability of CD4+ T cells to respond to TCR stimulation, which correlates with elevated levels of lactate in the blood. The contact-independent, non-specific suppression of T cell immunity through lactate production by ATDCs is a novel mechanism of action that distinguishes ATDCs from other cell-based immunotherapies currently under clinical investigation. In light of their strong tolerogenic potential *in vitro* and *in vivo*, we believe that ATDC therapy should be extended to other clinical trials with the aim of regulating the immune response.

17 - Foundational Research (New Targets and New Indications)

Intra-family transcriptional cross-talk and cell non-autonomous actions of intrinsic TGFβ/BMP system components in stem cells and their niches.

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We study how TGFβ/BMP signals are interpreted via co-operation of Smads with Smad-binding TFs, which mounts and fine-tunes proper transcriptional responses in cell differentiation in embryos and recapitulation of this developmental signaling in tissue/organ repair.
Our analysis of various TGFβ/BMP-family component knockout mice (receptors, Smad1/5, Zeb2) has revealed *in vivo* functions and action mechanisms of these proteins in early embryos and diverse cell types (neurons and glial cells, cardiac and skeletal myocytes, blood and lymphatic vasculature, and immune cells). During development, and in challenged adult mice (nervous system damage, cardiac infarct) this work identified compensatory mechanisms resulting from TGFβ/BMP intra-family cross-talk, as well as aberrant e.g. Wnt and Notch signaling in the mutant cells. Furthermore, it revealed cell non-autonomous effects of the studied intrinsic components. We will briefly illustrate these concepts.
One lesson learnt is that we need to study how TGFβ/BMP intra-system transcriptional regulation integrates into gene regulatory networks in stem cells and their progression of differentiation. This is elevant to both endogenous and *ex vivo* expansion of stem cells, and their differentiation. An overview of such regulations, incl. at single-cell level, is missing for TGFβ/BMP systems. For this, we use perturbation (esiRNA, CRISPR) of a prioritized list of 96 TGFβ/BMP components and multi-omics read-out in ESCs (and cerebral organoids) as a model. We integrate mRNA expression dynamics, gene-gene interactions inferred from single-component perturbations and single-cell mRNA-profiling, and develop technologies enabling simultaneous analysis of at least two omics techniques. These experimental combinations expose an intricate system of multi-level regulation whereby the majority of gene-gene interactions remarkably display mainly cell-stage specific behavior. Hence, interpretation of the consequences of single-gene perturbation or knockout in lineage-progressing cells, proposed for optimizing efficiencies and use in cell-based tissue/organ repair, should occur with caution regarding stage and transition.

18 - Foundational Research (New Targets and New Indications)

Transplantation of metabolically improved stem cell derived hepatocyte-like cells in the liver of uPA-SCID mice

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Background: *In vitro* and *in vivo* models to study liver diseases and toxicology rely on the use of primary human hepatocytes, hepatoma cell lines and mouse models, which all have certain limitations. Therefore, hepatocytes such as from pluripotent stem cells (hPSCs) are being investigated. Although protocols have been developed to generate hepatocytes from hPSC, most groups can only generate cells that are called hepatocyte-like cells (HLCs) as they remain immature, lacking major drug metabolizing enzymes (e.g. CYP3A4) and poorly repopulate the liver of immunodeficient mice with transgene/chemically induced liver toxicity.
Aim: To address this maturation problem, the Verfaillie lab has generated a hPSC line that overexpresses three liver-specific transcription factors (termed HC3x) in a doxycycline inducible manner as well as an optimised culture medium composition. Differentiation of HC3x-hPSCs in the optimised medium resulted in a more mature hepatocyte progeny, with increased production of albumin and functional CYP3A4 in comparison to control hPSC-HLCs. Here, we investigated the repopulation potential of the HC3x-HLCs in urokinase-type plasminogen activator (uPA)-SCID mice.
Methods: HC3x-hPSCs were differentiated towards HLCs using optimised medium conditions and were engrafted in the livers of uPA-SCID mice.
Conclusion: The liver of the uPA-SCID mice were successfully engrafted with HC3x-HLCs as was demonstrated via immunohistochemical staining for human mitochondria and human albumin. We will determine if the engrafted HC3x-HLCs can be infected by the hepatitis B virus, and also perform RNAseq analysis on the engrafted HC3x-HLCs to determine if their presence in an *in vivo* liver environment further promotes their maturation.

19 - Foundational Research (New Targets and New Indications)

Induced pluripotent stem cells (iPSC)-derived β cells for β cell replacement in diabetes

Valeria Sordi - IRCCS Ospedale San Raffaele, Italy

Background - iPSC can generate functional insulin-producing cells (iβ). However, gene expression profile and secretory function of iβ still need to be validated in comparison with adult β cells from organ donors, used in clinical islet transplantation. Moreover, selective killing of undifferentiated iPSC, potentially contaminating the graft and able to form teratoma, could increase the safety of iPSC therapy for diabetes.
Methods - The iPSC cell line CGTRCiB10 was obtained from the Cell and Gene Therapy Catapult, London. CGTRCiB10 was differentiated into iβ following pancreatic developmental stages and compared with donor pancreatic islets. The expression of marker genes of pancreas differentiation was measured through Taqman analysis. Single cell transcriptomics was performed with DropSeq technology. FACS and immunofluorescence for pancreatic markers (Pdx1, Nkx6.1, Insulin, Chromogranin A, PC1/3, insulin, glucagon,and somatostatin) were performed on iβ. Hormone secretion was measured using Luminex technology in basal conditions and after dynamic perifusion of glucose. Chimeric monoclonal antibody (mAb), which targets a specific marker of undifferentiated iPSC, was added during differentiation and cells were transplanted in immunodeficient mice.
Results - CGTRCiB10 iPSC differentiated into β cells with high efficiency (up to 95% Pdx1+, 70% Nkx6.1 and 40% Ins+ cells) and resulted positive for Pdx1, Nkx6.1, ChgA, PC1/3, INS, GCG, and SST. iβ basally secreted C-peptide, glucagon, and ghrelin and released insulin in response to stimuli. Single cell analysis revealed that iβ are remarkably similar to donor islets. Addition of mAb *in vitro* for 24h efficiently induced cell death in >80% undifferentiated iPSC, without affecting differentiating cells. Conclusions - We demonstrated that insulin-producing cells generated from iPSC recapitulate fundamental gene expression profiles and secretory function of native human β cells. Moreover, preliminary results obtained *in vitro* by using the proposed mAb indicate that it is possible to purify iβ. Ongoing experiments in mouse will further confirm if this strategy may increase the safety of iPSC-based cell therapy for diabetes.

20 - Foundational Research (New Targets and New Indications)

Generation of β cells from iPSC: the contribution of human pancreatic mesenchymal and endothelial cells

Valeria Sordi - IRCCS Ospedale San Raffaele, Italy

The field of cell therapy for the cure of diabetes is strongly in need of an infinite source of β cells. β cells can be differentiated *in vitro* from iPSC, but they retain an immature phenotype and function. Mesenchymal and endothelial cells can promote the development of β cells through the release of trophic factors. The aim of this study is to use pancreas-specific mesenchymal stem cells (pMSC) and endothelial cells (HIMEC) to support β cell differentiation and function.
The iPSC cell line CGTRCiB10, obtained from the Cell and Gene Therapy Catapult, London, was differentiated following pancreatic developmental stages. PMSC and HIMEC were cultured for 24h with iPSC differentiation media and conditioned supernatants added during differentiation. iPSC were differentiated in 4 conditions: control (Diff), adding pMSC from day 4 to 13 (M-Diff), adding HIMEC from day 14 to 25 (H-Diff); adding both pMSC and HIMEC (MH-Diff).
Survival, proliferation and phenotype of pMSC and HIMEC are not altered by culture in iPSC differentiation media. PMSC released high amount (>100 pg/ml) of IL-1RA, IL-6, IL-8, IL-12p40, Eotaxin, G-CSF, MCP-1, VEGF, GROα, HGF, MIF, SCGFβ, SDF1a, PAI-1; while HIMEC released high amount of IL-6, IL-8, G-CSF, MCP-1, GRO-α, PDGF-β, SCGF-β, MIF, PAI-1, Visfatin.
Gene expression analysis revealed the up-regulation of the pancreatic endoderm genes NKX2.2 (9,51 FC compared to Diff), NKX6.1 (6,55 FC) and INSULIN gene (2,68 FC) in M-Diff compared to control during the last steps of differentiation, while no improvements were observed in H-Diff.
Terminally differentiated cells resulted in 60% Pdx1 and 7% insulin positive in Diff, the percentage increased to 78% Pdx1 and 14% Ins in M-Diff, while no improvements were observed in H-Diff and MH-Diff. Dynamic insulin release assay revealed that terminally differentiated cells secreted a maximum of 1190 pg/ml of insulin in response to glucose in Diff, while 2362 pg/ml in M-Diff.
These results show that the presence of pMSC supernatants doesn't impair iPSC survival, rather it seems to help and support differentiation into mature β cells. Experiments with direct co-culture of iPSC and pMSC/HIMEC are ongoing.

21 - Foundational Research (New Targets and New Indications)

Induced pluripotent stem cell (iPSC) from a patient with monogenic diabetes for autologous β cell replacement

Valeria Sordi - IRCCS Ospedale San Raffaele, Italy

iPSC can differentiate into functional endocrine pancreatic cells. MODY-8 is a form of monogenic diabetes, characterized by a mutation in CEL (carboxyl-ester-lipase) gene, which leads to exocrine pancreas dysfunction and β cell failure. The relation between exocrine dysfunction and β cell death is unclear. We hypothesize that β cells are not affected by CEL gene mutation and therefore patient-specific iPSC offer the chance of MODY-8 treatment with autologous iPSC-derived β cell transplantation.

The aim of this study is to generate iPSC lines from MODY-8 patient, show their ability to differentiate in exocrine and endocrine pancreatic cells and test the function of differentiated cells.

iPSC were reprogrammed from skin fibroblast of a MODY-8 patient and characterized for pluripotency. The iPSC cell line CGTRCiB10, obtained from the Cell and Gene Therapy Catapult, London, was used as control during differentiation. MODY-8 iPSC were differentiated into pancreatic exocrine and endocrine pancreatic cells with protocols mimicking the stages of fetal pancreas development.

Six MODY8-iPSC clones have been isolated and characterized. MODY-8 iPSC are able to differentiate into exocrine cells (2% amylase/GP2+ cells) and β cells: terminally differentiated cells are Pdx1 (79±15%), Nkx6.1 (36±14%) and Insulin (13±5%) positive. CEL gene is not expressed in iPSC nor during any steps of endocrine differentiation. Perifusion data showed a response of MODY-8 iPSC-derived β cells in terms of insulin secretion: high glucose induced an insulin release of up to 1050 pg/ml, and up to 420 pg/ml in the presence of glucose+IBMX. Depolarizing stimulus induced a peak of insulin release (1924 pg/ml). In conclusion, MODY-8 iPSC-derived β cells seem capable of differentiation into exocrine and endocrine cell. β cell function appears preserved in mutated cells. Further characterization, including transplantation in diabetic mice, is ongoing, to provide robust evidence that CEL mutation does not affect differentiation and function of β cells. These results will set the basis for *in vitro* modeling of the disease and for personalized β cell replacement in patients with monogenic diabetes.

22 - Foundational Research (New Targets and New Indications)

Engineering of NK activating receptor ligands enhances immune compatibility of MHC-I-/- iPSC for cell therapy of type 1 diabetes

Lorenzo Piemonti - IRCCS Ospedale San Raffaele, Italy

Background and aims: Induced pluripotent stem cells (iPSC) represent a renewable source of pancreatic β cells. To escape T cell response upon allo-transplantation, previous works proposed disruption of B2M gene, abrogating surface expression of all class I molecules. However, MHC-I knocking-down triggers missing-self recognition of NK cells, nullifying this strategy. We aimed to inhibit NK lysis by the modulation of NK activating receptor ligands to enhance transplant compatibility of MHC-I-/- iPSC and their derivatives.

Materials and methods: The iPSC cell line CGTRCiB10 was obtained from the Cell and Gene Therapy Catapult, London. Expression of NK activating receptor ligands on human iPSCs was analyzed by qRT-PCR, IF and cytofluorimetry. CRISPR/Cas9-mediated gene editing was used to knock-out B2M, B7H3 and CD155 and to knock-in HLA-E genes. Differentiation of iPSCs into β cells was obtained following *in vitro* protocol mimicking embryonic pancreas development. Immunogenicity tests were performed by co-culturing engineered cells with CD8+ or NK cells, at different target-effector ratios.

Results: iPSC-derived β cells express high levels of NKp30-ligand B7H3 and moderate levels of DNAM-1-ligand CD155 and CD112. Cytotoxicity tests on wild type, B2M-/- and B2M-/-/HLA-E+/+ iPSC lines revealed that B2M-/- iPSCs escape CD8+, but not NK lysis; otherwise, forced expression of HLA-E avoids NK killing. Supposing that killing of B2M-/- iPSCs could be mediated by NKp30 and DNAM-1 receptors, we generated MHC-I-deprived B7H3-/-, CD155-/- and B7H3-/-/CD155-/- iPSC lines and tested their capability to evade NK response. All three lines resulted invisible to NK response. All gene edited iPSC lines maintained the ability to differentiate into pancreatic β cells.

Conclusion: Deletion of NK activating receptor ligands makes MHC-I-/- iPSCs resistant to NK recognition, contributing to offer new perspectives for using iPSC-derived β cells as next generation cell source for T1D treatment.

23 - Foundational Research (New Targets and New Indications)

Biofabrication of a Vascularized Islet Organ for Type 1 Diabetes

Lorenzo Piemonti - IRCCS Ospedale San Raffaele, Italy

Background: Islet transplantation is superior to extrinsic insulin supplementation in the treating severe Type 1 diabetes. However, its efficiency and longevity are limited by substantial islet loss post-transplantation due to lack of engraftment and vascular supply.
Material and Methods: To overcome these limitations, we developed a novel approach to bio-fabricate functional, vascularized islet organs (VIOs) *ex vivo*. We endothelialized acellular lung matrixes to provide a biocompatible multicompartment scaffold with an intact hierarchical vascular tree as a backbone for islet engraftment. Over seven days of culture, islets anatomically and functionally integrated into the surrounding bio-engineered vasculature, generating a functional perfusable endocrine organ.
Results: When exposed to supra-physiologic arterial glucose levels *in vivo* and *ex vivo*, mature VIOs responded with a physiologic insulin release from the vein and provided more efficient reduction of hyperglycemia compared to intraportally transplanted fresh islets. In long-term transplants in diabetic mice, subcutaneously implanted VIOs achieved normoglycemia significantly faster and more efficiently compared to islets that were transplanted in deviceless fashion.
Conclusion: We conclude that *ex vivo* bio-fabrication of VIOs enables islet engraftment and vascularization before transplantation, and thereby helps to overcome limited islet survival and function observed in conventional islet transplantation. Given recent progress in stem cells, this technology may enable assembly of functional personalized endocrine organs.

24 - Foundational Research (New Targets and New Indications)

Bioengineering of an iPSC derived vascularized endocrine organ for Type 1 Diabetes

Lorenzo Piemonti - IRCCS Ospedale San Raffaele, Italy

Background: Intrahepatic islet transplantation in patients with type 1 diabetes is limited by donor availability and lack of engraftment. To overcome these limitations, new sources of β cells and alternative sites are needed. Organ decellularization is an emerging strategy in organ regeneration. Based on our experience with decellularized rat lung as scaffold for the generation of Vascularized Islet Organ (VIO, lung scaffold repopulated by murine islets and HUVEC cells), we used the same platform to engineer an iPSC-based version.
Material and Methods: Rat lung was decellularized and seeded with iPSC derived β (iβ) and endothelial (iEC) cells, generating a Vascularized Endocrine Organ (VEO). VEO was cultured for 7 days in a customized bioreactor. Phenotype, function and vitality of iβ/iEC was assessed by flow cytometry, dynamic glucose perifusion and miR-375 expression analysis.
Results: iEC and iβ maintained *in vitro* their phenotype expressing endothelial (>95% CD31+/CD105+/CD73+/CD90- cells) and β-cell (>60% PDX1+/insulin+ cells) markers, respectively. VEO showed regenerated vascular network (CD31+) with iβ (insulin+) integrated. In VEO, the presence of the lung ECM is able to sustain iβ engraftment and survival and insulin secretion during maturation process (1 day vs 7 days). Insulin secretion was improved in mature VEO in comparison with standard 7 days of *in vitro* culture.
Conclusion: Our data demonstrated the plasticity of our platform (VIO) that was used to engineer the first human entirely iPSC-derived Vascularized Endocrine Organ (VEO).

25 - Foundational Research (New Targets and New Indications)

N-Glycosylation Profile of Undifferentiated and Differentiated Human Bone Marrow Mesenchymal Stem Cells

Marta Wieczorek - Charité Universitätsmedizin Berlin, Germany

Mesenchymal stem cells (MSCs) are multipotent cells that develop into several tissue types, migrate to diseased organs, have immunosuppressive properties, secrete regenerative factors and are easy to isolate and culture. The aim of this work was to characterize the cell surface N-glycome of MSCs and their differentiated progenies. For this purpose, human MSCs were first isolated from the bone marrow of donors and differentiated into adipocytes, chondrocytes and osteocytes. Then, cell surface glycopeptides were released from living cells and enzymatically cleaved N-glycans were analysed, after permethylation, using MALDI-TOF mass spectrometry. We were able to detect over 100 different N-glycans including high-mannose, hybrid, complex N-glycans as well as poly-N-acetyllactosamine chains.
Differentiation of MSCs into adipogenic direction was accompanied by an increased amount of biantennary fucosylated structures, a decreased amount of fucosylated as well as afucosylated tri- and tetraantennary structures, and an increased sialylation, which was corroborated by an upregulation of ST3GAL1 and a downregulation of FUT8 gene. The N-glycosylation profile of chondrogenically differentiated MSCs was investigated before and after isolation from their extracellular matrix (ECM) and compared to the profile of undifferentiated MSCs. In the presence of ECM, more high-mannose and less complex N-glycans were detected. In the absence of ECM, differentiation was accompanied with an increased amount of biantennary N-glycans. Cell surface N-glycosylation of chondrogenically differentiated MSCs was generally less branched than the one of undifferentiated MSCs. N-Glycans had higher amounts of core-fucosylation and LewisX epitopes. Osteogenic differentiation of MSCs was, in turn, characterized by an increased amount of biantennary structures and decreased amount of tri- and tetraantennary structures. The amount of fucosylated structures increased with differentiation and contained mainly core-fucose residues.
To conclude, the N-glycome of human MSC and their differentiated counterparts possess characteristic cell-type specific glycosylation features.

26 - Foundational Research (New Targets and New Indications)

Hypoxic preconditioning of adipose tissue-derived mesenchymal stem cells improves cells functionality and promotes protection against unfavorable environments in a diabetic rat-induced model.

Maria Dolores Carmona - Andalusian Network for Design and Translation of Advanced Therapies, Spain

Background: Adipose tissue-derived mesenchymal stem cells (ASCs) are multipotent cells useful in cell-therapy. It is known that hyperglycemia damage ASCs physiology. Hypoxia might represent a strategy to improve ASCs functionality.
Aim: We evaluated the *in vitro* effects of hypoxic-preconditioning on expanded-diabetic-ASCs analyzing their genetic-profile to improve the ASCs treatments in cell therapy Methods: ASCs were isolated from healthy (ASCs-C) and streptozotocin-induced diabetic (ASCs-D) Wistar rats. ASCs were expanded under normoxia conditions (21%O2) (ASCs-CN,ASCs-DN) or pre-conditioned for 48 hours under hypoxic condition (3%O2) (ASCs-CH,ASCs-DH). ASCs-populations were characterized by phenotype and differentiation capacity, population doubling, angiogenic, migration capacity and paracrine secretion were determined and a Gene-Chip microarrays were performed based on four comparative study groups: ASCs-CHvsASCs-CN, ASCs-DHvsASCs-DN, ASCs-DNvsASCs-CN and ASCs-DHvsASCs-CH. An enrichment analysis was performed using DAVID and cluster-Profiler and the protein-protein-interactions networks were constructed using STRING. Results: Hypoxia improved the ASCs-C and ASCs-D capacity to form tube-like structures. Interleukine-6 secretion were higher in ASCs-DN and ASCs-DH in comparison to ASCs-CN and ASCs-CH, respectively. Microarrays analysis showed that ASCs-DHvsASCs-CH comparative-group showed the greatest number of changes and ASCs-DHvsASCs-DN comparative-group the fewest.Enrichment analysis derived from ASCs-CHvsASCs-CN and ASCs-DHvsASCs-CH comparative-groups revealed an over-representation of terms and proteins related with angiogenesis modulation. Terms linked to mitosis were under-represented in ASCs-CHvsASCs-CN group. Enrichment analysis derived from ASCs-DNvsASCs-CN and ASCs-DHvsASCs-CH groups showed over-represented terms related with ASCs-malfunction and under-represented terms related with HIF-1α-stabilization.
Conclusion: Hypoxia pre-condition improves ASCs-C and ASCs-D functions and promotes their protection against unfavorable environments. However this pre-condition appears to be less effective in enhancing therapeutic features of ASCs-D in comparison to ASCs-C.

27 - Foundational Research (New Targets and New Indications)

Targeting microRNAs in cystic fibrosis (CF)

Roberto Gambari - University of Ferrara, Italy

Background. Cystic fibrosis (CF) is a lethal autosomal recessive genetic disease caused by a variety of mutations of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene. Since the demonstration that microRNAs are deeply involved in CF, great attention has been dedicated to possible alteration of CFTR gene expression by targeting those miRNAs involved in down-regulation of CFTR and associated proteins. In this case, Peptide Nucleic Acids (PNAs) appear to be of great interest, since they are capable of sequence-specific and efficient hybridization with complementary DNA and RNA.
Aim. To determine whether PNAs targeting microRNA involved in CFTR expression could enhance CFTR production in CFTR expressing cells.
Methods. RT-qPCR and Western blotting.
Conclusion. PNA-mediated inhibition of miR-145-5p and miR-101-3p (which down-regulate CFTR) leads to CFTR increase in Calu-3 cells. In addition to direct interaction with CFTR, miRNAs might regulate CFTR through binding to the 3'UTR of mRNAs coding CFTR regulators, such as NHERF1, NHERF2 and ezrin. Interestingly, PNA-mediated targeting of miR-335-5p, one of the miRNAs involved in NHERF1 regulation, was found to be associated with specific inhibition of miR-335-5p, increase of NHERF1 and increase of CFTR.
In addition to PNA-mediated targeting of CFTR-inhibiting miRNAs (anti-miRNA therapeutic approaches), miRNA-replacement therapy might also be considered. This might be a key strategy for inhibiting Pseudomonas aeruginosa dependent inflammatory responses, with relevant clinical implications. In this respect, miR-93-5p was demonstrated to be down-regulated during P.aeruginosa infection of CF cells. Accordingly, transfection of CF cells with pre-miR-93-5p was found associated with anti-inflammatory effects, including a decrease of IL-8 mRNA content and IL-8 protein release.

Acknowledgments: This work was supported by Fondazione Fibrosi Cistica (FFC), Project "MicroRNA Therapeutics in CF: Targeting CFTR and inflammation networks (MICRORNA-CF)" FFC#3/2016.

28 - Foundational Research (New Targets and New Indications)

Development of stem cell-based therapies for peripheral nerve injuries

Paul Kingham - Umeå University, Sweden

Background: Traumatic peripheral nerve injury (PNI) is common, with around 300,000 cases annually in Europe. PNIs can cause lifelong disability resulting from sensory, motor and/or autonomic deficits and intractable neuropathic pain, which has considerable social and economic impact. Next generation techniques of peripheral nerve repair, which aim to replace the current gold standard of nerve autograft, embrace the fields of tissue engineering and regenerative medicine and will likely incorporate cell and gene therapy approaches.
Aim: Our work combines stem cell technology and tissue engineering to create structures that mimic the key features of a nerve autograft, without the disadvantages of donor-site morbidity and the limited availability of donor tissue. The aim is to support and boost the endogenous regeneration provided by Schwann cells.
Methods and Results: Our earlier results showed that rodent adult stem cells can be efficiently differentiated into Schwann cell-like cells *in vitro*, and when transplanted *in vivo* they enhance axon regeneration and sensory neuron survival after injury. However, the Schwann cell properties are not consistently reproduced in human cells suggesting that this approach is not yet optimised for clinical applications. The long held belief that transplanted stem cells will directly rebuild damaged tissue is also now questioned. Despite showing positive effects of the stem cell transplantation, we have failed to detect long-term survival and integration of the cells. Rather, our results indicate that the stem cell secretome, released bioactive factors, underlie the therapeutic benefits of the cells. *In vitro* conditioning stimulation of the stem cells enhances release of a variety of neurotrophic and angiogenic factors. Furthermore, we have determined that extracellular vesicles, another component of the secretome, transfer mRNA and miRNAs to neurons, which enhances axon outgrowth.
Conclusions: We suggest that harnessing the properties of the stem cell secretome might be a useful adjunct for novel therapeutic approaches in the treatment of PNIs.

29 - Foundational Research (New Targets and New Indications)

Translating the STAT5 activation and repression code to advanced therapeutic strategies

Eleni Katsantoni - Biomedical Research Foundation Academy of Athens, Greece

Background: Signal transducer and activator of transcription 5 (STAT5) is a transcription factor that transduces signals from activated cell surface receptors to the nucleus to modulate transcription. STAT5 controls essential cellular functions and is encoded by two genes, Stat5a and Stat5b. STAT5 deficiency leads to severely impaired lymphoid development and perinatal death, and its constitutive activation is a hallmark of solid and hematologic malignancies.
Aim: Understanding STAT5 role in activation and repression of target genes is important for identification of biomarkers and designing of novel molecular strategies for therapeutic management of such disorders.
Methods: To understand the mechanisms of transcriptional activation and repression mediated by STAT5, we combined high-throughput genomics, transcriptomics and proteomics approaches. Using these approaches together with omics data integration methods, the full genome-wide map of STAT5 target genes was generated and a network of interacting proteins was defined.
Conclusion: Analysis of a STAT5a/LSD1/HDAC3 interactions network defined a dual function of LSD1 and HDAC3 on STAT5-dependent transcription (Nanou et al, 2017). Target genes of STAT5 are currently tested for their potential use as biomarkers in various hematologic malignancies, to contribute to the therapeutic management and stratification of the patients.
Furthermore inhibitors that block STAT5 and co-factors interactions are currently tested. It is anticipated that the use of target genes as biomarkers, and the targeting of co-factors or related interactions will contribute to efficient monitoring of advanced therapies and will provide novel targets for therapeutic interventions.

References: Nanou A, Toumpeki C, Lavigne MD, Lazou V, Demmers J, Paparountas T, Thanos D, Katsantoni E. The dual role of LSD1 and HDAC3 in STAT5-dependent transcription is determined by protein interactions, binding affinities, motifs and genomic positions. Nucleic Acids Res. 2017 Jan 9;45(1):142-154. doi: 10.1093/nar/gkw832. Epub 2016 Sep 19. PMID: 27651463.

30 - Foundational Research (New Targets and New Indications)

Characterization of aged dermal stem cell phenotype: implications for skin homeostasis

Ander Izeta - Biodonostia Health Research Institute, Spain

Functional decline of aged organisms is associated to tissue-specific stem cell impairment, which leads to homeostasis alterations. Dermal stem cells (DSC) lose regenerative capacity with age. The DSC pool includes Schwann cell precursors and perivascular cells, their stemness being mediated by Sox2 expression levels. We hypothesized that DSC activity may decrease during the skin ageing process as a consequence of Sox2+ cell population impairment. To test this hypothesis, we characterized *in vitro* and *in vivo* the DSC niches in young (2 month) vs aged (>18 month) C57BL/6 and Sox2EGFP mice (heterozygous for Sox2). As expected, cutaneous microvessel and nerve terminal density decreased with age, concomitant with diminished proliferation and differentiation capacities of aged-DSC. Moreover, Sox2 EGFP+ cell number decreased with age. Based on EGFP intensity, three Sox2+ cell subpopulations were defined: Sox2 high (Sox2hi), medium (Sox2med) and low (Sox2lo). Interestingly, in aged-DSC Soxhi cells were depleted and Sox2lo cell population enriched, indicating that Sox2 expression levels reduced with age. This reduction in aged-DSC resulted in loss in stemness capacities of Sox2+ cells, as measured by neural differentiation potential. Additionally, Sox2 heterozygosity resulted in adipogenic gene upregulation already in the young, and increased thickness of dermal white adipose tissue in old mice. Furthermore, histological analyses revealed impaired dermal extracellular matrix organization and composition. Altogether our results suggest that dermal homeostasis is compromised due to changes in aged DSC fate via modulation of Sox2 expression levels.

31 - Foundational Research (New Targets and New Indications)

Structural analysis of neuromuscular junctions in aging: effect of Sox2 heterozygosity

Ander Izeta - Biodonostia Health Research Institute, Spain

Aims: The neuromuscular junction (NMJ) is the synaptic interface through which motor neurons innervate fibers enabling muscle contraction. It is composed of nerve terminals, endplates (organized in characteristic pretzel-like structures), and terminal Schwann Cells (tSCs) that ensure functionality and maintenance of NMJs. During aging, synaptic activity is impaired, followed by muscle atrophy and declined muscle mass and function (sarcopenia). Pretzel structures dissolve and nerves disconnect from fibers. The transcription factor Sox2 is a key regulator of the Schwann cell lineage. Since Sox2 levels decrease during aging, we hypothesized that this decrease might impact the structure and function of tSCs capping NMJs during sarcopenia and aging. Methods: Skeletal muscles of young, adult and old WT and heterozygous Sox2GFP mice were analyzed by confocal microscopy. Qualitative and quantitative analysis of NMJs were performed. Results: We detected relevant degeneration in NMJs during aging. Differences in NMJ structure were observed in WT and Sox2GFP endplates during aging. A higher frequency of denervated-NMJs and NMJs without capping-tSCs was observed in Sox2GFP compared with WT. Conclusions: These results suggest an essential role of Sox2 in the maintenance of the NMJ-structure during aging, possibly through tSC modulation, and shed light into the mechanisms underlying sarcopenia.

32 - Foundational Research (New Targets and New Indications)

Using cartilage intermediate organoids for microengineering skeletal implants that exhibit predictive functionality and whole bone organogenesis potential

Ioannis Papantoniou - Katholieke Universiteit Leuven, Belgium

Background and novelty: The field of Regenerative Medicine and Tissue Engineering seeks to build functional tissues ultimately replacing failing organs, thereby curing the patient. The lack of living building blocks is a major hurdle in the manufacturing of human scale functional living implants. The ability to produce large populations of small functional tissue niches that could be used for bottom-up assemblies of larger tissues would constitute a major step towards addressing this bottleneck. This could be achieved by providing a living engineering medium able to yield predictive results upon implantation. Experimental approachIn the present study we produced, in a scalable manner, thousands "cartilage intermediate" organoids using adult progenitor cell populations. Cells in the microtissues appeared to undergo developmental processes *in vitro* mimicking those encountered in the embryonic growth plate and during fracture healing *in vivo*. Upon reaching a degree of "autonomy", as defined by genomic comparison to developmental controls, these microtissues were able to continue their biological program upon implantation, resulting in the formation of bone organs without inappropriate contaminating tissue structures. This capacity was robustly exhibited either when implanted as single organoids or in larger self-assembled multimodular tissues, independent of the implantation site. Strikingly critical size murine long bone defects (5 mm) were healed within natural physiological time scales with the development of a de novo bone marrow compartment while in all cases abundant implanted cells were present, demonstrating their critical role in the regenerative process. Results and discussion: With these advancements, we believe that we contribute towards an era were modular tissue implants possessing 'design specifications' could be produced at a scalable and robust manner. This paves the way for the mitigation of unmet clinical challenges of large non healing tissue defects such as critical size bone non-unions. These organoids could be viewed as a living 'bioink' allowing manufacturing of modular implants with intricate geometric features able to reach the clinic.

33 - Foundational Research (New Targets and New Indications)

2nd Generation Growth Factors for Clinical Applications

Joachim Nickel - University Hospital Würzubrg, Germany

Background: Several growth factors (GFs) are nowadays clinically used in order to treat diverse diseases such as forms of blood cancer but also non-union bone fractures. In every case the particular factor has to be applied together with proper carrier systems allowing a controlled release of the factor over a distinct period of time or the factor itself has to be modified e.g. in order to prevent a fast elimination by renal clearance. Both approaches aim to prevent a repeated application of the factor, which in case of a systemically acting factor would circumvent periodically applied injections/infusion by medical personal but in case of a locally acting factor would prevent multiple surgical interventions. Alternatively, these factors can be produced by autologous, genetically manipulated cells thus preventing immune reactions if transferred back into the patient but still being associated with (bio-)safety concerns.
Aim: In order to reduce the required GF doses it is essential to increase its bioactivity/cell signalling capacity, which is typically associated with the GF´s receptor binding affinity. The so generated GF variants can recombinant be expressed in autologous cells or, if the GF variant should be applied ectopically, linked to appropriate carrier structures.
Methods: Structural data and/or data obtained from mutation/interaction analyses allow a structure based design of a GF with superior cell signalling characteristics. The cDNAs encoding for the optimized GF can be introduced into the host cell utilizing lentiviral or adenoviral vectors. Alternatively, the cDNAs can be expressed in bacterial or higher eukaryotic expression systems and the proteins isolated and purified to homogeneity. In terms of a bacterial expression, we established protocols for the insertion of artificial amino acids allowing a site-directed immobilization of the GF via click-chemistry.
Conclusion: The optimal application form of GFs for clinical use is up to this date in scientific debate and the two forms described here certainly have their individual pros and cons. Nevertheless, both will benefit from a rational optimization of the growth factor´s bioactivity.

34 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Improving the therapeutic potential of lysosomal enzymes to treat CNS in lysosomal storage disorders

Rosanna Aiello - Tigem, Italy

Lysosomal Storage Disorders (LSD) are a large group of inherited genetic diseases caused by impaired activity of lysosomal enzymes leading to accumulation of undigested macromolecules within the lysosomes and thus cell dysfunction. The clinical manifestation is heterogeneous and neurological involvement represents a major problem. Our group has given evidences of how it is possible to increase CNS treatment efficacy in LSDs by either systemic or intrathecal gene therapy approaches employing engineered lysosomal enzymes opportunely modified to both enhance cross-correction capability and allow BBB crossing. We also want to modify lysosomal enzymes by inducing activating mutations to enhance their catalytic activity. To this aim we have developed and validated a fluorescence-activated cell-sorted (FACS) assay trough which is possible to screen in a high-throughput way randomly mutagenized lysosomal hydrolases to identify gain of function enzyme mutants. The overall goal of this research line is to develop gene therapy strategies with enhanced therapeutic potential and improved clinical translationality to treat CNS in multiple LSDs.

35 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Vascular regeneration by human pluripotent stem cells-derived endothelial cells

Gabor Foldes - Imperial College London, United Kingdom

Background: Atherosclerosis is one of the most common pathophysiologies of diseases. Given an ageing European population with an increasing prevalence of diabetes and hyperlipidaemia, the treatment of millions of patients will have an expanding impact on clinical and economic resource use. It was hoped that delivering viable vascular cells may transform the treatment of ischemic diseases with an anticipated impact rivalling the results of recent surgical revascularisation and device therapies. However, whilst feasibility results of the cardiovascular cell therapy trials were acceptable so far, inconsistent benefits and mortality outcomes limit their clinical application. This may be due to rapid clearance of the exogenously administered cells from the transplantation site. Aim: We believe that a combination of hPSC-derived endothelial cells (hPSC-EC) and biomatrices may be a promising tissue engineering approach to repair ischemic tissues. Methods: Our preferred strategy was decellularisation of the vessel wall from allogeneic or xenogeneic sources to develop a structurally sound 3D matrix and recellularise them with hPSC-EC. We obtained expandable populations of hPSC-EC which expressed mature endothelial markers. High-resolution SPECT/CT and PET/MRI showed that cells formed vascular networks in small animals *in vivo*. In a clinically relevant large animal model, hiPSC-EC-seeded onto decellularised vascular segments were functional as aortic grafts. Expression of angiogenic factors was induced during follow-up, suggesting that endothelial cells may undergo maturation. By quantifying the reendothelisation activity of hPSC-EC, we showed their good retention as well as a dynamic remodelling of the vessel wall with good maintenance of vascular patency. Conclusion: There is a need for new vascular engineering design of responsive, native-like conduits with the favourable properties of anti-thrombogenicity, and biomechanical stability. Adding exogenous cells to decellularised vessels may carry some costs in terms of complexity and regulatory hurdles but would also provide decisive functional benefits in endothelial regeneration and vascular therapy.

36 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Multicore, fast-clearing perfluorocarbon-loaded multimodal nanoparticles for stable ultrasound and 19F MRI applied to *in vivo* cell tracking

David Morrow - EATRIS, Netherlands

Functional decline of aged organisms is associated to tissue-specific stem cell impairment, which leads to homeostasis alterations. Dermal stem cells (DSC) lose regenerative capacity with age. The DSC pool includes Schwann cell precursors and perivascular cells, their stemness being mediated by Sox2 expression levels. We hypothesized that DSC activity may decrease during the skin ageing process as a consequence of Sox2+ cell population impairment. To test this hypothesis, we characterized *in vitro* and *in vivo* the DSC niches in young (2 month) vs aged (>18 month) C57BL/6 and Sox2EGFP mice (heterozygous for Sox2). As expected, cutaneous microvessel and nerve terminal density decreased with age, concomitant with diminished proliferation and differentiation capacities of aged-DSC. Moreover, Sox2 EGFP+ cell number decreased with age. Based on EGFP intensity, three Sox2+ cell subpopulations were defined: Sox2 high (Sox2hi), medium (Sox2med) and low (Sox2lo). Interestingly, in aged-DSC Soxhi cells were depleted and Sox2lo cell population enriched, indicating that Sox2 expression levels reduced with age. This reduction in aged-DSC resulted in loss in stemness capacities of Sox2+ cells, as measured by neural differentiation potential. Additionally, Sox2 heterozygosity resulted in adipogenic gene upregulation already in the young, and increased thickness of dermal white adipose tissue in old mice. Furthermore, histological analyses revealed impaired dermal extracellular matrix organization and composition. Altogether our results suggest that dermal homeostasis is compromised due to changes in aged DSC fate via modulation of Sox2 expression levels.

37 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

In vivo gene therapy strategies for preclinical models of human diseases affecting the central and the peripheral nervous system

Miguel Chillon - Vall d'Hebron Research Institute, Spain

Limitations of conventional pharmacological treatments can potentially be overcome by gene therapy strategies using viral vectors which may allow to achieve long-term therapeutic effect by a single-dose administration, restrict its action to specific cell types by using cell specific promoters or a particular vector, reach the central or the peripheral nervous system through a non-invasive route, or even cross the blood brain barrier, thus avoiding secondary effects of the drug in non-target organs or facilitating the administration of the treatment in the nervous system. In this context, our research group at the UAB-VHIR Joint Unit has 25 years of experience using a variety of gene therapy vectors in relevant preclinical models of different human diseases affecting different organs such as liver, skeletal muscle, lung or the central and peripheral nervous systems. Moreover, a battery of administration routes allows us to target many other tissues in preclinical mouse models. Here, we will present a brief summary of our latest results on relevant preclinical models for rare diseases such as muchopolysaccharidosis VII, megalencephalic leukodystrophy with cysts as amyotrophic lateral sclerosis; as well as non-communicable diseases like dementia, Alzheimer's Disease and multiple sclerosis; or pathologies affecting the PNS as diabetic neuropathy or peripheral nerve regeneration. Extensive knowledge of molecular, physiological, and functional phenotyping of the treated animals, together with biodistribution and biosafety analysis of the vectors will be shown.

38 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Generation of preclinical models and use of gene therapy strategies for the treatment of LMNA associated congenital muscular dystrophies

Ignacio Pérez de Castro - Instituto de Salud Carlos III, Spain

LMNA-associated congenital muscular dystrophy (L-CMD) is a rare disease with no cure, associated with mutations in LMNA and characterized by progressive muscle weakness, atrio-ventricular conduction system anomalies, cardiac tissue fibrosis and respiratory failure. Our main goal is to find new therapeutic approaches for the treatment of L-CMD. In order to achieve this, we have generated cell and mouse models with a genetic modified LMNA exon4. In addition, we have explored the therapeutic potential of homology-directed independent integration (HITI) mediated by CRISPR/Cas9 in the treatment of L-CMD. We have generated a new, KI mouse model for the expression of LmnaR249W, one of the main mutations found in L-CMD patients. This model recapitulates the cardiac phenotype found in L-CMD patients. Also, we have obtained a collection of exon4, Lmna mutants in C2C12 myoblasts that have been used to study the role of exon4 on LMNA function, the effect of deleting, partially or fully, exon4 and the pathogenic mechanisms associated with R249W. Finally, using HITI, we have been able to integrate a wild-type, LMNA minigene in human myoblasts carrying the R249W mutation. The edited myoblasts were characterized by the inhibition of the expression of the mutant allele, the recovery of wild-type nuclear morphology and the rescue of the myogenic differentiation.In summary, we have generated cell and mouse models that can be used to better understand L-CMD and test new therapeutic strategies. In addition, we have obtained a proof of concept for the therapeutic potential of HITI technology for the treatment of L-CMD.

39 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Tumor infiltrating lymphocytes in pediatric patients: analysis, expansion under GMP conditions and initial clinical experience

África González - FIB Hospital del Niño Jesús, Spain

Adoptive transfer of tumor infiltrating lymphocytes (TILs) were the first cellular immunotherapy to mediate regression of mainly melanoma tumor and more than 20% of patients have remained disease free for many years. There is not much development of TILs for pediatric cancers. We are setting up conditions to explore the clinical use of TILs in children with refractory solid tumors. Surgically removed tumors of rhabdomyosarcoma, neuroblastoma and Ewing sarcoma patients from Hospital Universitario del Niño Jesús were transported to the GMP Facility. Culture of TILs were performed after digestion of tumor tissues in enzymatic digestion medium and in some cases, from tumor fragments.
Following Rosenberg´s protocol cells were cultured in p24 plates during 14 days in RPMI supplemented with 6000UI IL-2. Then, a Rapid Expansion Protocol was performed with OKT3 and feeder stimulation during another 14 days. At different days post culture, viable cells were analyzed by multicolor flow cytometry to characterize immune cell composition using a panel for several immune cell populations. Proliferation ability and cytokine expression of some expanded TILs were also analyzed. Results from the different patients varied in terms of total expansion and TILs subpopulations but we were able to efficiently expand TILs from pediatrics tumors samples. Three patients were infused with TILs products at range doses of 200x10e6 cells for P-001, 108x10e6 cells for P-003 and 2000x10e6 cells for P-005. No adverse event was detected in these treated patients, but fever in close relatioship with IL-2 administration.

40 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Improved T Cell Activation Bioassays for Development of Bispecific Antibodies and Engineered T Cell Immunotherapies

Axel Johann - Promega, Germany

Immunotherapy aims to boost a patient's own immune system to fight disease. The strategy is aimed at inducing, strengthening or engineering T cell responses have emerged as promising approaches for the treatment of cancer and autoimmune disease. Here we describe a platform of T cell activation bioassays for the development of CD3 bispecific antibodies and engineered T cell immunotherapies. Specifically, we developed two bioluminescent reporter-based bioassays to measure T cell activation via TCR/CD3 or TCR/CD3 plus CD28 co-stimulation.These assays reflect the Mechanism of Action (MoA) of biologics designed to engage and stimulate T cell activation to attack target disease cells and provide consistent and reliable measurement of potency of pathway activation for anti-CD3 bispecific Ab and CAR-T cell activity.

41 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Geometry and strain sensing dictate YAP-dependent mechanical signaling in human cardiac stromal cells

Maurizio Pesce - Centro Cardiologico Monzino IRCCS, Italy

Background: Cardiac stromal cells are the most abundant cell type present in the heart, where they have an important homeostatic function in matrix renewal and electromechanical coupling. These cells may have a role in stiffening of the myocardial MatrixAim: Given the known role of the Hippo pathway in cell-based mechanosensation, we characterized the conditions for activation of the YAP-dependent transcriptional pathway with reference to geometry/strain sensing using human the 'cardiosphere', a known cardiac niche organoid model. Methods: Human cardiospheres (C-Sp) and C-Sp-derived cells (CDCs) were obtained from fragments of atrial appendages from patients admitted for AoC bypass. C-Sps and CDCs were cultured and analysed by molecular tools and quantitative imaging, using already published methods. Conclusions: CDCs plated onto stiff (E > 50kPa) culture plates exhibited high ratios of nuclear/cytoplasmic YAP, as compared to cells plated on substrates with lower stiffness (E ~ 10kPa). Analogously, cells with a stretched cytoplasm/nuclear shape in the border regions of the 3D organoids exhibited a higher YAP nuclear localization level and expression of fibrotic markers compared with cells with a more round geometry in the center of the spheres. To uncouple the cytoskeleton tensioning-dependent YAP nuclear shuttling, we treated CDCs plated onto stiff substrates with blebbistatin and Y27632. This showed a fully reversible YAP relocation from the nucleus to the cytoplasm and a reversion of canonical target genes (CYR61, ANKRD1 and CTGF) expression. These results establish a relationship between pro-pathologic evolution of human adult cardiac stromal cells and activation of mechanosensing-dependent pathways. They further provide criteria for reducing the impact of cardiac fibrosis and Heart Failure.

42 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Pro-fibrotic evolution of human saphenous vein progenitors by mechanical stress is mediated by a YAP/TEAD transcriptional circuitry

Maurizio Pesce - Centro Cardiologico Monzino IRCCS, Italy

Background: Implantation of saphenous vein (SV) graft in coronary position determines vessel wall remodeling and intimal hyperplasia. We have recently found that cell mechanosensing may play a crucial role in this pathology. Aim: We investigated the response of saphenous vein progenitors (SVPs) to mechanical stimulation mimicking the cyclic wall strain determined by SV implantation into coronary position. Methods: human-derived SVPs were isolated with a MACS based protocol using a positive selection for CD34 and a negative depletion of CD31+ cells. Cells were subjected to uniaxial strain (10% elongation; 1Hz) for 24 and 72 hrs using the FlexCell system, followed by cell analysis by Immunofluorescence and RNA extraction for RNA-sequencing analysis Conclusions: A gene enrichment analysis of transcripts up/downmodulated by mechanical stress revealed an involvement of the HIPPO/YAP/TEAD transcriptional circuitry in cells stimulated with the cyclic strain. In keeping with this hypothesis, mechanical stimulation increased significantly the nuclear translocation of the YAP protein. Treating cells with drugs interfering with the actomyosin cytoskeleton (Forskolin) determined a completely reversible relocation of YAP protein from the nucleus. This event coincided with decreased expression of canonical target genes (CYR61, ANKRD1 and CTGF), as verified by Q-RT-PCR. The use of a molecule preventing association of YAP/TAZ complex with DNA binding protein TEADs (Verteporfin) caused a similar downregulation of YAP targets without interfering with cytoskeleton tensioning. The two inhibitors also inhibited the motility of SVPs in migration assays. Together, these findings demonstrate the susceptibility of human SVPs to elongation/compression forces and show the specific role of YAP-dependent transcription in activation and recruitment of SVPs in mechanical activation of SV pathologic process.

43 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

A drug combination administered via an implantable, polymeric delivery system improves endogenous regeneration in a rat model of spinal cord injury (SCI).

Laura Calzà - University of Bologna, Italy

Introduction: SCI is a incurable condition, in which a cascade of cellular and molecular events triggered by inflammation and excitotoxicity impairs endogenous regeneration, namely remyelination and axonal outgrowth. We designed a treatment solution based on an implantable biomaterial (electrospun PLLA) loaded with ibuprofen and triiodothyronine (T3) to counteract inflammation, thus improving endogenous regeneration. METHODS: PLLA scaffolds were prepared by electrospinning technology. The drug-loaded PLLA has been characterized in term of drug release over 14 days (HPLC for ibuprofen; UPLC-MS/MS for T3). *In vivo* efficacy has been tested by implanting the drug-leaded-PLLA in the rat model of T8 contusion spinal cord injury (N=15). As control, drug-unloaded PLLA was implanted in lesioned rats (N=15). The BBB score for locomotion and gait analysis were used as efficacy primary end-points, flow cytometry for tissue cell composition, immunohistochemistry and computerized image analysis for remyelination and axonal protection evaluation. Results: we observed the expected locomotion recovery starting at day 7 (BBB score). In PLLA-implanted rats, the recovery stabilizes at 21PLD, so that non further improvement is observed. On the contrary, in PLLA+Ibu+T3 rats a significant treatment-effect is observed ($F(7,216)=7.349$, $P=0.0073$). This positive effect was also confirmed by the step sequence regulatory index in the gait analysis at post-lesion day 49 ($p=0.0097$). The glutamate release at 24 hours and 8 DPL is reduced PLLA+Ibu+T3-compared to PLLA-implanted rats ($p<0.05$; $p<0.01$, respectively), such as the estimated lesion volume (rostro-caudal $p=0.0101$; dorso-ventral $p=0.0041$). The myelin and 200-neurofilament-positive area-fraction is higher in PLLA+Ibu+T3 implanted rats ($p<0.05$; $p<0.01$, respectively), where the % of astrocytes is significantly reduced ($p<0.05$). Discussion & Conclusions: The implant of PLLA electrospun scaffold loaded with ibuprofen and T3 significantly improves the endogenous regeneration (i.e. remyelination and axonal outgrowth), leading to an improvement of the functional locomotion outcome in the spinal cord contusion injury.

44 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Amniotic membrane-derived mesenchymal stromal cells affect B cell recruitment and distribution in lungs of mice with bleomycin-induced pulmonary fibrosis

Anna Cargnoni - Centro di Ricerca "E. Menni" Fondazione Poliambulanza Istituto Ospedaliero, Italy

Background: The role of inflammation in the pathophysiology of pulmonary fibrosis remains controversial, and continuous research is focused in understanding the interplay between inflammatory and fibrotic processes.We have previously shown that treatment with cells derived from amniotic membrane reduced bleomycin-induced lung fibrosis in mice. Aim: In this study, we explored the effects of amniotic-derived mesenchymal stromal cells (hAMSCs) on lung inflammatory populations of mice with bleomycin-induced lung fibrosis, and possibly correlate these effects with their ability to reduce pulmonary fibrosis. Methods: We performed a longitudinal analysis of the effects of hAMSCs on changes in alveolar and lung immune populations induced by intra-tracheal instillation of bleomycin. Immune cells collected through broncho-alveolar lavage were examined by flow-cytometry and lung tissues were used to study gene expression of markers associated with different immune cell types. Results: hAMSC treatment transiently increased alveolar levels of T regulatory cells (Treg) and lung expression of Treg marker Foxp3, while hAMSCs did not affect levels of monocyte-derived macrophages, but reduced their antigen-presentation potential and increased polarization toward M2 phenotype. For the first time, we observed that hAMSC treatment markedly reduced B cell alveolar levels and lung expression of the specific marker B220. Furthermore, we observed the formation of lymphoid aggregates, comprised of B and T cells in variable proportions, in bleomycin-challenged lungs that expand in numbers and size during the course of lung injury. Interestingly, hAMSCs blocked the formation, the expansion and B cell enrichment of lymphoid aggregates. Conclusion: Since B cell aggregates have been found associated with various respiratory diseases including idiopathic pulmonary fibrosis, and have been proposed to sustain chronically evolving inflammation in the fibrotic process, the ability of hAMSC treatment to control B cell recruitment and reduce the formation of lymphoid aggregates in bleomycin-induced lung injury can contribute to limit the progression of fibrotic lesion.

45 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Cellular virotherapy increases tumor-infiltrating lymphocytes (TILs) and CD8+ activation in murine immunocompetent models

Laura Hidalgo, Álvaro Morales - Instituto de Salud Carlos III, Spain

Oncolytic virotherapy uses virus designed to selectively replicate and kill cancer cells. In contrast to an intratumoral or intravenous administration, our group uses mesenchymal stem cells (MSC) as cellular vehicles to transport the oncolytic adenovirus to the tumor site. This viroimmunotherapy, named Celyvir, has been already applied to children in a human clinical trial and a veterinary trial with good clinical responses. Despite this promising results, development of more realistic immunocompetent animal models are still necessary for the proper study of the treatment. Here we have developed and validated a murine version of Celyvir (mCelyvir) using mouse MSC infected with the murine oncolytic adenovirus dlE102 in immunocompetent mouse models of renal adenocarcinoma and melanoma. In both models, mCelyvir significantly reduced tumor growth by 50% and induced changes in number and activation of tumor-infiltrating lymphocytes (TILs). In conclusion, our cellular virotherapy shows antitumor activity based on the activation of the immune system

46 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

AKT and JUN are Differentially Activated in Mesenchymal Stem Cells After Infection with Human and Canine Oncolytic Adenoviruses

Laura Hidalgo, Álvaro Morales - Instituto de Salud Carlos III, Spain

There is increasing evidence about the use of oncolytic adenoviruses (Ads) as promising immunotherapy agents. We have previously demonstrated the clinical efficiency of using mesenchymal stem cells (MSCs) as cellular vehicles for oncolytic Ads, human ICOVIR5 or canine ICOCAV17, respectively. Considering the better clinical outcomes of canine Celyvir, in this study we searched for differences in MSC cellular responses to Ad infection that may help understand the mechanisms leading to higher antitumoral immune response. In other primary cell types Ads infection activate the NF-κB pathway and interferons and pro-inflammatory cytokines secretion. However, our findings indicates that human ICOVIR5 activates AKT and JUN in both human and canine MSCs whereas canine ICOCAV17 does not, suggesting that ICOCAV17 induces a more limited host response in MSCs which may be related to the better clinical outcome. This mechanism of action would be imitated by selecting specific human MSC in base to their limited host response after Ads infection.

47 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Gene replacement therapy for peripheral demyelinating neuropathies

Alexia Kagiava - The Cyprus Institute of Neurology and Genetics, Cyprus

Loss of function mutations in the GJB1 gene, encoding gap junctions protein connexin32 (Cx32), cause X-linked Charcot-Marie-Tooth (CMT1X) disease, one of the commonest forms of inherited demyelinating peripheral neuropathy. In order to develop a translatable gene therapy approach, we cloned the Mpz.Egfp (mock vector) and Mpz.GJB1 (full vector) expression cassettes driven by myelin protein zero (Mpz) promoter into the AAV transfer plasmid and used the AAV9 vector serotype to target Schwann cells. We further tested the ability of a similar vector driven by a short (0.41 kb) part of the Mpz promoter (mini-Mpz) to drive Schwann cell targeted expression. Following lumbar intrathecal injection of the AAV9-Mpz.Egfp and the mini-Mpz vector in 2-month old wild type (WT) mice, EGFP reporter gene expression was detected in the perinuclear compartment of Schwann cells in lumbar roots, sciatic and femoral nerves. After delivery of the AAV9-Mpz.GJB1 therapeutic vector into 2-month old Cx32 knockout (KO) mouse model of the disease, Cx32 expression was detected in the paranodal non-compact myelin areas of myelinated fibers. We then performed a post-onset treatment trial in which 6-month old Cx32 KO mice, after the onset of the neuropathy. Outcome was assessed at 8 and 10 months of age by behavioral, electrophysiological and morphological analyses comparing mock or therapeutic vector injected groups. We observed improved motor performance and sciatic nerve conduction velocities, as well as improved myelination and reduced inflammation in PNS tissues of treated mice. Our study provides evidence that a clinically translatable AAV9-mediated gene therapy approach targeting Schwann cells could be potentially used for the treatment of CMT1X, even after the onset of the disease. Furthermore, other demyelinating CMT neuropathies could be treated using a similar Schwann cell-targeted approach.

48 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Combined in silico and *in vitro* human tumor test systems on a decellularized tissue matrix to understand therapeutic mode of actions for translation into the clinic

Gudrun Dandekar - University Hospital Würzburg, Germany

Background: Arising strategies against cancer demand more sophisticated models to understand parameters for permanent success and to know which patients benefit. Reliable models are an urgent need as conventional models such as 2D and animals show high failure rates.Aim: Our aim is to understand the complex interplay between different cell types and signaling dependencies by improved human in silico and 3D tissue models to develop efficient and long-lasting intervention strategies. This should lead to tumor signatures that allow patient-stratification. Methods: We set up in silico topologies of signaling networks for lung cancer that integrate specific driver mutations. These can be utilized for dynamical modeling of drug responses. On a decellularized matrix derived from porcine jejunum (BioVaSc-TERM® technology) we generate solid tumor models that show a better predictivity than 2D and animal models due to more physiologic growth conditions. As unique features the preserved basement membrane and vessel structures allow to investigate metastasis formation and immune cell migration. Drug responses can be translated to the in silico model to optimize the connectivity and integrated signaling nodes to experimental data. We could demonstrate CAR T-cell efficacy in solid tumor models for lung and breast cancer, but not complete eradication in deeper tumor tissue layers. Bioreactor cultures allow enhanced tissue formation and testing of relevant parameters such as T cell exhaustion over several weeks. Models can be expanded by further cell types such as immune cells, endothelial cells, healthy fibroblasts and Cancer Associated Fibroblasts (CAFs) to investigate safety aspects and efficacy in a more immunosuppressive microenvironment and to develop strategies against therapy resistance.Conclusion: By combination of in silico and tissue engineered tumor models and their optimization, we would like to contribute to the Restore consortium mode of action studies that should lead to hypothesis driven therapeutic intervention strategies and patient stratification. In the advent of NGS this is a pre-requisite for efficient translation of academic research to the clinic.

49 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Optimising TALEN-mediated CCR5 knockout in human T cells for large-scale production

Lea-Isabell Schwarze - University Medical Center Hamburg-Eppendorf, Germany

For 2017 the World Health Organisation estimated that about 36.9 Mio people worldwide were infected with HIV and that about 900.000 people died of AIDS-related illnesses. In our lab we recently developed a novel TALEN, namely CCR5-Uco-TALEN, to target the chemokine-receptor 5 (CCR5), the co-receptor of M-tropic HIV-1 strains. Knockout of CCR5 in autologous CD4+-T cells could protect T cell subsets from de novo infection and aid HIV-positive patients to control their viremia. We have now improved safety of our CCR5-Uco-TALEN by exchanging the wildtype homodimeric FokI-binding domain against a codon-optimised, obligatory heterodimeric FokI-variant. This reduces the number of possible off-targets drastically, while CCR5 on-target cutting rates remain within the same range. Furthermore, we optimized *in-vitro* production of CCR5-Uco-hetTALEN mRNA by introducing a poly(A) tail into the production plasmid, which reduces the batch to batch differences in mRNA production, as well as the costs, especially for large-scale production. In order to meet regulatory requirements, we developed new ddPCR-based assays to evaluate large deletions and translocations caused by the CCR5-Uco-hetTALEN at the on-target side CCR5. Most importantly, we are developing a protocol at the CliniMACS Prodigy from Miltenyi to separate, activate, treat and expand CD4+ T cells. Preliminary results show cell counts of up to 5x10^9 cells after an expansion phase of 9 days post electroporation with CCR5-Uco-hetTALEN, as well as high gene editing frequencies of >75% at the CCR5 on-target site. In additional validation steps we will be finalizing our protocol and preparing for GMP-grade production.

50 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

3D Bioprinted Colon Tissue for Drug Screening and Disease Modeling

Daniel Cermeno - Hospital La Paz, Spain

Drug discovery is a long, complex process with growing difficulty. In average, new drugs take 10 years from invention to approval, and less than 12% of new candidates entering Phase1 are finally approved. This fact is especially problematic for advanced therapy medicinal products (ATMP), which in general are taking longer to approve, and raise more major objections than other medicines. Evidence shows that the regulatory strategy employed is in some cases sub-optimal, in particular the use of scientific advice. One reason for such poor translation from drug candidates to successful clinical use is a lack of model systems that accurately recapitulate tissue function of human organs, and their response to drug compounds. The so-called organoids are three dimensional (3D) culture models, which have the ability to mimic the spatial and chemical attributes of native tissues, and have now proven to provide better results for drug screening compared to 2D cultures and animal models. However, there are several limitations in current organoid fabrication technologies, such as uncontrolled size, poor reproductively, and inadequate complexity. Here, we describe a high-throughput 3D bioprinting model of colon tissue that fills the gaps found in the previous mentioned systems, and presents high efficacy levels for pharmaceutical studies.Patient biopsies were disected to obtain colonic crypts, which are then emedded in a platelet enriched hydrogel, and bioprinted following a multilayered biomimetic approach. Then, a co-culture system was introduced by bioprinting a mixture of collagen and cancer-associated fibroblasts in lattice patterns. This resulted in the formation of the characteristic mucosa found in the gastrointestinal tract. Our models are positive for colon cancer stem cell markers CD133, EpCAM, and Lgr5, and represent the necessary ECM reorganization for colon cancer development. Finally, we demonstrate that our approach enables translational applications, such as activating and expanding tumor-reactive T cell populations, and predicting patient-specific treatment outcomes.

51 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Development of a personalized 3D *in vitro* microfluidic co-culture system to study tumor-lymph node interactions and interventions in non-small cell lung cancer

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Introduction: There is an unmet need to develop precision immune-oncology tools for clinical use. Real-time monitoring of primary tumors in an *ex vivo* setting that recapitulates the dynamic tumor-host interactions, through appropriate dynamic model systems is lacking. Existing models, including circulating tumor cells and patient-derived xenografts require weeks to months to be generated and lack the autologous tumor immune microenvironment. Aim: To establish the conditions to allow co-culture of primary lung tumors with autologous mesothoracic lymph nodes that is a preliminary step before analyzing their interactions and possible effects on drug responses. Methods: We have adapted a 3D microfluidic device developed by Jenkins et al in AIM Biotech to the short-term co-culture of patient-derived tumor and autologous lymph node fragments. Following mechanical dissociation of fresh lung tumor and mesothoracic lymph node specimens, < 130 μm fragments were dispersed in growth-factor reduced hydrogel (Matrigel) (0.1g tissue/ml) and accommodated in the central channel of the device at a 1:1 ratio. The lateral channels were filled with medium, allowing alimentation of the co-culture. Cell co-culture was validated by studying phenotypic characteristics (light microscopy, live/dead stain and immune cell profiles by Confocal). Results: The chip developed mimics the tumor microenvironment and sustains lymph node cells within the hydrogel scaffold. High-resolution imaging shows robust proliferative responses of lymph node cells to tumor fragments and measurable tumor killing. Conclusion: We have validated our co-culture system at the phenotypic level. We will now continue with studying properties of immune cells (cytotoxicity, secretome) and personalized drug responses.

52 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Brain delivery of human amniotic mesenchymal stromal cells protects aged traumatic brain injured mice promoting protective astrocytic polarization

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Background. Traumatic brain injury (TBI) shows a second peak of incidence in the elderly, associated with worse outcome. Preclinical studies have investigated the effects mesenchymal stromal cells (MSC) as a potential therapy for the TBI treatment in young adult. But little is known about the potential effects on the aged population. Aim. To assess the efficacy of human amniotic MSC (hAMSC) in TBI aged mice infused either intracerebroventricularly (ICV) or intravenously (IV). Methods. Aged C57Bl/6 male mice (15-18 months) were subjected to sham or TBI by controlled cortical impact. PBS (control) or hAMSC were infused ICV (150.000 hAMSC in 5µl of PBS) or IV (106 hAMSC in 200µl of PBS) 24 hours post TBI. Sensorimotor deficits were assessed weekly up to 5 weeks (w), by neuroscore and beam walk tests, and cognitive functions were evaluated at 4w, by novel object recognition (NOR) test. Mice were sacrificed at 5w for histopathological analysis to evaluate lesion volume and neuronal death (Nissl staining), vessel density (CD31 marker), astrogliosis (GFAP marker) and microgliosis (CD11b marker). A group of animals were sacrificed at 3 days post TBI to evaluate toxic (A1: Serping 1, H2-T23, H2-D1, Ggta1) and protective (A2) astrocytes (Tgm1 and Clcf1) by RT-PCR analysis in the contusional cortex. Results. hAMSC ICV, but not IV, treated mice showed an early and persistent reduction of sensorimotor deficits (up to 5w post TBI), and an improvement of recognition memory compared to controls (TBI PBS). TBI hAMSC ICV treated mice showed a decrease neuronal death and an increase microvessel density in the contusional cortex compared to controls. No effect was on overall glial TBI groups. However, mRNA gene expression analysis at early stages (3 days post-TBI) showed a selective downregulation of genes associated to proinflammatory astrocytic activation. Conclusions. Local but not systemic hAMSC infusion is protective in aged mice. hAMSC, when infused ICV modulate the inflammatory microenvironment reducing proinflammatory astrocytic activation thus contributing to the improvement of functional outcome.

53 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

NKG2D CAR T cells as novel therapeutic approach to cure pediatric cancer

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In collaboration with: TEDDY Background: NKG2D ligands (NKG2DL) are expressed in different pediatric tumors including sarcoma and acute leukemia. Interactions between NKG2D receptor on NK cells and NKG2DL on tumor cells are essential for the NK cell elimination of Tumor Initiating Cells. However, NK cell-based therapies show limitations like poor *in vivo* expansion and rapid exhaustion. Engineering T cells to express a NKG2D CAR may overcome these limitations and become an effective therapy for pediatric cancer patients. Aim: The aim of this study has been to obtain preclinical evidence to support that a NKG2D CAR T cell-based therapy is an effective treatment for some pediatric cancers. Additionally, we have developed an automated manufacturing protocol to obtain NKG2D CAR T cells for clinical use in CliniMACs Prodigy that has led to the safe infusion of NKG2D CAR T cells into two pediatric patients suffering from refractory acute leukemia. Methods: The expression of NKG2DL was analyzed in leukemia and osteosarcoma primary cells and cell lines by flow cytometry and RT-PCR at different stages of the disease. The cytotoxicity of NKG2D CAR T cells was explored *in vitro* by performing 4h-Europium-TDA conventional assays. The *in vivo* efficacy of NKG2D CAR T cells was explored in a murine model of human osteosarcoma. NKG2D CAR T cells were manufactured in CliniMACs Prodigy device and infused into two patients suffering from refractory/relapsed acute lymphoblastic leukemia. Conclusions: Leukemia and osteosarcoma cells expressed NKG2DL and were sensitive to NKG2D CAR T cells lysis *in vitro*. AML and T-ALL cell lines were more susceptible to NKG2D CAR T cell lysis compared to B-ALL cell lines. In the osteosarcoma murine model, only those mice treated with NKG2D CAR T cells showed delayed tumor growth and increased survival. Automated manufacturing of clinical-grade NKG2D CAR T cells using CliniMACS Prodigy is feasible and reproducible. NKG2D CAR T cells infusions are essentially safe and their therapeutic effect relies on lymphodepleting conditioning, NKG2DL expression on tumor cells and sNKG2DL.

54 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Generation of iPSC-derived organoids with oncogenic mutations: A powerful tool for gene discovery and therapy

Ali Turhan - Institute National de la Santé et de la Recherche U935 and University Paris Saclay, France

Recent developments of organoid technology from different adult tissues offer novel and unprecedented perspectives in the field of modelling organ development. Organoid research has major applications in cancer research and therapy. iPSC represent also a highly relevant platform for generation of organoids. In our center we have generated iPSC from the somatic tissues of patients with germline hereditary cancers. We report here the generation of an iPSC line from a patient with type 1 papillary renal carcinoma (PRCC) with hereditary c-MET mutation. We designed a 3D culture system to induce the differentiation of c-MET iPSC into renal organoids. We demonstrated that iPSC-derived organoids expressed markers of renal progenitors; and transmission electron microscopy analyses confirmed the presence of tight junctions in tubular structures. c-MET iPSC have the potential to generate teratoma and organoids expressing carcinoma markers. We then performed a gene profiling of c-MET-mutated iPSC and compared it to that reported from patients with c-MET-mutated type 1 PRCC. Strikingly, a similar signature was found with identification of 11 genes expressed among c-MET embryonic bodies and shared with genes expressed in PRCC according to the papillary renal cell carcinoma database. Among these, BHLHE40 and KDM4C, well-known genes to be involved in PRCC, were found to be overexpressed preferentially in primary PRCC tumors with c-MET mutation. These results demonstrate that c-MET mutated patient-derived-iPSC can be used to generate organoids with cancer-like features and they represent the proof of concept of the feasibility of this approach as a novel platform for cancer modeling.

55 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Ability of Mesenchymal Stem Cells to attenuate Macrophage cell paralysis in late stage Sepsis

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Introduction: Sepsis is the result of a life-threatening complication to infection, leading to the dysfunctional release of proinflammatory cytokines. The only somewhat effective treatment is to treat the infection using antibiotics, removing the source of infection where possible, and individual organ support. Patients who survive this hyper-inflammatory stage of sepsis can progress to late stage characterised by immunosuppression due to monocytic cell paralysis and T-cell exhaustion. Mesenchymal stem cells (MSC) have been shown to have immunomodulatory properties and previous investigations by our lab group have shown that MSCs are effective in combating E. coli-induced early stage sepsis by reducing bacterial numbers, enhancing the immune system, and leading to positive outcomes in terms of tissue damage and mortality. Other groups have shown that MSCs are supportive in late stage sepsis to T-cells.Aim: We aimed to develop both an *in vivo* and *in vitro* prolonged sepsis model and subsequently show that MSCs would restore immune cell homeostasis in late sepsis.Methods: We established an *in vivo* model of prolonged sepsis by administering intratracheal K. pneumonia cultures to CD rats. MSCs were administered after 2 days and immune cell and cytokine profiles of the blood and BAL analysed after a further 3 days. An endotoxin tolerant cell culture was established using immune cell lines and isolated white cells from blood and BAL exposed to increasing levels of endotoxin *in vitro*. MSC conditioned media was produced from MSCs isolated from three different tissue sources and cells were treated for 24h post induction of tolerance. Phagocytosis, ELISA and Western blot were used to analyse the shift toward restoration of immune-competence. Conclusion: MSCs have the ability to restore immune homeostasis *in vivo* and *in vitro* as demonstrated by a restoration of functionality in cells isolated from treated animal models and also in cells induced to become tolerant *ex vivo*. MSCs from each source could comparatively restore their function and conditioned media was as effective as co-culture *in vitro.*

56 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Generation of a glioblastoma model using c-met-mutated iPSC-derived neuronal organoids

Ali Turhan - Institute National de la Santé et de la Recherche U935 and University Paris Saclay, France

Glioblastoma (GBM) is a highly aggressive brain tumor with poor prognosis. Current experimental models, using either human or mouse cell lines, are not representative of the complex features of GBM. Overexpression of c-met gene, is one of the molecular features of GBM and MET signaling has been shown to be involved in the regulation of GBM stem cell renewal via Wnt/beta-catenin pathways. In addition, MET expression correlates with progression-free and overall survival. We generated an iPSC line from a patient with c-met mutation with increased levels of phospho-Met and we asked the feasibility of generating a glioblastoma-like organoid model. We report here that c-met-mutated iPSC aggregates spontaneously and differentiate into dopaminergic neurons more rapidly than control iPSC aggregates at 60, 90, and 120 days. Gene expression profiling of c-met-mutated iPSC aggregates at day +90 shows neuronal- and glioblastoma-related genes, reproducing a genomic network described in primary human GBM. Likewise, transmission electronic microscopy confirmed the presence of structures reported in GBM, such as excess of intermediate filaments. We then asked whether the organoids expressed some of the phenotypic markers of primary human GBM such as glial fibrilarry acidic protein (GFAP) . The c-MET-mutated organoids, as compared to control, were highly positive for GFAP expression. Finally, the use if temozolomide (TMZ) *in vitro* showed a preferential cytotoxicity of this drug in c-met-mutated organoids as compared to controls. This study shows the feasibility of generating GBM-like organoid model using c-met-mutated iPSC aggregates and its potential future use in cancer modeling *in vitro*.

57 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Non-clinical translational study of irradiated umbilical cord mesenchymal stromal cells as biological adjuvant with radiotherapy.

Rosario Sánchez Pernaute - Andalusian Network for Design and Translation of Advanced Therapies, Spain

Background: Radiotherapy induces direct cytotoxicity and activates tumor cells that in turn exert a tumor suppressor action through bystander and abscopal effects. Low dose radiation can also activate MSC, triggering the release of anti-tumor cytokines and other molecules with cytotoxic activity, that may affect tumor burden or distant metastasis, enhancing the therapeutic effect of radiotherapy. Aim: To demonstrate the efficacy and safety of clinical grade human IR-MSC as adjuvant therapy for melanoma. Methods: NOD scid gamma (NSG) mice (n = 24) were injected subcutaneously with 10^6 cells from the human melanoma cell line A375 and treated with radiotherapy alone or combined with clinical grade human MSC from umbilical cord irradiated with 2Gy. Mouse weight, tumor volume and survival rate were measured for 35 days. To test biodistribution (n = 30) and toxicity (n = 28), mice were inoculated by tail vein injection with 10^5 IR-MSC and 10^5 MSC, IR-MSC or transformed MSC (tMSC), as a positive control of tumor potential, respectively. Organs were collected at different time points, from 1 to 90 days, to detect the presence of MSC and morphological changes using histological analysis and qPCR for the detection of human Alu sequences. Conclusions: Activated clinical grade human IR-MSC significantly improve the therapeutic effect of radiotherapy in melanoma tumor-bearing mice. The cells initially migrate to lung and liver but are not detected after 90 days in the host. These preclinical studies suggest that our cell product is safe and effective, and thus could be proposed as an adjuvant treatment for melanoma.

58 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Germinal Zone Neural Stem Cells from hemorrhagic fluid of preterm infants

Rosario Sánchez Pernaute - Andalusian Network for Design and Translation of Advanced Therapies, Spain

Background: Intracerebroventricular hemorrhage is a common cause of morbidity and mortality in premature infants. The rupture of the germinal zone into the ventricles entails loss of neural stem cells and disturbs the normal cytoarchitecture of the region, compromising late neurogliogenesis and causing long-term disabilities. Aim: To develop an autologous cell therapy medicinal product for preterm infants with intraventricular hemorrhage. Methods: We established the methodology to isolate neural stem cells from the hemorrhagic cerebrospinal fluid, evacuated through minimally invasive therapeutic neuroendoscopic lavage in 8 consecutive cases of severe intracerebroventricular bleeding. We analyzed the expression of neural stem cell markers and their *in vitro* differentiation potential using flow cytometry, immunofluorescence and confocal microscopy. We performed transcriptomic analyses and bioinformatics to define their unique profile related to their origin in the ganglionic eminences of the ventral forebrain. Finally, we examined the safety profile *in vitro*, and, *in vivo*, through injection of CD133+ purified cells into the brain of nude mice and histological analysis at 6 months. Conclusion: This novel source of neural stem cells poses no ethical concerns, as the fluid is usually discarded, and could be useful for the development of an autologous therapy for preterm infants, aiming to restore late neurogliogenesis and attenuate neurocognitive deficits. Furthermore, these cells represent a valuable tool for the study of human germinal zone biology and the final stages of human brain development.

59 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

The evolution of preclinical testing through novel humanized precision disease models

Niki Karagianni - Biomedcode Hellas SA, Greece

Background: In search for advanced therapies for human disease, the translational value of preclinical work is unquestionable. There is however a pressing need to develop animal models that closely reflect the pathogenic mechanisms as well as the complexity of the human disease.

Aim and Methods: Biomedcode develops advanced mouse models with high predictive power for the therapeutic efficacy of human therapeutics. These models are humanized for key pathogenic players such as TNF, TNFR1, IL17, RANKL, IL23p19 and others and, either spontaneously or following induction, develop chronic inflammatory conditions such as arthritis, IBD, osteoporosis, spondyloarthritis, psoriasis etc. Biomedcode's arthritis mouse models have provided the *in vivo* proof-of-concept and supported the successful IND filing of several novel and biosimilar human therapeutics, including Infliximab (Remicade®) whose anti-TNF therapeutic activity was first validated in the human TNF transgenic arthritis mouse models.

Conclusion: Using standardized procedures and high end *in vivo* and *ex vivo* read outs, Biomedcode maximizes the scientific output from each experimental model closely following the 3Rs and maximizing the translational value of the models. As we now move to the era of advanced therapies, it is critical to use optimized mouse models that guarantee similarity to human disease and allow the accurate transition from bench to bedside and back. Optimized mouse models will also allow the validation of preclinical testing approaches based on organoid or microphysiological systems, supporting thus combined preclinical testing of advanced therapies and application of the Replacement principle.

60 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

CAST-Seq, a novel preclinical genotoxicity assay, enables qualitative and quantitative insights in chromosomal aberrations in gene-edited human hematopoietic stem cells

Julia Klermund - Medical Center University of Freiburg, Germany

Genome editing with designer nucleases has shown great promise but also revealed the risk of genotoxicity caused by the insertion of mutations or chromosomal aberrations. Recently developed methods to identify genome-wide off-target activity confirmed those perils but also revealed the restrictions of these assays, including insufficient sensitivity or specificity, or the failure to detect gross chromosomal aberrations. To overcome these limitations, we established CAST-Seq, a method capable of detecting directly in clinically relevant cell types, aberrations derived from on- and off-target activity of CRISPR-Cas nucleases or TALEN, including large deletions, inversions and translocations. Moreover, we detected novel on-target activity mediated aberrations, such as homology-mediated translocations, acentric and dicentric translocations between homologous chromosomes, and large deletions. Validation of the results by deep sequencing and digital PCR confirmed the quantitative nature of CAST-Seq and revealed new insights into the DNA repair kinetics and dynamics in human stem cells.

61 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

STARSTEM: NanoSTARs Imaging for Stem Cell Therapy

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Mesenchymal stem/stromal cells (MSCs) have proven potential for cell-mediated therapy in many diseases such as osteoarthritis (OA). However, a major challenge to clinical translation is a lack of understanding about cell engraftment in tissues and their biological activity in situ. STARSTEM aims to develop a nanotechnology-enhanced optoacoustic imaging (OAI) platform. The core novelty of this approach is a gold nanoparticle, shaped like a star (the nanostar), which amplifies signal response in OAI. STARSTEM will be capable of tracking MSCs and MSC-derived extracellular vesicles (EVs) labeled with nanostars at unprecedented depth and sensitivity. While a large proportion of transplanted stem cells undergo apoptosis after local delivery and are cleared by the immune system, there is limited knowledge about cells that survive, their rates of engraftment, biodistribution or the cell/host interaction that results in a therapeutic outcome (i.e. mechanism of action). Using OAI, STARSTEM will monitor biodistribution and engraftment of cells as well as functional markers of healing, including vascularization and oxygen saturation. The STARSTEM technology begins with optimization of the nanostar production process. MSC and MSC-derived EVs will then be tagged with nanostars, deployed in a mouse model of OA and tracked over time using OAI. This will then be extended to a large animal (ovine) model of OA as the selected pre-clinical model. Data generated will support an application for a phase-1 clinical trial of nanostar-enhanced OAI in humans.

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62 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Recombinant Tetraspanin Decorated Extracellular Vesicles for Regenerative Medicine

Madhusudhan Reddy Bobbili - University of Natural Resources and Life Sciences, Austria

Introduction: Extracellular vesicles (EVs) emerged as an important mode of cell-to-cell communication in both normal and pathological conditions by transferring the cargo from donor cell to recipient cell. EVs derived from MSCs and other cell types transfer molecules with reparative and anti-inflammatory properties to recipient cells. Over a decade, lot of research has been done to understand the omics, mode of secretion and uptake mechanisms. However, trafficking of EVs *in vivo* is still poorly understood. Methods: We used recombinant tetraspanin (CD81 with C-terminus snorkel tag (1)) as a tool to understand trafficking of EVs *in vivo*. As a first step, we established a method for isolating functional EVs carrying CD81snorkel-tag from stably expressing cells *in vitro*. This method uses a combination of anti-HA (hemagglutinin) affinity matrix and PreScission protease to isolate EVs from cell culture supernatants without damaging the integrity of the EV membrane. Results: EVs isolated by this method are further characterized by using multiplex bead-based flow cytometry assay (2) and electron microscopy. The multiplex bead-based assay results showed us that we were able to pull out EVs carrying only snorkel-tag from a mixture of EVs from different sources. In addition, functionality of recombinant EVs was confirmed by CRE-LoxP method (3). Furthermore, as a proof of concept we are working on delivery of CD81snorkel-tag mRNA to mouse liver using charged lipodoid nanoparticles and isolating recombinant EVs from mouse plasma using our method. Discussion: Presence of snorkel-tag on EVs doesn't affect the EV protein surface signature. In addition, it enables us to track EVs *in vivo* and also isolate specific EV from biological fluids when expressed under tissue-specific promoter. EVs present many advantages over stem cells in regenerative medicine. However, biodistribution and beneficial effects of MSC derived EVs are under investigation. Our method provides complete knowledge of EV cargo and their communication *in vivo* and their great therapeutic potential may allow their use as novel delivery vehicle for drugs and genes in regenerative medicine.

63 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

laminin-functionalized 3D silk scaffold maintains expansion, stemness and differentiation potential of human pluripotent stem cells

Therese Kallur - BioLamina, Sweden

Tissues are 3D formations of cells integrated in an extracellular matrix (ECM) with specific sites for cell anchorage offering positional and instructive information regulating cell behavior. Thus, to culture authentic cells from which real biological questions can be answered, environmental context is pivotal.

Laminins comprise a family of 16 unique heterotrimeric glycoproteins present in the body. Laminins are tissue specific, supply a natural environment for all cell types, and influence cell adhesion, differentiation, migration, phenotypic stability, and cell functionality. As such, recombinant laminins are biologically relevant ECM protein substrates that can be used to mimic the *in vivo* cell niche *in vitro*.

Silk 521 is a biomaterial made of recombinant silk, which is functionalized with human recombinant laminin 521 protein (Biolaminin 521). This material supports integration and proliferation of human pluripotent stem cells *in vitro* and serves as a viable base for the development of subsequent lineage-specific differentiation in a 3D format.

Human PSCs (2 hESC lines and 1 hiPSC line) seeded in the Silk 521 scaffold integrate, migrate, and form small colonies at day 1 post seeding and retain pluripotency. Human PSCs in the Silk 521 scaffold could be cultured using different types of medium, expressed pluripotent markers, and differentiated to all three tissues germ layers. On the contrary, cells seeded in silk only (control), could hardly integrate into the foam and the cells did not amplify at all, regardless of media or cell line used, and regardless of the presence of ROCKi. Together these data highlight the biological effects of the laminin substrate within the Silk 521 biomaterial.

In sum, Silk 521 is a biorelevant 3D system that is biodegradable and non-immunogenic, and thus ideal for cell culturing and many other biomedical applications.

64 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Proteomic analysis reveals angiogenic and immunomodulatory function for placental stromal cell-derived extracellular vesicles

Dirk Strunk - Cell Therapy Institute Paracelsus Medical University, Austria

Allogeneic regenerative cell therapy has shown surprising results despite lack of engraftment of the transplanted cells. Their efficacy was so far considered to be mostly due to secreted trophic factors. We hypothesized that extracellular vesicles (EVs) can also contribute to their mode of action. Here we provide evidence that EVs derived from therapeutic placental-expanded (PLX) stromal cells are potent inducers of angiogenesis and modulate immune cell proliferation in a dose-dependent manner.

Crude EVs were enriched >100-fold from large volume PLX conditioned media via tangential flow filtration (TFF) as determined by tunable resistive pulse sensing (TRPS). Additional TFF purification was devised to separate EVs from cell-secreted soluble factors. EV identity was confirmed by western blot, calcein-based flow cytometry and electron icroscopy. Surface marker profiling of tetraspanin-positive EVs identified expression of cell- and matrix-interacting adhesion molecules.

Differential tandem mass tag proteomics comparing PLX-EVs to PLX-derived soluble factors revealed significant differential enrichment of 258 proteins in purified PLX-EVs involved in angiogenesis, cell movement and immune system signaling. At the functional level, PLX-EVs and cells inhibited T cell mitogenesis. PLX-EVs and soluble factors displayed dose-dependent proangiogenic potential by enhancing tube-like structure formation *in vitro*.

Our findings indicate that the mode of PLX action involves an EV-mediated proangiogenic function and immune response modulation that may help explaining clinical efficacy beyond presence of the transplanted allogeneic cells.

65 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Translational science programs at NIH to accelelrate advanced therapies: the Somatic Cell Genome Editing program and Rare Diseases Clinical Research Netwrok

Philip John (P.J.) Brooks - National Institutes of Health, United States

This presentation will discuss several programs at National Center for Advancing Translational Sciences (NCATS), NIH focused on accelerating translation of advanced therapies for the benefit of patients and society. The Somatic Cell Genome Editing (SCGE) program, supported by the NIH Common Fund, is focused on the development of quality tools to accelerate implementation of effective and safe genome editing in human patients. The largest component is the development of technologies to deliver genome editors to disease relevant somatic organs and cell types, including the nervous system, liver, lung, and muscle. A novel aspect of the program is a requirement for independent validation of new delivery technologies. Other components of the program include new *in vitro* biological systems for testing safety and efficacy of genome editing in human cells, reporter animal models to detect genome editing in any cell type, and new genome editing systems. These research tools will be made widely available to the research community to accelerate new therapies.

The NIH Rare Diseases Clinical Research Network (RDCRN) is supported by a collaboration of multiple NIH Institutes. The RDCRN is a network of approximately 20 different research consortia, many of which include clinical sites in Europe. Each focused on a group of three or more related diseases. The network also includes a data coordinating center to disseminate the clinical data, and requires strong partnerships with patient advocacy groups. Because the lack of natural history data is often a limiting factor for clinical trials in rare diseases, longitudinal natural history studies are also a requirement for each funded project. The third cycle of the RDCRN is nearing completion, with cycle 4 projects to be announced by the fall of 2019.

Lastly, in view of the pressing need to develop treatments for the thousands of known rare diseases, I will also discuss emerging NCATS projects focused on accelerating clinical trials in rare disease by using platform approaches, such as AAV vector based gene therapy, which have the potential benefit multiple rare diseases in parallel.

66 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Stepwise maturation of human iPSC into clonogenic immunosuppressive mesodermal stromal progenitors identified extra-hematopoietic immunomodulatory role of DOCK proteins

Dirk Strunk - Cell Therapy Institute Paracelsus Medical University, Austria

Stromal cells are important components of all mammalian tissues contributing to organ integrity (fibroblasts/stroma) and vascular stability (pericytes) in addition to their enigmatic niche function. Their inherent immunomodulatory capacity attracted particular attention resulting in the initiation of hundreds of clinical trials, mainly testing their trophic immunomodulatory potency. Key stromal immunomodulation mechanisms are still not completely understood.
Here we show that mesodermal stromal cells (MSC) derived from brachyury-expressing iPSC progeny undergo a stepwise maturation eventually re-acquiring phenotype, clonogenicity, gene expression kinetics and function of their parental stromal cells.
Induced pluripotent stem cells (iPSC; Sendai/OKSM) were generated from healthy human bone marrow and umbilical cord blood-derived fibroblasts. After mesodermal induction, stromal differentiation was induced by platelet-derived growth factors under animal serum-free conditions. Immunophenotype, clonogenicity, gene expression, differentiation capacity and immunomodulatory function were compared to parental MSC.
Under feeder-free defined conditions, iPSC differentiated into expandable and cryo-preservable CD73+/CD105+/Tra-1-81- early iPS-MSC lacking immunosuppressive potential. Successive passaging was required for reaching mature phenotype and immunomodulatory competence over time, while maintaining clonogenicity comparable to parental MSC. Sequential RNAseq revealed acquisition of a spectrum of immune response-related genes significantly expressed in mature iPS-MSC and resembling parental MSC immune-gene expression. Among those, dedicator of cytokinesis (DOCK) genes stand out because mutations cause severe combined immunodeficiency (SCID; NEJM 2015). Interestingly, SCID patient-derived fibroblast lines harboring bi-allelic DOCK mutations showed significantly reduced immunomodulatory capacity. This provides first evidence for an extra-hematopoietic immunomodulatory role of DOCK proteins in stromal cells that was previously considered to be restricted to immune cells.

67 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

A non-union sheep model to predict the bone-forming potency of Advanced Therapy Medical Products as translational model to the clinic

Marina Maréchal - Katholieke Universiteit Leuven, Belgium

Large bone defects and non-unions remain a major challenge in the clinic. In these patients, the use of the gold standard, autologous bone, is often not possible due to limited availability of graft material. Most of the currently used treatments require re-interventions, exposing patients to lengthy and invasive procedures such as distraction osteogenesis. The use of Advanced Therapy Medicinal Products (ATMPs) might overcome this unmet medical need.
One of the key hurdles in evaluating ATMPs is establishing suitable models that closely mimic the patient. In view of bone engineering, we developed two sheep models following the rules of the "Pentaconcept", i.e. implanting an ATMP consisting of a combination of carrier, growth factor(s) and osteoprogenitor cells, in a mechanically stabilized biological chamber.
The first sheep model consists of a fresh critical size tibial defect of 3 cm. A biological chamber is formed using a polymethylmetacrylate (PMMA) spacer and guaranteed proper containment for the ATMP. The second sheep model is a real non-union model, established by first creating a 4.5 cm defect filled with mostly fibrotic tissue to mimic a clinically observed compromised environment. Debridement of the non-union tissue and implantation of PMMA generates a biological chamber (Masquelet) and after 6-7 weeks, the ATMP is implanted.
The results indicate that in the critical size model, there is no difference in bone ingrowth and bridging of the defect using growth factor coated scaffolds vs combinations of growth factors with periosteum derived progenitor cells. Remarkably, in the non-union model, cell-based ATMPs results in substantial bone formation in 6 out of 8 sheep, with complete bridging of the defect in 3 out of the 8 sheep. ATMPs without cells, however, only show some relevant bone formation in 2 out of 8 sheep and none of them bridges the gap.
In summary, tissue-engineering strategies are promising but have so far underdelivered in clinics. Our preclinical studies in a compromised environment allow for proper testing of ATMPs in a more clinically relevant animal model and may therefore better predict the clinical outcome in a well-defined patient.

68 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Molecular Profiling of Human Regulatory T Cell Subsets Supports a Linear Differentiation Model and Identifies a Novel Regulatory T Cell Population with Stem Cell-like Properties

Jacqueline Wendering - Charité Universitätsmedizin Berlin, Germany

Thymic-derived CD4+CD25highFoxP3+ regulatory T cells (tTREG) represent a unique T cell lineage with the ability for negative regulation of inflammation. Whereas the concept of linear differentiation of immunological memory subsets within pro-inflammatory T cell compartments is well defined, it remains unclear whether tTREG show similar subset plasticity with various sub-populations displaying distinct functional features. The presence of persistently expanding antigen-specific tTREG, protecting against aberrant immune responses, implies the existence of tTREG memory. However, the lack of definitive markers impedes the phenotypic and functional classification of memory tTREG. The differentiation and memory formation of effector T cells is defined by rigid molecular pathways and lineage regulators for inducing epigenetic changes. In addition, classical cell surface markers may be used to broadly classify T cell subsets of distinct differentiation states. Here, we applied conventional memory T cell-defining marker profiles of CCR7 and CD45RA expression to tTREG. Phenotypically, we demarcated naïve-, central memory- and effector memory-like tTREG compartments as functionally distinct subsets within the bulk tTREG population. Intriguingly, we identified most diverse T cell receptor usage in phenotypically naïve-like tTREG, followed by phenotypically memory-like tTREG subsets in a linear pattern. Epigenetic profiling revealed highest DNA methylation in tTREG with early differentiated phenotypes. Furthermore, we could identify a novel stem cell memory-like tTREG subset with superior suppressive function compared to other tTREG subsets. Positive correlation of conventional T cell and tTREG subsets may demonstrate a synchronized memory formation in the effector and regulatory compartment. Our findings may have direct implication for adoptive tTREG therapy.

69 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Functionalized hydrogels to amplify the paracrine effects of transplanted mesenchymal stromal cells and to enhance skeletal muscle regeneration

Sven Geissler - BCRT, Charité Universitätsmedizin Berlin and Julius Wolff Institute, Germany

The control of stem cell function is one of the main goals for the development of biomaterials for cell-based therapies. Especially, cell-cell interactions within the biomaterials are of crucial importance and, for example, strongly influence the paracrine function of mesenchymal stromal cells (MSCs) on skeletal muscle cells. From a clinical perspective, the use of nanoporous hydrogels is very advantageous because they can be minimal-invasively injected and adapt to the defect.
However, such hydrogels affect cell-cell interactions and thereby reduce the therapeutic (paracrine) effect of MSCs. Here, we hypothesized that the cell-cell interaction in the hydrogel via the N-cadherin binding motif (HAVDI) improves the paracrine function of MSC, increases its sensitivity to bioactive cues, and improves its therapeutic benefits.
We show that 3D culture in microporous (~ 80-120 µm) hydrogels that passively promote cell-cell interactions sensitizes MSCs to growth factors, particularly IGF-1. IGF-1 enhances the paracrine secretory activity of MSC, and the application of secreted factors to myoblasts effectively stimulates their migration and differentiation. In contrast, the paracrine activity of MSCs encapsulated in nanoporous (~ 5-20 nm) hydrogels remains unchanged. Blocking N-cadherin on MSCs abolishes the stimulatory effects of IGF-1 in microporous but not nanoporous hydrogels. The role of N-cadherin in the regulation of MSC function was further examined and the biomaterials were functionalized with the HAVDI peptide sequence (extracellular domain of N-cadherin) to mimic corresponding cell-cell interactions. MSCs encapsulated in nanoporous HAVDI gels, but not in gels functionalized with a scrambled sequence, show increased paracrine activity in response to IGF-1. In summary, we uncovered the impact of biomaterial environments that either promote or inhibit cell-cell interactions on MSC's paracrine activtity. Our results show how the regenerative potential of MSCs can be influenced and maximized by specific interactions with their matrix, neighboring cells, and soluble factors in their microenvironment.

70 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Visual restoration: preclinical validation of retinal prosthesis and optogenetic therapy in non-human primates

Serge Picaud - Institut de la Vision, France

Blindness can result from the loss of photoreceptors in age-related macular degeneration or in retinal dystrophies like retinitis pigmentosa. Electrical activation of residual retinal neurons can restore some visual perception in blind patients. However, current retinal prostheses do not provide face recognition or autonomous motion in an unknown environment. To reach these goals, we have now assessed a new photovoltaic retinal prosthesis and optogenetic therapy on non-human primates for their translations towards clinical trials.

We demonstrated that individual retinal ganglion cells can be selectively activated by only one electrodes and not its neighbors. Then, introducing photovoltaic implants in the subretinal space, we produced blind spots allowing us to assess visual perception generated by their infrared activation on the blind retina. The behavioral study in awake non-human primates indicated that activation of a single unit of the implant was sufficient to trigger a visual saccade.

To reach cellular resolution of visual restoration, we assessed optogenetic therapy in non-human primates. Expression of the microbial opsin, ChrimsonR, enabled us to activate retinal ganglion cells despite synaptic blockers suppressing the natural responses. Response properties were compatible with video rate stimulations and perception of letters.

Infrared photovoltaic prostheses are in clinical trials for blind patients with dry age-related macular degeneration while optogenetic therapy has entered into clinical trials for blind patients with retinitis pigmentosa.

71 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Genetic insulators designed for use in hPSCs: Proof of principle in inducible cell lines to produce mature GMP grade megakaryocytes *in-vitro*, and platelets for transfusion

Cedric Ghevaert - University of Cambridge, United Kingdom

A caveat to engineering techniques recently employed in translational laboratories is that eukaryotes also have elaborate defence systems to protect their genomes from alien transcriptional units. Insertion triggers transcriptional silencing and so dwindling expression and loss of therapeutic effect.

Aim: The development of silencing resistant constructs has suddenly become vital to avoid this threat to the success of cellular therapies by the addition of insulator sequences to abrogate this unwelcome complication.

Methods: We have applied this to the *in-vitro* production and maturation of megakaryocytes (MKs), the precursors of blood platelets offering clinical potential for the treatment of patients with thrombocytopenia and other bleeding disorders. We have published a robust system known as forward programming (FOP) for efficient production of large numbers of highly pure megakaryocytes (MKs) from hPSCs, by ectopic expression of three transcription factors GATA1, TAL1 and FLI1 (3TFs). A crucial limitation was reliance on lentiviral vectors. We have since shown that genetic engineering of hPSC lines to introduce an inducible system drives MK forward programming as efficiently as the original lentiviral approach.

The Opti-OX system uses a TET-ON-3G, reverse tetracycline-controlled transactivator (rtTA) constitutively expressed through a CAG promoter targeted to the ROSA26 genomic safe harbour (GSH). The 3TFs are introduced as a polycistronic cassette conditionally expressed under the control of a Tet-ON promoter inserted into a second putative GSH, the AAVS1 locus. Upon induction with doxycycline the TRE becomes transcriptionally active but is silenced promptly with rapid loss of transgene expression due to epigenetic silencing leading to suboptimal forward programming. We show specifically designed insulators maintain transgene expression for longer leading an improvement in MK output.

Conclusion: In the context of this inducible system, the AAVS1 locus is not a “safe harbour”.

Addition of insulators prevents epigenetic silencing, thereby rendering the AAVS1 a true safe harbour even during cell differentiation when the TRE is transcriptionally active.

72 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Precision gene-engineering meets cancer immunotherapy: Targeted insertion of chimeric antigen receptors into the T-cell genome using a novel, virus-free CRISPR/Cas9 knock-in strategy

Justus Weber - University Hospital Würzburg, Germany

Background: Immunotherapy with gene-engineered CAR-T-cells is a transformative cancer treatment. In conventional CAR-T-cell products, gene-transfer is accomplished with viral vectors that are associated with genotoxicity, a risk for insertional mutagenesis, and substantial effort and expense of vector production and handling. To overcome these limitations,we established virus-free targeted insertion to direct the CAR-transgene into defined genomic loci in human T-cells.
Results: We validated CRISPR gRNAs targeting the endogenous T-cell-receptor (TCR), and generated CAR knock-in T-cells. We confirmed TCR knock-down and CAR knock-in by flow cytometry and on the genomic level. Notably, CAR knock-in T-cells displayed i. lower and more homogeneous CAR surface expression, ii. activation-dependent CAR regulation, and iii. reduced levels of baseline activation and exhaustion marker expression compared to T-cells with random genomic insertion. CAR knock-in T-cells conferred specific cytotoxicity, cytokine secretion, and outperformed conventional (random insertion) CAR-T-cells in antigen-dependent proliferation. These data were confirmed with several CAR-constructs of relevance in hematologic (CD19) and solid tumor indications (ROR1/2). Preliminary data also suggest that targeted CAR insertion into the TCR locus is advantageous to the PD1 locus, likely because it enables T-cells to regulate CAR expression physiologically, in sync with their activation status. Accordingly, we consistently found improved viability and lower rates of AICD in knock-in CAR-T-cells after a tumor challenge.
Conclusion: We are presenting virus-free targeted CAR insertion into a genomic safe harbor as a strategy to augment safety and performance of CAR-T-cell therapy; and consider precision genomic engineering as a key enabling technology in the field of ATMPs.

73 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Producing megakaryocytes from iPSCs for clinical-grade platelet production

Cedric Ghevaert - Biodonostia Health Research Institute, Spain

Platelets are the cells responsible for clotting and patients with inherited bone marrow failure or clotting deficiencies are often reliant on platelet transfusions. Platelet units are ABO blood type and Rhesus D matched and rare units are often in short supply. Megakaryocytes are the multinucleated cells which produce platelets. In the lab, we have developed a protocol for the forward programming of induced pluripotent stem cells (iPSCs) into megakaryocytes and subsequent platelet production using only three instructive transcription factors and two cytokine cocktails. This work details the progress of this project towards the clinic. We aimed to identify clinical grade iPS lines which reproducibly produced mature, functional megakaryocytes. We also developed an inducible system for the overexpression of the patterning transcription factors. Finally, we also aimed to construct a bioreactor for efficient platelet release from the megakaryocytes to generate clinical grade platelet units. First of all, clinical grade pluripotent stem cell lines were sourced and tested for their ability to produce mature megakaryocytes. Next, a doxycycline-inducible system for transgene expression was stably inserted into the pluripotent stem cells and tested. Finally, we developed a dual flow bioreactor which allows the megakaryocytes to release platelets. In this project, we identified several clinical grade cell lines which efficiently and reproducibly generate large quantities of mature megakaryocytes. We also developed an inducible system for transgene overexpression, eliminating the need for lentiviral transduction and generating a safer transfusion product. In addition, we developed a collagen scaffold-based bioreactor for the release of functional platelets from the megakaryocytes, as the final step towards the production of clinical grade platelet units from iPSCs. The forward programming approach could thus provide a safe supply of blood products in areas where disease burden might be a concern for the selection of blood donors. It could supplement current platelet supply and obviate the need to cross match platelet units by using genetically modified immune silent iPSCs.

74 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Micro actuators based on dielectric and magnetoactive elastomers

Johannes Ziegler - Fraunhofer Institute for Silicate Research, Germany

The Center Smart Materials (CeSMa) at the Fraunhofer ISC develops adaptive materials, whose mechanical properties can be controlled by external influences. The subject of research and development are, besides magnetorheological elastomers (MRE) which harden in the magnetic field due to the interaction of magnetic dipoles, electroactive polymers based on silicone rubber.

MREs are composite materials of magnetically polarizable particles in a soft elastomeric matrix, which are characterized by an overlap of magnetic and elastic forces. When a magnetic field is applied, the elastomer stiffens and the MRE is deformed due to magnetic forces of attraction. This enables, for example, a valve function or pump to be actuated. Dielectric elastomer actuators (DEA) consist of alternating layers of conductive and insulation rubber material. By applying a high voltage on the conductive electrode layer the soft dielectric layer gets compressed by the electrostatic pressure and the actuator deforms. Due to the high design diversity in the production process and the possibility of building up multilayer stacks the kind and level of actuation can be adapted to a variety of applications.

This novel actuator materials offer a wide range of biomedical applications like micro pumps and controllable valves for media like gases and fluids and for the mechanical and electrical stimulation of tissue engineering.

75 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Experimental models systems for the development of personalized therapeutic strategies for beta-thalassemia

Alessia Finotti - University of Ferrara, Italy

Background. We have developed *in vitro* and *in vivo* experimental model systems for personalized therapy of thalassemia, including K562 cell lines and transgenic animals carrying the human beta-globin gene with the β039, β+IVSI-6 and β+IVSI-110 mutations. In order to screen for fetal hemoglobin (HbF) inducers, we have developed and characterized K562 cellular biosensors carrying enhanced green fluorescence protein (EGFP) and red fluorescence protein (RFP) genes under the control of the human γ-globin and β-globin gene promoters, respectively. These dual-reporter erythroid cell lines are suitable to identify the induction ability on the transcription of γ-globin and β-globin genes. To develop a cellular screening system for the identification of BCL11A inhibitors (a strong repressor of γ-globin gene transcription), we produced 12 K562 cell clones with integrated copies of a BCL11A-XL expressing vector, producing different levels of BCL11A-XL. A clear inverse relationship between the levels of BCL11A-XL and the extent of hemoglobinization induced by a panel of HbF inducers was found. The HbF inducer mithramycin was able to rescue the ability to complete the erythroid program, even in K562 cell clones expressing high levels of BCL11A-XL, suggesting that BCL11A-XL activity is counteracted by mithramycin.

Aim. To develop experimental model systems to test gene editing approaches and identify novel readthrough molecules.

Methods. We have produced a K562 cell line stably transfected with a GFP expressing vector containing a stop codon within the GFP sequence.

Conclusion. These model systems were found to be useful to identify novel globin inducers and read-through molecules and to develop PNA-based strategies to perform gene editing with an efficiency approaching the CRISPR-Cas9 system. The validation of the developed protocols will be performed also using a β-thalassemia cellular BioBank, at present constituted by cryopreserved erythroid precursor cells from 129 β-thalassemia patients and a total of 765 vials.

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76 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

A macrocyclic multivalent tetraargininocalix[4]arene as a non-covalent vector for peptide nucleic acids (PNAs), microRNAs and antagomiRNA molecules

Alessia Finotti - University of Ferrara, Italy

Background. Calixarenes were shown to be suitable for the efficient delivery of nucleic acids. We have demonstrated the delivery of plasmid DNA using, as scaffold for vector molecules, the macrocyclic calix[4]arene in cone geometry. This makes possible the display of two spatially well-defined regions, one apolar at the lower rim and one polar at the upper rim. The clustering on the macrocycle of only four units of basic amino acids, such as arginine and lysine, gave rise to new non-viral vectors for DNA cell transfection, more potent than commercial transfecting agents.
Aim. To determine the delivery ability of a macrocyclic multivalent tetraargininocalix[4]arene 1 used as non-covalent vector for Peptide Nucleic Acids (PNAs), antagomiRNA molecules and miRNA mimics.
Methods. Cell lines transfections with Calixarene 1 and nucleic acids analogues like PNA, antagomiRNAs and miRNA mimics.
Conclusion. We obtained high delivery efficiency, low cytotoxicity, maintenance of the biological activity and ease preparation of the transfection formulation, suggesting this vector as a universal delivery system for this class of nucleic acid analogues. As far as PNAs, these data are relevant because the major drawback of PNAs in the low rate of cellular uptake. PNAs have been proposed as antisense molecule and triple-helix forming agents. As far as antagomiRNA molecules and miRNA mimics, these molecules have been proposed for miRNA therapeutics. MicroRNAs (miRNAs or miRs) are short non-coding RNA molecules, which act as gene regulators by repressing translation or by inducing the cleavage of target RNA transcripts. Emerging evidence suggests that the altered expression of miRNA may be involved in the pathogenesis of several severe human diseases, opening new avenues in the field of therapeutic strategies, i.e. miRNA targeting or miRNA mimicking. In conclusion, the efficient and not toxic delivery of PNAs, premiRNA and antimiRNA molecules is a promising feature of tetraargininocalix[4]arene vectors.

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77 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

In vitro test system for novel compounds to treat local neuropathic pain

Nurcan Üçeyler - University of Würzburg, Germany

Background: Diseases of the small caliber nerve fibers lead to excruciating focal neuropathic pain in many patients, for which targeted treatment is challenging.
Aim: We aim at generating innervated three-dimensional (3D) *in vitro* skin models entirely produced using patient-derived skin and neuronal cells. These personalized innervated skin models will be used for *in vitro* assessment of novel topically applicable analgesics against focal neuropathic pain. Such 3D skin models will revolutionize pre-clinical testing of potential novel analgesics and open many avenues for in depth pathophysiological investigation of neuropathic pain pathophysiology and the contribution of cutaneous nociception.
Methods: We extract and cultivate skin fibroblasts and keratinocytes from diagnostic skin punch biopsies and generate 3D *in vitro* skin models. These models are then innervated using patient-derived sensory neurons which are generated from the same skin punch biopsy fibriblasts via induced pluripotent stem cells. Innervated models are then used for in depth analysis of cell-cell interactions and cell-nerve fiber interactions. Also, electrophysiological analysis of sensory neuron activation is assessed by patch-clamp techniques. Assessments are performed at baseline comparing 3D models from patients with defined neuropathic pain syndromes such as small fiber neuropathy and healthy controls. Further, 3D innervated skin models are used for the application of novel and potentially analgesic compounds and effects are investigated using the electrophysiological read-out of patch-clamp analysis.
Conclusion: To the Restore consortium, we provide an entirely human, personalized 3D innervated *in vitro* skin model that will revolutionize the pre-clinical assessment of novel analgesic compounds against neuropathic pain and build the soli.

78 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Perfusable *in vitro* models for to investigate the efficacy of cell therapeutics

Florian Groeber-Becker - Fraunhofer Institute for Silicate Research, Germany

Background: Tissue vascularization plays a vital role in many diseases such as cutaneous wound healing, graft versus host disease, tumor progression and has gained increasing attention as target for novel drug candidates. Moreover, the vascularization is one of the key factors allowing prolonged shelf life and the size of bioengineered tissues.
Aim: We aim to develop vascularized tissue models based on decellularized xenogeneic matrix or plastic compressed collagen hydrogel.
Method: To generate vascularized tissue models two different technologies have been developed either based on decellularized xenogeneic matrixes (BioVaSc-TERM®) or printed collagen hydrogels. Since, both technologies require a strong engineering part to facilitate a physiological perfusion of tissue models; also, novel bioreactor systems have been developed. Using the BioVaSc-TERM® platform, we succeeded in the generation of vascularized skin, liver models and bone models. Here we could demonstrate in a proof of concept study that the vascular system can be used to dissipate allogenic activated human T cells in skin models to simulate aspects of acute graft versus host disease.
Conclusion: We can provide to the RESTORE consortium two technology-platforms to generate different vascularized tissue for mechanistic studies in preclinical studies.

79 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Immunosuppressant-resistant regulatory T cell products for advanced adoptive T cell therapy in solid organ transplantation

Ghazaleh Zarrinrad - Charité Universitätsmedizin Berlin, Germany

To date, end-stage organ failure inevitably requires solid organ transplantation (SOT), which needs continuous immunosuppression to prevent allograft rejection. Classical immunosuppressive therapy is difficult to balance and causes many adverse effects. Recently, adoptive T cell therapy of regulatory T cells (Treg) was applied to SOT recipients and classical immunosuppressive therapy was successfully reduced in these patients. Though, baseline chemical immunosuppression was not completely abolished. Chemical immunosuppression suppresses in addition to pathologic allogen-specific effector T cells also the transferred Tregs as the target structures are important for both, effector T celland Treg function.
In the present study, we developed a GMP-compatible production process for manufacture of Treg products, which are resistant to a classically used immunosuppressant. Therefore, we sorted Treg from peripheral blood mononuclear cells using fluorescently activated cell sorting technology in a closed system. After an initial round of expansion, the Treg were rendered resistant to a particular immunosuppressant by knockout of the cell-intrinsic adaptor protein required for function of the particular immunosuppressant using a CRISPR-Cas9-based vector-free system. Following another expansion phase, the immunosuppressant-resistant Treg products were phenotypically, functionally and qualitatively analyzed *in vitro*, revealing promising results.
Our strategy represents a GMP-compatible minimally manipulative gene-editing approach, which may help to further promote the success of adoptive Treg therapy in the SOT setting.

80 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Safety of Cell Therapy Products: *In vitro* Methods to Assess the Tumorigenicity of Human Cell-Based Therapeutic Products - International Multi-Site study

Beata Surmacz-Cordle - Cell and Gene Therapy Catapult, United Kingdom

Human pluripotent stem cells (hPSC) have the potential to revolutionize regenerative medicine. However, there are concerns associated with hES/iPSC-derived products, in particular, the possibility of residual undifferentiated PSCs persisting in the final product which could lead to tumorigenicity. Currently, there is no globally accepted consensus on the evaluation of methods for tumorigenicity *in-vivo* or *in-vitro*. This results in a high variability of data presented in regulatory submissions and difficulty in interpretations. A public-private partnership (PPP) initiative to evaluate the existing methods for detection of undifferentiated cells and transformed cells is critically important not only for product developers but also for regulatory authorities and patients.

To address this challenge Health and Environmental Sciences Institute's Emerging Issues Committee (HESI) launched in 2016 a multi-sector collaborative sub-committee, the Cell Therapy - TRAcking, Circulation, & Safety (CT-TRACS), which provides a platform for developers, researchers, regulators, imaging specialists and other stakeholders to interact, discuss challenges and identify best practices in the safety assessment of these. An International Multi-Site Study call is set up to test *in-vitro* methodologies for tumorigenicity assessment of cell therapy products.

Here we will present an international collaborative project to evaluate *in-vitro* testing methods focusing on (1) the detection of LIN28 using ddPCR and (2) the development of a highly efficient culture assay. The two objectives, among many, for the Multi-Site study are to develop better, standardized *in-vitro* models for predicting tumorigenicity and to aid researchers, developers and regulators assess the safety of products with more confidence and contribute to faster/earlier decision-making.

81 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

In vivo CRIPSR/Cas9 Ldha inhibition as universal treatment for Primary Hyperoxaluria

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Sequence-specific endonucleases, specially CRISPR/Cas9 systems, have substantially increased the efficiency and specificity of genome editing, enabling the precise modification of the genome. Genome editing technologies not only facilitate deciphering the contribution of a specific mutation(s) in particular genetic disease but also represent an invaluable tool for the development of innovative therapeutic strategies for genetic diseases. Our previous results clearly indicate that CRISPR/Cas9-mediated substrate reduction therapies (SRTs) targeting glycolate oxidase (GO) represents a promising therapeutic option for PH1. In this work, we have extended the use of this *in vivo* genome editing strategies to target other enzymes of the glyoxylate metabolism that would be widely applicable not only for PH1 but also for other PH subtypes. Thus, we have developed CRISPR/Cas9 systems targeting Ldha gene that encodes the hepatic lactate dehydrogenase (LDH). A single administration of AAV8 therapeutic vectors drastically reduced LDH levels in the liver of PH1 and PH3 mice, reducing urine oxalate levels and kidney damage without toxic symptoms. Genome wide off-target analysis revealed the safety of this approach with no indel detection in the liver of treated animals. Altogether, our data provides evidence that *in vivo* genome editing technologies would provide new tools for improved and more universal therapeutic approaches for PH.

82 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

CRISPR/Cas9 gene editing for the correction of FOXG1 mutations in mitotic and post-mitotic cells

Sergio Daga - University of Siena, Italy

Mutations in FOXG1 gene cause the congenital variant of Rett syndrome, the most severe form of Rett spectrum disorders. The gene encodes for a transcriptional regulator and both under- and over-expression cause disease in humans. We thus reasoned that adding a normal copy of the gene under a not native regulator would be a risky therapeutic strategy while gene editing would be much more effective. We present here the successful application of an Adeno-Associated viruses (AAV)-coupled CRISPR/Cas9 system in patient-specific human cellular models, namely fibroblasts and neurons differentiated from induced Pluripotent Stem Cells (iPSCs). We have engineered a two-plasmid system to correct two different FOXG1 mutations, c.688C>T (p.(Arg230Cys)) and c.765G>A (p.(Trp255*)). Mutation-specific sgRNAs and donor DNAs have been selected and cloned together with an mCherry/GFP reporter system. Cas9 flanked by sgRNA recognition sequences for auto-cleaving has been cloned in a second plasmid. The system has been designed to be ready for *in vivo* delivery via AAVs. We demonstrated that different serotypes have different efficiency in different target cells, the best being either AAV9 in fibroblasts and iPSC-derived neurons or AAV2 in iPSCs. mCherry+/EGFP+ fibroblasts and neurons isolated by Fluorescent Activated Cell Sorting and analyzed by Next Generation Sequencing have shown efficient gene editing via Homology-Directed Repair in both cell types, with 20-35% of mutated alleles correctly reverted to the wild type sequence, outlining the relevant potentiality of the approach for Rett syndrome therapy.

83 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Development of a Scalable platform for AAV manufacturing

Juline Guenat - Cell and Gene Therapies Catapult, United Kingdom

AAV vectors are an appealing tool for both *ex-vivo* and *in-vivo* gene therapy. The number of AAV gene products entering early and late phase clinical trials is significantly on the increase. High cost of goods, low process yield, and poor product characterisation are all metrics that require substantial development and improvement within this emerging field. Currently most AAV vectors are manufactured using an adherent process and the demand currently outstrips capacity. Whilst switching from a classical 2D approach to a suspension process using single use bioreactors might be appealing to meet the requirement in term of doses and patient number for late stage of development (e.g Phase III study), this can come at the expense of laborious and costly comparability study. Hence, a reliable, low risk manufacturing platform delivering at the desired scale should be identified early on during development. Therefore, it becomes clear that there is a need to develop the next generation of upstream platform processes using a suspension cell line in STRs. Following Quality by design principles, we sought to develop this platform for AAV manufacturing. Using a scale down model, we investigated the impact of a broad range of process parameters using a design of experiment approach on AAV productivity. Scalability of the newly designed process as well as the impact of our USP on full capsid enrichment during our purification process has been investigated. Overall, our latest efforts in developing an end to end scalable suspension platform for AAV manufacturing will be presented.

84 - Preclinical Models and Technologies (Focus on Human-on-a-chip)

Raman Trapping Microscopy for label-free and fast quality control of cell products

Karin Schütze - CellTool GmbH, Germany

Introduction: Raman microscopy is based on interaction of focused laser light with molecules. It provides label-free and non-destructive information about structure as well composition of bio-molecules within a cell and thus is used to characterize cell types, to analyse cell status, or to monitor cell reaction on drugs or environmental impact.
Aim: Implementing a method for efficient and fast quality control of cell products, tissue models and autologous grafts.
Method and results: We use a combined Raman-Trapping-Microscope based on 785nm Raman excitation. A few µl of a cell sample is pipetted into a small channel of a common fluidic slide. Raman spectra are taken in a highly automated manner. RTM was used to monitor cellular differentiation and cell reaction on culture conditions. Furthermore, RTM could follow stem cell recovery after thawing, assure that tissue models lack from tumor cells, and prove quality of skin grafts. In blood products such as erythrocyte and thrombocyte concentrates RTM could detect cellular cross contamination as well as structural changes of molecules corresponding to storage time. Simultaneous laser trapping features allow to capture and analysis even small cells and particles such as bacteria or exosomes within their physiological solution to check contamination or provide early diagnosis of disease, respectively.
Conclusion: RTM works label-free requiring less than 500 cells for reliable results. Thus, it has the potential to become a standard for fast, efficient and highly reliable quality control of advanced therapeutic medicinal product, tissue model or blood products. RTM is showing a great potential in fast microbial identification and extracellular vesicles measurements such as exosomes.

85 - Manufacturing Technologies (Including Product Characterisation and Automation)

ISOCell PRO: Advanced Cell and Gene Therapy Isolator

Cristina Zanini - Euroclone S.p.A., Italy

Good Manufacturing Practice (GMP) compliant procedures are a prerequisite for cell production inclinical application and clean rooms are ideal zones for cell therapies production. The clean rooms useful for clinical application require high running and maintenance costs andneed trained operators and strict procedures to prepare the rooms and the people involved in theprocesses. This requires huge efforts in terms of facilities, personnel training and quality control. To provide a streamlined workflow environment reducing the set up andrunning costs of cell therapy products we have used ISOCell PRO, a Cell Therapy Grade A Isolatoralternative to the use of GMP class A Biological Safety Cabinet in a class B clean roomenvironment. Indeed, ISOCell PRO is a Closed System that requires Grade D surrounding environment. ThePositive Pressure Isolator guarantees Grade A environment in the working area with asepticconditions according to GMP. A CO2 incubator is integrated, what makes the system easilyvalidated at affordable costs. Decontamination process is automatic, fast, safe and economicallyaffordable and no need for special operators' clothes.Actually, SwissMedic completed the validation of a GMP process involving the use of ISOCellPRO units settled at the Centre de Production Cellulaire (CPC-CHUV - Lausanne). The CPC in compliance with GMP produces tissues and cells necessary to the treatment of burnpatients ensuring the production of autologous skin (the patient is the donor and the recipient of itsown cells). Our experience suggests this workstation as a possible alternative to the classic clean room due toits small size and the simplification of the working and maintenance operative procedures. Inconclusion this kind of isolators may represent an interesting solution in the perspective of the moreand more strong request for costs reduction of GMP in clinical application.

86 - Manufacturing Technologies (Including Product Characterisation and Automation)

Bioinspired Manufacturing of hPSC-derived cardiomyocytes for application in Cardiovascular Regenerative Medicine

Margarida Serra - Instituto de Biologia Experimental e Tecnológica, Portugal

In vitro differentiation of human pluripotent stem cells into cardiomyocytes (hPSC-CMs) is a crucial process to enable their application in cell therapy and drug discovery. Nevertheless, despite the remarkable efforts over the last decade towards the optimization of cardiac differentiation protocols, there are some technological challenges remaining, including the low scalability and differentiation yields. Additionally, generated hPSC-CM are still immature, closely reminiscent of fetal/embryonic cells in what regards phenotype, structure and function. Our work aims to overcome this hurdle by devising bioinspired and integrated strategies to improve the generation and functionality of these hPSC-CM. The key research line has been focused on the development of novel cell culturing strategies that recreate environmental conditions excelling hPSC proliferation as well as their differentiation/maturation into functional hPSC-CM, through metabolic and process understanding. In particular we have been designing processes based on expertise in 3D cell culture, bioreactor technology, co-culturing approaches and microencapsulation strategy. Noteworthy, we also applied robust multi-parametric techniques including advanced "-omics" technologies (proteomics, transcriptomics, metabolomics and fluxomics) as complementary analytical tools to support bioprocess understanding and optimization as well as to unveil the mechanism of action of cell therapy products.

87 - Manufacturing Technologies (Including Product Characterisation and Automation)

Viral vector manufacturing for preclinical assays

Miguel Chillon - Vall d'Hebron Research Institute, Spain

Viral vectors are widely used tools for gene transfer and gene expression in preclinical and clinical assays. Their use is an attractive choice given their high transduction efficiency, and the ease and flexibility to genetically express or inhibit one gene or a combination of genes in specific areas and periods of time, while avoiding compensation phenomena or other drawbacks associated with transgenic animal models. Up to date, at least 20 different types of viral vectors have been used in clinical assays. Among them retrovirus (Moloney murine leukemia virus - MoMLV), adenovirus, Herpes virus, lentivirus (LV) and adenoassociated virus (AAV) are the ones most frequently used. However, despite the availability of standardized procedures for their application in both *in vitro* and *in vivo*, and their low risk level when used in a controlled setting, the production of viral vectors requires the application of specialized techniques, access to expensive equipment and biological safety laboratories. In this context, the Viral Vector Production Unit (UPV) is a vector core specialized in the cloning, production and purification of state of the art different adenovirus (human and canine) and adeno-associated virus (AAV) serotypes for public research groups and private companies all over the world. Here we will present our expertise: (i) in vector design and construction to selective express or inhibit specific genes as well as to include state of the art techniques as genome editing or optogenetics (ii) in development of new vectors; (iii) in quality control assays; and (iv) in process development and process optimization of upstream and downstream manufacturing processes.

88 - Manufacturing Technologies (Including Product Characterisation and Automation)

Scalability strategies for cell manufacturing

Gloria Carmona Sánchez - Andalusian Network for Design and Translation on Advanced Therapies, Spain

Cell-based therapeutic products are being consolidated as a conventional treatment, as highlighted by the recent rise of phase I-III clinical trials, its incorporation into healthcare systems and the increase in commercialization. The AND&TAT coordinates a network of GMP laboratories, belonging to the Andalusian Public Health System, which make it possible to offer these innovative therapies to the population. Currently, most ATMPs used are being manufactured using manual methods (flasks or cell-factories). These time-consuming methodologies, require highly-qualified human resources, imply a scalability bottleneck and will not be able to accommodate future needs. We have evaluated the different available technologies to prepare the System for the expected increase in cell production demand. Automated 2D planar technologies, comparable to 2D-manual methods, could be design with limited surface, appropriate to autologous treatment, or in multiple size modules, being the easier transferring option. Packed-bed system represents the most optimized space alternative due to 3D cell distribution, favoring niche environment but, affecting harvesting efficiency and cell potency. Microcarrier suspension culture platforms require a thorough process of optimization in each scale-up step; moreover, aggregation, shear stress and detachment are critical in those devices.In conclusion, given the heterogeneity of cell types, doses, and disease indications, there is not a "one-size-fits-all" standardized manufacturing platform which could be viewed as a solution.Any platform scale will require an exhaustive transfer process from manual manufacturing protocol to automation, and should be chosen according to the specific medicinal product requirements and individual needs.

89 - Manufacturing Technologies (Including Product Characterisation and Automation)

Two Novel, Bioluminescent Assays for the Selective Detection of Target Cell Killing in Mixed Cultures

Axel Johann - Promega, Germany

Killing of target cells in a mixed-cell population is a key attribute of biologics products, with the ability to track the fate of select populations of cells critical for the development of novel immunotherapeutics. We have developed two approaches to selectively measure the death of target cells mediated by effector cells in applications such as CAR-T therapies or bi-specific antibodies. The two assay platforms offer workflow flexibility to support all stages of the drug discovery and development process. Both approaches detect the release of labeled intracellular proteins from target cells upon cell death, using NanoBiT® technology, a protein complementation reporter in which two subunits interact to form an active luciferase enzyme. The two assay platforms offer flexibility for rapid screening in discovery and lot release potency assays of cell therapy development.

90 - Manufacturing Technologies (Including Product Characterisation and Automation)

End-to-end platform for human pluripotent stem cell manufacturing

Nelsen-Salz Birgit - Lonza, United States

Industrialization of stem-cell based therapies requires innovative solutions to close the gap between research and commercialization. Allogeneic cell therapy indications that target large patient populations need scalable cell production platforms to reliably deliver the cell quantities needed during the various stages of development and commercial supply. Human pluripotent stem cells (hPSCs) are a key intermediate cell therapy product which serve as the source material for generating therapeutic cell types. We have developed a closed, automated and scalable bioreactor platform capable of sustaining high fold expansion of hPSCs. Such a platform could facilitate in-process monitoring and integration of online monitoring systems leading to significantly reduced labor requirements and contamination risk. We demonstrate high fold (>60X) hPSC expansion in suspension in a controlled 3L bioreactor using perfused xeno-free media without the need of intermediate cell passaging, thus reducing labor and process steps. Cell harvest and release from microcarriers are done in a closed steps, following by a closed downstream processing step of cell concentration. The hPSCs can be cryopreserved to generate a bank of cells or further processed as needed. Cryopreserved cells can be thawed into a 2D tissue culture platform or a 3D bioreactor to initiate a new expansion phase or to be directly differentiated to the clinically relevant cell type. The expanded hPSCs express hPSC-specific markers, have a normal karyotype and the ability to differentiate to the cells of the three germ layers by directed differentiation (cardiomyocytes, endoderm precursor cells and neural stem cells). These data show that high quality hPSCs can be generated in large quantity in a cGMP-compatible, closed and automated platform.

91 - Manufacturing Technologies (Including Product Characterisation and Automation)

Development of new analytical solutions for quality testing of ATMPs throughout manufacture

Beata Surrmacz-Cordle - Cell and Gene Therapy Catapult, United Kingdom

The global market for ATMPs has seen continual year on year growth with over 900 cell and gene therapy companies and over 1000 clinical trials to date. Majority of the therapies are looking to advance from phase 2 to phase 3 and commercialisation. With an increase in the number of patients predicted to be treated a concurrent expansion of GMP manufacturing sites has been apparent across the continents. It is currently predicted that autologous therapies will produce as much as 500 batches of products a year with expected increase to 1000's of batches in the coming years. Analytical methods used to assess the quality of the batches are complex, timely and performed manually utilising highly trained operators. This results in an increased demand on analytical throughput for in process and release quality testing to GMP compliance. It is therefore anticipated that Quality Control (QC) will become a bottleneck, slowing the ability of companies to release products and hindering commercial success. Obviating this will require a step change in QC provision, opening up opportunities for both automation and new disruptive technologies. We have developed a modelling tool allowing to design requirements for numbers of operators, pieces of equipment and lab space required within QC environment to support maximum capacity and efficiency of autologous product testing. The data indicates clear bottlenecks with regards to the FTEs needed to perform required testing. We are now looking into solutions such as automation and microfluidics to streamline the analysis with an integrated software system (Synthace/Antha) which will allow for control over automated lab workflows.

92 - Manufacturing Technologies (Including Product Characterisation and Automation)

The autostem platform as an exemplar for automated production of adherent regenerative cells at scale

Mary Murphy - National University of Ireland Galway, Ireland

Large scale generation of GMP-compliant therapeutic cells is necessary to progress regenerative therapeutics worldwide. Mesenchymal stromal cells (MSCs) represent an effective therapy for many indications with some in advanced clinical assessment or approved for patients. The AUTOSTEM pipeline fully automates the production of clinically compliant adherent MSCs on a multi litre scale, including closed introduction of bone marrow, cell expansion, formulation, and cryopreservation (https://youtu.be/rFD-Ojvya5c). The platform has three areas with appropriate biosafety levels: 1) transfer of materials 2) Grade D for establishment of cultures and expansion to the scale required in closed bioreactors and 3) Grade A for open processing for steps such as cell harvesting and cryopreservation. Cells are transferred from grade A-D through a transfer hatch and from grade D-A using an innovative fluid transfer device. Two robots enable all processes and transport of cells/materials between stations. All processes are executed automatically in a manner that is GMP-ready with closed, sterile expansion of hundreds of doses possible. All equipment including bioreactors, cell counters, centrifuges and other processing systems are linked to a central control system responsible for adaptive process control. Novel devices developed include pumping and sampling stations, decappers, and an automated pipette. The phenotypic fidelity of MSCs produced in the platform has been validated both *in vitro* and *in vivo*. The AUTOSTEM platform is also adaptable for clinical production of any cell or cell-derived product.

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93 - Manufacturing Technologies (Including Product Characterisation and Automation)

Comparability of MSC manufacturing protocols for clinical trials

Melissa van Pel - Leiden University Medical Center, Netherlands

Mesenchymal stromal cells (MSC) are clinically applied for their immunomodulatory and regenerative capacities. Despite the high number of clinical trials that have been performed thusfar, comparison of results obtained from these trials is hampered by the heterogeneity in MSC manufacturing protocols. Therefore, understanding the impact of differences in production methods between centers on characteristics of the cell therapy product and harmonisation of QC-tests are key in making MSC therapy available for larger groups of patients. MSC generation involves the isolation of bone marrow mononuclear cells (BM-MNC) using (ficoll) density centrifugation and subsequent expansion steps in T-flasks. After passaging the cells three times, MSC are harvested and cryopreserved in a typical product size of 300 x106 cells. Previously, changes in our clinical MSC manufacturing protocol have been implemented, including changes in antibiotics and in providers of raw materials such as ficoll and albumin. Here, we directly compare >30 real-time bone marrow-derived MSC manufacturing procedures performed in our center on the characteristics of the cells products. We compared all individual steps of our manufacturing methods pre -and post manufacturing changes, including release tests such as identity, purity and potency as well as in-process monitoring tests and in-process controls. Despite the high similarity in production methods, differences were observed in BM-MNC subsets following density gradient separation (ficoll). Moreover, changes in the antibiotics used at culture initiation significant decrease in the population doubling level and in a decreased drug product yield at passage 2. Despite these differences, identity and purity of these drug products were comparable. In conclusion, minor changes in clinical manufacturing protocols may impact on drug product yield and thus hamper comparability of results obtained in clinical trials. Therefore, detailed harmonisation of manufacturing protocols between centers is key in bringing new regenerative cell therapies to patients.

94 - Manufacturing Technologies (Including Product Characterisation and Automation)

Addressing the challenges of controlled, scalable and affordable expansion of hpscs for therapeutic use

Terri Gaskell - Cell and Gene Therapy Catapult, United Kingdom

Pluripotent stem cell technologies herald a disruptive clinical potential for allogeneic cell therapies. However, Cost of Goods and process complexity are key challenges for the commercialisation and competitiveness of these hPSC derived allogeneic therapy. Addressing these challenges will require investment in development and implementation of process engineering automated solutions, advanced analytical technologies for process monitoring and control, and sophisticated supply chain and transport logistics. Here, we summarise the hPSC Programme of The Cell and Gene Therapy Catapult. This project aims to design automated processing solutions for controlled, scalable, and affordable expansion of pluripotent stem cells in 2D and 3D culture systems. Work is centred around using starting materials compliant with industry-standards, baseline affordable processes for scalable expansion in 2D and 3D culture, and process integration strategies to provide solutions for culture intensification and integrated downstream steps (DSP) of cell concentration and washing. The processes developed will be used as foundations for the next stage of process development towards seamless expansion and differentiation processes integrated with downstream solutions. In addition, novel chemistries and replacement of key medium compounds are examples of our strategic focus for the next-generation of COGs reduction to develop affordable manufacture of allogeneic cell therapy products in large scale.

95 - Manufacturing Technologies (Including Product Characterisation and Automation)

Magnetic nanoparticles for immunogenic cell death and mobilization of dendritic cells

Maria Tsoumakidou - BSRC Alexander Fleming, Greece

Background. We have generated innovative magnetic nano-flowers (mNFLs) for hyperthermia induction and cancer cell death at low concentrations. Immunogenicity of cell death depends on dendritic cells (DCs), but is hampered by DC exclusion and/or suppression at tumor sites. Chemokines that could be used to attract DCs cannot be easily directed at tumor sites and diffuse quickly when injected in situ. Immune adjuvants become poorly immunogenic and toxic when administered systematically and in high dosages. Aim. We aimed to interrogate i) the immunogenicity of mNFL-induced cancer cell death and ii) the biological activity of DC targeting chemokines encapsulated in magnetic nanoparticles. Our ultimate goal is to develop an innovative immunotherapeutic approach based on the combination of magnetic hyperthermia with DC-targeting magnetic nanoparticles. Methods. mNFLs were administered i.v. in tumor-bearing mice. In one set of experiments mice were exposed to alternate magnetic field (hyperthermia) or sham. In another set of experiments magnetic nanocarriers of CCL4 or naked CCL4 were intratumorally injected into mice. Tumors were lysed and analyzed by FACS. Results. Albeit there were signs of T cell responses (increased granzymeB, low T regulatory cells), hyperthermia alone did not increase CD8+ T cell infiltration and failed to rescue T cells from exhaustion, associated with poor mobilization (numbers, phenotype) of DCs. CCL4-containing magnetic nanocarrriers effectively mobilized DCs and T cells at tumors. Conclusion. There is a limited immunotherapeutic potential of magnetic hyperthermia as stand-alone treatment, which points to the need for combinatorial treatment approaches with agents that target dendritic cells at tumors.

96 - Manufacturing Technologies (Including Product Characterisation and Automation)

High Content Analysis Reveals Distinct Morphological Profiles Of Human MSCs Under Different Culture Conditions

Marilina Piemontese - National University of Ireland Galway, Ireland

The number of clinical trials employing Mesenchymal Stem Cells (MSC) for the treatment of human diseases has grown substantially in the last decade. In this context new characterisation tools for cell and media manufacturing process are required. With this in mind, we sought to develop an automated high-content imaging assay to characterize the morphological profile of MSC in response to different media formulations. To do this, 1.5x103 human MSC were seeded in the absence (SF) or presence of 10% FBS (SC) into a 96-well plate ON. Cells were stained with Hoechst and CellMask Green dye, followed by fixation. Wells were imaged on an Operetta automated confocal plate instrument or on a Cytell instrument. Morphological descriptors were calculated on a single-cell level using Harmony or CellProfiler software by first identifying the nuclei (Hoechst), defining the cell boundaries (CellMask Green), and finally quantifying cell morphological features.High content analysis of high resolution images indicated that MSC growing in absence of serum displayed distinct morphological features compared to SC MSC. Specifically, in the absence of serum cells showed a smaller and more circular morphology, with less protrusions compared to SC MSC, which displayed an elongated shape, with numerous cellular protrusions. These phenotypic differences were expressed for instance by a significant higher circularity index and lower form factor by SF MSC, which measure respectively the overall roundness and spreading of a cell. Both automated workflows were able to successfully cluster the two experimental groups based on several morphological descriptors, allowing the generation of a morphological profile. Of note, the assay produced similar morphological profiles using MSC from two donors. Taken together, we report the development of a high-content assay to readily capture and analyse the morphological profile of MSC grown in different culture conditions and quantitatively showed that serum influences MSC morphology. Importantly, the assay may represents a straightforward strategy to benchmark MSC preparation and media formulations during production processes for therapeutic application

97 - Manufacturing Technologies (Including Product Characterisation and Automation)

Manufacturing of bioartificial hiPSC-based islets for Diabetes treatment

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Background: Diabetes mellitus type I is a chronic metabolic disease characterized by pathological increased blood glucose resulting from the destruction of insulin-secreting β-cells of the pancreatic islet. Islet transplantation represents a promising therapy but major drawbacks comprising donor shortage or loss of the natural islet niche and therefore limited graft survival exist. Aim: We are aiming to consider the fact that islet survival and function intensely relies on the interplay of all cell types including not only hormone-releasing cells but also other components such as the vascular and/or the nervous system of the islet. As the pancreatic islet is at the center of islet transplantation success, we hypothesize that bioartificial hiPSC-based substitutes have to closely resemble native islet characteristics. Therefore, we work on the development of pancreatic islet organoids (POIs) from hiPSCs with *in vivo*-like characteristics regarding islet size, structure and cellular composition combining endocrine islet cells with a vascular and neuronal system.Methods: We establish protocols for standardized up scaling of hiPSC maintenance culture in 3D. To this aim, we recently developed a hiPSC-specific suspension bioreactor platform with an on board microscopy unit that allows live monitoring of hiPSC culture and thereby quality assessment. For the derivation of islet-specific cell types, we apply state-of-the-art differentiation protocols that allow cell generation and islet organoid formation in 3D. Especially, long-term culture of islet organoids in suspension bioreactors are hypothesized to improve the maturity of cells, one drawback of current 2D-differentiated hiPSC-derived pancreatic cells. Standardized control assays including gene and protein expression profiling but also functional assessment of islet organoids for hormone-releasing capacities are performed to characterize POIs.Conclusion: We are aiming to develop GMP-graded islet organoids based on GMP-qualified hiPSC cell lines and protocols to engineer pre-clinical islet models that serve as platform for standardized and automated ATMP development for clinical translation.

98 - Manufacturing Technologies (Including Product Characterisation and Automation)

Scale-up of lenti viral vector manufacturing

Alexander Tappe - Sartorius Stedim Biotech GmbH, Germany

Clinical grade lentiviral vectors are often produced in HEK293T cells by transient transfection under static conditions, e.g. in cell factories. At best such processes are difficult to scale-up, but essentially they cannot meet the vector demand for cell therapies in a cost efficient manner. To reduce the manufacturing cost of said vectors we transferred an adherent HEK293T cell line that had been cultivated with serum-containing media to suspension cultivation under serum-free conditions.

Afterwards a media benchmarking in parallelized 15 ml stirred tank bioreactors was performed to identify the best starting medium formulation. The transfection and cultivation conditions have been optimized using the same system with a design of experiments approach, which resulted in an increase in productivity from 1.4E05 to 2.6E05 TU/ml.

In a second step we scaled up the process to 250 ml and then 10L bioreactors using tip speed as the scale-up criteria. No loss of productivity and infectivity was observed. The infectious titer was measured via antibody staining for all experiments. Based on this data it will be possible to scale up lentiviral manufacturing up to 2,000 L in commercially available single-use bioreactors.

99 - Manufacturing Technologies (Including Product Characterisation and Automation)

3D cultures for calreticulin detection in cell membrane following ionizing radiation treatment

Devid Maniglio - Univerisity of Trento, Italy

Calreticulin (CRT) is an endoplasmic chaperon molecule with many different functions. CRT sequestrates Ca2+, binds with unfolded proteins and prevents those proteins to be exported from the endoplasmic reticulum to the Golgi apparatus. CRT, like other chaperones, can translocate from the cytosol to the cell surface, particularly during endoplasmic reticulum stress induced by drugs, microbial stimuli or high energy radiation exposure. Following radiation, CRT becomes a very strong signal, a lighthouse that can be used for targeting specific drugs or radionuclides. Delivery of radionuclides in situ could help to overcome radio-resistance problem, like hypoxia, or to target the high resistant cancer stem cells. In this work potential selective targeting of in membrane overexpressed Calreticulin after proper antibody screening and after ionizing radiation treatment have been evaluated. Either 2D standard culture and 3D culture conditions have been used as conditions, the first for initial screening, the second for better mimicking *in vivo* tissue architecture. For the experiments B16 cell line were used. 3D porous scaffolds were made of methacryloil gelatin obtained by chemical functionalization of pig skin derived gelatin. Porosity was obtained by N2O gas foaming, a recently developed technique suitable for large porosity one-step scaffold fabrication and combined with 365 nm UV light crosslink to achieve scaffold stability.

100 - Manufacturing Technologies (Including Product Characterisation and Automation)

Fibrin hydrogel as a potential platform for the delivery of extracellular particles and vesicles

Heinz Redl - Ludwig Boltzmann Institut für Traumatologie, Austria

Backgground: Various approaches and applications in regenerative medicine use hydrogels due to its unique properties, among them its biocompatibility. Previously, we showed that endothelial cells form a vascular-like structure when embedded and co-cultured with mesenchymal stromal cells (MSC) in the biological hydrogel fibrin. The proangiogenic effect is assumed not only to be derived from direct cell-to-cell contact but also from MSC-secreted extracellular vesicles (EVs). Aim: The aim of this study is to investigate if there is any effect of fibrin on the extracellular particle (EP) release of the cells and if the hydrogel could be used as a delivery platform for EVs. Methods: Adipose derived stromal cells (ASC) and GFP-expressing human umbilical vein endothelial cells (GFP-HUVEC) were cultured either individually or in co-culture for 7 days. For 3D cultivation, fibrin matrices with 2.5 mg/mL fibrinogen and 0.2 U/mL thrombin were prepared. EPs were collected for 48h in EBM-2 (without serum). The size and number of EPs was measured by nanoparticle tracking analysis and Annexin V+ EVs were detected by flow cytometry. Results :Differences in particle size and amount were detected when measuring supernatants derived from 2D or 3D co-cultures. Further, more particles were detected for ASC cultured in fibrin then for HUVEC, whereas only small differences in sizes were observed. Conclusions: The data indicate, that either a difference in the adsorption of the different cell-derived particles by the hydrogel or the release of EPs itself could lead to the differences between the properties of EPs derived from ASC, HUVEC or the co-culture. In further studies, the capabilities of fibrin as a delivery platform for EVs derived from MSCs and induced pluripotent stem cells will be investigated to achieve a supporting 3D environment for the network formation of EC.

101 - Manufacturing Technologies (Including Product Characterisation and Automation)

Additive manufacturing by Melt Electrowriting for mature bioartificial cardiac tissues

Manuel Mazo Vega - Cima Universidad de Navarra, Spain

Background: The outbreak of the human induced pluripotent stem cells (hiPSC) technology has not so far materialised into a tangible application, either on drug testing or as therapy, mainly due to the immaturity of the obtained phenotypes. The application of Additive Manufacturing strategies, together with novel biomaterials constitutes one of the most promising strategies to overcome this roadblock.
Aim: Our aim is to combine composite-material strategies with Additive Manufacturing by Melt Electrowriting to create human iPSC-based engineered cardiac tissues.
Methods: Melt electrowriting (MEW) was used to 3D print a fibrous reinforcement. hiPSC-CMs were generated by biphasic modulation of the Wnt pathway, followed by a metabolic enrichment. Cell were embedded in matrigel, casted on MEW scaffolds and maintained for 4 weeks, after which gene expression, structure and functionality were analysed.
Results: Scaffolds were printed in medical grade poly-ε-caprolactone (PCL) to a diameter of 10 ± 2 µm. Differentiation yielded cultures of >80% cTnT+ cells which were successfully incorporated to the scaffolds. The formed microtissues were able to contract macroscopically for the duration of the experiments (4 weeks). The matrix was able to align the hiPSC-CMs in the direction of the fibres. Analysis showed a more mature functionality as compared with 2D controls on matrigel. This was related to an increased expression in ventricular myosins (MYL2/MYL7) as well as fast-conducting connexins (GJA1/GJC1 and GJA5/GJC1). Finally, in order to provide a more controlled hydrogel for the generation of these composites, we synthesized GelMA, a smart hydrogel capable of controlled crosslinking under visible light irradiation.
Conclusions: All in all, we have planted the seeds for the generation of mature cardiac microtissues.

102 - Manufacturing Technologies (Including Product Characterisation and Automation)

Composite scaffold as cell delivery system for treatment of Limb Ischemia

Michele Carrabba - Univeristy of Bristol, United Kingdom

Background: Cell Therapy has been explored as potential of critical Limb Ischemia (LI) in alternative to traditional interventional approaches. Despite the initial promising results, cell therapy showed limitations in cell retention and absence of supportive environment.
Aim: We propose the design and development of a novel cells delivery system based on a hybrid scaffold able to stimulate reparative angiogenesis embedding vascular cells (pericytes and endothelial cells) in a mouse model of peripheral LI.
Methods: The scaffolds were designed with the shape of channels and fabricated with Biomaterial-Ink technique.
Polycaprolactone (PCL) 10%(w/v) and Poly-lactic-co-glycolic acid (PLGA) 15%(w/v) were used to compare the effect of different mechanical properties. The synthetic structures were then covered with a layer of gelatin (GL) electrospun nanofibers, with adhesive features similar to those of the natural ECM. Adventitial Pericytes (APCs), isolated from saphenous vein, were seeded at 10,000 cells/cm2 onto PCL- and PLGA-based scaffold. On top of the confluent layer of APCs, a patterned co-culture of APCs and HUVECs were then bioprinted. APCs and HUVECs, in ratio 1:4 and density of 4million/ml, were loaded in a hydrogel solution made of 6% Sodium Alginate and 13% F127 Pluronic, extruded in lines with a Bio-Ink. The approach was validated implanting the hybrid scaffolds, with and without cells, in mice with unilateral LI.
Conclusions: Mechanical properties of the composite scaffolds showed significant differences depending on the material, with the PCL displaying values closer to the femoral artery. *In vitro* assays showed good performances for both PCL and PLGA. Viability of Bioprinted APCs and HUVECs was observed always around 70%, and the aligned pattern was maintained. Based on these results, PCL- and PLGA-based scaffold with and without cells were implanted around the occluded femoral artery. The group with implanted cellularized PLGA-AG/PL showed faster recovery of blood flow compared with vehicle (11 vs. 18 days, respectively, p=0.01) in the Doppler Flowmetry assessment and the highest increase of number of arterioles in the ischemic area.

103 - Manufacturing Technologies (Including Product Characterisation and Automation)

Addressing the Manufacturing Challenges of Mesenchymal Stromal Cell-based Therapies through Bioengineering

Claudia Lobato da Silva - Instituto Superior Técnico Universidade de Lisboa, Portugal

The robust and scalable cell manufacturing for the cost-effective delivery of safe and potent cell-derived Advanced Therapy Medicinal Products (ATMPs) relies on process engineering tools to understand the impact of cellular features (biological, biochemical, etc) on cell product function and performance, and how do process variables influence the critical quality attributes of the cell product.
Mesenchymal stromal cells (MSC) have been widely exploited as potential therapeutic agents for several conditions due to their intrinsic regenerative and immunomodulatory properties, as well as high proliferative capacity *ex-vivo*, ease of isolation, multilineage differentiation ability and low immunogenicity. Large cell doses (>1x106 cells/kg) have been required for clinical implementation of MSC-based therapies and the success in obtaining those cell numbers starting from different human tissues such as bone marrow (BM), adipose tissue (AT) or umbilical cord matrix (UCM) is dependent on efficient *ex-vivo* expansion protocols able to comply with GMP. We have established the clinical-grade expansion of human MSC in scalable microcarrier-based bioreactors using serum-/xenogeneic-free (S/XF) culture components and have demonstrated the potential to maximize cell productivity by changing different culture parameters including microcarriers, tissue source and bioreactor configuration. Recently, increasing evidence has proposed extracellular vesicles (EVs) as exosomes, as mediators of many of the MSC-associated therapeutic features. In this context, we have adapted the previously established platform for the manufacturing of human MSC to allow the reproducible production of MSC-derived EVs with promising characteristics for biomedical settings (i.e. more homogeneous size-distribution profiles and higher EV concentration, when compared to static culture, as determined by nanoparticle tracking analysis).

104 - Manufacturing Technologies (Including Product Characterisation and Automation)

Biohybrid Implants - Fostering Clinical Translation by Textile Reinforcement

Stefan Jockenhövel - RWTH Aachen University, Germany

Regenerative Medicine has promised to overcome the limitations of conventional implants with the potential to remodel, to self-repair and specifically for the pediatric applications to grow with the child. In the past two decades, many successful pre-clinical trials have demonstrated the potential of tissue-engineered implants, but the number of translated products to the clinic are very limited. This is due to the high complexity of the production process and the need to control the complex adaptive behavior of the patient-individualized cell source in the process.
While the classical tissue-engineered implant has primarily focused on a complete autologous solution, the biohybrid approach is looking for a balance combination of technical and biological components with regard to (i) a high (re)producibility by the technical component and (ii) an optimal hemo/biocompatibility by the biological component.
Because “human beings are textile-reinforced composites”, textile engineering offers a multi-scale toolbox for mimicking and supporting tissue engineered constructs. We have demonstrated the use of textile fibers to create anisotropic tissue constructs in cardiovascular and respiratory tissue engineering.
The presentation will give an insight in the “evolution” of cardiovascular and respiratory tissue-engineered implants from complete autologous towards biohybrid textile reinforced constructs as key technology for a (re)producible and herewith transferable implant into clinic.

105 - Manufacturing Technologies (Including Product Characterisation and Automation)

Strategies for the Expansion of Human Induced Pluripotent Stem Cells as Aggregates in Single-Use Vertical-Wheel Bioreactors

Joaquim M.S. Cabral - Instituto Superior Técnico Universidade de Lisboa, Portugal

Background: Human induced pluripotent stem cells (hiPSCs) have been regarded as an enormous breakthrough for medicine, due to their ability to generate virtually all types of cells in the human body. One of the great bottlenecks in the usage of these cells for Regenerative Medicine applications is their expansion to clinically-relevant quantities. The Vertical-Wheel bioreactors present a novel configuration, whose vertical agitation allows for homogeneous mixing conditions inside the vessel, while conveying less shear stress to the cells when compared to traditional alternatives.
Aim: This work reports by the first time, the expansion of hiPSCs as floating aggregates in the Vertical-Wheel bioreactors, while also demonstrating different strategies to increase the performance of the culture and allowing for the harvest of higher cell numbers at the end of culture
Methods and Results: Cultures were performed in the PBS Biotech MINI 0.1 bioreactor with 60 mL of working volume. Two different culture media were tested, mTeSR1 and mTeSR3D, in a repeated batch or fed-batch mode, respectively, as well as dextran sulfate (DS) supplementation. A maximum cell density of (2.3±0.2)×106 cells·mL−1 and a volumetric productivity of (4.6±0.3)×105 cells·mL−1·day−1 were obtained after 5 days with mTeSR1+DS, resulting in aggregates with an average diameter of 346±11 µm. The generated hiPSCs were analysed by flow cytometry and qRT-PCR and their differentiation potential was assayed, revealing the maintenance of their pluripotency after expansion.
Conclusion: The results here described present the Vertical-Wheel bioreactor as a promising technology for hiPSC bioprocessing. The specific characteristics of this bioreactor, namely in terms of the innovative agitation mechanism, and this system overall has the potential to comply easily with cGMP due to the single-use bioreactor system and the lack of matrices of any kind for cell adherence.

106 - Manufacturing Technologies (Including Product Characterisation and Automation)

A sound-induced technology for multiscale orchestration of functional vessels networks

Tiziano Serra - AO Research Institute Davos, Switzerland

Cell patterns are important for studying morphogenesis, unraveling biophysical mechanisms, and in the development of novel tissue engineering approaches. Surface acoustic wave (SAW) technologies, based on Faraday wave principle, enable the generation of spatially orchestrated particulate systems (cells, spheroids, inorganic aggregates). Patterns shape can be tuned on demand by varying a set of parameters, such as sound frequency, amplitude, chamber shape. Here we propose the use of a SAW-based technology to create precise and reproducible microvascular networks formed by interconnected and perfusable vessels. To do that, spheroids formed by human umbilical vein endothelial (HUVECs) and human mesenchymal stem cells (hMSCs) are patterned in few seconds within an extracellular matrix-like hydrogel. Then, HUVECs sprouting from the patterned spheroids and self-organization into micro-vessels will finish the work. Hierarchically shaped vessels with a multiscale organization (meso-micro scale) can be integrated into a fluidic chip where perfusion can be performed in a reproducible manner with a controlled flow rate.

107 - Manufacturing Technologies (Including Product Characterisation and Automation)

Advanced Manufacturing of human pluripotent stem cells and their progenies for ATMP production

Robert Zweigerdt - Medizinische Hochschule Hannover, Germany

Background: The clinical translation of human pluripotent stem cell (hPSC) progenies depends on robust, GMP-compliant processes for producing specific cell types at therapeutically relevant quality and quantity. Instrumented stirred-tank bioreactor (STBR) technology allows for the systematic development and upscaling of cell production processes. These systems ensure homogeneous distribution of cells, nutrients and gases in suspension culture and enable the continuous monitoring of parameters required for process optimization.
Aim and Methods: By adapting STBRs towards hPSC-specific needs, we have recently enabled hPSC cultivation as matrix-free cell-only aggregates in suspension culture at chemically defined conditions. This approach substantially reduces costs and lowers regulatory hurdles related to the GMP-requirements of process components. Subsequently, we showed that suspension-based hPSC culture is compatible with lineage-directed differentiation into cardiomyocytes, endothelial cells, hepatocytes, macrophages and megakaryocytes. It will highlight how >90% lineage purity can be achieved by process modification inducing superior reproducibility and higher cell yields in parallel to process upscaling.
Conclusion: Much more potential for process development remains. By taking full advantage of automated process control abilities in STBRs and by combining multidisciplinary expertise in stem cell biology with industrial biotechnology, we will here demonstrate a leap forward in hPS cell bioprocessing. Presenting unpublished data, we will novel strategies enabling hPSC cultivation at 10-fold higher (!) cell density compared to the current state in the field. This represents a new level regarding the standardized, commercially viable production of human pluripotent stem cells for both autologous as well as allogeneic ATMPs.

108 - Manufacturing Technologies (Including Product Characterisation and Automation)

Cost of Decentralized CAR T Cell Production in a Non-Profit Setting

Stefan Eichmüller - Deutsches Krebsforschungszentrum, Germany

Background: Chimeric Antigen Receptor (CAR) T cell therapy is a promising immunotherapy with high acquisition costs, which has raised affordability and sustainability concerns. Furthermore, the current centralized production paradigm for the T cells is less than satisfactory. Our study provided a cost estimation for an alternative production mode, decentralized T cell production, in a non-profit setting in Germany.
Methods: We first identified the work steps and main activities in the production process. Then we determined the fixed cost and variable cost. Main cost components included personnel and technician salaries, expenditure on equipment, a clean room, as well as production materials. All costs were calculated in 2018 euros.
Results: For a clean room with one CliniMACS® Prodigy machine, annual fixed costs summed up to approximately €438,000 ($490,000). The variable cost per production was roughly €35,000 ($39,000). At the maximum capacity of one machine, total cost per product would be close to €60,000 ($67,000). As shown in the scenario analysis, if three machines were to be installed in the clean room, per production cost could be as low as €45,000 (roughly $50,000). If a cheaper alternative to lentivirus was used, per production total cost could be further reduced to approximately €33,000 (roughly $37,000).
Conclusions: Decentralized T cell production might be a less-costly and more efficient alternative to the current centralized production mode that requires a high acquisition cost.

109 - Manufacturing Technologies (Including Product Characterisation and Automation)

Generation of universal platelets from HLA-null human pluripotent stem cell (hPSC) lines

Cedric Ghevaert - University of Cambridge, United Kingdom

Background: Alloimmunisation, the process of developing an immune response to "non-self" (foreign) antigens after exposure to genetically different cells or tissues, increases in occurrence in patients undergoing transplant or repeated transfusions; and in multiparous women. Human Leukocyte Antigens (HLA) expressed on cells, such as platelets, distinguish between "self" and "non-self" antigens. Patients that develop HLA-alloimmunisation and require transfusions to prevent bleeding, undergo HLA-typing to receive HLA-compatible platelets from a restricted pool of recallable donors. However, locating a perfect match is not always possible and partial matches result in shorter survivability of circulating platelets.
Aim: Platelets are formed and released from Megakaryocytes (MKs). Our aim is to develop HLA-null hPSC lines to produce functional MKs, which would then generate 'HLA-universal donor' platelets. These platelets would circumvent alloimmunity concerns and help alleviate the cost of transfusion management for HLA-alloimmunised patients.
Methods: CRISPR-Cas9n technology was employed with guides targeted to the β2-microglobulin (B2M) locus to generate HLA-knock out (KO, null) hPSC lines. Whole genome sequencing was performed to analyse the effect of CRISPR-mediated HLA-KO in hPSCs. MKs and platelets differentiated from HLA-KO hPSC lines by either doxycycline-induction or the forward programmed method (PMID 27052461) were characterised with flow cytometry and immunofluorescence microscopy.
Conclusion: We have successful generated HLA-null hPSC lines. These lines maintain pluripotency (TRA+/ SSEA+) in chemically defined cultures and do not express HLA in the presence of interferon gamma (IFNγ), a known inducer of HLA proteins. Genome sequencing analysis reveals no obvious off-target effects or significant changes in mutation signatures. HLA-KO hPSCs can be differentiated into highly purified MKs that are polyploidy and generate proplatelets and platelets. Our work here progress towards delivering an alternative and universal platelet source, with the potential of using these cells as site-specific delivery vehicles of components with added clinical benefits.

110 - Manufacturing Technologies (Including Product Characterisation and Automation)

Development of Protocols towards GMP-compliant Genome Engineering of hiPSCs

Ulrich Martin - LEBAO Hannover Medical School, Germany

Background: Future cellular and *ex vivo* gene therapies will require safe and GMP-compliant protocols for generation of human induced pluripotent stem cells (hiPSCs). Moreover, GMP-compliant protocols will also be necessary for subsequent genetic correction of disease specific mutations and targeted introduction of therapeutic genes, selection markers or suicide genes.
Aim: It was aim of our study to develop robust protocols for reprogramming of peripheral blood cells and subsequent targeted gene editing under defined animal-component-free conditions.
Methods and Results: Here we present a protocol for manufacturing of GMP-compliant human iPSC lines from peripheral blood CD34pos cells (Haase et al.; 2019; Stem Cell Research). Using TALEN mRNAs we were also able to target the Adeno-Associated Virus Site 1 locus (AAVS1) in such hiPSCs via donor-mediated homologous recombination under GMP-compliant conditions.
Conclusion: This method represents the basis for the establishment of GMP-compliant genetically corrected hiPSC lines that may also carry different therapeutically relevant transgenes.

111 - Manufacturing Technologies (Including Product Characterisation and Automation)

Osteoclasts differentiated from human iPS cells as a test system for gene therapeutic approaches for autosomal recessive osteopetrosis

Uta Rössler - BCRT, Charité Universitätsmedizin Berlin, Germany

Autosomal recessive osteopetrosis (ARO) patients suffer from brittle bones, severe anemia, immune deficiency, and variable central nervous system problems. They usually die during childhood. Causative mutations abolish bone resorption by osteoclasts. Currently, the only curative treatment for ARO is allogenic hematopoietic stem cell transplantation, which still harbors significant risks. Here, we report on an *in vitro* model based on human induced pluripotent stem cells (hiPSCs) for testing alternative treatment strategies.

Using hiPSCs from a control subject and an ARO patient, an efficient protocol for differentiation of osteoclasts from hiPSCs was established. The expression profile and surface markers of hiPSC-derived precursors and osteoclasts showed high similarities to CD14+ monocytes and usual PBMC-derived osteoclasts, respectively. A more detailed comparison revealed a higher fusion rate of osteoclasts from control hiPSCs leading to enlarged osteoclasts that preferentially resorbed pits instead of longer trenches unlike CD14+ monocyte-derived osteoclasts. Most important, ARO hiPSC-derived osteoclasts were incapable of bone resorption in contrast to control osteoclasts. The establishment of additive gene transfer strategies is the first step in the direction of a personalized treatment for ARO patients improving success rates and prognosis. Hence, we developed a therapeutic Sleeping Beauty transposon construct and reached transfection rates of up to 85% and stable integration in 21% of hiPSCs.

112 - Manufacturing Technologies (Including Product Characterisation and Automation)

The CULTAINER - A standard container-based facility solution for GMP-compliant ATMP manufacturing

Hans-Georg Eckert, Claudia Papewalis - Valicare, Germany

Valicare GmbH, a 100% subsidiary of Robert Bosch Packaging Technology GmbH, offers GMPcompliance services since 2002. We are a team of experienced GMP experts with backgrounds in various disciplines of natural sciences and engineering and a passion for innovative Advanced Therapeutic Medicinal Products (ATMPs). Facilitating the translation of novel therapeutic approaches into marketable ATMPs of pharmaceutical quality is our mission. Thus, we support our customers during all phases from R&D to GMP and market access.

Transferring the complexity of novel ATMPs into GMP-compliant manufacturing for clinical trials represents one of the major time consuming challenges on the way from bench to bedside. We aim to address this bottleneck by offering a ready-to–manufacture IMP-package, named CULTAINER, constituted by a container-based manufacturing facility, including the successful manufacturing authorization certification procedure for the investigational ATMP (iATMP) of our customers. Furthermore, we will tackle regulatory and ATMP GMP-compliance issues, such as documentation, qualification and validation, process and analytical method development, validation and transfer, automation, market access and financing strategies. The CULTAINER concept is generic and thereby fast in realization. It can be applied to different product classes and easily be customized to specific needs.

Our CULTAINER concept combines expert knowledge with experienced executive support and can thereby catalyze critical steps to bring innovative ATMPs to the patient. During this journey, Valicare is your competent partner with a strong industrial background in a network with common goals.

113 - Manufacturing Technologies (Including Product Characterisation and Automation)

MSC-EVs protect mice from Graft-versus-Host Disease pathology in a preparation dependent manner

Verena Börge - University Hospital Essen and University of Duisburg-Essen, Germany

Extracellular vesicles (EVs) harvested from supernatants of humane adult bone marrow-derived mesenchymal stem/stromal cells (MSCs) can suppress acute inflammatory cues in a variety of different diseases, including Graft-versus-Host Disease (GvHD) and ischemic stroke, and promote regeneration of affected tissues. Following a successful clinical treatment attempt of a steroid refractory GvHD patient, we intend to optimize MSC-EV production strategies for further clinical applications. As we observed functional differences of independent MSC-EV preparations *in vitro*, we aimed to set up an *in vivo* GvHD model for the more advanced functional testing of different MSC-EV preparations. To this end we set up a bone marrow transplantation mouse model in which endogenous bone marrow was myeloablated by ionizing irradiation (IIR). GvHD was induced by the transplantation of major histocompatibility mismatched allogeneic spleen-derived murine T cells. If not treated otherwise, myeloablated mice developed severe GvHD symptoms. The GvHD symptoms were effectively suppressed if MSC-EV preparations were applied at 3 consecutive days, which exerted immune modulatory effects in a mixed-lymphocyte reaction assay. MSC-EV preparations lacking *in vitro* immune modulating activities within the MLR assay, however, hardly improved the symptoms of the GvHD mice. Thus, our results demonstrate that not all MSC-EV preparations harvested from adult bone marrow-derived MSCs are functional equivalent. Thus, successful transplantation of MSC-EVs into the clinics requires a platform allowing identification of MSC-EV preparations with sufficient therapeutic, most probably immune modulating activities.

114 - Manufacturing Technologies (Including Product Characterisation and Automation)

External Quality Control Program in Advanced Therapies

Rosario Sánchez Pernaute - Andalusian Network for Design and Translation of Advanced Therapies, Spain

Quality controls used for the manufacture and release of Advanced Therapies Medicinal Products (ATMPs) remain at an early stage of development and, given the intrinsic nature of the specific cell-based preparations, are subject to human factor issues without technical standardization. For this reason, adopting the experience from the clinical diagnostic field, a specific External Quality Control Program has been created with the aim of harmonizing and guiding the manufacturers to develop safe and effective ATMPs. Once each 6 months, manufacturers attached to the program shall receive a rationally-prepared sample, which is virtually identical to the one that the other participants shall receive. By using their routine analytical methods, they will have to perform the quality controls each manufacturer had voluntarily subscribed and upload the results to the program website. Each one of them shall be able to download a final report, that takes into account the confidentiality of the participants, where their particular results are statistically compared to the others' results. The program guarantees the absolute anonymity of both the participants and the individual results. Manufacturers can select which quality controls they would like to perform among the following initially considered: Microbiological control of cell-based preparations (sterility test), Endotoxins, Mycoplasmas detection, Immunophenotyping, Gram and Calcofluor white staining, Thawing yield, in-house Cellular growth promotion and Karyotype. Other test could be included if required.

115 - Manufacturing Technologies (Including Product Characterisation and Automation)

Standardized GMP-compliant scalable production of human pancreas organoids

Lorenza Lazzari - Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy

Background: Organoids are three-dimensional *in vitro*-grown cell clusters that recapitulate key features of native organs. In regenerative medicine, organoid technology represents a promising approach for the replacement of severely damaged organs, such as the pancreas in patients with type 1 diabetes. Although the development of pancreas organoids (hPOs) from human pancreas biopsies has been demonstrated to be feasible, it is not yet possible to produce a clinically relevant number of hPOs for biobanking from a single biopsy. A larger quantity of starting material is needed.
Aim: Our goal was to establish a Good Manufacturing Practice (GMP)-compliant protocol for the automated generation of biobank-appropriate, ready-to-use hPOs.
Methods: Starting from discarded pancreatic tissues, we developed a large-scale process for obtaining clinically relevant quantities of undifferentiated organoids, obviating enzymatic digestion and operator-dependent pancreatic ducts picking steps. In addition to karyotyping and growth curve analysis, we also obtained an hPO transcriptional profile and immunophenotype, including acinar, ductal, and pancreas progenitor marker expression data.
We made modifications to two critical small-scale research-protocol steps without affecting hPO isolation efficiency or growth: use of automated mechanical dissociation and elimination of the picking procedure. We observed that our hPOs were heterogeneous, containing numerous acinar, ductal, and pancreatic progenitor cells, but no endo.

116 - Manufacturing Technologies (Including Product Characterisation and Automation)

Chemical defined, biomimetic matrices for serum-free culture of MSC and beyond

Richard Wetzel - denovoMATRIX GmbH, Germany

The natural cellular environment is a complex interplay of extracellular matrix (ECM) components such as glycosaminoglycans (GAGs) and proteoglycans, soluble factors and cell-cell interactions. Current matrices facilitating *in vitro* culture of stem cells are either poorly defined ECM extracts or single component polymers that lack the natural complexity. We have developed a library of self-assembling matrices that are suitable for expansion of stem cells for therapeutic applications. By combining GAGs with biofunctional peptides, these matrices present multiple essential cues of the natural ECM in a chemically defined manner. Our technology enables a screening approach to identify the relevant composition for the cell type of choice. Screening the material library led to a candidate coating supporting growth of mesenchymal stromal cells (MSC) in the absence of serum. Long-term culture studies showed enhanced cell proliferation in animal-free media for up to 40 population doublings. We further identified another matrix that facilitates the maintenance and proliferation of induced pluripotent stem cells (iPSC). iPSC growth was studied and maintained for 3 passages while preserving their differentiation capacity. After neuronal induction of iPSC-derived neural precursor cells, the maturating neurons switched their biomatrix preferences. Analysis of the differentiation efficiency provided evidence that the incorporated GAGs supported neuronal development. Stability tests on FGF2 demonstrated that GAG-containing matrices maintain FGF2 activity for up to 3 days. Thus, the presented matrices provide optimal conditions for the expansion and differentiation of adult and induced stem cells. The developed matrices are biologically relevant, modular, chemically defined and scalable and thus enable defined cell culture protocols for stem cell research, drug-development and cell therapy applications.

117 - Clinical Implementations (Including Reimbursement Models)

The TWO Study - A Phase II Trial of Regulatory T Cell Therapy in Renal Transplantation

Johanna Hester - University of Oxford, United Kingdom

Transplantation is the most effective treatment for end-stage kidney disease. Yet despite major improvements in immunosuppression, long-term transplant survival rates remain poor. Excellent short-term survival rates are achievable with tacrolimus and mycophenolate mofetil (MMF) combination therapy, but this is associated with side-effects that include nephrotoxicity and neoplasia. There is, therefore, a need to minimise immunosuppression while retaining the excellent graft survival rates. Regulatory T cell (Treg) therapy has shown promise in early clinical studies and may promote conditions that allow immunosuppression reduction. We have recently completed a Phase I study in renal transplant recipients to assess the feasibility and safety of Treg therapy (the ONE Study). This dose escalation trial has demonstrated that the production of clinical-grade Treg is feasible and that there are no adverse reactions following administration. We are now running a 5-year randomised Phase IIb immunosuppression minimisation trial in living donor renal transplant recipients, assessing the ability to achieve immunosuppression monotherapy with the use of expanded polyclonal Treg therapy post-transplantation. The primary outcome will be the incidence of acute rejection episodes at 18 months post-transplantation. Patient peripheral blood and protocol biopsies will also be subjected to in-depth immunological analyses to detect the presence of Tregs and the effects of therapy.

118 - Clinical Implementations (Including Reimbursement Models)

Chemokine systems to enhance the potency of Advanced Therapies

Tino Vollmer - BCRT, Charité Universitätsmedizin Berlin, Germany

What contributes to the function of Advanced Therapies (AT)-products when transferred *in-vivo*? In T lymphocyte-based AT-products the specificity of cells plays a pivotal role. By current strategies, this can be achieved either by chimeric antigen receptors (CAR) or engineered T cell receptors that redirect T lymphocytes to the target. However, despite recognition of the target, this does not ensure efficient trafficking to the locus of interest, neither does it ensure survival of transferred T lymphocytes. E.g. solely 1% of transferred CAR-T lymphocytes reach the tumor site when applied to an animal model (Albelda et al., 2014). To break the barriers, such as the immune-suppressive tumor milieu, it is of interest to boost the intrinsic capacity of T lymphocytes to migrate and to sustain in the tissue environment.First, I aim to give insights into our current approach to identify a relevant chemokine receptor-ligand interplay for T lymphocytes in solid cancer. Here, we found that a specific receptor variant-ligand axis was associated with tumor rejection in muscle-invasive bladder cancer. We confirmed a functional role of this axis by inducing expansion of protective CD8+ memory stem T cells (TSCM) *in-vitro.* This indicates that chemokine systems may be relevant for long-lived CD8+ T cell function supporting an effective anti-tumor response. Further, I will highlight our next steps to translate the mechanisms of this specific receptor-ligand interplay into a reshaping approach for AT against cancer.Second, I aim to give an overview about strategies to implement dual receptor approaches in AT-products. How can we employ receptor-ligand-matching to enhance *in-vivo* function of T lymphocytes? E.g. besides anti-tumor function, chemokine systems may be exploited for regulatory T lymphocytes (TREG) to home into the transplanted organ to elicit local suppressive function. Enhancing the tissue-specific function and survival of T lymphocytes may be key to induce an efficient *in-vivo* response of AT irrespective of the cellular approach or disease of interest. Eventually, to endow T lymphocytes with a holistic functional capacity may restore the outcome of the patient.

119 - Clinical Implementations (Including Reimbursement Models)

Public Healthcare System as key player in leveraging advanced therapy supply chains

Rosario Sánchez Pernaute - Andalusian Network for Design and Translation of Advanced Therapies, Spain

In the last years, we have witnessed a considerable R&D+i growth in the field of Advanced Therapies Medicinal Products (ATMPs): cell therapy, gene therapy and tissue engineering. Many global leader companies and public research institutions have been investing large amounts of money in what it is considered a highly profitable global market, with prominent commercial opportunities for the treatment of a wide range of diseases.Up to now, a total of 14 ATMPs have been approved by the European Medicines Agency. However, this so-called revolution of healthcare is not a path of roses. Marketed European ATMPs have faced several difficulties reaching the commercialization approval and afterwards; all those approved from the first marketed ATMP in 2009-2013 have been withdrawn. In this regard, the selected business models of these ATMPs have proved to be unrealistic exploitation plans, mostly due to poor supply chain & reimbursement strategies and, subsequently, unsuccessful patient access, which has led to a lack of reimbursement options and total economic failures for the industry. In addition, the number of authorized ATMPs is expected to grow, which could entail a significant burden for Public Healthcare Systems (PHSs). Taking as a paradigm the Spanish Model for Organ Transplantation, we propose here a potential supply chain strategy for commercial ATMPs by harnessing the established operational and administrative structures within PHSs and exploring new public-private partnership opportunities with the industry. Thus, a sustainable approach is envisaged by sharing expertise and resources in order to achieve a cost-effective, patient-centered objective.

120 - Clinical Implementations (Including Reimbursement Models)

Advanced Therapy Medicine, Oncolytic Virotherapy, Mesenchymal Stem Cells

Manuel Ramirez - Hospital del Niño Jesús, Spain

Background: Our group is developing a new strategy for metastatic tumors based on oncolytic virotherapy administered by autologous mesenchymal cells obtained from bone marrow. Results of a first clinical trial and a compassionate use program have shown a very high safety profile. Most of the treated patients did not present clinical response while a small group did. Aims: We have carried out a comprehensive study of the advanced therapy medicine (ATM) to find determinants associated with the clinical outcome. Results: The study of the ATM's transcriptome from a first cohort of patients identified biological processes in the carrier cells that differentiated responders from non-responders (viral infection and nucleic acid metabolism; immune regulatory routes; cell-cell adhesion; mitosis; cell metabolism; response to stress; and bone marrow fibrosis). Next, we used an approach based on systems biology and neural networks to identify candidates responsible for the differences found. The mesenchymal cells of the responding patients expressed significantly lower levels of the MAVS and NDRG1 genes compared with those of the non-responders. Both genes are related to the cellular antiviral response and cellular metabolism. The identified molecules were validated in a second independent cohort of patients. Conclusions: These results point to possible pretreatment biomarkers and the possibility of optimizing the ATM to manufacture a universal version of the medicinal product.

121 - Clinical Implementations (Including Reimbursement Models)

Sirolimus-mediated induction of fetal hemoglobin in beta-thalassemia: from laboratory experimental evidences to clinical trials

Roberto Gambari - University of Ferrara, Italy

Background. The mTOR inhibitors rapamycin (sirolimus) and everolimus were found by our group to be strong inducers of production of fetal hemoglobin (HbF) by erythroid precursors (ErPCs) from beta-thalassemia patients. In beta-thalassemia, the induction of HbF production is clinically relevant, due to its ability to mimic to some extent HbA function.
Aim. Sustain the possible use of sirolimus in clinical trials by a more complete analysis of 57 ErPC cultures from 37 beta-thalassemia patients.
Methods. The production of HbF was studied by high-performance liquid chromatography (HPLC) and the results confirmed at the mRNA level by real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).
Conclusion. (a) Sirolimus increases HbF in cultures from beta-thalassemia patients with different basal HbF levels (the cultures from 51.4% of the patients were responsive to sirolimus treatment); (b) the cultures from 37.8% of the patients were not responsive to both sirolimus and hydroxyurea (HU); (c) sirolimus was able to induce HbF in 46.15% of the cultures not responsive to HU; (d) sirolimus displayed higher efficiency than HU in 57.14% of the cultures responsive to both sirolimus and HU; (e) 42.86% of HU treated cultures displayed HbF induction higher than sirolimus. The possibility to propose mTOR inhibitors for *in vivo* pilot clinical studies was also sustained by the recent Orphan Drug Designation (ODD) of sirolimus by the European Medicinal Agency (EMA) for the treatment of beta-thalassemia (code EU/3/15/1585) and of SCD (code EU/3/17/1970). ODD of sirolimus as HbF inducer in beta-thalassemia and SCD was also obtained by the U.S. Food and Drug Administration (FDA). At present two clinical trials are ongoing, one funded by Wellcome Trust (NCT03877809) the other by AIFA.

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122 - Clinical Implementations (Including Reimbursement Models)

Regulatory Framework for Innovation Assessment in Advanced Therapies in Europe

Rosario Sánchez Pernaute - Andalusian Network for Design and Translation of Advanced Therapies, Spain

Regulation (EC) No 1394/2007 establishes a centralized marketing authorization procedure for Advanced Therapy Medicinal Products (ATMPs) when they are intended to be placed on the market or industrially prepared. However, the competent authorities of each Member State (Regulatory Medicines Agencies and/or HTA bodies) are responsible for the reimbursement of each ATMP in its territory, meaning that reimbursement models vary from country to country. Therefore, there is a lack of a harmonized, consistent and transparent evaluation procedure for ATMP pricing and reimbursement. Among the European reimbursement models, there are some key factors that influence on the final reimbursement decision. In all cases therapeutic benefit, patient's benefit and unmet medical needs are taken into consideration. Another relevant aspect, especially in the advanced therapy field, would be the innovative value of the product. In this sense, different Regulatory Agencies have taken different approaches. Here we remark Italian, German, French and Swedish approaches to their innovation assessment.

123 - Clinical Implementations (Including Reimbursement Models)

A prospective three-track decision framework to determine the product value of new atmps

Rosario Sánchez Pernaute - Andalusian Network for Design and Translation of Advanced Therapies, Spain

Marketed European Advanced Therapy Medicinal Products (ATMPs) have faced several difficulties reaching the market authorization and afterwards. In fact, all those approved from the first marketed ATMP in 2009 till 2013 have been withdrawn. Market authorization principally focus on achieving a positive therapeutic effect whilst ensuring patients' safety. Consequently, other essential aspects that guarantee market success are not generally considered and, nowadays, lead to major bottlenecks in the field of ATMPs. This fact, together with the cost-constrained healthcare systems, highlights the need of prospective procedures to determine whether or not the ATMP represents a good investment from either commercial or healthcare perspectives.We propose here a pre-assessment with three decision-making criteria to better analyse the product value of the ATMP and facilitate the establishment of a go/no-go decision criteria for further developmental stages. This value assessment should be performed early enough to the translational process and would encompass three methodologies related to measuring its innovative potential, social impact and early economical evaluation (EEE):

TRACK 1: Innovative potential. A quantitative method developed by the University of Alcalá de Henares in collaboration with the Spanish Ministry of Health, Social Services and Equality would allow to assess the innovation level of ATMPs based on two major criteria: value of therapeutic innovation and safety, and impact on therapy and care resources.

TRACK 2: Social impact. Social Return on Investment (SROI) is the most internationally used method to measure social impact since it involves project stakeholders to create an impact map which allows to analyse the social changes generated, and to transform them into a monetary value.

TRACK 3: EEE. By means of Headroom Analysis, we would incorporate demand-side reimbursement process (decision to buy) into supply-side investment decision (decision to develop), determining the maximum reimbursable price of the innovative ATMP.

124 - Regulatory Science and Clinical Trials

Treatment of steroid refractory acute graft-versus-host disease with allogenic adipose tissue-derived mesenchymal stromal cells. A multicenter phase I/II clinical trial

Fabiola Lora Ulgar - Andalusian Network for Design and Translation of Advanced Therapies, Spain

Acute graft-versus-host disease (aGvHD) is a significant cause of morbidity and mortality following allogenic hematopoietic stem cell transplantation. Immunosuppression with corticosteroids forms the basis of first-line therapy, producing responses in less than 50% of patients with aGvHD. Mesenchymal stromal cells (MSC), based on their immunomodulatory properties, may play a key role in the treatment of the disease. MSC suppressive effect on T-cell function has been shown to be only transient. Therefore, the efficacy of MSCs could be increased when administered sequentially. Additionally, preclinical studies suggest that MSCs obtained from adipose tissue show an immunomodulatory potential at least as strong as MSC obtained from bone marrow, and a greater proliferative potential. On this basis, a phase I/II, single arm, open-label, multicentre study to assess the safety and efficacy of a sequential dose infusion (0,7-1 x 106 MSC/kg, days +1, + 4, + 11, + 18) of allogenic adipose tissue-derived MSC in patients with SR-aGvHD (Grade II-IV) was designed and is currently ongoing. After the first ten patients had received the MSCs, an interim analysis was carried out. Twelve serious adverse events were reported, none of which MSCs related. Only one severe infection occurred, deemed unrelated to MSCs, which resolved without sequelae. No suspected unexpected serious adverse reactions occurred. Neither infusion-related toxicity was observed.

125 - Regulatory Science and Clinical Trials

Safety, Feasibility and trends of Efficacy of Intravenous Injection of Autologous Adipose-Derived MSCs in Patients with ALS: A phase I-IIa multicenter randomized triple blind placebo controlled trial

Fabiola Lora Ulgar - Andalusian Network for Design and Translation of Advanced Therapies, Spain

Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with rapidly progressive disability and gradually or abrupt progressive muscular paralysis and atrophy with a short survival of 2-5 years. Mesenchymal stem cell therapy has been proposed as a promising approach for treatment of ALS. Objective: Evaluate the safety and trends of efficacy of intravenous administration of 3 doses of autologous mesenchymal stem cells (MSC) from adipose tissue in patients with ALS. Methodology: A phase I-IIa, multicenter, controlled, triple-blind clinical trial, randomized in four groups, has been conducted. Forty subjects were randomized into one of the following arms: placebo group, 1 million MSC /kg, 2 million MSC / kg, and 4 million MSC / kg. The manufacturing process of the Investigational Medicinal Product (IMP) was carried out in the Cell Manufacturing Unit of the Regional University Hospital in Malaga (coordinated by AND&TTA). Preliminary results: The efficacy data are currently being analyzed. Secondary outcome measures were determined by means of changes in the ALSFR-R, in forced vital capacity and in muscle bulk estimated by MRI. No related deaths occurred. A total of 12 adverse reactions were related to the infusion procedure or IMP: 8 infusion site phlebitis, 2 infusion site thrombophlebitis, and 2 deep venous thrombosis.Conclusion: Prospective studies with a larger sample and longer-term follow up, are urgently required to assess the clinical benefits of cell-based therapy including improvement in disease progression and quality of life and prolongation of survival in people with ALS.

126 - Regulatory Science and Clinical Trials

CD20 CAR-TIME: CD20 CAR transduced T cells for individualized melanoma therapy

Michael Apel - Miltenyi Biotec GmbH, Germany

In this presentation we will provide initial clinical results from a phase I CAR T cell trial in Melanoma (MB-CART 20.1 for Melanoma; NCT03893019; multicenter phase I trial for treatment of patients with metastatic melanoma).
17,000 patients are diagnosed with malignant melanoma in Germany every year. 3,000 patients die as a consequence of the disease. Conventional therapy options such as dissection, chemo- or radiotherapy show only limited success and are often accompanied by severe adverse reactions.
In the BMBF-funded project CD20 CAR-TIME, CAR T cells will be applied to treat melanoma patients in a clinical study. Moreover, this project consortium will explore how automation with the CliniMACS Prodigy can make this personalized form of therapy available to a large number of patients.
Background: It has been shown by Hinrich Abken that CD20 is expressed on melanoma tumour cells and that CD20 targeting can eradicate tumours (Schmidt et al. 2011). Hence, this concept is investigated in the CD20 CAR-TIME trial that Miltenyi Biotec is sponsoring.
Approach: CD20 CAR T cell products for the clinical trial are manufactured in accordance with GMP guidelines in Ulrike Köhl's lab at Hannover Medical School (MHH) and in the Miltenyi's GMP facility in Bergisch Gladbach, Germany. QC (quality control) and tracking of the cell products is monitored closely at both sites.
Concomitant research carried out in the lab of Hinrich Abken at the Regensburg Center for Interventional Immunology investigates the *in vivo* functionalities of CAR T cells, e.g., their persistence, cytotoxic and proliferative capacity.
The first dosing group is presently being treated and we will present data from manufacturing, quality control, treatment and monitoring.

References: Schmidt, P. et al. (2011) Eradication of melanomas by targeted elimination of a minor subset of tumor cells. PNAS 108: 2474-2479.
https://www.cd20-car-time.de/index.html

127 - Regulatory Science and Clinical Trials

First-in-human" clinical trial employing adoptive transfer of autologous thymus-derived Treg cells (thyTreg) to prevent graft rejection in heart-transplanted children

Rafael Correa-Rocha - Instituto de Investigación Sanitaria Gregorio Marañón, Spain

Background: Immune allograft rejection remains the main obstacle to reach successful transplants. Transfer of regulatory T cells (Treg) has acquired growing interest in attempts to prevent rejection. However, the limited number and the differentiated phenotype of Tregs isolated from blood constitute important drawbacks for the effectiveness of this strategy. We have explored the use of the thymic tissue, which is routinely discarded during pediatric cardiac surgery, as an alternative source of Tregs to be used as cellular immunotherapy in heart-transplanted children.
Methods: We developed a novel GMP-compatible protocol to obtain massive amounts of thymus-derived Tregs (thyTreg) from thymuses discarded from infants. A ""first-in-human"" clinical trial (phase 1/2a) will be initiated in 2019 to test the safety and effectiveness of the adoptive transfer of autologous thyTregs in heart-transplanted children.
Results: ThyTreg produced with our protocol showed a very high purity, with >95% of CD25+Foxp3+ cells. Importantly, the number of thyTreg cells obtained from one single thymus reached values of more than 10x109 (billions) cells, which would be enough to prepare hundreds of therapeutic doses. The final product of thyTreg showed adequate phenotype and Foxp3 stability, and the final thyTreg product showed a very high suppressive capacity, decreasing the proliferation of CD4+ and CD8+ T cells by more than 80%. Infants younger than 2 years old included in the waiting list for a heart transplant will be enrolled in the clinical trial. A single dose of autologous thyTreg cells will be infused back to the children at day 7-10 post-transplant. The rest of the thyTreg doses will be cryopreserved for potential reinfusions to the patient.
Conclusion: Massive quantities of highly suppressive and pure Thy-Tregs obtained with our novel GMP-compatible protocol are suitable to be employed as cellular immunotherapy to prevent rejection in heart-transplanted children. At the present year, we will initiate the first clinical trial to test the safety of the procedure, the feasibility and the effect of the thyTreg therapy in the context of solid organ transplantation.

128 - Regulatory Science and Clinical Trials

Non-clinical and clinical development of autologous ATMPs

Oliver Pullig - Fraunhofer Translational Center Regenerative Therapies, Germany

Background: A lot of promising scientific results for advanced therapy medicinal products (ATMPs) remain in an early development status. This is due to a lack of abilities to bridge the gap to GMP manufacturing or to contribute extraordinary amounts of means, resources and time to achieve GMP compliance and to be ready for the performance of efficacy studies or regulatory required GLP-conform safety studies.
Aim: TLC-RT investigates new ways in the treatment of particular diseases of the joints, the immune system and the cardiovascular system as well as tumors and wound healing. Our aim is the GMP conform translation of autologous tissue-engineered products such as cartilage implants or cellular products combined with innovative biomaterials to improve tissue and organ properties (EU projects VascuBone - tissue-engineered vascularized scaffolds for human application, BIO-CHIP – GMP manufacturing of two tissue-engineered combination products, HemAcure - advanced pre-clinical translation of genetically modified cells). Finally, due to our close connection to the University Hospital Würzburg our final goal is to support, facilitate and accelerate translation of ATMPs as an Academic Translational Hub.
Methods:

- Preparation, support and assistance of manufacturers in regulatory consultations and applications
- Implementation of regulatory requirements for QM and DMS
- Implementation of regulatory requirements for GMP, GLP, GCP manufacture and testing of medical devices and ATMPs incl. risk-based approaches and automatization
- Biodistribution and tumorigenicity studies incl. genetically modified cells according to GLP standard
- Fabrication of vascularized, implantable 3D ATMPs for hosting of cells, medical defect coverage, and replenishment (BioVaSc-TERM™)

Conclusion: TLC-RT provides important tools for the translation of ATMPs. Furthermore, within our own non-clinical and clinical development of different autologous ATMPs we identified a variety of roadblocks and found solutions to get rid of them. Consequently, our wide-ranging experiences and competences can have a strong impact to accelerate, facilitate and strengthen the ATMP development in Europe.

129 - Regulatory Science and Clinical Trials

Young people's involvement in paediatric drug development

Annagrazia Altavilla - Espace Ethique PACA-Corse and TEDDY, France

Background: In 2007 the Paediatric Regulation (No 1901/2006) came into force to improve the health of children and young patients (CYP) in Europe, by facilitating the development and availability of medicines for the paediatric populations, and to foster the investment of the pharmaceutical industry in this field. Despite the increase in the number of clinical trials, about one third have failed to be completed due to lack of recruitment. Recruitment to clinical trials in CYP has population specific challenges including smaller numbers of patients, study design and feasibility, patient recruitment and retention, consent and assent of CYP, suitable formulations and small bio-samples collection. In recent years, many initiatives have been carried out in order to encourage paediatric patients' engagement and the implementation of adequate health policies also in the field of paediatric research. In this context TEDDY, the European Network of Excellence for Paediatric Clinical Research has provided valid tools in the promotion of this aspect in the European policy and increased the social awareness on the importance of the paediatrics and CYP involvement in clinical research.
Aims: To describe that many of the challenges in recruiting patients for paediatric clinical trials can be addressed by actively involving CYP in study design through the forum of a Young Persons' Advisory Group (YPAGs) so their insights can inform successful drug trial design and implementation.
Methods: YPAGs with a remit to support the design and conduct of paediatric drug trials are increasing across Europe. We will share the impact that YPAGs are having on the design and development of drug trials across Europe and at various points in the research process, from setting research priorities to disseminating research findings.
Conclusion: Key challenges to recruitment and retention in paediatric trials remain an issue but working alongside young people through the forum of a YPAG in partnership with regulators, life science industries and other stakeholders is a continued effort to improve the quality and acceptability of study procedures for children and families.

130 - Regulatory Science and Clinical Trials

hPSCreg's Clinical Study Database for hPSC-derived Cell Therapies

Nancy Mah - BCRT, Charité Universitätsmedizin Berlin, Germany

The generation of human pluripotent stem cell (hPSC) lines at multiple sites, such as different kinds of research facilities (e.g. core facilities, individual research laboratories, biobanks) lends to a high degree of variability in the availability of donor information and the characterization and production process generating PSC lines. Coupled with additional variability introduced by cross-border regulatory regions and cultures, it is difficult to compare and evaluate lines that originate from diverse sources. To make hPSC data FAIR (Findable, Accessible, Interoperable and Re-usable), the Human Pluripotent Stem Cell Registry (hPSCreg; https://hpscreg.eu) collects a wide range of PSC-related data in standard formats, including ethical provenance, evidence of pluripotency, and genetic constitution. The standardized collection of these key data enables an informed assessment of registered hPSC lines for their applications in research and clinical translation by academia, regulatory bodies, and industry. To further monitor the success of hPSC-based cell therapies from their source hPSC cells, hPSCreg has created a clinical study database specifically for clinical applications of hPSC-derived cells (https://hpscreg.eu/browse/trials).

131 - Ethics, Economics, Big data and AI

Developing a Broadly-Applicable Imaging Platform for Optimizing Cell Therapies in Solid Tumours

David Morrow - EATRIS, Netherlands

The introduction of immunotherapy, particularly immune cell therapies, have transformed the therapeutic landscape in recent years. Following the success of Kymriah®, all eyes now turn towards large patient groups with solid tumour cancers and whether the success of CAR-Ts can be reproduced in these patients. However, solid tumours represent a much greater challenge as these tumours incorporate mechanisms designed to keep T cells out due to the tumour microenvironment and before any such strategy can be comprehensively evaluated *in vivo*, a means to track these cells in situ for safety and efficacy and monitor their success is fundamental. Our project aims to develop a broad platform, that incorporates non-invasive *in vivo* imaging and predictive modelling for the optimisation of cell therapies, that can be integrated in existing ATMP supply chains. A multimodal imaging platform will be implemented, building on clinical-grade and clinically approved nanoparticles, to provide the ATMP developer a quantitative, non-invasive early stage prognostic indicator for cell therapy efficacy. We propose to develop, apply and exploit a multimodal clinical and preclinical cell tracking platform taking advantage of state-of-the art customisable imaging nanotechnology in addition to other relevant modalities.
This cell labelling will be integrated into existing clinical ATMP supply chains where a consensus regulatory pathway for imaging agents in ATMPs with imaging agents will be established. Our Project includes leading academics from the EU research infrastructure for translational medicine, EATRIS, clinicians, large industrial partners, SMEs, and international organisations, so we can reach all major relevant research groups. This will enable us to build an innovative, robust, standardised platform. Although we will apply the platform to CAR T cell therapies, the impact of our platform will extend to other types of cell therapies.

132 - Ethics, Economics, Big data and AI

Early Health Technology Assessment to Optimise Project Design of Advanced Therapies

David Morrow - EATRIS, Netherlands

Successful development of advanced therapies towards clinical application requires a different approach to exploratory research in the project design, execution and management of the project. A variety of elements such as technical feasibility (e.g. manufacturing and scale-up), regulatory clarity for product classification or Freedom to Operate are additional critical success factors beyond the scientific excellence. To successfully navigate this complex path towards the patient, researchers need to access a broad range of expertise, facilities and resources in a goal-oriented fashion. In this context, EATRIS with ZonMw (Netherlands Organisation for Health Research and Development) has developed an early Health Technology Assessment framework specifically designed to support the translation of high potential projects. This framework is based on a pilot performed on four projects funded in 2017 by ZonMw. For this pilot, the project proposals were assessed using the 9 dimensions proposed by the EUNetHTA Core Model® (Version 3.0) and with the input of HTA experts at Vita-Salute San Raffaele University.The assessment showed that not all dimensions considered in EUNetHTA Core Model® are suitable for early stage development projects. However, it was perceived by ZonMw that such early feedback on projects was highly valuable to the Principal Investigators of the projects to inform on the product development strategy and go/no-go decision criteria and more efficient allocation of resources.The final Early HTA framework implemented at ZonMw starts with the submission of the project proposal by the researcher, followed by a light assessment using an adapted version of the EUNetHTA Core Model® topics and identification of knowledge gaps in the proposal to be addressed during the project's lifespan. The third phase involves full HTA performed towards the project's completion.The Early HTA service is now included in the EATRIS centralised service catalogue for academic projects together with its feasibility assessment and regulatory support services.

133 - Ethics, Economics, Big data and AI

Ethics and advanced therapies/emerging technologies: towards a new model of governance

Annagrazia Altavilla - Espace Ethique PACA-Corse and TEDDY, France

Background: Despite the variety of legal instruments, the EU framework is far from being homogeneous, and not all the steps in the translational pipeline are equally addressed from a legal/regulatory point of view. Regarding the use of new/emerging technologies (e.g. gene editing), at present, any clear ethical/legal/regulatory framework exists, because of the lack of agreement due to ethical, societal, scientific and economic issues. Ambiguities and heterogeneity are factors to be seen as slowing down the innovation process and raising ethical issues, especially for "vulnerable population" such as children. Aim: To develop a more adequate model of governance including the ethical/regulatory assessment of advanced therapies/new technologies in accordance with ethical/legal norms and fundamental rights, taking into account peculiarities of children. Methods: In the light of ethical norms and fundamental rights as well as of literature trends, different phases of the research pipeline will be analysed to identify uncovered and/or unsolved issues to be dealt in an ethical/regulatory perspective. Conclusion: The analysis carried out underlines the need to rethink the ethical assessment of advanced therapies/new technologies, in the light of a comprehensive "translational approach" (based on the tree main pillars: Benchside, Bedside and Community). To promote enhancements in prevention, diagnosis and therapies, particularly for vulnerable populations. The need to combine disciplines, resources, expertise and techniques within these pillars is also stressed. Finally, it is proposed to develop a new model of governance involving the main stakeholders and including a specific paediatric common service aimed at addressing ethical/legal/social/regulatory issues and at developing adequate initiatives in this field.

134 - Ethics, Economics, Big data and AI

Big Data and Machine Learning: new horizons in the field of personalized medicine and advanced therapies

Elisabetta Volpe - Fondazione per la Ricerca Farmacologica “Gianni Benzi” Onlus and TEDDY, Italy

Background. In the last 15 years we have witnessed a revolution in the Healthcare sector, which is being transformed by our growing ability to record massive amounts of patient data (e.g. genetic, environmental and lifestyle information, electronic health records, sensor and medical imaging data). In this era of Big Data, application of Artificial Intelligence (AI) and of Machine Learning (ML), as one of its most important branches, provides researchers with new tools and perspectives that are fundamental to developing non-traditional therapies such as advanced therapies (AT), and therapeutic approaches, such as personalized medicine (PM) and precision medicine.
Aim. The goal of this work is to frame the importance of ML in this innovative context and to take a look at the biggest opportunities and challenges. Moreover, we focused on evaluating the sustainability of the regulatory framework in relation to the ML innovation and on considering how appropriate it would be to issue new regulatory recommendations.
Methods. A comprehensive approach, consisting of electronic database consultation and specific websites searching, as well as complementary web searches, was used to collect literature analysing Big Data use in Healthcare and its technical, ethical, legal and social implications, such as data sharing, information overload, semantic integration approach and genomic privacy, with special reference to paediatric applications as the future goal.
Conclusion. Our investigation clarified the many areas in which ML could be employed, including clinical trials of cell therapies, gene therapies and tissue engineering, planning of effective recruitment and retention of patients, with high impact in fields like paediatrics and rare diseases. These findings tie in with the European Medicines Agency (EMA) draft 'Regulatory Science to 2025', a Reflection Paper that underlines the need for a more flexible regulatory system, a correspondingly better integration between science and technology, a new paradigm for PM, innovative therapeutics and regulatory procedures for AT.

135 - Ethics, Economics, Big data and AI

Catching them early: ethics and economy of prenatal and childhood application of ATMPs

Carsten Werner Lederer - The Cyprus Institute of Neurology and Genetics and TEDDY, Cyprus

Background: Advanced therapy medicinal products (ATMPs) differ from small-molecular drugs in many aspects. ATMPs are plagued by higher production cost and, even for rare disease targets, by limited production capacity. Additionally, high development costs contrast with still vague reimbursement and discount models for one-off curative or infrequent palliative treatments. In this context, prenatal or paediatric application, while still technically, ethically and legally challenging, may reduce production cost to a fraction and usually promises higher efficiency than application in adults.
Aim: This study sets out to present data on prenatal, perinatal and paediatric application of ATMPs, with emphasis on findings in informative disease models, on a comparison of cost and efficiency with application in adults, and on preconditions and legal and ethical impediments to clinical translation.
Methods: This is a selective review of the latest ATMP developments in prenatal technology, of (part-)paediatric clinical trials, of pricing information for GMP-grade ATMP components, and of extant regulations applicable to/adaptable for ATMPs.
Conclusion: Diagnostic and prognostic advances allow ever-earlier informed application of ATMP products. More efficient, more affordable therapy is possible by in utero or paediatric application, with vastly reduced cell and vector requirements for selected ATMP applications. Besides improving affordability, efficiency and use of GMP resources, early application is fundamental to treatment of many as yet untreatable diseases with pre- or perinatal onset. There is thus every incentive for ATMPs to redress the gap, apparent in the TEDDY European Paediatric Medicines Database, for paediatric vs adult medication. Critical work remains to address ethical and safety concerns for the young or unborn patient, in particular where data from adult studies are absent. However, as successful studies accumulate and the underlying technologies and their ethical and legal framework conditions become established, prenatal and paediatric application of ATMPs promises competitive and ground-breaking treatments and the resolution of many challenges in the field.

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