HELLENIC SOCIETY
OF GENE THERAPY AND REGENERATIVE MEDICINE

www.generegther.gr

6th Congress of the Hellenic Society of Gene Therapy and Regenerative Medicine

May 26-28, 2023

Cotsen Hall
American School of
Classical Studies
Athens

FINAL PROGRAM

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Welcome message,

*On behalf of the **Hellenic Society of Gene Therapy** and **Regenerative Medicine,***

Dear society members, our international colleagues and friends,

*I would like to formally invite and welcome you to the **6th Annual Meeting of the Hellenic Society of Gene Therapy and Regenerative Medicine** to be held in **Athens**, the historical capital of **Greece**, from **26-28 May 2023**.*

This will be the first meeting of our society conducted by full in-person attendance after the global outbreak of COVID-19, providing again to the participants networking opportunities with top researchers and healthcare professionals from Greece and all over the world. The gene and cellular therapy fields have made significant progress over the last few years and the number of Advanced Therapy Medicinal Products being approved is rising, having drastically changed the whole treatment paradigm.

The educational and scientific program of our meeting is expected to keep us up to date on revolutionary science and research and trigger spirited discussions and debates. The latest clinical advances of gene therapy and regenerative medicine as well as the current regulatory and financial challenges of advanced therapies will be presented by experts in the field and discussed. In addition, the oral and poster sessions will give the opportunity to young scientists and junior faculty to present their research and productively interact with their peers.

The success of the Congress depends on your support and contributions and I take the opportunity to invite all of you who are interested in the fields of gene therapy and regenerative medicine to join our society.

We look forward to meeting in person, sharing knowledge, promoting networking, discussing the latest advances of gene/cell therapy & regenerative medicine and of course, enjoying Athens!

Sincerely,

Evangelia Yannaki
President of Board of Directors
and Organizing Committee

General Information

The **6th Congress of the Hellenic Society of Gene Therapy and Regenerative Medicine** will take place from 26 to 28 May 2023, at Cotsen Hall of the American School of Classical Studies in Athens.
The entrance to Cotsen Hall is at **9, Anapiron Polemou str., Kolonaki**.

Registration

The delegates who wish to attend the Meeting with their physical presence, should complete their online registration.
Entrance will be controlled by an electronic badge scanning system. The badge will be given to delegates upon their arrival, by the Congress Secretariat. It will also be sent to them by e-mail upon completion of their registration.

Registration – Payment Methods

Registration can be completed in the following ways:

- **Online, using a credit or debit card:**
  Access to the electronic registration platform of the Meeting is available through the website of the Hellenic Society of Gene Therapy and Regenerative Medicine, **www.generegther.gr** and of the Conference Secretariat, **www.vitacongress.gr**.
  The online registration and payment system using debit or credit card is fully encrypted and directs you to a secure bank page, where the card will be debited automatically.
- **Bank deposit:**
  Payment by deposit in the following bank account. Participation Form and a copy of the back transfer transaction should be sent by e-mail to: **info@vitacongress.gr**.

  Bank account:
  Bank: ALPHA BANK
  Account Name holder: V. VOURAZERIS & SIA G.P.
  Account Number: 194002330000040
  IBAN: GR0501401940194002330000040
  BIC/SWIFT: CRBAGRAA

Congress Secretariat

VitaCongress V. Vourazeris & Co O.E.
4 Papadiamantopoulou street & Vas. Sofias, 11528 Athens, Greece
Tel.: +30 2107254360, Fax.: +30 2107254363
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Registration Fee

Category	Physical and Virtual Presence	Virtual Attendance only
Doctors	120 €	50 €
Biologists, Biochemists, Other Medical Professionals	50 €	20 €
Doctors in Residency, Postdoctoral Fellows	30 €	15 €
Nurses, Technologists	30 €	15 €
Undergraduate Students, Postgraduate Students, PhD Students	Free Participation	Free Participation

**The above costs are subject to VAT where applicable.*

Registration includes Congress attendance and certificate.

Participation Form

FIRST NAME: LAST NAME:

AFFILIATION:

DEPARTMENT:

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E-mail: TEL. NUMBER:

Category	Physical and Virtual Presence	Virtual Attendance only
Doctors	120 €	50 €
Biologists, Biochemists, Other Medical Professionals	50 €	20 €
Doctors in Residency, Postdoctoral Fellows	30 €	15 €
Nurses, Technologists	30 €	15 €
Undergraduate Students, Postgraduate Students, PhD Students	Free Participation	Free Participation

**The above costs are subject to VAT where applicable.*

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Access to the electronic registration platform of the Meeting is available through the website of the Hellenic Society of Gene Therapy and Regenerative Medicine, **www.generegther.gr** and of the Conference Secretariat.

Students wishing to attend the Meeting should send by e-mail to **g.kokka@vitacongress.gr** the participation form and a copy of their student ID or relevant certificate from their school.

The participation form is also available in word format on the website of the Hellenic Society of Gene Therapy and Regenerative Medicine, **www.generegther.gr** and of the VitaCongress Secretariat, **www.vitacongress.gr**.

DATE SIGNATURE

Acknowledgements

We would like to warmly thank the following sponsors:

PROGRAM

Friday, 26 May 2023 - Morning

08.30-09.30 **Working Groups**

09.30-11.00 **Educational I: things you need to know***

Chair: **Alexandros Spyridonidis - Evgenios Goussetis**

09.30-10.00 **Viral vectors: gains and lessons learned from clinical use**
Nicholaos P. Anagnou

10.00-10.30 **Gene editing: CRISPR and beyond**
Carsten Lederer

10.30-11.00 **CAR T cells: the present and the future**
George Vassilopoulos

11.00-11.30 Coffee break

11.30-13.30 **Educational II: things you need to know***

Chair: **Achilles Anagnostopoulos - Eleni Papadaki**

11.30-12.00 **Tumor-infiltrating lymphocytes**
Panayotis Tsirigotis

12.00-12.30 **T regulatory cells**
Alexandros Spyridonidis

12:30-13:00 **Mesenchymal cells**
Maria Roubelakis

13.00-13.30 **GMP contamination control strategies**
Anastasia Papadopoulou

13.30-15.00 Lunch break and e-poster exhibition

*Session in Greek

Friday, 26 May 2023 - Afternoon

15.00-17.00 Gene Therapy progress for common and rare diseases

Chair: **Evangelia Yannaki - Nicholaos P. Anagnou**

15.00-15.30 **Metabolic diseases**
Fatima Bosch

15.30-16:00 **Hemophilias**
Edward Tuddenham

16.00-16.30 **Inherited retinal diseases**
Stylianos Michalakis

16.30-17.00 **HSCs and neural repair**
Kevin Kemp

17.00-17.30 **Coffee break**

17.30-19.00 Neurosciences - Targeting the brain

Chair: **Rebecca Matsas - TBA**

17.30-18.00 **Modeling Parkinson's disease with patient-derived iPSCs: spotlight on neuron-glia interactions**
Florentia Papastefanaki

18.00-18.30 **Neuro-immune communication at the choroid plexus**
Aleksandra Deczkowska

18.30-19.00 **Gene regulation networks in neural development and cancer progression: novel therapeutic targets in brain tumors and diseases**
Panagiotis Politis

19.00-19.30 **Coffee break**

19.30-20.15 Keynote lecture

Chair: **Evangelia Yannaki**

The long journey to cure thalassemia: allogeneic transplantation, gene transfer and gene editing
Franco Locatelli

20.30 Welcome reception

Saturday, 27 May 2023 - Morning

08.00-09.00 **Working Groups**

09.00-11.00 **CAR T cells and beyond**

Chair: **Anastasia Papadopoulou - Ifigeneia Tzannou**

09.00-09.30 **Dressing up viruses to fool cancer: novel approaches or precision oncolytic immunotherapy**
Vincenzo Cerullo

09.30-10.00 **Multi-targeting CAR T cells for Multiple Myeloma**
Maria Themeli

10.00-10.30 **TCR-redirected T cells for solid tumors**
Eliana Ruggiero

10.30-11.00 **CAR T cells for systemic lupus erythematosous**
Dimitris Mougiakakos

11.00-11.30 **Coffee break**

11.30-13.00 **CAR T-cell point-of-care manufacturing and real-world evidence in Europe**

Chair: **Ioanna Sakellari** - **Evgenios Goussetis**

11.30-12.00 **Germany: Point-of-care CAR T cell manufacturing**
Michael Schmitt

12.00-12:30 **Greece: CAR T cells- real world data**
Anna Vardi

12.30-13:00 **Spain: The case of CAR ARI-001: development and clinical data**
Manel Juan

13.00-14.15 **Lunch break and e-poster exhibition**

: *Virtual Participation*

Saturday, 27 May 2023 - Afternoon

14.15-16.15 New tools and developments I

Chair: **Nicholaos P. Anagnou - Carsten W. Lederer**

14.15-14.45 **Base editing**
Annarita Miccio

14.45-15.15 **Epigenetic editing**
Angelo Lombardo

15.15-15.45 **Next generation globin vectors**
Nikoleta Psatha

15.45-16.15 **Novel cellular targets and delivery strategies for hematopoietic stem/progenitor cell gene therapy**
Luca Biasco

16:15-16:35 Coffee break

16:35-18:00 Oral Presentations and Awards

Chair: **Achilles Anagnostopoulos, Rebecca Matsas, Maria Roubelakis, Nicholaos P. Anagnou**

16.35-17.40 Oral Presentations: Gene and Cell Therapy

1. **DIVERSE IMMUNOGENETIC PROFILE OF PATHOGEN-SPECIFIC T CELLS IN THE CONTEXT OF ADOPTIVE IMMUNOTHERAPY**

C. Pantazi[1,2,3], E. Vlachonikola[2,3], A. Vardi[1], E. Zotou[1,3], K. Koukoulias[1], I. Vallianou[1], M. Giannaki[1], Z. Boussiou[1], P. Christofi[1,3], G. Karavalakis[1], A. Papalexandri[1], M. Yiangou[3], I. Sakellari[1], A. Chatzidimitriou[2,4], A. Papadopoulou[1], E. Yannaki[1,5]
[1]Gene and Cell Therapy Center, Hematology Department – Hematopoietic Cell Transplantation Unit, "George Papanikolaou" Hospital, Thessaloniki, Greece, [2]Institute of Applied Biosciences (INAB), Centre for Research and Technology Hellas (CERTH), Thessaloniki, Greece, [3]School of Biology, Department of Genetics, Development and Molecular Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece, [4]Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden, [5]Hematology, University of Washington, Seattle, USA

Saturday, 27 May 2023 - Afternoon

2. **SAFETY AND EFFICACY OF PENTAVALENT-SPECIFIC T-CELLS FOR THE TREATMENT OF OPPORTUNISTIC INFECTIONS POST HAPLO-IDENTICAL TRANSPLANTATION**

Z. Bousiou[1], I. Kyriakou[1], G. Karavalakis[1], C. Pantazi[1], M. Liga[2], I. Vallianou[1], M. Giannaki[1], K. Koukoulias[1], E. Zotou[1], I. Batsis[1], A. Vardi[1], A. Papalexandri[1], F. Kika[1], A. Spyridonidis[2], I. Sakellari[1], A. Papadopoulou[1], E. Yannaki[1]
[1]G. Papanikolaou Hospital, BMT and Gene Therapy Unit, Thessaloniki, Greece, [2]University Hospital of Patras, Patra, Greece

3. **GENETIC MANIPULATIONS OF EPLIN/LIMA1 EXPRESSION DELINEATE ITS ROLE AS A MOLECULAR THERAPEUTIC TARGET FOR CERVICAL CARCINOMA**

E. Drakopoulou[1]*, E. Kalafati[1]*, K. Goula[2], S. Moraitis[2], D. Valakos[3], G. Vatsellas[4], N.P. Anagnou[1], K.I. Pappa[1,5]
[1]Cell and Gene Therapy Laboratory, BRFAA, Athens, [2]Department of Pathology, Alexandra Hospital, Athens, [3]Molecular Biology Laboratory and [4]Greek Genome Center, BRFAA, Athens, [5]First Department of Obstetrics and Gynecology, University of Athens School of Medicine, Athens, Greece
**Equal participation*

4. **IN VIVO BASE EDITING TO INDUCE A -113A>G HPFH MUTATION RESULTS IN EFFICIENT HBF REACTIVATION IN HUMANIZED MOUSE MODELS OF B-HEMOGLOBINOPATHIES**

K. Paschoudi[1,3], C. Li[2], M. Giannaki[3], A. Georgakopoulou[2], P. Christophi[3], C. Piperidou[1,3], I. Mavrikou[3], A. Papadopoulou[3], E. Siotou[3], E. Vlachaki[4], I. Sakellari[5], N. Psatha[1], A. Lieber[2], E. Yannaki[2,3]
[1]School of Biology, Aristotle University of Thessaloniki, Greece, [2]University of Washington, Seattle, USA, [3] Gene and Cell Therapy Center, Hematology Department, "G. Papanikolaou" General Hospital, Thessaloniki, Greece, [4] Hematology clinic, "Hippokration" General Hospital, Thessaloniki, Greece, [5] Hematology clinic, "G. Papanikolaou" General Hospital, Thessaloniki, Greece

Saturday, 27 May 2023 - Afternoon

5. A NOVEL GENE THERAPY VECTOR FOR BETA-HEMOGLOBINOPATHIES WITH A SELECTIVE EXPANSION CASSETTE

A. Kirtsou[1,2,3], A. Panagiotou[1,2], K. Paschoudi[1,2], P. Sova[5], C. Beta[1,2], I. N. Vasiloudis[1,2], C. Piperidou[1,2], T. Papayannopoulou[6], E. Yannaki[2,6], N. Psatha[1]

[1]Department of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]Gene and Cell Therapy Center, Hematology Department, George Papanicolaou Hospital, Thessaloniki, Greece, [3]Department of Medicine, Democritus University of Thrace, Alexandroupoli, Greece, [5]Altius Institute for Biomedical Sciences, Seattle, [6]University of Washington, Seattle

6. EFFECTIVE TARGETING AND KILLING OF MYELOMA CELLS BY THE IFNB/HF LENTIVIRAL VECTOR

E. Kalafati[1], E. Drakopoulou[1], T. Bagratuni[2], E. Terpos[2], E. Tsempera[1], M.K. Angelopoulou[3], F.L. Cosset[4], E. Verhoeyen[4,5], K. Konstantopoulos[3], E. Papanikolaou[6], N.P. Anagnou[1,6]

[1]Laboratory of Cell and Gene Therapy, Biomedical Research Foundation of the Academy of Athens, Greece; [2]Plasma Cell Dyscrasia Unit, Department of Clinical Therapeutics, University of Athens School of Medicine, Alexandra Hospital, Athens, Greece; [3]Department of Hematology and Bone Marrow Transplantation Unit, University of Athens School of Medicine, Athens, Greece; [4]International Centre for Infectiology Research, INSERM, U-1111, Université de Lyon, Lyon, France; [5]INSERM, U-1065, Université Côte d'Azur, Nice, France; [6]Laboatory of Biology, University of Athens School of Medicine, Athens, Greece

7. PRECLINICAL VALIDATION OF HBB$^{IVSI-110(G>A)}$-SPECIFIC GENE EDITING AS ADVANCED THERAPY FOR THALASSEMIA

P. Patsali[1], N. Psatha[2,5], K. Paschoudi[2,5], P. Papasavva[1], N. Papaioannou[1], B. Naisseh[1], P. Christofi[2,5], S. Christou[6], M. Sitarou[6], A. Pirovolaki[6], M. Hadjigabriel[6], T. Athanasopoulos[7], Claudio Mussolino[3,4], T. Cathomen[3,4], M. Kleanthous[1], E. Yannaki[5], C. W. Lederer[1].

[1]Department of Molecular Genetics Thalassamia, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, [2]School of Biology, Department of Genetics, Development and Molecular Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece, [3]Center for Chronic Immunodeficiency, Medical Center – University of

Saturday, 27 May 2023 - Afternoon

Freiburg, Freiburg, Germany, [4]Institute for Transfusion Medicine and Gene Therapy, Medical Center – University of Freiburg, Freiburg, Germany, [5]Gene and Cell Therapy Center/Hematology-Hematopoietic Cell Transplantation Unit, G. Papanikolaou Hospital, Thessaloniki, Greece, [6]Thalassaemia Centre, Cyprus Ministry of Health, Cyprus, [7]Vector Innovation, Vector Development, Product Development & Supply (PDS), Cell & Gene Therapy (CGT) at GSK, London, United Kingdom

17.40-18.00 Awards

8. DEVELOPMENT OF A TAILORED, CELL-FREE THERAPEUTIC APPROACH BASED ON MSC-EXOSOMES FOR ACUTE HEPATIC FAILURE

BEST ORAL PRESENTATION

A. Psaraki[1,2], M. Makridakis[3], C. Nikokiraki[1], A. Stamatopoulou[1], F. Korkida[1], Z. Dorezi[1], K. Georgila[1], E. Michalopoulos[3] R. Gramignoli[4], S. Sakellariou[5], M. Xilouri[6], A.G. Eliopoulos[1], C. Stavropoulou-Giokas[3], A. Vlahou[7], M.G. Roubelakis[1,2]

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9. A SYNTHETIC ERYTHROID ENHANCER SUCCESSFULLY REPLACES MICROLCR IN GENE THERAPY VECTORS FOR BETA-HEMOGLOBINOPATHIES

BEST ORAL PRESENTATION

K. Paschoudi[1,2], A. Kirtsou[1,2], P. Sova[3], G. Georgolopoulos[3], F. Papadopoulos[1,2], A. Pantou[1,2], G. Bolis[2] A. Panagiotou[1,2], J. Vierstra[3], T. Papayannopoulou[4], E. Yannaki[2,4], N. Psatha[1]

[1]School of Biology, Aristotle University of Thessaloniki, Greece, [2]Gene and Cell Therapy Center, Hematology Department, "G. Papanikolaou" General Hospital, Thessaloniki, Greece, [3]Altius Institute for Biomedical Sciences, Seattle, [4]University of Washington, Seattle

Saturday, 27 May 2023 -Afternoon

BEST POSTER PRESENTATION

P1. CONTROL HUMAN IPSC-DERIVED ASTROCYTES RESCUE THE DEGENERATIVE PHENOTYPE OF P.A53T-ASYN IPSC-DERIVED NEURONS GENERATED FROM PARKINSON'S DISEASE PATIENTS

O. Apokotou[1], C. Paschou[2], A. Kollias[2], E. Taoufik[2], R. Matsas[2], F. Papastefanaki[1,2]

[1]*Human Embryonic and Induced Pluripotent Stem Cell Unit, Hellenic Pasteur Institute, Athens, Greece*

[2]*Laboratory of Cellular and Molecular Neurobiology-Stem Cells, Hellenic Pasteur Institute, Athens, Greece*

18.00-19.10 Special lectures

Chair: **Stylianos Michalakis - Evangelia Yannaki**

18.00-18.35 Tailoring AAV vectors for in vivo gene therapy
Hildegard Büning

18.35-19.10 In vivo gene therapy: hemoglobinopathies and beyond
Andre Lieber

19.10-20.10 General assembly

20.30 Speaker's dinner

Sunday, 28 May 2023 - Morning

08.15-09.30 Poster and Oral presentations

Chair: **Eleni Katsantoni, Erasmia Taoufik**

08.15-08.30 Did-you-see-my-poster?

P2. INVESTIGATING THE ROLE OF THE CILIARY ASSOCIATED PROTEIN-*AHI1* IN CORTICAL DEVELOPMENT USING ANIMAL MODELS AND HUMAN BRAIN ORGANOIDS

L. Mouratidou[1,2†], P. Nti Kostantzo[2,3†], P. Politis[4], S. Taraviras[5], S. Cappello[6], C. Kyrousi[1,2]

[1]1st Department of Psychiatry, Medical School, National and Kapodistrian University of Athens, Greece, [2]UMHRI University Mental Health, Neurosciences and Precision Medicine Research Institute "Costas Stefanis", [3]Athens International Master's Programme in Neurosciences, Department of Biology, National and Kapodistrian University of Athens, Greece, [4]Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, Greece, [5]Department of Physiology, Medical School, University of Patras, Greece, [6]Department of Developmental Neurobiology, Max Planck Institute of Psychiatry, Munich, Germany

[†]These authors contributed equally to this work

P3. THROUGH THE TBX5 LABYRINTH; CARDIOMYOCYTE PRECURSORS IN THE INJURED ADULT MAMMALIAN HEART

P. Siatra[1], I. Kokkinopoulos[2]

[1]Department of Internal Medicine III, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, [2]Immunobiology and Developmental Biology Laboratories, Center for Translational Research Biomedical Research Foundation of the Academy of Athens, Athens, Greece

P4. MIRK/DYRK1B KINASE CONTROLS THE GENERATION AND THE COLUMNAR ORGANIZATION OF SPINAL MOTOR NEURONS VIA SONIC HEDGEHOG PATHWAY

N. Kokkorakis[1], P.K. Politis[2], V. Nalbadi[1], L. Zagoraiou[2], R. Matsas[1], M. Gaitanou[1]

[1]Laboratory of Cellular and Molecular Neurobiology-Stem Cells, Hellenic Pasteur Institute, [2]Center of Basic Research, Biomedical Research Foundation of the Academy of Athens

Sunday, 28 May 2023 - Morning

P5. EVALUATION OF INDUCTION OF FETAL HEMOGLOBIN SYNTHESIS BY GENOME EDITING OF CIS- AND TRANS-ACTING COMPONENTS OF THE B-GLOBIN LOCUS

P.L. Papasavva[1], N.Y. Papaioannou[1], L. Koniali[1], B. Naiisseh[1], S. Christou[4], M. Sitarou[4], M.N. Antoniou[5], T. Cathomen[2,3], M. Kleanthous[1,+], C.W. Lederer[1,+], P. Patsali[1,+]

[1]Department of Molecular Genetics Thalassemia, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, [2]Institute for Transfusion Medicine and Gene Therapy, Medical Center – University of Freiburg, Freiburg, Germany, [3]Center for Chronic Immunodeficiency, Medical Center – University of Freiburg, Freiburg, Germany, [4]Thalassemia Centre, Cyprus Ministry of Health, Cyprus, [5]Department of Medical and Molecular Genetics, King's College London, London, United Kingdom

[+]These authors contributed equally to this work

P6. CELL-INTRINSIC PATHOLOGICAL CHARACTERISTICS IN A53T-ASYN IPSC-DERIVED ASTROCYTES FROM PARKINSON'S DISEASE PATIENTS

C. Paschou[1], A. Kollias[1], K. Charmpi[1], O. Apokotou[2], P. Handris[1], M. Samiotaki[3], G. Panayotou[3], E. Taoufik[1], R. Matsas[1], F. Papastefanaki[1,2]

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P7. IN VIVO MICROGLIAL BIN1 DELETION FOLLOWING LPS-INDUCED NEUROINFLAMMATION REGULATES MICROGLIA PROLIFERATION AND INFLAMMATORY RESPONSE

M. Margariti[1], E. Papadimitriou[1], I. Thanou[1], A. Pelletier[2], M.A. Roussaki[1], E. Xingi[3], M. Avloniti[5], V. Kyrargiri[5], M. Costa[2,4], D. Thomaidou[1]

[1]Hellenic Pasteur Institute, Neural Stem Cells and Neuro-imaging Group, Department of Neurobiology, Athens, Greece, [2]Institut Pasteur de Lille, Univ. Lille, Inserm, CHU Lille, Lille, France, [3]Hellenic Pasteur Institute, Light Microscopy Unit, Athens, Greece, [4]Federal University of Rio Grande do Norte, Brain Institute, Natal, Brazil, [5]Hellenic Pasteur Institute, Laboratory of Molecular Genetics, Microbiology Department, Athens, Greece

Sunday, 28 May 2023 - Morning

P8. EFFICIENT EPIGENETICALLY-MEDIATED REACTIVATION OF GAMMA GLOBIN EXPRESSION IN AN IMMORTALIZED HUMAN ERYTHROID PROGENITOR CELL LINE

F. Papadopoulos[1,2], K. Paschoudi[1,2], A. Pantou[1,2], T. Intzou[1,2], E. Yannaki[2,3], N. Psatha[1]
[1]School of Biology, Aristotle University of Thessaloniki, Greece, [2]Gene and Cell Therapy Center, Hematology Department, "G. Papanikolaou" General Hospital, Thessaloniki, Greece, [3]University of Washington, Seattle

08.30-09.30 Oral presentations: Regenerative Medicine

10. CHEMICAL INJURY BY CHEMOTHERAPEUTIC AGENT ARA-C INDUCES MYELIN DYSFUNCTION AND ECTOPIC NEUROBLASTS' RECRUITMENT IN THE BRAIN

I. Thanou[1], P.N. Koutsoudaki[2], M. Margariti[1], A. Haroniti[1], M. Fourmouzi[1], S. Havaki[2], V. G. Gorgoulis[2], D. Thomaidou[1]
[1]Hellenic Institute Pasteur, Neurobiology Department, Athens, Greece
[2]National and Kapodistrian University of Athens, School of Medicine, Laboratory of Histology-Embryology, Athens, Greece

11. ESTIMATION OF THE HLA HOMOZYGOUS CORD BLOOD UNITS (CBUS) FOR THE PRODUCTION OF INDUCED PLURIPOTENT STEM CELLS (IPSCS) FOR TRANSPLANTATION AND REGENERATIVE MEDICINE PURPOSES OF GREEKS

H. Latsoudis[1], I. Vasilopoulou[1], I. Mavroudi[2], A. Xagorari[3], T. Chatzistamatiou[4], E.F. Sarri[4], D. Papaioannou[3], I. Gontika[2], I. Fragiadaki[2], D. Sotiropoulos[3], C. Stavropoulos-Giokas[4], H.A. Papadaki[2]
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Sunday, 28 May 2023 - Morning

12. BIOFABRICATION OF 3D BIOPRINTED VASCULAR-LIKE STRUCTURES FUNCTIONALIZED WITH PLATELET-RICH PLASMA FOR ENDOTHELIAL TISSUE REGENERATION

V. Platania[1], N.N. Tavernaraki[1], I. Gontika[2], E. Fragiadaki[2], N. Triantopoulou[3], H.A. Papadaki[2], K. Alpantaki[4], M. Vidaki[3,5], M. Chatzinikolaidou[1,6]

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13. EFFECT OF STEM CELL DERIVED MICROPARTICLES ON VIABILITY, APOPTOSIS AND DIFFERENTIATION PROGRAM OF HEMOPOIETIC CELLS

A. Xagorari[1], K. Sianidou[2], F. Deligianni[3], Z. Katana[3], A. Kouvatsi[3], I. Sakellari[1], A. Kritis[2], D. Sotiropoulos[1]

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Sunday, 28 May 2023 - Morning

14. TISSUE-ENGINEERING OF INTERVERTEBRAL DISC FROM HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS IN 3D-PRINTED SCAFFOLDS

N. Gkantsinikoudis[1,2], S. Koltsakidis[3], P. Prodromou[3], K.E. Aggelidou[1,2], S. Kapetanakis[4], E. Tsiridis[5], I. Magras[6], D. Psalla[7], G. Kazakos[8], D. Tzetzis[3], A. Kritis[1,2]
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15. HIF-1A RESTRICTS CARDIOMYOCYTE REGENERATION

A. Daiou[1†], K. Petalidou[1†], K.E. Hatzistergos[1*]
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09.30-11.00 New Tools and developments II

Chair: **Georgios Vassilopoulos - Nikoleta Psatha**

09.30-10.00 **mRNA therapeutics - Development of a novel PTD-mediated IVT-mRNA delivery platform**
Lefkothea Papadopoulou

10.00-10.30 **Small molecules and novel vector design to enhance Cell & Gene Therapy applications**
Takis Athanasopoulos

10.30-11.00 **Bioinformatics: using gene regulatory maps to navigate gene therapies**
Grigorios Georgolopoulos

11.00-11.20 Coffee break

Sunday, 28 May 2023 - Morning

11.20-12.50 UCB and Bone Marrow as sources for regenerative and immune cellular therapies

Chair: **Aikaterini Stavropoulou Gkioka - Damianos Sotiropoulos**

11.20-11.50 **UCB-derived platelet rich plasma (PRP): potential therapeutic applications**
Stathis Michalopoulos

11.50-12.20 **Induced pluripotent stem cells from homozygous umbilical cord blood units for regenerative medicine purposes**
Eleni Papadaki

12.20-12.50 **Clinical Potential of MSC-EVs and Translational Challenges**
Bernd Giebel

12.50-14.20 Regenerative medicine

Chair: **Maria Roubelakis - Rebecca Matsas**

12.50-13.20 **The neural stem cell niche**
Stavros Taraviras

13.20-13.50 **Corticotropin-releasing hormone in human brain development**
Yasemi Koutmani

13.50-14.20 **Cancer models in vitro**
Apostolos Klinakis

14.20-14.45 Light lunch

14.45-16.15 Advanced therapies: regulations and economics

Chair: **Konstantinos Konstantopoulos - Nicholaos P. Anagnou**

14.45-15.15 **Priority Medicines: support from EMA for unmet medical needs**
Paschalia Koufokotsiou

15.15-15.45 **Hospital exemption**
Maria Gazouli

15.45-16.15 **The economics of ATMPs**
Dyfrig Hughes

16.15-16.30 Concluding Remarks

Chairs - Speakers

Achilles Anagnostopoulos	Chief Scientific Director, TheraCell Laboratories, Athens, Greece
Nicholaos P. Anagnou	MD, PhD, Professor of Biology, Laboratory of Biology, University of Athens School of Medicine and Group Leader, Laboratory of Cell and Gene Therapy, Foundation for Biomedical Research of the Academy of Athens (IIBEAA), Athens, Greece
Takis Athanasopoulos	VP, Gene Therapy and Vector Technology, Vigeneron GmbH, Munich, Germany
Luca Biasco	Executive Director, Hematopoietic Stem Cell Gene Therapy, Sana Biotechnology, 100 Technology Square, Cambridge, MA 02139 - Executive Director, SANA Biotechnology, Cambridge, MA, USA Visiting Professor, University College London (UCL), London, UK
Fatima Bosch	Professor of Biochemistry and Molecular Biology and Director of the Center of Animal Biotechnology and Gene Therapy at the Universitat Autònoma de Barcelona
Hildegard Büning	Hannover Medical School (MHH), Germany
Vincenzo Cerullo	Professor of Biological Drug Development, Faculty of Pharmacy, Head of Drug Research Program, HiLIFE fellow, Translational Immunology Programme, University of Helsinki, Finland
Aleksandra Deczkowska	Brain-immune communication, Department of Neuroscience - Department of Immunology, Institute Pasteur, Paris
Maria Gazouli	Professor of Biology – Nanomedicine, Medical School, National and Kapodistrian University of Athens, Greece
Grigorios Georgolopoulos	Chromatin/gene regulation / Bioinformatics / Data analysis
Bernd Giebel	PhD, University Hospital Essen, Germany
Evgenios Goussetis	Pediatrician-Hematologist, Director, Bone Marrow Transplant Unit, General Children's Hospital "Agia Sofia", Athens, Greece
Dyfrig Hughes	PhD, Professor in Pharmacoeconomics & Director of Research. Centre for Health Economics & Medicines Evaluation. School of Medical and Health Sciences, Bangor University, Wales
Manel Juan	Immunologist, Servei d' Immunologia, CDB-Hospital Clínic, Plataforma de Inmunoterapia HSJD-Clínic, Barcelona, Spain
Eleni Katsantoni	Investigator C at Biomedical Research Foundation of the Academy of Athens
Kevin Kemp	PhD, FHEA, MSc, BSc. - Senior Lecturer in Neuroscience, Bristol Medical School University of Bristol, Bristol, UK

Chairs - Speakers

Apostolos Klinakis	PhD, Investigator - Professor Level
Konstantinos Konstantopoulos	Emeritus Professor of Pathology-Hematology EKPA, Hematology Clinic, "Mitera" Hospital, Marousi-Athens, Greece
Paschalia Koufokotsiou	Policy officer-Pharmaceutical and Health Policy, DG Sante, European Commission, Brussels, Belgium
Yasemi Koutmani	PhD, Research Scientist at Biomedical Research Foundation of the Academy of Athens, Greece
Carsten Werner Lederer	PhD, Laboratory Scientific Officer and Assistant Professor, Molecular Genetics Thalassaemia Department, The Cyprus Institute of Neurology and Genetics and The Cyprus School of Molecular Medicine, Nicosia, Cyprus
Andre Lieber	Professor Medical Genetics, Adjunct Professor Pathology, University of Washington, Division of Medical Genetics, Seattle, USA
Franco Locatelli	President, National Council of Health, Full Professor of Pediatrics, Catholic University of the Sacred Heart, Director, Department of Pediatric Hematology and Oncology, IRCCS Ospedale Pediatrico Bambino Gesù, Rome, Italy
Angelo Lombardo	Group Leader, Epigenetic regulation and targeted genome editing, San Raffaele - Telethon Institute for Gene Therapy (SR-Tiget), Professor of Tissue Biology, Vita-Salute San Raffaele University Medical School, Milano, Italy
Rebecca Matsas	PhD, Research Director, Head, Department of Neurobiology, The Hellenic Pasteur Institute, Athens, Greece
Annarita Miccio	PhD, Lab Director, Laboratory of Chromatin and Gene Regulation during development, Paris Cité University, Institute of Genetic Diseases Imagine, INSERM, UMR 1163
Stylianos Michalakis	Professor, Department of Ophthalmology, University Hospital, LMU Munich, Germany
Stathis Michalopoulos	PhD, Staff Research Scientist - Associate Professor Level, Foundation for Biomedical Research of the Academy of Athens (IIBEAA), Athens, Greece
Dimitrios Mougiakakos	Prof. Dr. Med., Director, Department of Hematology, Oncology, and Stem Cell Transplantation, University of Magdeburg, Germany
Eleni Papadaki	MD, PhD, Professor of Haematology, University of Crete School of Medicine, Head of the Department of Haematology, University Hospital of Heraklion, Heraklion, Crete, Greece

Chairs - Speakers

Anastasia Papadopoulou	PhD, Biochemist, PhD, Production Manager, Gene and Cell Therapy Center, Hematology - HCT Unit, "G. Papanikolaou" Hospital, Thessaloniki, Greece
Lefkothea Papadopoulou	Professor of Pharmacology, Department of Pharmacognosy-Pharmacology, Department of Pharmacy, Aristotle University of Thessaloniki, Greece
Florentia Papastefanaki	PhD, Staff Research Scientist, Laboratory of Cellular and Molecular Neurobiology – Stem Cells, Human Embryonic and Induced Pluripotent Stem Cell Unit, Hellenic Pasteur Institute, Athens, Greece
Panagiotis Politis	Group leader – Researcher B' (Associate Professor level), Center of Basic Research, Biomedical Research Foundation of the Academy of Athens
Nikoleta Psatha	PhD, Assistant Professor, Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, Greece
Maria Roubelakis	Associate Professor of Biology and Regenerative Medicine of the Laboratory of Biology, School of Medicine, National and Kapodistrian University of Athens, Affiliated Research Scientist, Biomedical Research Foundation, Academy of Athens, Greece
Eliana Ruggiero	Project Leader, Experimental Hematology Unit, San Raffaele Hospital, Milano, Italy
Ioanna Sakellari	Haematologist, Director and Head, Haematology Department - BMT Unit, "G. Papanikolaou" Hospital, Thessaloniki, Greece
Michael Schmitt	Professor for Cellular Immunotherapy, Head of GMP laboratory (Good Manufacturing Practice Facility), Manufacture of vaccines and cellular products for human use in clinical investigation studies, Head of Cellular Immunotherapy Working Group, Heidelberg, Germany
Damianos Sotiropoulos	MD, Hematologist Director, Dept. of Hematology, Bone Marrow Transplantation Center, Cord Blood Bank, "G. Papanikolaou" Hospital, Thessaloniki, Greece
Alexandros Spyridonidis	Professor, MD, PhD, Director of Bone Marrow Transplantation Unit, Institute of Cell Therapy, CBMDP Donor Center, MSc Cell and Gene Therapies, University of Patras, Patra, Greece
Aikaterini Stavropoulou-Gkioka	Immunologist, Director, Umbilical Cord Blood Bank, Medical Biological Research Foundation of the Academy of Athens, Athens, Greece
Erasmia Taoufik	Neuroimmunologist, Principal Investigator, Hellenic Pasteur Institute, Athens

Chairs - Speakers

Stavros Taraviras	PhD, Professor, Department of Physiology, Medical School, University of Patras, Patra, Greece
Maria Themeli	Assistant Professor, Department of Haematology, Amsterdam University Medical Centers, Location VUmc, Cancer Center Amsterdam, The Netherlands
Panagiotis Tsirigotis	Associate Professor in Haematology, Department of Second Propedeutic Internal Medicine, National and Kapodistrian University of Athens, School of Medicine, "Attikon" Hospital, Athens, Greece
Edward Tuddenham	MD, Emeritus Professor of Haemophilia UCL, Katharine Dormandy Haemophilia Centre, Royal Free Hospital, UK
Ifigeneia Tzannou	Hematologist, Curator, Hematology - Lymphoma Clinic and Bone Marrow Transplantation Unit, General Hospital of Athens "Evaggelismos", Athens, Greece
Anna Vardi	Hematologist, PhD, Hematology Department - HCT Unit, "G. Papanikolaou" Hospital, Thessaloniki, Greece
George Vassilopoulos	MD, PhD, Professor of Haematology - Internal Medicine, University of Thessalia, University Hospital of Larissa, Group Leader, Laboratory of Cell and Gene Therapy, Foundation for Biomedical Research of the Academy of Athens (IIBEAA), Athens, Greece
Evangelia Yannaki	Director, Gene and Cell Therapy Center, Hematology Department-HCT Untit, "G. Papanikolaou" Hospital, Thessaloniki, Greece Affiliate Professor, University of Washington, Seattle, USA

ORAL ABSTRACTS

8. DEVELOPMENT OF A TAILORED, CELL-FREE THERAPEUTIC APPROACH BASED ON MSC-EXOSOMES FOR ACUTE HEPATIC FAILURE

BEST ORAL PRESENTATION

A. Psaraki[1,2], M. Makridakis[3], C. Nikokiraki[1], A. Stamatopoulou[1], F. Korkida[1], Z. Dorezi[1], K. Georgila[1], E. Michalopoulos[3] R. Gramignoli[4], S. Sakellariou[5], M. Xilouri[6], A. G. Eliopoulos[1], C. Stavropoulou-Giokas[3], A. Vlahou[7], M. G. Roubelakis[1,2]

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Aim: Acute hepatic failure (AHF) is a rapid deterioration of liver function, leading to a high mortality rate. Liver transplantation is the gold-standard therapy with limitations related to donor organ shortage and life-long immunosuppressive therapy. Our previous published studies, indicated that factors released by fetal mesenchymal stromal cells (MSCs) and their hepatocyte progenitor-like (HPL) cells, could induce liver repair and down-regulate the systemic inflammation in CCL_4-mice, by secretion of IL-10 and Annexin A1. In our recent studies, we showed that exosomes (EXO) derived from fetal MSCs represent an alternative bio-therapeutic for improving AHF phenotype in vivo. Herein, we present a novel concept in AHF therapy, based on the *modified* secreted EXO by Umbilical Cord MSCs (UC-MSCs) in an AHF mouse model.

Materials & Methods: The data presented here, demonstrated the therapeutic effects of isolated EXO in ex vivo and in vivo experimental models, mimicking AHF disease. Firstly, EXO derived from UC-MSCs were collected, characterized and injected intrahepatically into CCL_4-induced mice. We further analyzed the EXO proteomic content and we showed that MEFG-8 protein was enriched in EXO. Functional analyses, including administration of recombinant MEFG-8 protein into CCL_4-induced mice, proteomic analysis of MFGE-8 treated liver tissues and silencing studies using a lentiviral system for diminishing the MEFG-8 levels into EXO, were additionally performed.

Results: UC-MSC-EXO decreased the inflammation and improved the liver phenotype by promoting the oval cell proliferation in CCL_4 induced mice. LC-MS/MS proteomic analyses showed that MEFG-8 was enriched in EXO and facilitated AHF rescue by suppressing PI3K signaling. In addition, administration of recombinant MFGE-8 protein managed to reduce liver damage in CCL_4-mice. LC-MS/MS proteomic analyses from livers of MFGE-8 treated CCL_4-mice indicated the presence of proteins, mediating efferocytosis (such as Pklr, Gstt1, Gstt3, Fggy, Mthfd1,

Dcxr, Slco1b2 and Abcc6), as well as others related to autophagy (such as ATG5, ATG7, LC3B and Beclin-1). Clinically, MEFG-8 expression was decreased in biopsies of liver tissue from patients with AHF. To further delignate the function of MEFG-8 protein, we applied a tailored therapeutic approach for AHF involving the application of UC-MSC-EXO with modified levels of MEFG-8 in their cargo.

Conclusion: In conclusion, EXO derived from UC-MSCs improved liver phenotype in CCL_4-induced mice and promoted oval cell proliferation. More specifically, we investigated the function of MFGE-8 in liver damage in CCL_4-induced mice. Further, we performed LC-MS/MS analysis in the MFGE-8-treated mouse livers and identified its specific role in efferocytosis and autophagy. In this study, a proof-of-concept is provided for a tailored, multidisciplinary and cell-free, alternative strategy for AHF (Figure 1) and important new mechanistic information on the reparative function of progenitor cells in the liver.

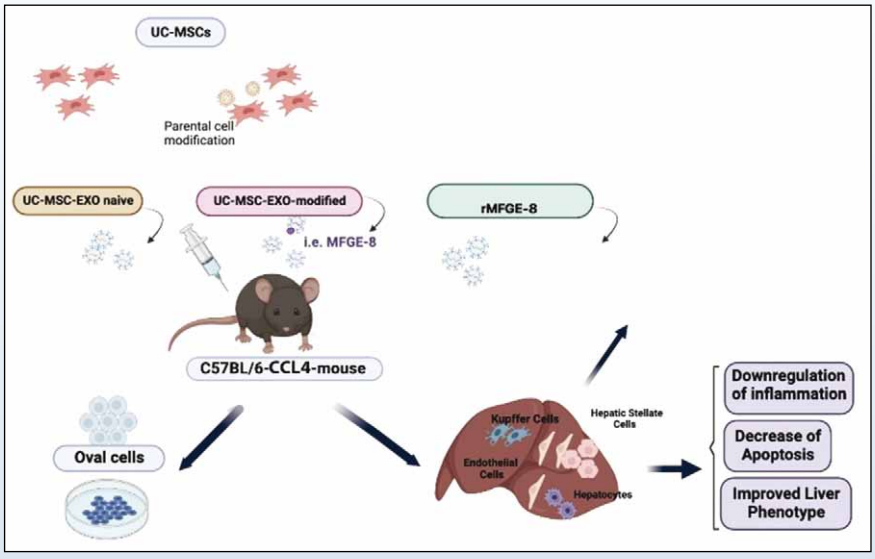

Figure 1. AHF therapeutic scheme based on exosome and MFGE-8 administration.

9. A SYNTHETIC ERYTHROID ENHANCER SUCCESSFULLY REPLACES MICROLCR IN GENE THERAPY VECTORS FOR BETA-HEMOGLOBINOPATHIES

BEST ORAL PRESENTATION

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Introduction: Current gene therapy approaches for beta-hemoglobinopathies have recently achieved promising results in patients with thalassemia major and sickle cell disease. Not overlooking the successful outcomes, the incorporation of a long (~3kb) truncated β-globin LCR to drive transgene expression negatively affects viral titers and transducibility of hematopoietic stem cells (HSCs). In a previous study, we uncovered a series of novel, compact (300bp) and potent erythroid enhancer sequences. These elements display erythroid-specific activity and temporal specificity. Two of these enhancers, regulating the endogenous expression of PVT1 and PPARA, displayed high activity at early and late stages of erythroid maturation, respectively.
Aim: We theorized that the combination of the aforementioned enhancers could: i) increase the expression levels above those observed for the individual sequences ii) ensure high transgene expression throughout erythroid differentiation (ED) iii) reduce the size of the current gene therapy vectors for beta-hemoglobinopathies. To this end, we explored the activity of the concatenated PVT1-PPARA enhancer during ex vivo (ED) of human HSCs. Subsequently, we sought to compare this enhancer concatemer (cEnh) to the individual enhancers and different versions of clinically used μLCR. Furthermore, cEnh and the optimal μLCR were incorporated into two different viral vectors encoding either a BCL11A-shRNA or a gamma globin expression cassette.
Materials and Methods: Firstly, to estimate the enhancer activity of each element we incorporated PVT1 and PPARA individually into a lentiviral vector encoding a reporter gene (GFP) and subsequently transduced CD34+ cells from healthy donors. The cEnh and the most efficient μLCR enhancer were cloned into either a BCL11A-shRNA or a gamma globin lentiviral vector. The efficiency of these vectors was tested in $CD34^+$ cells from thalassemic patients, previously enrolled in mobilization studies. Following transduction, the cells were cultured in ED medium and in semi-solid methylcellulose medium. During erythroid differentiation, cell growth rate, reactive oxygen species and cell morphology were assessed. At the end of erythroid differentiation, γ-globin expression was evaluated by FCM and HPLC.
Results: The two novel enhancers displayed a synergistic or additive effect in combination as we observed a significantly increased GFP expression compared to either enhancer alone reaching similar or even higher expression levels compared to both μLCR sequences. Thalassemic CD34+ cells transduced with the shRNA and gamma globin vectors displayed similar colony formation and cell growth rate during ex vivo ED whereas the cEnh-shRNA- and μLCR-shRNA-vector-transduced cells resulted in comparable frequencies of HbF+ cells and increased over

the untransduced cells (41.05±5.83% cEnh and 35.5±2.51%μLCR versus 21.3±3.25% untransduced group). Notably, cEnh performed better than μLCR when combined with a gamma globin transgene, in terms of HbF expression (39.97±6.81% cEnh and 21.97±2.87% μLCR versus 21.65±3.95% untransduced group). The increased gamma globin levels resulted in a distinctive morphological improvement coupled with reduced oxidative stress levels.

Conclusions: Overall, our data demonstrate that the replacement of μLCR by a synthetic concatenated PVT1-PPARA enhancer in clinically applicable vectors is feasible, successfully leading to efficient transgene expression and HbF induction in thalassemic erythroid cells, thus opening up the path to the development of a new generation of globin vectors.

1. DIVERSE IMMUNOGENETIC PROFILE OF PATHOGEN-SPECIFIC T CELLS IN THE CONTEXT OF ADOPTIVE IMMUNOTHERAPY

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Background. Opportunistic infections are among the most fatal complications in patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT). Adoptive transfer of pathogen-specific T cells (pSTs) recognizing pathogen-derived peptides through their endogenous T-cell receptor (TR) represents an attractive treatment of viral and fungal infections post allo-HCT. However, the TR repertoire of *ex vivo* generated pSTs for immunotherapy has not yet been fully elucidated.

Aim. We analysed the TR beta-chain gene repertoire by next-generation sequencing (NGS) of two immunotherapy products under evaluation in phase I/II clinical trials: i) tri-virus specific T cells (*tri-VSTs*) targeting cytomegalovirus (CMV), Epstein Barr virus (EBV) and BK virus (BKV) (EudraCT:2014-004817-98) and ii) pentavalent-specific T cells (*penta-STs*) additionally targeting adenovirus (ADV) and the fungus *Aspergillus fumigatus* (AF) (EudraCT:2020-004725-23).

Methods. GMP-grade tri-VSTs and penta-STs were manufactured after exposure of peripheral blood mononuclear cells (PBMCs) from immunocompetent donors to CMV, EBV, BKV ± AdV and AF overlapping peptides and a 10-day culture. Specificity of donor-derived cell products and patient-derived PBMCs was assessed by IFN-γ Elispot. TCRB sequencing was performed in the whole T cell products (tri-VSTs:n=2, penta-STs:n=4), their corresponding pathogen-specific cell subsets (n=26) post immunomagnetic IFN-γ enrichment and in patient PBMCs (n=26). Immunogenetic analysis was performed by RT-PCR amplification of TRBV-TRBD-TRBJ rearrangements according to the BIOMED-2 protocol and paired-end NGS (Miseq/NextSeq). The NGS sequences after length and quality filtering were submitted to IMGT/HighVQUEST for annotation. Metadata analysis and clonotype computation (TRB rearrangements using the same TRBV gene and identical CDR3 amino acid sequence) were based on a validated purpose-built bioinformatics platform (*tripr*).

Results. Tri-VSTs and penta-STs provided a diverse TR repertoire consisting of 6,580-33,863 unique clonotypes/sample (median:22,530) and demonstrated high clonality levels with the median frequency of the major clonotype being 6.48% (range:2.23%-20.7%). The clonotypes identified in the enriched subpopulations were subjected to strict filtering: i) ≥10 read counts, ii) higher frequency in the enriched subpopulations over the unselected product as defined by the greater frequency (fold increase) of each post-enrichment clonotype than the medi-

an fold increase of all enriched clonotypes. The applied criteria resulted in 9,799 clonotypes, of which 8,749 were present in a single specific-cell fraction, arguably suggesting that they are pathogen-specific (CMV-specific:2,768, EBV-specific:1,763, BKV-specific:2,003, ADV-specific:1,363, AF-specific:852). Indeed, several of those identified virus-specific clonotypes could be tracked *in vivo* in two patient-derived PBMCs up to 15 weeks post tri-VST infusion with ranging frequencies 0.002%-12.7% (Pt1: 32 CMV-specific, 25 EBV-specific and 29 BKV-specific & Pt2: 20 CMV-specific, 31 EBV-specific and 31 BKV-specific clonotypes). Importantly, their presence *in vivo* was correlated with a decrease in the corresponding viral load, an increase in the frequency of circulating virus-specific T cells and ultimately, clinical response. **Conclusions**. Overall, our findings elucidate the diverse immunogenetic profile of *ex vivo* generated pSTs, identify potential pathogen-specific clonotypes conferring protection against infections and provide a novel method to track *in vivo* the pSTs. Prospectively, the identification of optimal TRs that mediate clinical responses may serve as a stratification tool for patients at risk for suboptimal responses and help to select the best candidates for adoptive immunotherapy.

Funding was provided by Research, Technology Development and Innovation State Aid Action "RESEARCH-CREATE-INNOVATE" (T2ΕΔΚ-02437).

2. SAFETY AND EFFICACY OF PENTAVALENT-SPECIFIC T-CELLS FOR THE TREATMENT OF OPPORTUNISTIC INFECTIONS POST HAPLO-IDENTICAL TRANSPLANTATION

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Background Opportunistic infections present a major cause of morbidity and mortality after allogeneic transplantation. Conventional therapies are limited and associated with toxicity and resistance development. Adoptive transfer of pathogen-specific T cells (pSTs) has recently emerged as an appealing alternative strategy. We present the results of an on-going phase I/II study, evaluating the safety and efficacy of donor-derived pentavalent-specific T cells (penta-STs) simultaneously targeting cytomegalovirus-CMV, Epstein Barr virus-EBV, BK virus-BK, adenovirus-AdV and *Aspergillus fumigatus*-AF (*EudraCT2020-004725-23, NCT05471661*).

Methods Penta-STs were generated after pulsing blood mononuclear cells from immunocompetent donors with peptides spanning immunogenic EBV *(LMP2, EBNA1, BZLF1)*, Adv *(Hexon, Penton)*, CMV *(pp65, IE1)*, BK *(Large T, VP1)* and AF *(Gel1, SHMT, Crf1)* antigens and 10-day culture with IL4+7. The specificity of cell products and the circulating specific T-cells were assessed by Elispot. Penta-STs were infused at $2x10^7/m^2$/dose in haplo-transplanted (with post-transplant cyclophosphamide) patients developing an infection from the targeted pathogens and receiving no or <0.5mg/kg methylprednisolone at the time of infusion.

Results So far, 11 GMP-grade products have been generated. The final products were expanded ~5.4 fold over baseline reaching on average 162±27x10^{6}cells. They were enriched in CD3$^+$cells(93±1%) and comprised of CD4$^+$(59±6%) and CD8$^+$cells(34±6%) expressing central(52±7%) and effector memory markers(32±8%). All penta-STs were specific against the pathogens for which the donor was seropositive; 9/11 presented activity against CMV[912±241 spot forming cells (SFC)/2x10^5], 11/11 against EBV(1023±210 SFC/2x10^5), 11/11 against Adv(1243±195 SFC/2x10^5), 11/11 against BK(476±103 SFC/2x10^5) and 9/11 against AF(151±41 SFC/2x10^5). To date, 6 patients received penta-STs for single (EBV n=2, CMV n=1, BK n=1), double (CMV+EBV n=1) and triple viral infection (CMV+EBV+BK n=1) that were developed at median 90 days post-transplant. No patient had ADV or AF infection. Four patients were on corticosteroids at the time of infusion due to GVHD(n=3) and ciclosporin toxicity(n=1). Penta-ST infusion was safe, with no infusion-related toxicity, aGVHD occurrence or cGvHD exacerbation, attributed to the cells. From a total of 10 infections (including 2 EBV diseases), there were 9 complete responses (EBV n=3, CMV n=3, BK n=3) and 1 partial response (EBV). Best response was achieved at median 3.5 weeks post-infusion and correlated with expansion of circulating respective pSTs. Importantly, 2/10 infections (EBV, BK) resolved without additional drug therapy and a patient with EBV-PTLD received only a short course of anti-CD20 treatment due to rapid and complete resolution of lymphadenopathy post-infusion. Notably, at a median follow up of 5.5 months(3-12) post infusion, no reactivations from the targeted pathogens were developed, except of 2 patients who reactivated viruses other than those they had received the cells for; CMV and EBV were reactivated in a patient whose product was CMV seronegative donor-derived and another one with poor in vivo expansion of EBV-specific T cells who received a 2nd infusion, respectively **Conclusions** Although preliminary, our data show that pST therapy is a feasible and safe option for patients at high risk for opportunistic infections after allogeneic transplantation, potentially minimizing the need for drug therapy and preventing reactivations

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3. GENETIC MANIPULATIONS OF EPLIN/LIMA1 EXPRESSION DELINEATE ITS ROLE AS A MOLECULAR THERAPEUTIC TARGET FOR CERVICAL CARCINOMA

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Aim of the study: Remodeling of the cell cytoskeleton occurs during carcinogenesis. Among the proteins involved in this process, EPLIN (Epithelial Protein Lost in Neoplasia) or LIMA1, rep-

resents an important cytoskeleton regulator, exhibiting an aberrant downregulation in epithelial tumors. However, its mechanisms of action in human neoplasia have not been systematically elucidated. In our previous high resolution proteomic analysis (Oncol Rep 42:1441-50, 2019), we documented a statistically significant decreased expression in HeLa, SiHa and C33A cervical cancer lines compared to the normal cervical keratinocyte HCK1T line. Based on the above, the aim of this work was to further investigate the functional role of EPLIN in cervical cancer through a series of *in vitro* genetic manipulations of its expression by i) selectively silencing its expression in the normal HCK1T keratinocytes, ii) induction of its overexpression in the C33A cancerous cells and iii) performing systematic immunohistochemical analysis on a large series of clinical samples covering the full spectrum of cervical carcinogenesis.

Materials and Methods: EPLIN expression was silenced by transduction with the specific shLIMA1 lentiviral vector, while forced overexpression was performed by transfection with the pRP-EGFP/Puro-EFS>hLIMA1 vector. After documenting the efficient silencing and overexpression of EPLIN, their effect was assessed on proliferation, migration, invasion, and colony-forming ability, as well as by transcriptome analysis with next-generation RNASeq technology. For immunohistochemical analysis, 89 representative clinical samples of all stages of cervical carcinogenesis were used, employing tissue microarrays.

Results: Transduction with the shLIMA1 lentiviral vector into normal HCK1T cells resulted in a 92% (n=7) decrease of EPLIN expression at the transcriptional level accompanied by a 136% *increase* in migration and invasion (p=0.07, n=7) and 294% (p=0.03, n=6), respectively. Bioinformatic analysis of the transcriptome, revealed a decrease in the expression of genes related to the cell cycle, with a significant percentage of them related to the formation of the mitotic spindle. In addition, EPLIN silencing led to an increase in the expression of genes related to cell-cell interaction with the extracellular matrix, conferring cells with mesenchymal features and linking them to the process of epithelial-to-mesenchymal transition (EMT). a program enhancing mobility, invasion, metastasis and resistance to apoptosis. In contrast, forced overexpression of EPLIN by 548% in the C33A cancer cells (n=4), resulted in statistically significant *reduction* of cell proliferation (p=0.05, n=3) and ability for invasion (p=0.03, n=3). Immunohistochemical analysis disclosed reduced expression of EPLIN in cancerous cervical epithelium compared to normal, while in hyperplastic epithelium documented a correlation of expression with the degree of hyperplasia. However, in squamous cell carcinomas, a statistically significant decrease of EPLIN expression was established (p=0.013), which correlated with the advanced clinical stage. Finally, in the majority of adenocarcinomas, an absence of expression was observed (p<0.0001).

Conclusions: These data document for the first time that EPLIN and its abberant expression in cervical cancer is involved in cell proliferation and metastasis, besides to its role as a structural protein. The resulting reversible phenotypes upon its manipulation in the present study, provide the impetus to harness its expression as a novel gene therapy approach.

4. IN VIVO BASE EDITING TO INDUCE A -113A>G HPFH MUTATION RESULTS IN EFFICIENT HBF REACTIVATION IN HUMANIZED MOUSE MODELS OF B-HEMOGLOBINOPATHIES

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Introduction. Genome editing approaches have led to new therapeutic opportunities for gene therapy of β-hemoglobinopathies, including the in situ correction of β- globin mutations or the induction of HbF reactivation. Most of these strategies involve nucleases inducing double-strand DNA breaks, which may cause critical on- and off-target effects. In contrast, the recently developed base editors (BE) efficiently install precise nucleotide substitutions, without creating DSBs. Given that the hereditary persistence of fetal hemoglobin (HPFH) greatly ameliorates the clinical phenotype of β-thalassemia and sickle cell disease (SCD), the introduction of HPFH-related mutations using BE seems to be a safer and potentially efficient approach.

Aim: the installation of a -113A>G HPFH mutation in $CD34^+$ hematopoietic stem cells (HSCs) from β-thalassemia and SCD patients by ex vivo and in vivo base editing in a humanized mouse model, using a non-integrating, tropic to human HSCs via CD46, HDAd5/35++ vector, expressing a highly efficient adenine base editor (ABE8e). To further expand the transduced cells, our vector contained also an $mgmt^{P140K}$ gene allowing for enrichment in edited cells after O^6BG/BCNU treatment.

Materials and Methods. Following 48-hour transduction, and (±)O^6BG&BCNU treatment, patient CD34+ cells were seeded in erythroid differentiation (ED) medium. The editing rate was evaluated by NGS and the γ-globin expression by flow cytometry and HPLC. For the in vivo experiments we generated xenotransplantation mouse models by transplanting NBSGW mice with $CD34^+$ cells from either thalassemic or SCD donors to establish human, disease-associated hematopoiesis. Six weeks post transplantation, HSPCs from the chimeric bone marrow (bm) were successfully mobilized to the periphery by G-CSF(250μg/kgX6 days) and Plerixafor(50μg/kgX4 days). Shortly after the last dose of plerixafor, the mice were intravenously injected with HDAd-ABE allowing selective transduction of the human HSCs. Six days after the in vivo transduction, one course of in vivo selection with (±) O^6BG/BCNU was administered and the mice were sacrificed three months post in vivo transduction.

Results. The -113A>G conversion rates reached ~60% after selection in vitro. At the end of ED, the percentage of HbF+ cells in enucleated red cells was significantly higher in the HDAd-ABE8e-transduced over the untransduced cells ($\%HbF^+/NucRed^-$cells; Thal:42%, SCD:45%) while the selection further increased their frequency ($\%HbF^+/NucRed^-$cells Thal:60%, SCD: 85%). More-

over, the γ-globin chains reached levels of up to 34%, relative to β- and α-globin chains, translating to improved erythropoiesis and disease characteristics, including sickling prevention in SCD. The in vivo transduction with HDAd-ABE8e/selection with O6BG-BCNU didn't negatively affect human HSCs engraftment reaching up to 60% hCD45+cells in the thalassemic humanized model and leading to human multi-lineage representation in the bm. Additionally, the in vivo transduction resulted in increased rates of HBF^+cells within the $hGlyA^+$cell population of the chimeric bm post in vivo transduction (up to 43%), being further increased post in vivo selection (up to 60%).

Conclusions. Overall, we present a novel approach for effective in vivo gene editing to enhance HbF reactivation which can potentially overcome several barriers of the current ex vivo gene editing (including leukapheresis and myeloablation) and allow a wider application of gene therapy for hemoglobinopathies.

5. A NOVEL GENE THERAPY VECTOR FOR BETA-HEMOGLOBINOPATHIES WITH A SELECTIVE EXPANSION CASSETTE

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Backround: Gene therapy has recently been established as an effective curative approach for a variety of genetic hematopoietic diseases, including beta-hemoglobinopathies. Despite the promising results in gene therapy trials for beta-thalassemia and sickle cell disease, there are several important limitations of the currently used gene therapy vectors minimizing the overall outcome of the approach. Among these, the relative inefficient transduction of long term HSCs especially in lower vector copy numbers (VCN) and the possibility of integration site-related gene silencing over time or insertional mutagenesis have yet to be resolved.

Aims: Scope of this study is to design and assess a new lentiviral vector for the gene therapy of beta-hemoglobinopathies. The novel vector comprises of a short-hairpin RNA (shRNA) targeting the HbF suppressor BCL11A, a new, compact, erythroid specific enhancer driving the transcription of the transgene, a recently identified insulator with enhancer-blocker and barrier activity and the F36VMpl selection cassette, a fusion protein able to ignite a growth signal in response to a chemical inducer of dimerization (AP20187). The addition of the new erythroid enhancer in combination with the dual insulator aim to improve the safety and long-term efficacy profile of the vector and the incorporation of the selective expansion cassette aims to improve therapeutic outcomes in low VCNs.

Methods: The shRNA-BCL11a-F36VMpl vector was constructed with the addition of a GFP re-

porter gene for normalization purposes. HUDEP-2 cells as well as human healthy donor and sickle cell disease patient derived CD34+ were transduced at low MOIs and subsequently cultured in the presence or absence of AP20187. Transduction, selective proliferation, and expression of γ-globin in erythroid cells was evaluated.

Results: The addition of the selection cassette did not affect the produced viral titers ($36x10^6$ TU/ml vs $16x10^6$ TU/ml, in the control vector respectively). In HUDEP-2 cells, the addition of AP20187 led to a significant (29%) increase of transduced cells on day 12 of the culture compared to the minus-AP20187 condition (81%±0.45 vs 52%±1.57). In addition, the percentage of HbF+ cells in F36VMpl-cells was 35% compared to 8% in untransduced cells. The transduction rate in CD34+ cells ranged from 1-65%, depending on the MOI used. With the addition of AP20187 to the erythroid culture of CD34+ cells, an increase of transduced cells in the total population was observed, starting from a 3-fold and reaching a 8-fold increase at the lowest MOI. In addition, γ-globin/ β-globin ratio in both normal and SCD CD34+ cells was increased by AP20187 addition (normal CD34+: 0,34 +AP20187 vs 0,19 -AP20187 / SCD CD34+: 0,6 +AP20187 vs 0,41 -AP20187).

Summary: We have so far shown that the shRNA-BCL11a-F36VMpl viral vector can achieve an erythroid-specific silencing of BCL11A with a subsequent activation of fetal hemoglobin and has the ability to selectively expand the corrected cell population. Since AP20187-mediated activation of F36VMpl allows for progenitor cells to be dramatically expanded both in vitro and in vivo without toxic side effects, we theorize that this vector might overcome the inefficiency of ex vivo gene therapy correlated with low transducibility.

6. EFFECTIVE TARGETING AND KILLING OF MYELOMA CELLS BY THE IFNB/HF LENTIVIRAL VECTOR

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Aim of the study: Multiple myeloma (MM) represents a malignant plasma cell disorder characterized by severe clinical manifestations and complications. Despite significant advances in the treatment of MM which had led to higher rates of response, patients still encounter relapses while overall survival remains low. In the context of developing novel therapeutic approaches for MM, we investigated the efficacy of an IFNβ-expressing lentiviral vector, pseudo-

typed with the measles virus H and F glycoproteins (IFNβ/HF) both in myeloma cell lines and in $CD138^+$ cells isolated from MM patients at the time of diagnosis. Moreover, we investigated the putative mechanisms of apoptosis and autophagy in IFNβ-mediated cell death and the synergistic action of IFNβ/HF with current therapeutic agents.

Materials and Methods: Cytotoxicity was evaluated using the CCK-8 assay. Apoptosis and cell cycle were estimated by AnnexinV/7-AAD and PI staining, respectively. Expression of apoptotic genes was determined by qPCR and a Human Apoptosis Antibody Array. IFNs secretion and its paracrine action were determined by ELISA and Transwell® co-culture. Autophagy gene expression was assessed employing qPCR and confocal microscopy. Neutralizing antibody activity was determined by flow cytometry utilizing GFP/HF.

Results: IFNβ/HF-transduction at multiplicity of infection (MOI) 1, led to a dramatic reduction of cell survival followed by a marked increase of apoptosis: 90.3% ($p \leq 0.001$) in H929, 74.1% ($p \leq 0.001$) in JJN3, 91.82% ($p = 0.004$) in U266, and 92.59 % ($p = 0.002$) in RPMI-8226 cells. Notably, the increase of Caspases 3 and 9 highlighted the crucial role of the intrinsic apoptotic pathway in the IFNβ-induced apoptosis, whereas a concomitant increase in proteins such as Endo G and HtRA, involved in the caspase-independent apoptosis pathway, was observed. Regarding the investigation of autophagy, a significant decrease in Beclin-1, Atg4b, and Atg5 was detected. Following Transwell® co-culture, IFNβ secreted by the transduced cells, was capable of significantly decreasing the viability of *untransduced* cells. Furthermore, IFNβ/HF efficiently transduced primary cells, increasing IFNβ and IFNγ secretion and decreasing cell survival, with a 51.8% increase of apoptosis ($p = 0.01$) and induction of cell cycle arrest ($p = 0.002$). Interestingly, the synergistic action of IFNβ/HF at a low MOI of 0.1 combined either with bortezomib ($p = 0.025$), carfilzomib ($p = 0.0392$), or lenalidomide ($p = 0.0465$) enhanced the myeloma cell cytotoxicity *in vitro*, documenting the feasibility of combinatorial gene therapy to improve the therapeutic outcome. Lastly, the neutralizing antibody titer against lentiviral vectors pseudotyped with measles virus glycoproteins was found to be at low levels, and therefore, IFNβ/HF can overcome the immunological barrier using a higher MOI.

Conclusions: These data document that IFNβ/HF exerts its cytotoxic action and induces apoptosis via the intrinsic apoptotic pathway. This efficacy is further enhanced in a synergistic action when combined with current therapeutic agents. Thus, the novel IFNβ/HF vector represents a promising therapeutic candidate as a selective antitumor agent for the treatment of MM.

7. PRECLINICAL VALIDATION OF HBB$^{IVSI-110(G>A)}$-SPECIFIC GENE EDITING AS ADVANCED THERAPY FOR THALASSEMIA

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Purpose of the study: β-Thalassemia is a common single-gene disorder worldwide caused by deficient production of β-globin. One of the most prevalent β-thalassemia mutations is HBB$^{IVSI-110(G>A)}$, which creates an aberrant intronic splice site in β-globin. This mutation has a relative carrier frequency of 76% in Cyprus and above 20% in many EU countries. To address this mutation, a proof of concept for an efficient mutation-specific therapy was established using designer nucleases, including CRISPR/Cas9 RNA-guided nuclease (RGN) and TALENs. Specific DNA cleavage of the intronic mutation allows the prevailing non-homologous end joining mechanism to destroy the aberrant splice site and restore normal splicing and HBB expression. This approach achieved clinically relevant efficiencies on patient-derived primary cells. The project aims to take this approach forward for preclinical assessment of edited cells in vitro and in vivo in chimeric NOD, B6, SCID Il2rγ$^{-/-}$Kit$^{W41/W41}$ (NBSGW) mice, developed to support engraftment of human hematopoietic stem cells (HSCs) without irradiation. The goal is to validate the suitability of the therapy for clinical trials regarding efficacy, safety, and long-term repopulation (LTR) potential of modified cells. The study also aims to compare the mutation-specific approach with a universal therapy targeting the BCL11A enhancer element for the induction of HbF, currently in clinical trials for β-thalassemia and sickle cell disease

Material and Methods: RGN and TALENs were delivered via nucleofection to mobilized HBB$^{IVSI-110}$ patient-derived HSCs as ribonucleoprotein complexes (RNPs) and in vitro transcribed mRNAs, respectively, both targeting the HBB$^{IVSI-110}$ mutation and as RNPs targeting the BCL11A enhancer element (sg1617 RGN). The therapeutic potentials of the genome editing tools were assessed in vitro with induced erythroid differentiation (ED) cultures, in which correction was evaluated at the DNA (on- and off-targeting, Sanger sequencing), protein (RP-HPLC) and late-stage ED levels (flow cytometry), in clonogenic assays for erythroid and myeloid lineage potential and in vivo with xenotransplantation of the treated HSCs in NBSGW mice for the assessment of the LTR potential of the edited cells 16-weeks post-transplantation (flow cytometry).

Results: Overall, both mutation-specific designer nucleases let to high on-targeting (IVSI-110

RGN 89%, TALENs: 68%) with undetected off-targeting, whereas BCL11A enhancer targeted disruption was low (16%). RP-HPLC analysis of the in-vitro ED cultures, showed functional correction of HBB-like globin proportion in the mutation-specific edited populations (HBB; IVSI-110 RGN: 69%; TALENs: 54% vs Untreated: 21%) and a minor increase of HBG in the sg1617 RGN edited population relative to untreated (HBG; sg1617: 78% vs Untreated: 72%). There was a clear correction of late-stage ED in the mutation-specific edited populations and genome editing did not affect the erythroid and myeloid lineage potential of the edited HSCs. Importantly, analysis of BM chimerism in xenotransplanted NBSGW mice showed high engraftment for all samples (hCD45+: 75% and hCD34+ves: 10%).

Conclusions: Even though, analysis on the biosafety of the RGN- and TALEN-based mutation-specific approach is still in progress, the current data indicates IVSI-110 RGN as the most promising approach for clinical application, since therapeutic levels were achieved while the erythroid and myeloid-lineage and LTR capacity of the edited HSC population was maintained.

10. CHEMICAL INJURY BY CHEMOTHERAPEUTIC AGENT ARA-C INDUCES MYELIN DYSFUNCTION AND ECTOPIC NEUROBLASTS' RECRUITMENT IN THE BRAIN

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Purpose of this study: Adverse side effects of chemotherapy that impair brain function have been recently anticipated. Reduction of Neural Stem Cell (NSC) proliferation rates in adult brain neurogenic zones, white matter degeneration and brain inflammation are thought to contribute to the appearance of the 'Chemobrain effect'. The aim of this study was to investigate the effect of brain chemical injury induced by Arabinoside-C (Ara-C) on adjacent neurogenic and non-neurogenic brain areas and its relation to the functional outputs of 'Chemobrain'.

Material and Methods: Multiple stereotaxic intraventricular injections of Ara-C were performed to induce chemical injury in the brain of adult mice. Spatio-temporal distribution of NSCs, neuronal and glial cells was characterized using immunohistochemistry at different time points (4, 15 days and 6 weeks) following the chemical lesion. Brain microstructure was evaluated using transmission electron microscopy (TEM).

Results: Our data revealed that Ara-C induces myelin dysfunction and myelinated axon loss accompanied by an imbalance of the oligodendrocyte genealogy. We also observed a strong neuroinflammatory response with extensive astro- and microgliosis around ventricular and septal walls and adjacent brain parenchyma and presence of CD3+ T cells in the Subventricular Zone (SVZ) and choroid plexus, suggesting a disruption of the blood–CSF barrier. Ara-C infusion leads to persisting ependymal cell layer disruption and impaired neurogenesis in the SVZ neurogenic niche. Furthermore, it triggers the ectopic presence of Doublecortin+ (DCX+) neuroblasts in the adjacent non-neurogenic striatal parenchyma, the majority of which cluster

inside myelinated white matter tracts. Our observations indicate that neuronal progenitors' cell fate decisions and maturation state are largely determined by their spatial distribution, with more differentiated newly generated neurons outside myelin structures and migrating neural progenitors inside myelin bundles.

Conclusions: Our study demonstrates that Ara-C induces significant changes in the brain microstructure, which may contribute to the 'Chemobrain effect'. The imbalance of oligodendrocyte genealogy, ependymal cell layer disruption, impaired neurogenesis, and presence of ectopic DCX+ neuroblasts inside myelinated white matter tracts are the potential mechanisms underlying this effect. Our ongoing studies are aimed to further elucidate the lineage trajectories, origin and molecular profile of ectopic neuroblasts. This analysis will provide valuable insights into mechanisms of regeneration and repair following chemotherapy-induced brain injury.

11. ESTIMATION OF THE HLA HOMOZYGOUS CORD BLOOD UNITS (CBUS) FOR THE PRODUCTION OF INDUCED PLURIPOTENT STEM CELLS (IPSCS) FOR TRANSPLANTATION AND REGENERATIVE MEDICINE PURPOSES OF GREEKS.

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Background/Objectives: The allogeneic hematopoietic stem cell transplantation depends on the HLA compatibility between recipient/donor. The high genetic diversity and molecular complexity of HLA, is largely responsible for the difficulty in identifying appropriate donors for patients in need. An off-the-shelf approach to overcome these obstacles is to generate iPSCs from donors homozygous for common HLA-A,-B,-C,-DRB1 haplotypes. Besides transplantation, these cells may play an important role in the field of regenerative medicine. Here, we calculate the number of HLA-homozygous CBUs from the three Public Cord Blood Banks (PCBBs) of Greece that might be used to generate iPSC lines (haplolines) for transplantation and other medical needs of the Greek population.

Methods: 2,525 CBUs (Papanikolaou Hospital, n=1,599; BRFAA, n=590; University Hospital of Heraklion, n=336) were typed for HLA Class-I (*-A,-B,-C*) and Class-II (*-DRB1*) loci using 2nd field NGS. Hapl-o-Mat was used to estimate maximum likelihood haplotype frequencies based on the high resolution allelic frequencies of three (A, B, DRB1) and four (A, B, C, DRB1) HLA genes from 1,599 and 926 samples, respectively. The selection of HLA homozygous CBUs was based on a 3- and 4-locus match. The concordance of the HLA-A,-B,-DRB1 or the -A,-B,-C,-DRB1 alleles

of the estimated homozygous haplotypes in both homozygous and heterozygous recipients with the relevant HLA haplotype was used to calculate the matching coverage both for the total cohort and for the stratified by the PCBBs data. To estimate the number of haplolines needed to cover approximately 100% of the Greek population we used an iterative algorithm with all the inferred haplotypes of the Greek PCBBs. The cumulative coverage of Greeks was estimated by dividing the number of matched samples in each iteration by the total cohort size. **Results:** 1,621 corrected (1/2n) 3-locus HLA haplotypes were inferred for the cohort (n=2,525), with 5 haplotypes presenting at ≥1%. Even though 5 CBUs were homozygous for 3 haplotypes, we selected only the top A*02:01~B*18:01~DRB1*11:04 (2.83%) as a potential iPSCs haploline candidate based on the criterion of presence in ≥2 samples. While the estimated total coverage was 6.93%, a considerable variation (p=0.00124) was revealed following cohort stratification by PCBBs (BRFAA: 9.32%, Papanikolaou: 6.88%, Crete: 2.98%). The 4-locus HLA analyses resulted in 935 inferred and corrected haplotypes with 3 being frequent in more than 1% of the 926 samples. The extended 4-locus haplotype A*02:01~B*18:01~C*07:01~DRB1*11:04 was again the most frequent (2.32%) and present in 2 homozygous CBUs. The estimated total matching coverage was 4.88%, ranging between 6.46% and 1.51% for BRFAA and Crete (p=0.00116), respectively. The iterative algorithm revealed that 100 3-locus or 4–locus haplolines are required to cover 68.93% or 64.63% of the Greek population resulting in the approximate 100% matching coverage with 513(99.96%) and 343(99.35%) 3-locus and 4 –locus haplolines, respectively. **Conclusion:** These results signify the importance of establishing iPSCs repositories from individuals homozygous for frequent HLA haplotypes to cover the HLA diversity of the Greek population. While the concept of iPSC haplolines/haplobanks is still in its early stages, it is anticipated as an effective approach in transplantation and regenerative medicine.

12. BIOFABRICATION OF 3D BIOPRINTED VASCULAR-LIKE STRUCTURES FUNCTIONALIZED WITH PLATELET-RICH PLASMA FOR ENDOTHELIAL TISSUE REGENERATION

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Introduction: One of the most popular biofabrication techniques is 3D bioprinting which can be used to produce personalized constructs for tissue engineering (TE). Gellan gum (GG) is a natural

biocompatible polysaccharide with angiogenic properties (Li, Z., Carbohydrate Polymers (2022) 290, 119469) In our study, we combined GG, laponite (Lap) and platelet-rich plasma (PRP) aiming to enhance the endothelial regeneration through the synergistic effects of their individual properties. Laponite has the ability to form porous three-dimensional networks mimicking the extracellular matrix structure, and PRP delivery of growth factors stimulates the endothelial cell proliferation and migration, offering a composite bioink for cell growth and support (Woodfield, T., Comprehensive Biomaterials II (2017), pp 236). The sustained release of these growth factors from the laponite-PRP composite material over time can provide a continuous source of stimulation for the cells, leading to more effective tissue engineering strategies for endothelial tissue regeneration.

Materials and method: Four different blends, 0.5%Lap-1%GG-25% v/v PRP, 0.5%Lap-1%GG, 1%Lap-1%GG-25% v/v PRP and 1%Lap-1%GG were combined with Wharton jelly mesenchymal stem cells (WJ-MSCs) and bioprinted into hollow tubes. The swelling and mechanical properties of the four compositions have been determined. Biological evaluation of the constructs includes the live/dead staining, the evaluation of ECM formation by visualization of the produced collagen and glycosaminoglycans, and the endothelialization markers PECAM-1 and vWf by immunohistochemistry. In vivo immunological response of the bioprinted tubes was examined in C57BL6 mice subcutaneously after 14 days.

Results: Biofabricated constructs were designed with an inner diameter of 3 mm and a wall thickness of 1 mm. Stress/strain analysis revealed the elastomeric properties of the hydrogels with Young modulus values of 10 MPa. Increasing the Lap concentration led to a non-significant decrease of swelling ratio from 93% to 91%. Live/dead assay revealed cell viability of at least 76% from days 3 to 21 for all compositions. On day 3 the 0.5%Lap-GG and the 1%Lap-GG presented 85% and 88% viability respectively, followed by an increase over 99% on day 7 for both bioinks. After 21 days the 0.5%Lap-GG maintained viability level up to 99%, while a slight decrease was observed for 1%Lap-GG. Gradual increase of GAGs accumulation and collagen production through days 7, 14 and 21 indicate the promotion of the ECM formation. *In vitro* functional evaluation depicts the expression and membranous localization of PECAM-1 from day 7. Granular intracellular localization of vWF was detected after 2 weeks. *In vivo* subcutaneous implantation indicated the absence of any adverse immunological reactions.

Conclusion: The results revealed the expression of both vWF and PECAM-1 by WJ-MSCs entrapped in all four construct compositions with significantly higher expression of vWF in the presence of PRP. Cell-laden Lap-GG bioinks were used to fabricate vessel-like structures of high shape fidelity, high cell viability, high elasticity and swelling properties. The biological effect of PRP/GG/Lap bioinks has been validated towards endothelial tissue regeneration. The integration of PRP improved the cellular behavior and promoted endothelial differentiation. No adverse reactions of the implanted constructs in mice have been observed.

13. EFFECT OF STEM CELL DERIVED MICROPARTICLES ON VIABILITY, APOPTOSIS AND DIFFERENTIATION PROGRAM OF HEMOPOIETIC CELLS

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Purpose: Microparticles (MPs) are extracellular vesicles sized 0.1–1μm, released from plasma membrane. MPs from different cell types circulate in disease states and can affect normal cell functions, but processes such as apoptosis or cell activation underlying their release are unclear. Our previous study showed that CD34+ microparticles (CD34+MPs), derived from hemopoietic stem cells (HSC) are detected in umbilical cord blood (UCB), which is an alternative source for hematopoietic cell transplantation (HCT), carry miRNAs (Xagorari, et al., 2019). However, the impact of MPs on allo-HCT remains unclear. Therefore, the aim of this study was to evaluate the effect of CD34+MPs on the HSC differentiation program by hemopoietic colony-forming unit (CFU) assays. Additionally, has been analyzed their effect on the cell viability and the expression of apoptotic genes in promyelocytic leukemia cell line HL60 and in mononuclear cells (MNCs) of UCB.

Methods: UCBs inappropriate for transplantation due to low volume were collected after informed consent. CD34+MPs were isolated from the plasma of UCBs by centrifugation and purified by immunomagnetic separation (Miltenyi Biotec). MNCs from CBUs were isolated by ficoll-density gradient centrifugation. HL60 cells and MNCs were cultured in liquid culture for 24 hours with or without CD34+MPs with and without Fetal bovine serum. Cell viability was determined by trypan blue exclusion. Quantitative reverse transcription polymerase chain reaction (RQ-PCR) was performed for BCL2 and FAS genes. Additionally, proteome analysis for apoptotic proteins was carried out. Furthermore, MNCs cultured in liquid culture without growth factors for 1 or 24 hours with or without CD34+MPs and then were seeded in semisolid cultures in the presence of a cocktail of growth factors for colony forming unit granulocyte/macrophage (CFU-GM), burst forming unit erythroid (BFU-E) and Colony-Forming Unit-Granulocyte/Erythrocyte/Monocyte/Megakaryocyte (CFU-GEMM) colony growth.

Results: The viability of HL60 and MNCs incubation with CD34+MPs was decreased significantly after 24 hours. Furthermore, to analyze the apoptotic effect of CD34+MPs on the hemopoietic cells RQ-PCR was performed. The expression of the antiapoptotic BCL2 and the apoptotic FAS was increased in the presence of CD34+MPs in HL60 after 24 hrs incubation. In MNCs increased the expression of BCL2 but decreased the expression of FAS. The expression profile of both apoptosis-related genes was independent of the FBS addition excluding the possibility

of autophagy. Proteome profiler confirmed the previous results in HL60 and in MNCs although FAS was not expressed. In order to examine the effect of CD34+MPs on the HSC differentiation, CFU assay was performed showing that CD34+MPs decreased the number of CFU-GM and slightly CFU-GEMM. In contrast CD34+MPs promote the growth of BFUs.
Conclusion: The results of this study suggest that the viability of normal and malignant hemopoietic cells decreases in the presence of CD34+MPs by regulating the expression of apoptotic related genes. Stem cell derived microparticles may serve as a potential regulator of hemopoietic differentiation program.

14. TISSUE-ENGINEERING OF INTERVERTEBRAL DISC FROM HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS IN 3D-PRINTED SCAFFOLDS

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Aim: Degenerative Disc Disease (DDD) represents a clinical entity with remarkable associated morbidity and mortality in recent years. Despite the increasing incidence and socioeconomic burden of DDD, available data regarding pathogenesis and currently implemented therapeutic modalities, remain scarce. Furthermore, these modalities offer symptomatic treatment, being not capable of reversing the natural progression of disease (Gantsinikoudis et al., Tissue Eng Part B Rev. 2022 Aug;28(4):848-860). Within this framework, replacement of compromised Intervertebral Disc (IVD) with a viable construct, manufactured via Tissue-Engineering (TE) methods, has been rendered feasible in current literature, theoretically featuring promising outcomes over conventional methods (Gantsinikoudis et al., Tissue Eng Part B Rev. 2022 Aug;28(4):848-860, Nesti LJ et al., Tissue Eng Part A. 2008 Sep;14(9):1527-37, Hudson KD et al., Tissue Eng Part A. 2017 Apr;23(7-8):293-300. Aim of this study is to construct a Tissue-Engineered IVD (TE-IVD) with proper implantation of Adipose-Derived Mesenchymal Stem Cells (ADMSCs) in 3D-printed scaffolds, performing an *in vitro* and an *in vivo* evaluation.

Materials and Methods: Nine (n=9) ovine cervical spine segments (C3-C4, C4-C5 and C5-C6) were initially harvested from three female sheep of "Chiotikon" species being subjected to morphometrical, biomechanical and histological analysis in order to attain baseline values for manufacturing of TE-IVD constructs. Morphometrical analysis of dissected IVDs was performed with digital caliber, whereas biomechanical assessment included conduction of uniaxial cyclic compression, creep and compressive strength testing in controlled environment. Histological evaluation was performed with routine hematoxylin-eosin, Masson's trichrome and Alcian blue staining in light microscope. After acquisition of baseline values, portions of IVD scaffolds were distinctly constructed; Annulus Fibrosus (AF) was manufactured via 3D-printing Polycaprolactone (PCL) scaffolds, whereas Nucleus Pulposus (NP) was constructed with appropriate concentration of fibrin glue scaffolds. Human-ADMSCs will be subsequently implanted in AF and NP scaffolds, being co-cultured in controlled conditions for 27 days. Whole TE-IVD constructs will therefore be subjected to morphometric, biomechanical and histological analysis, according to the same described protocol. These constructs will be subsequently implanted into twelve (12) sheep of "Chiotikon" species. Ovine models will be evaluated via clinical examination, radiologic evaluation (X-ray, Computed Tomography) and *ex vivo* (biomechanically and histologically) in 1, 3, 6 months and 2 years postoperatively.
Results: Mean antero-posterior and transverse diameters of isolated ovine physiological discs were 24.4 ± 2.6 mm and 24.4 ± 2.1 mm, respectively, featuring both a gradual increase in a cranio-caudal direction. Cyclic compression testing demonstrated that preconditioning was observed in the last cycles, whereas critical failure point calculated at 15.5 MPa. Histologic assessment demonstrated the sparsely organized internal NP in contrast to the well-organized architecture with concentric collagen lamellae in external AF. The above information was implemented towards Computer Aided Designing (CAD) the ovine TE-ID scaffold. The finalized scaffold was 3D printed using polycaprolactone a biocompatible and biodegradable polymer widely accepted in tissue engineering.

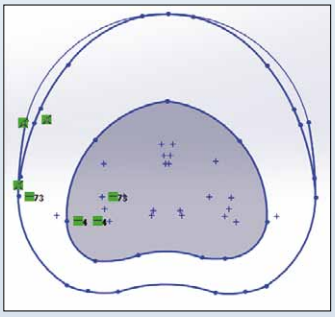

Figure 1. Computer aided design of the C3-C4 ovine ID. This served as a prototype for 3D printing of the final scaffold.

Conclusions: Tissue-Engineering of Intervertebral Disc via implantation of ADMSCs in 3D-printed scaffolds represents a feasible procedure with promising outcomes, considering the advantages associated with use of ADMSCs in contrast with other cell types.

15. HIF-1A RESTRICTS CARDIOMYOCYTE REGENERATION

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Introduction: Heart diseases remain the main cause of morbidity and mortality, due to the limited regenerative capacity of postnatal cardiomyocytes (CMs). Previous research suggested a paradoxical mechanism, whereby switching CM metabolism to glycolysis through hypoxia-mediated stabilization of HIF-1α, may activate postnatal CM proliferation and regeneration.

Objectives: To examine the role of HIF-1α-mediated glycolysis in CM regeneration.

Methods: One-day-old (D1) BALB/c mice were randomized to sham or cryoinjury- induced heart regeneration, followed by daily subcutaneous injections of PBS (Ctrl), the HIF-1α inhibitor YC-1, the HIF-1α inducer $CoCl_2$, and either a low (LMet) or high (HMet) dose of the cyclic AMP inhibitor Metformin, for 7 or 21 days. Hypoxia was evaluated with Pimonidazole staining (PIM). CM mitosis in D7 mouse hearts was examined using confocal immunofluorescence and Western blot analyses, while scar size was assessed by Picrosirius red staining, 21 days post-cryoinjury.

Results: Confocal microscopy and Western blot analyses showed O_2-independent HIF-1α stabilization in developing and regenerating CMs, indicated by the lack of PIM and nuclear HIF-1α co-localization. Inhibition of HIF-1α or glycolysis by YC-1 (p=0.03) or LMet (p=0.0009), respectively, enhanced neonatal CM mitosis; whereas induction of HIF-1α by $CoCl_2$ (p=0.07), or glycolysis by HMet (p=0.08), respectively, did not. Consistently, at 21 days post-injury, $CoCl_2$ (p=0.008) or HMet (p=0.15) hearts exhibited increased or comparable to Ctrl scar sizes, respectively; whereas, in YC-1 (p=0.5) or LMet (p=0.01) hearts it was comparable to or smaller than Ctrl, respectively.

Conclusions: Our study highlights an O_2-independent HIF-1α glycolytic mechanism which is activated in the developing and regenerating heart to constrain cardiomyocyte proliferation. Thus, transient, pharmacologic inhibition of HIF-1α or glycolysis could represent a novel cardiac regenerative medicine therapeutic strategy.

e-POSTER ABSTRACTS

P1. CONTROL HUMAN IPSC-DERIVED ASTROCYTES RESCUE THE DEGENERATIVE PHENOTYPE OF P.A53T-ASYN IPSC-DERIVED NEURONS GENERATED FROM PARKINSON'S DISEASE PATIENTS

BEST POSTER PRESENTATION

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Aim of the Study: Parkinson's disease (PD) is characterized by progressive loss of midbrain dopaminergic neurons resulting in motor and non-motor symptoms. The histopathological disease hallmark is the presence of intraneuronal protein inclusions, termed Lewy bodies and Lewy neurites (Deplats et al., 2009). Approximately 10% of PD cases are associated with mutations in specific genes, such as the p.A53T α-synuclein (αSyn) mutation (G209A in the SNCA gene), causing a familial form of PD with early onset and severe phenotype. While the disease mechanisms remain largely unresolved, cell reprogramming provides a unique human setting for studying PD mechanisms. The aim of our study is to investigate the contribution of non-neuronal cells and their interactions with neurons in PD and uncover novel disease targets for therapy.

Materials And Methods: We have previously established an induced pluripotent stem cell (iPSC)-based neuronal model from patients harboring the p.A53T mutation, which displays disease-associated phenotypes, including protein aggregates, axonal pathology, and compromised network connectivity (Kouroupi et al., 2017). Here, we generated ventral midbrain-patterned iPSC-derived astrocytes and developed a co-culture system of p.A53T or control neurons on either p.A53T or control astrocytes at all possible combinations, and examined their reciprocal interplay.

Results: We observed compromised neuronal viability and impaired neuritic outgrowth of both control and p.A53T neurons when co-cultured with p.A53T astrocytes while the degenerative phenotype of mutant neurons was exacerbated in co-culture with mutant astrocytes, presenting prominent intraneuronal accumulation of protein aggregates and other typical PD histopathological hallmarks including Lewy body-like formations, Lewy neurites, and retraction bulbs. Interestingly, these phenotypes were significantly reversed when p.A53T-αSyn neurons were cultured on control astrocytes.

Conclusions: Our data support a critical role of mutant astrocytes in the neurodegeneration process and a remarkable ability of healthy astrocytes in rescuing neurodegeneration of mutant neurons. The underlying molecular/cellular pathways are being characterized.

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P2. INVESTIGATING THE ROLE OF THE CILIARY ASSOCIATED PROTEIN-*AHI1* IN CORTICAL DEVELOPMENT USING ANIMAL MODELS AND HUMAN BRAIN ORGANOIDS

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The cerebral cortex is one of the most complex structures, and malfunctions during its development lead to severe brain disorders such as the malformations of cortical development (MCDs) in humans. Previous studies show that disruption of the primary cilium, a small organelle serving as a cellular antenna, can lead to microcephaly a subclass of MCDs, suggesting its potential role in human cortical development. While primary cilia in mice regulate the cell cycle of neural progenitor cells (NPCs) and affect neuronal migration, their role in human brain development is vague. Interestingly, mutations in the ciliary-associated gene *AHI1* have been identified in the MCD polymicrogyria, however, its role in cortical development has not yet been identified. Thus, we aim to investigate the role of primary cilia, using *AHI1* as a candidate gene, during cortical development and scrutinize the mechanisms which upon disruption might lead to polymicrogyria. By comparing published single-cell RNA sequencing datasets, we first demonstrated species-specific and cell-type-specific differences of *AHI1* expression. Then, to dissect its role *in vivo*, we provoked ectopic *AHI1* overexpression or silencing in the developing mouse cortex. Our findings indicate that *AHI1* manipulation alters both the numbers and the position of NPCs and neurons in the developing mouse cortex. Knowing that primary cilium plays a key role in murine cortical development we sought to investigate the primary cilia in *AHI1* manipulated animals and we found that primary cilia length, orientation and numbers are being disrupted, suggesting that cilia's function is also disrupted. Given the differential species-specific expression of *AHI1,* we then manipulated *AHI1* expression in human brain organoids. Our preliminary data showed a reversed effect in human NPCs and neurons than the murine ones which comes to an agreement with the differential species-specific expression of *AHI1*. The results of this study will give insight into the role of primary cilia in human cortical development, as well as the key mechanisms regulating MCDs.

P3. THROUGH THE TBX5 LABYRINTH; CARDIOMYOCYTE PRECURSORS IN THE INJURED ADULT MAMMALIAN HEART

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Purpose of the study: The single curative measure for heart failure patients is total heart transplantation, which is limited due to a shortage of donors, the need for immunosuppression and economic costs. Therefore, there is an urgent unmet need for identifying specific cell populations capable of cardiomyocyte regeneration.
A reliable source for cardiac cells is that of cardiac progenitor cells. These, have been identified in the embryonic heart, while their presence in the adult mammalian heart remains a matter of a debate; it has been shown that the c-kit+ adult heart progenitor cell population does not confer to any form of regenerative potential even after a decade of research and millions being spent in an elusive adult cardiac progenitor. Current markers for the identification of cardiac stem cells have not been reliable, to date.
Methods and Results: We have shown previously that the key early cardiac transcription factor T-box 5 (Tbx5) is expressed in the earliest cardiac progenitor cells populations in the developing heart, "locking" them into a unipotent cardiomyocyte-only fate. By employing a developmental approach in an adult heart injury model, we have detected the presence of a resident ventricular T-box-5-expressing cell population that resembles cardiomyocyte precursors, both in surface marker expression and single-cell transcriptomics. An important feature of these cells is their enrichment in neural developmental gene pathways, upon injury.
The Tbx5 transcriptional network is important not only for initiating early cardiac specification, but also to prime the regenerative mechanism in adult lower vertebrates. The importance of this TF in mammalian heart regeneration has not been examined in detail, primarily because of the lack of specific molecular markers able to distinguish between CPC progenies or any CPC re-activation events.
Conclusions: Our findings indicate that re-expression of Tbx5 may tag cardiomyocyte precursors after adult heart insult, which resemble more neonatal rather than embryonic cardiac progenitor/precursor cells. Moreover, the transcriptome of the in vivo Tbx5-expressing cardiomyocyte precursors in the injured heart seem to become enriched for signalling pathways engaged by the central nervous system. These data suggest that cardiomyocyte-like precursors are present in the adult injured mammalian heart, probably hindered by the microenvironment; yet they can be manipulated to initiate a cardiac regeneration program.

P4. MIRK/DYRK1B KINASE CONTROLS THE GENERATION AND THE COLUMNAR ORGANIZATION OF SPINAL MOTOR NEURONS VIA SONIC HEDGEHOG PATHWAY

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Objective: Spinal motor neurons (SpMNs) control diverse motor tasks including respiration, posture, and locomotion. SpMNs are organized into median (MMC), lateral (LMC), hypaxial (HMC), and preganglionic (PGC) motor columns respectively. LMC MNs at the brachial and lumbar level of the spinal cord subdivide into medial LMC (LMCm) and lateral LMC (LMCl) columns innervating the muscle limb ventrally and dorsally, respectively. Here, we intend to elucidate the role of the Mirk/Dyrk1B dual-specificity minibrain kinase in the generation, survival, and columnar organization of SpMNs in the developing spinal cord.

Materials and Methods: We performed *gain-and-loss-of-function* and *phenotype rescue studies* by applying *in ovo* unilateral electroporation at E2 chick embryonic spinal cord, while the *in vitro* pharmacological inhibition of Dyrk1B kinase activity in E12.5 embryonic mouse primary spinal motor neuron (SpMN) cultures is in progress.

Results: Functional integration and cross-talk between Mirk/Dyrk1B kinase and Sonic hedgehog/Gli, PI3K/mTOR/AKT, and MEK/ERK signaling pathways affect cellular and molecular processes in development, physiology, and pathology. We revealed a novel role for Mirk/Dyrk1B kinase in the generation of SpMNs in the embryonic chick spinal cord by regulating the Sonic hedgehog (Shh) pathway. Using *in vivo gain-and-loss-of-function and phenotype rescue* approaches in E2 chick spinal cord and its subsequent analysis at E4, we found that Dyrk1B overexpression promoted at E4 cell cycle exit and neuronal differentiation in a cell-autonomous manner, while in a non-cell autonomous manner Dyrk1B overexpression promoted increased apoptosis specifically in the MN domain, followed by a dramatic loss of p2, pMNs, and p3 progenitors, as well as of post-mitotic motor neurons (MNs) and V2a interneurons (INs). This intense ventral phenotype of Dyrk1B overexpression suggested the involvement of Shh signaling. In agreement, real-time RT-qPCR analysis revealed that Dyrk1B overexpression in the E2 chick spinal cord reduces dramatically Shh and Gli3 mRNA levels at E4. At E6, the loss of MNs is selectively reflected in reduced LMCm MNs that innervate the muscle limbs ventrally. In phenotype rescue experiments, the compromised Shh signaling, due to Dyrk1B overexpression, was restored by using AZ191 compound, a specific DyrK1B kinase inhibitor, or SAG agonist of Smoothened (SMO), which activates the Shh pathway. Both compounds resulted in the restoration of p2, pMNs, and p3 progenitors, as well as of LMCm MNs. The specific effect of Dyrk1B in LMCm MNs could be explained by our finding that Shh is expressed exclusively by LMCm MNs at the E6 chick spinal cord. Pharmacological inhibition of Dyrk1B kinase activity in E12.5 mouse MNs primary cultures is in progress.

Conclusions: In conclusion, Mirk/Dyrk1B kinase acts as a transcriptional suppressor of the Sonic hedgehog pathway, thus regulating the number of p2, pMNs, and p3 progenitors that are

under the strong influence of Shh gradient, and especially controls the generation and survival of SpMNs as well as their columnar organization in the LMCm column.

P5. EVALUATION OF INDUCTION OF FETAL HEMOGLOBIN SYNTHESIS BY GENOME EDITING OF CIS- AND TRANS-ACTING COMPONENTS OF THE B-GLOBIN LOCUS

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The reactivation of γ-globin can ameliorate the clinical phenotype of β-hemoglobinopathies by functional compensation of β-globin deficiency and anti-sickling action of fetal hemoglobin (HbF). Most importantly, it constitutes a universal therapy approach that can potentially be applied to all β-hemoglobinopathy patients, irrespective of genotype.

This work focuses on genetic modification of globin expression regulators and the β-globin locus as potential therapeutic approaches for β-hemoglobinopathies by reactivation of γ-globin. To this end, we employed the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR associated protein 9 (Cas9) system to abolish expression of two known γ-globin gene repressors, BCL11A and ZBTB7A (*trans* editing), and a dual-targeting single RNA-guided CRISPR/Cas12a system to create a large (7.4-kb) β-δ intergenic deletion at the β-globin locus (*cis* editing). Tools were delivered as ribonucleoprotein (RNP) complexes by nucleofection of human umbilical cord blood-derived erythroid progenitor-2 (HUDEP-2) cells and primary thalassemic CD34+ cells. Edited cells were assessed for on-target editing efficiency by Inference of CRISPR Edits (ICE) and digital polymerase chain reaction (dPCR), and analysed for globin and hemoglobin expression after induction of erythroid differentiation, by high-performance liquid chromatography (HPLC) and by flow cytometry for intracellular HbF expression. The study suggests that generation of the 7.4-kb cis deletion at the β-locus, relying on the highly efficient non-homologous end-joining (NHEJ) repair mechanism of double-strand breaks (DSBs), may lead to higher HbF levels than the disruption of trans-acting components also involved in other essential functions of hematopoiesis, as are the γ-globin repressors BCL11A and ZBTB7A. Analysis of clonal populations of HUDEP-2 cells bearing the intended edits are underway to confirm findings in bulk edited cell populations.

P6. CELL-INTRINSIC PATHOLOGICAL CHARACTERISTICS IN A53T-ASYN IPSC-DERIVED ASTROCYTES FROM PARKINSON'S DISEASE PATIENTS

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Aim: Astrocytes, the most abundant cells in the human brain play critical roles in neuronal health while they can exert neuroprotective or neurotoxic effects upon disease. However, their involvement in Parkinson's disease (PD) has only recently started being appreciated, contrary to the long-running intensive research on neuron-intrinsic dysfunction. PD is characterized by the progressive loss of midbrain dopamine neurons whereas the histopathological disease hallmark is the presence of intracellular protein inclusions rich in α-synuclein (αSyn). 5-10% of PD cases have been linked with mutations in specific genes, such as the αSyn gene SNCA (Oliveira et al., 2021). pA53T-αSyn (G209A in SNCA) is the best-characterized mutation causing a severe, early-onset familial form of PD (Polymeropoulos et al., 1997; Kouroupi et al., 2017). Our aim is to investigate the effect of pA53T-αSyn mutation in astrocytes to unveil novel potential PD mechanisms and therapeutic targets.

Materials and Methods: We have previously established an induced pluripotent stem cell (iPSC)-based neuronal model of PD from patients harboring the p.A53T-αSyn mutation, exhibiting disease-associated phenotypes, including intraneuronal protein aggregates, axonal pathology, and reduced synaptic connectivity. To investigate the role of astrocytes in PD, we generated ventral midbrain-patterned astrocytes from pA53T-αSyn patient and control iPSCs.

Results: Both control and PD iAstrocytes express typical lineage markers and functional characteristics, including IL-6 upregulation upon cytokine stimulation, calcium transients, and phagocytosis. αSyn expression was detected at low levels in control induced (i)Astrocytes whilst PD iAstrocytes displayed cytoplasmic accumulation of pathological αSyn species, prominent protein aggregates, and cytoplasmic vacuolization. Dysregulation of genes associated with the unfolded protein response, such as BiP and ATF4, were also noted at basal conditions and after thapsigargin-triggered ER stress. Of relevance, autophagy was found among the affected pathways by proteome profiling of PD versus control iAstrocytes. Further, since astrocytes are known to effectively phagocytose protein aggregates like those containing αSyn pathological species thereby protecting neurons, we used pHrodo-*E. coli* and noted that iAstrocytes possess phagocytic capacity. Yet, the clearance of endocytosed cargo is hampered in PD iAstrocytes, as indicated by the observed accumulation of enlarged cargo-loaded vesicles in their cytoplasm.

Conclusions: Our data demonstrate that the p.A53T-αSyn mutation causes intrinsic malfunctions in astrocytes, related with proteostasis and clearance mechanisms that may have a critical contribution in PD neuronal pathology.

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P7. IN VIVO MICROGLIAL BIN1 DELETION FOLLOWING LPS-INDUCED NEUROINFLAMMATION REGULATES MICROGLIA PROLIFERATION AND INFLAMMATORY RESPONSE

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Introduction – Purpose of the study: Genome-Wide Association Studies have identified several Single Nucleotide Polymorphisms (SNPs) strongly associated to increased risk of developing LOAD, many of which are related to microglia activation. SNPs in the locus harboring Bridging Integrator 1 (BIN1) gene show the strongest association with AD, after Apolipoprotein E. BIN1 is a member of the Bin/Amphiphysin/Rvs (BAR) family of adaptor proteins implicated in cell membrane modelling dynamics. Although, its role in neurons has been studied both in vitro and in vivo, the role of BIN1 in microglial activation state and its contribution in LOAD pathology remains to be clarified. Our goal is to investigate the effect of microglia-specific Bin1 deletion on mouse brain under homeostatic and inflammatory conditions.

Materials – Methods: We have developed a conditional double transgenic Cx3CR1Cre-ERT2//Bin1fl/fl mouse, in which BIN1 is knocked out in microglial cells. Then, we challenged the BIN1-cKO mice with a single dose of LPS to induce an inflammatory response. In our model, we used immunohistochemical analysis to characterize the forebrain molecular phenotype, and performed single nucleus RNA-Seq to reveal novel targets related to microglial BIN1. Finally, we confirmed our findings from the sequencing by protein detection and real-time RT-PCR.

Results: Our snRNA-Seq analysis resulted in different microglia subpopulations, which have been alter among our experimental conditions. Importantly, after the LPS treatment we observed the enrichment of a subpopulation that has the capacity to proliferate and a subpopulation that exhibits IFN-type I inflammatory response in the BIN1-cKO animals. Further analysis based on these data lead us to observe an increased KI67+ microglia population, indicating the enhanced proliferative capacity in the inflammatory environment. Lastly, we observed upregulation on genes such as stat1, irf7, Ifi204, mki67, top2a, that can be correlated with these exact subpopulations.

Discussion: According to our analysis, LPS treatment affects various signaling pathways regu-

lated by microglia differently in Bin1 cKO and control animals. The deletion of BIN1 resulted in the enrichment of two arising populations with district molecular and phenotypical characteristics. To confirm these findings, we conducted subsequent real-time RT-PCR and immunohistochemical analysis, and we are currently analyzing the resulting subpopulations in further detail.
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P8. EFFICIENT EPIGENETICALLY-MEDIATED REACTIVATION OF GAMMA GLOBIN EXPRESSION IN AN IMMORTALIZED HUMAN ERYTHROID PROGENITOR CELL LINE

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Introduction: Over the last few years, the development of genome editing approaches for beta-hemoglobinopathies enabled the targeted introduction of genomic tools to either correct point mutations or induce disease-modifying mutations, such as the reactivation of gamma globin to cure β-hemoglobinopathies. Despite the promising results that have emerged from clinical and preclinical genome editing applications, limitations still exist due to the generation of potentially harmful off– and on-target effects, related to the induction of double strand brakes (DSB). Recently, the recognition of the crucial role of epigenome modifications in the tight regulation of gene expression as well as the development of custom-designed epigenome editors (epi-editors) has enabled the development of alternative and less invasive approaches for targeted gene regulation.
Aim: Aim of the present study is to address the feasibility of epigenetically mediating gamma globin reactivation.
Materials and Methods: First, we generated a HUDEP-2 cell line stably expressing a deactivated Cas9 protein (dCas9) fused with the Krüppel-associated box (KRAB) effector domain. We then designed guide RNAs (gRNAs) targeting 3 genes known to regulate HbF. Specifically, we used 2, 1 and 3 gRNAs targeting the promoter of ZBTB7A, the erythroid enhancer of BCL11A, and the recently identified HBG-suppressor, ZNF410, respectively. The HUDEP-2-dCas9-KRAB cells were transduced with each lenti-gRNA vectors individually and cell expansion, differentiation and HbF expression were assessed at different time points.
Results: Transduction with the lenti-guide vectors didn't alter the cell growth rate and the erythroid differentiation potential of the dCas9-KRAB HUDEP-2 cells. The expression of the gRNA resulted in efficient suppression of ZNF410, LRF and BCL11A expression that was translated into significantly increased gamma globin levels within the transduced population ($p \leq 0.05$). Among the three targeted transcription factors (TFs), the epigenetic

silencing of ZNF410 induced higher levels of HbF+ cells over the other two TFs (ZNF410: 36±5.6% HbF+ cells , LRF: 31.8±2.11% HbF+ cells, BCL11A: 25.7±2.07% HbF+ cells versus 16±2.5% HbF+ cells in untransduced group, p≤0.05).
Conclusions: Overall, our data demonstrate that the epigenetic suppression of the three gamma globin regulators, BCL11A, LRF, ZNF410 is feasible and effective, leading to significant HbF induction in HUDEP-2 cells. Future studies in primary cells will solidify these data.

P9. DISRUPTED HIPPOCAMPAL NEUROGENESIS FOLLOWING BRAIN CHEMICAL LESION BY CHEMOTHERAPEUTIC AGENT ARA-C

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Purpose of this study: A wide range of cognitive deficits, which all fall under the umbrella term "ChemoBrain", have been reported in cancer patients after receiving chemotherapy. Confusion and memory loss are only a few of the effects of Chemobrain which have been attributed to hippocampal dysfunction. As the hippocampus is one of the sites of adult neurogenesis, the aim of this project is to investigate the response of the neurogenic niche of the hippocampus after the administration of Arabinoside-C (Ara-C), an antimitotic agent commonly used in chemotherapy.
Material and Methods: Two intraventricular injections of Ara-C were performed with a 2-days interval followed by 4 BrdU injections to assess the proliferation dynamics of the hippocampal neurogenic niche. Quantification and characterization of the different Neural Stem Cells (NSC) populations were performed by immunochemistry at 3 time-points (4, 15 days and 6 weeks) following Ara-C administration. Additionally, real-time PCR was conducted to explore alterations in the gene expression of the cell populations of the niche.
Results: Our study revealed that the intraventricular administration of Ara-C leads to the disruption of neuronal cell lineage dynamics in the subgranular zone (SGZ) of the dentate gyrus of the hippocampus. This was indicated by the reduction of DCX+ neuroblasts, by contrast to the observed increased trend of the non-proliferating pool of Sox2+ cells at the time points of 4 and 15 days. At 6 weeks, this observation was reversed, since the number of DCX+ neuroblasts increased as compared to the control group. Furthermore, the morphology of DCX+ neuroblasts was also affected, since their dendritic projections were lost or disoriented, a phenomenon that was evident from 15 days and endured 6 weeks after the chemical insult. Alongside with the disorientation, an abnormal migration deeper into the granule cell layer (GCL) was also evident at 6 weeks. Lastly, the transient upregulated expression of Notch effectors Hes1 and Rbpj together with Hopx, all pointed to a stallment of neuronal lineage progression.
Conclusions: In this study, we report that intraventricular administration of the chemotherapeutic agent Ara-C, leads to disrupted neuronal lineage progression in the dentate gyrus of the hippocampus, accompanied by abnormal migration of neuroblasts deeper into the GCL,

most probably due to the disoriented localization of their dendritic projections. Our ongoing studies aim to provide more clarity on the neuronal lineage trajectories, as well as the specific cause of dendritic atrophy and investigate whether these mismigrating neuroblasts can integrate into the hippocampal niche.

P10. PML REGULATES THE SPECIFICATION AND POTENTIATES THE SURVIVAL OF NEURAL STEM CELLS

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Purpose of study: The Promyelocytic Leukemia Protein (PML) originally characterized as a tumor suppressor is the core organizer of cognate nuclear bodies (PML-NBs) that regulate various physiological processes in different cell types. In embryonic (ESCs) and induced pluripotent (iPSCs) stem cells we have recently reported that PML is required for maintenance of the naïve and acquisition of the induced pluripotency state, respectively. Although previous studies suggest that PML regulates neuronal plasticity, cognitive function and toxic poly Q protein aggregates clearance there is no conclusive evidence for a role in neuroprotection or neurodegeneration. Recent studies report PML as a hub gene for Alzheimer's Disease (AD) and that PML bodies co-localize with the carboxy-terminal region of Amyloid Precursor Protein (APP-CT) in correlation with AD pathophysiology. In this work we examine the role of PML in neuronal cell specification and survival from neurotoxic stress. We also examine the role of PML in AD employing the 5xFAD mouse model.

Materials and Methods: Cortical embryonic neural stem cells (eNSCs) were isolated from E13.5 C57BL/6 WT and Pml-/- mice. Neuronal differentiation of ESC was driven by monolayer culturing in N2/B27 supplemented DMEM/F12 media. NSC neuronal and astrocyte differentiation proceeded by removal of EGF and FGF and addition of 1% FBS, respectively. Cell survival was evaluated by the MTT assay and apoptotic activity with annexin staining. C57BL/6 WT and C57BL/6 5xFAD prefrontal cortex and hippocampus sections were stained with antibodies and analyzed with Leica SP8 inverted microscope and Las software.

Results: PML is required for the neuronal specification of ESC since we observed a dramatic reduction of neurons when the PML KD cell line was used. In eNSCs the absence of PML influences the differentiation pathway choice favoring neuronal and inhibiting astrocyte lineage. We examined cell survival of NSCs (WT and KO PML) following oxidative, genotoxic or amyloid stress. PML -/- eNSCs are defective in mounting a response towards Rotenone and Etoposide. Moreover, in the presence of beta-amyloids we observed a significant reduction in viability. PML KO eNSCs present lower mitochondrial potential and higher ROS levels compared to the WT, reinforcing the hypothesis that key mitochondrial mechanisms are disrupted when PML is absent. Using a mouse model of familial AD (5xFAD) we observed a reduction of nucle-

ar PML expression whereas in later stages of AD pathology, PML is also expressed in the cytoplasm. We hypothesize that different PML isoforms have different functions in the course of AD.
d. Conclusions: We propose that PML

- is required for neuronal specification of ESC and eNSC.
- shows a neuroprotective function *in vitro* by enhancing the defence against β-amyloid and apoptotic stress and sustaining the mitochondrial integrity.
- is expressed differently in correlation with the pathological stage of 5xFAD mouse suggesting an involvement in AD progression.

P11. DEVELOPMENT OF MECHANO ACTIVE SCAFFOLDS FOR ENHANCED OSTEOGENIC DIFFERENTIATION

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Introduction: Bone is a highly dynamic tissue that undergoes continuous mechanical forces through lifetime. Mechanical stimuli applied on scaffolds resembling a part of the human bone tissue could have major effect on osteogenesis (Florencio-Silva, R, Biomed Res Int, 2015. 2015: p. 421746). Poly(3,4-ethylenedioxythiophene) (PEDOT) is a piezoelectric material that responds to mechanical stimulation producing an electrical signal that promotes the osteogenic differentiation of pre-osteoblastic cells via opening of voltage-gated calcium channels (Guex, A.G., Acta Biomaterialia, 2017. 62: p. 91-101.). The focus of our research was to examine the biological behavior of the mouse calvaria osteoblastic precursor cell line MC3T3-E1 when seeded onto lyophilized piezoelectric PEDOT/PVA/gelatin scaffolds, cultured under osteogenic conditions and undergoing uniaxial compression.
Materials and method: Two different concentrations of PEDOT (0.15% w/v and 0.10% w/v) were combined with a 5% w/v PVA- 5% w/v gelatin homogenized solution, were casted into 96well-plate, freeze dried and crosslinked with 2% v/v (3-glycidyloxypropyl)trimethoxysilane and 0.025% w/v glutaraldehyde. Non-crosslinked scaffolds of all compositions were also evaluated for comparison. The elastic modulus of the scaffolds was measured by uniaxial compression test. Degradation rates for the various scaffold compositions after 7, 14 and 21 days were also evaluated. MC3T3-E1 ($4x10^4$ cells/scaffold) were seeded onto the scaffold and allowed to proliferate for 3 days in culture. Followingly, the scaffolds were subjected to uniaxial compression with a frequency of 1 Hz and a strain of 10% of the scaffold height (500 μm). Cyclic mechanical compression was applied for 1 h at the dynamic culture every second day for 21 days and the results were compared to those of a static culture. The loading parameters were selected to resemble the in vivo loading situation (Schreivogel, S, J. Tissue Eng Regen Med, 2019. 13(11): p. 1992-2008). The viability of cells seeded on the PEDOT/PVA/gelatin scaffolds were determined by means of the PrestoBlue™ cell viability assay. Live/dead staining was con-

ducted and visualized via confocal microscopy. The morphology was monitored via scanning electron microscopy (SEM). Furthermore, measurements of the alkaline phosphatase (ALP) activity were carried out to determine the effect of the piezoelectric scaffolds and the uniaxial compression on the osteogenesis.

Results: PEDOT/PVA/gelatin scaffolds presented favorable mechanical properties for bone tissue engineering. Their elastic modulus ranged between 1.2 and 4.5 MPa. The degradation rates of the non-crosslinked scaffolds were higher than those of the crosslinked ones. At day 14, the crosslinked scaffolds indicate a mass loss of 10% while the non-crosslinked ones exceed the value of 17%. The cell viability indicates an increase of cell number over time, while SEM images display well-spread cells. After 3 and 7 days, the ALP activity is higher in the dynamic culture compared to the static one. Moreover, energy dispersive spectroscopy analysis revealed the presence of calcium phosphate in the elaborated extracellular matrix.

Conclusion: Our results indicate that the PEDOT/PVA/gelatin scaffolds support the adhesion, proliferation, and osteogenic differentiation of the pre-osteoblastic cells under mechanical stimulation. The scaffolds have favorable physicochemical and biological properties for bone tissue engineering and other load-bearing tissues to amplify matrix production via mechanical stimulation.

P12. NANOBODY-MEDIATED INHIBITION OF T-CELL ACTIVATION; BINDING SPECIFICITY STUDIES

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The ability to selectively and effectively inhibit T-cell function is crucial for developing personalized treatments for T-cell-mediated diseases, as well as for advancing organ transplantation and T-cell immunotherapies. Many efforts are currently focused on identifying compounds that can manipulate T-cell activation. Lck, a protein tyrosine kinase belonging to the Src family kinases (SFKs), is necessary for initiating T-cell signaling and subsequent T-cell activation, making it an attractive target for small molecule inhibitors. However, developing potent and selective Lck inhibitors has been challenging due to the structural similarities among SFK members in their catalytic centers. This project aims towards the selective downregulation of T-cell signaling and function via specific inhibition of Lck through intracellularly expressed nanobodies (Nbs). These Nbs can recognize a poorly conserved regulatory region of Lck, preventing its localization to its natural locations in the plasma membrane, a prerequisite for the kinase to become enzymatically active.

Screening experiments of 30 different Nbs have identified one top candidate (Nb*S*) that can specifically bind Lck by co-immunoprecipitation (Co-IP) experiments in an exogenous co-transfection system. The present work focuses on assessing the specificity of Nb*S* for Lck, compared

to other SFK members. We have performed Co-IP and confocal microscopy experiments with wild-type SFKs and chimeric Lck constructs and verified that Nb*S* is highly specific for Lck. These data identify intracellularly expressed Nbs as promising agents for highly selective SFK inhibition. Our next goal is to evaluate the Nbs' ability to attenuate T-cell signaling and function through specific Lck inhibition.

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P13. EPIGENETIC SILENCING OF THE GLUCOCORTICOID RECEPTOR TO BROADEN THE APPLICABILITY OF ANTIGEN-SPECIFIC T-CELL IMMUNOTHERAPY

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Background-Aim: Adoptive immunotherapy (AI) with pathogen-specific T cells (pSTs) represents an attractive alternative therapy for opportunistic infections after allogeneic hematopoietic cell transplantation (allo-HCT). However, patients receiving high-dose steroids, who are the most vulnerable to life-threatening infections, are being deprived from the benefits of AI due to the suboptimal function of adoptively transferred T cells in the presence of steroids. We have recently reported the generation of steroid-resistant pSTs, by genetic disruption of the glucocorticoid receptor (GR) gene (NR3C1) using CRISPR/Cas9 editing. To minimize the possibility of unpredictable genomic changes by gene editing, we here, aimed to investigate whether GR-resistant cells could be developed by precision epigenome editing. **Methods:** We developed a clustered regularly interspaced short palindromic repeats interference (CRISPRi) system to epigenetically edit the T2 lymphoblastic cell line and confer resistance to steroids. Seven guide RNAs (gRNAs) were prepared to target the proximal NR3C1 promoter (a transcription start site and CpG island between exons 1&2) and delivered as pooled library by lentiviral vectors into T2 cells expressing a catalytically-inactive Cas9 (dCas9) fused to a transcriptional repressor, either DNMT3A methyltransferase or Krüppel associated box (KRAB) domain. Transduced T2 cells were subsequently incubated in the presence or absence of 10^{-4}M dexamethasone (DEX) for 7 days. Cells transduced with an "empty" viral vector expressing dCas9 but no gRNA (empty vector) were used as negative control. **Results.** T2 cells were initially transduced and selected to express DNMT3A or KRAB, then co-transduced with a lentiviral vector encoding the 7 different gRNAs at an MOI<1 and incubated with or without DEX. In the presence of DEX and relative to their untreated counterparts, the proliferation of emp-

ty vector-transduced DNMT3A-expressing T2 cells was strongly inhibited while the inhibition of proliferation of DNMT3A-expressing T2 cells edited with the pool of gRNAs, was significantly lower from day 5 onwards ($p \leq 0.0001$), suggesting an at least partial, functional resistance to DEX. Unlike DNMT3A-GR-edited cells, the proliferation of KRAB- expressing T2 cells edited with the same pool of gRNAs was strongly inhibited, similar to the empty vector-transduced cells, suggesting that DNA methylation of the NR3C1 promoter region plays a key role in the repression induction of the GR gene. **Conclusion**. Overall, we present a proof-of-concept, feasibility study of epigenetically disrupting the expression of GR by a DNA methyltransferase to selectively confer resistance to steroids. Further studies on screening additional gRNAs and transcriptional repressors or multiplex targeting towards GR inactivation are in process, so as to identify an optimized epi-editing tool to be subsequently tested in primary T cells.

P14. IDENTIFICATION OF NOVEL GAMMA-GLOBIN REPRESSORS THROUGH A CUSTOM CRISPR KNOCKOUT SCREEN AND VALIDATION STUDIES FOR THE TREATMENT OF B-HAEMOGLOBINOPATHIES

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Purpose of the study: Reactivation of γ-globin for the production of foetal haemoglobin (HbF) can ameliorate β-thalassaemia and sickle cell disease. Although therapeutic strategies involving addition of a functional β-globin gene or genome editing for γ-globin reactivation are promising, the high cost and limited availability, together with safety and efficacy issues still prevent their widespread use. Pharmacological targeting of negative regulators of γ-globin expression, such as BCL11A and LRF, is another popular strategy. However, the multiple regulatory roles of these proteins, as well as the difficulty in specifically targeting them, are proving problematic. Hence, we want to identify and validate novel γ-globin repressors, which may prove to be more amenable druggable targets.

Materials and Methods: We have performed a custom CRISPR/Cas9 knockout screen in HUDEP2 cells, targeting 293 genes selected from previously published literature, which are suspected to affect HbF expression. HUDEP2 cells showing high HbF expression levels were isolated using FACS after staining against HbF and the targeted genes were identified through NGS. The shortlisted targeted genes are being validated individually through CRISPR/Cas-mediated knockouts (lentiviral transduction and/or nucleofection). The editing efficiency is tested using ICE (Inference of CRISPR Edits), while loss of candidate gene expression is tested using immunoblotting. HPLC and immunoblotting are used to investigate the effect of the knock out on γ-globin expression levels.

Results: The CRISPR/Cas9 knockout screen identified seven potential γ-globin repressor genes,

which scored as highly during the screening process as some of the well-known γ-globin regulators. The three most promising candidate genes have been selected for further validation and functional studies. One gene encodes for a protein involved in ion transport and iron homeostasis, the other gene is a transcriptional regulator and the last gene plays a central role in chromatin remodelling and acts as a transcriptional regulator. So far, we have been unable to validate the screening results, with knock-outs leading to minor or no increases in γ-globin gene expression.

Conclusion: A number of reasons could contribute to the validation inconsistencies we experience, which we need to investigate. These include exon skipping caused by the CRISPR-generated mutations, alternative splicing of the targeted mRNA or on-target mRNA mis-regulation. Our work, if successful, could potentially identify new HbF regulators, which may provide novel therapeutic targets for the treatment of β-haemoglobinopathies.

P15. INVESTIGATING ALPHA-SYNUCLEIN MEDIATED COORDINATION OF RNA METABOLISM

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Parkinson's disease (PD) is a severe neurodegenerative disorder. The disease is linked to the aggregation of a small amyloid protein, alpha-synuclein (aSyn), which is implicated in synaptic vesicle trafficking and neurotransmitter release, with an as yet undefined role. In PD, aSyn is found in brain inclusions in neurites and in Lewy bodies. A well characterized mutation of aSyn (G209A), encodes for A53TαSyn protein that exhibits faster aggregation kinetics and is directly linked to the familiar type of PD. In this study, we use a toolkit of neuronal cell line with stable expression of A53TαSyn, primary hippocampal neurons from transgenic A53T mice and patient derived hiPSC-neurons. Our transciptomics and proteomics analysis of hiPSC derived neurons revealed altered expression levels of core molecules involved in RNA metabolism linked to A53T mutation. Combining "-omics" approaches with high end microscopy and single molecule RNA FISH (smFISH), we aim in investigating how the expression of A53TαSyn affects RNA dynamics in neurons. Initial data bridge aSyn biology to RNA granule organization and imbalanced metabolism of RNA machinery triggered by the presence of A53T aSyn in cellular models of PD.

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P16. MICROGLIAL SIGNATURES IN AN IN VIVO FAMILIAL PARKINSON'S DISEASE MODEL

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Parkinson's disease (PD) is the second most common neurodegenerative (ND) disorder presenting a wide range of manifestations including motor impairment, memory loss, cognitive decline and psychiatric symptoms. Despite extensive research, the pathophysiology of PD is still unknown, and the lack of therapeutic options presents a huge clinical unmet need. A common denominator of most ND disorders is synaptic dysfunction that is caused by changes in synaptic structure and function, which can be either the cause or the effect of the disease etiology. Recent data in neuropsychiatric disorders suggest a neurodevelopmental origin for synaptic dysfunction which contrasts with a decades-long axiom, that synaptic dysfunction is among the end-results of ND. This contradicting theory was recently supported by the discovery of unexpected commonalities in the operating mechanisms in both developmental and ND conditions. These findings suggest that synaptic dysregulation is the result of improper glial-neuronal interactions, not only in adulthood, but as early as critical embryonic and postnatal developmental time-points for the neuronal circuits, where the importance of microglia is well established. Understanding the role of microglia early in ND can improve our understanding and open a path for new therapeutic targets.

In this study, we exploit scRNA seq to gain an insight into the role of microglia on progressive neurodegeneration, using a mouse model (M83) carrying the familial PD mutation in the alpha-synuclein gene (pA53T-αSyn). Specifically, we performed scRNA-seq in microglia from M83 transgenic mice brains (P30 and 5 months old) and compared them with wild-type control littermates to identify how the neuronal pA53T-αSyn expression alters microglia subpopulations and PD associated signaling pathways, prior to symptoms onset. In parallel, we perform extensive immunocytochemical characterization of microglia in multiple regions of M83 mice at respective time points, but also in earlier stages of microglial development stages, including the pre-microglia stages (E14-17 & P7-10), to identify the effects of neuronal pA53T-αSyn on microglia during the early brain developmental stages and disease progression. Finally, findings from scRNA seq analyses are validated by analysis of the spatiotemporal pattern of selected αSyn affected microglia-associated molecules, using smRNA-FISH and immunodetection in M83 mice.

Our study utilizes single-cell-level techniques in the familial PD αSyn A53T model to shed light on the role of microglial-neuronal interactions during the pre-symptomatic stages of PD development and disease progression. Using this non-neurocentric approach, we characterize the involvement of microglia on PD progression, disease-related subpopulations, and potential therapeutic disease modifying targets in PD.

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P17. PARACRINE EFFECTS OF ASTROCYTES IN PARKINSON'S DISEASE PATHOLOGY: A STUDY USING IPSC-DERIVED NEURONS AND ASTROCYTES CARRYING THE P.A53T-ASYN MUTATION

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Aim of the study: Synucleinopathies are a group of neurodegenerative disorders characterized by misfolded α-Synuclein (αSyn) aggregates in the central and peripheral nervous systems. In Parkinson's disease (PD) one of the best-researched mutations is G209A in the SNCA gene resulting in the pathological p.A53T-αSyn protein. While most research work had focused on neuron-intrinsic deficits and degeneration, studies introducing neuron-glia interactions in PD pathology have recently started emerging. Astrocytes, the most abundant cells in human brain, play critical roles in neuronal health while they can exert neuroprotective or neurotoxic effects upon disease. Recent studies demonstrate that astrocytes may contribute to neuronal health status through their secretome (Yang, Y. et al., PNAS 2022, *119*(29), e2110746119; Barbar, L. et al., Neuron 2020, *107*(3), 436–453.e12). Our aim is to elucidate whether there are interactions between astrocytes and neurons carrying the p.A53T-αSyn mutation that are facilitated by secreted mediators and contribute to PD pathology.
Materials and methods: We used our previously established induced pluripotent stem cell (iPSC)-based neuronal model from patients harboring the p.A53T mutation, which displays disease-associated phenotypes, including protein aggregates, axonal pathology, and compromised network connectivity and additionally generated and characterized ventral midbrain-patterned iPSC-derived astrocytes. To investigate the paracrine mechanisms underlying the midbrain neuron-astrocyte interactions, both control and p.A53T neurons were treated with control and p.A53T astrocyte conditioned media (ACM) at all possible combinations.
Results: Our data show that control neurons treated with conditioned medium from p.A53T-αSyn astrocytes (PD ACM vs control ACM) display reduced neuronal viability with an increased vulnerability of tyrosine hydroxylase-positive dopaminergic neurons, and increased levels of total neuronal aSyn. Moreover, treatment of p.A53T-αSyn neurons with conditioned medium from control astrocytes (control ACM vs PD ACM) reduces the number of detected intraneuronal protein aggregates, ameliorates neurite network extension, and increases neuronal survival.
Conclusions: Our results indicate that p.A53T-αSyn mutant astrocytes contribute to the neurodegeneration process whereas control astrocytes mitigate the pathological phenotype of

PD neurons and that these effects are mediated at least partially through their secretome in a paracrine fashion. The study of the underlying molecular/cellular pathways is ongoing.

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