ABSTRACT BOOK

UPDATES ON GENETICS AND EPIGENETICS OF HEMATOLOGICAL MALIGNANCIES: FROM KNOWLEDGE TO APPLICATIONS

20-22 November, 2024

Josep Carreras Leukaemia Research Institute (IJC)

Badalona, Barcelona

MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES
AGENCIA ESTATAL DE INVESTIGACIÓN
EXCELENCIA SEVERO OCHOA
04/2024 - 03/2030
Josep Carreras LEUKAEMIA Research Institute

TABLE OF CONTENTS

WELCOME

We are pleased to welcome you to the Symposium "**Updates on Genetics and Epigenetics of Haematological Malignancies: From Knowledge to Applications**". Organized by Josep Carreras Leukaemia Research Institute, the meeting will be held at the headquarters of the Josep Carreras Leukaemia Research Institute, Badalona, Barcelona, from **20th-22nd November 2024**.

During the symposium, world-class experts will share their views and thoughts on their journey against cancer from multiple points of view: cell biology, immunology, genetics, epigenetics and computational biology, among others. Together, we will gather an interdisciplinary representation of the finest researchers, from the basic and clinical sciences, to present their latest publications and discuss with their peers openly.

The latest technologies like single cell analysis, next generation sequencing and genome editing will be also key players of the event, with experts on the field commenting on the endless possibilities they open for research and diagnosis.

We hope that the conference will meet all expectations and that fruitful discussions and new collaborations will be established as a result.

Warm regards,

Laura Belver, Anna Bigas, Marcus Buschbeck and Manel Esteller.

PROGRAMME

UPDATES ON GENETICS AND EPIGENETICS OF HEMATOLOGICAL MALIGNANCIES: FROM KNOWLEDGE TO APPLICATIONS

Wednesday, 20 November

13:30 – 14:30 Registration

14:30 – 14:40 Welcome - **Manel Esteller**
Josep Carreras Leukaemia Research Institute (IJC), CIBERONC

14:40 – 15:25 Opening Lecture, introduced by Manel Esteller
Ross Levine
Memorial Sloan Kettering Institute, New York, USA
"Deciphering and Targeting Clonal Evolution to AML"

15:30 – 16:30 **Session 1 - Stem Cells and Premalignancy**
Session Chair: Gregoire Stik
Senior lectures (20+10)
Michael Milsom
German Cancer Research Center (DKFZ), Heidelberg, Germany
"Pre-malignant programming of hematopoietic stem cells during aging and inflammation"

Florian H. Heidel
Hannover Medical School (MHH), Germany
"Epigenetic regulators as targets and effectors of cell signaling"

16:30 – 16:45 Selected Short talk (10+2)
Michael Scherer
German Cancer Research Center (DKFZ), Heidelberg, Germany
"Unraveling clonal identity and cell state in hematopoiesis from single-cell DNA methylation data"

16:45 – 17:10 Group Photo and Coffee break

17:15 – 17:30 Selected Short talk (10+2)
Cristina Lopez-Rodriguez
Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona, Spain
"Fine-tuning of hematopoiesis under inflammatory stress"

17:30 – 18:00 Senior lecture (20+10)
Vincenzo Calvanese
Josep Carreras Leukaemia Research Institute (IJC), Catalonia, Spain
"Chromatin and transcriptional modulation in human blood stem cell self-renewal"

18:00 – 19:30 IJC Poster session "get to know us", Cocktail and Networking

PROGRAMME

Thursday, 21 November

09:00 – 11:00 **Session 2 - Premalignancy, Immune Niches and Disorders**

Session Chair: Marcus Buschbeck

Senior lectures (20+10)

George Vassiliou
Wellcome-MRC Cambridge Stem Cell Institute, Cambridge, UK
"Clonal haematopoiesis and leukaemia prevention"

Bruno Paiva
Clinica Universidad de Navarra, Centro de Investigación Médica Aplicada (CIMA), Spain
"Immunogenomic research in monoclonal gammopathies"

Alberto Orfao
University of Salamanca, CIBERONC, Spain
"Oncogenetic pathways and immune response in systemic mastocytosis: from bone marrow mastocytosis to advanced disease subtypes"

Brigitte Schlegelberger
Hannover Medical School (MHH), Germany
"How to deal with genetic predisposition to hematologic malignancies"

11:00 – 11:20 Coffee break

11:30 – 13:00 **Session 3 - Biology and Treatment of Myeloid Neoplasms**

Session Chair: Manel Esteller

Senior lectures (20+10)

Iannis Aifantis
New York University (NYU), New York, USA
"Mapping of tumor-immune system interactions in myeloid neoplasms"

Matteo Della Porta
Humanitas Cancer Center, Milan, Italy
"A single cell multi-omics approach to understand the mechanisms of leukemic evolution in patients with myelodysplastic syndromes"

Courtney DiNardo
MD Anderson, Houston, USA
"Individualizing AML Therapy: Best Practices and Novel Therapeutics in 2024"

13:00 – 13:15 Selected Short talk (10+2)

Alessandra Ferrelli
The Francis Crick Institute, London, UK
"Developing a humanised in vivo model to study myeloproliferative neoplasms"

13:15 – 14:00 Lunch and Poster Session

PROGRAMME

Thursday, 21 November

14:00 – 15:00 **Session 4 - Epigenetics and Chromatin Dynamics in Hematological Malignancies**

Session Chair: Biola M. Javierre

Senior lectures (20+10)

Manel Esteller

Josep Carreras Leukaemia Research Institute (IJC), CIBERONC, Catalonia, Spain

"Epigenetics and Epitranscriptomics in Hematological Malignancies"

Christoph Plass

German Cancer Research Center (DKFZ), Heidelberg, Germany

"Enhancer hijacking in acute myeloid leukemia"

15:10 – 15:55 Selected Short talk (10+2)

Andreas Lennartsson

Karolinska Institutet, Solna, Sweden

"Targeting Polycomb in the treatment of KMT2A-rearranged acute myeloid leukemia"

Alba Maiques-Diaz

Clínic Foundation for Biomedical Research - August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain

"Chronic lymphocytic leukemia hijacks germinal center B cell transcriptional and epigenetic mechanisms to proliferate"

Ruslan Sadreyev

Massachusetts General Hospital, Boston, USA

"AML aggressiveness is driven by chromatin remodeling and expression changes of core regulators"

16:00 – 16:15 Coffee break

16:20 – 17:20 **Session 5 - Biology and Treatment of Acute Leukemias**

Session Chair: Laura Belver

Senior lectures (20+10)

Jan Cools

KU Leuven, Center for Human Genetics, Belgium

"Transcriptional deregulation in T-cell acute lymphoblastic leukemia"

Maria L. Toribio

Centro de Biología Molecular "Severo Ochoa", Madrid, Spain

"Pre-TCR targeting as a Selective Immunotherapy for T-cell Acute Lymphoblastic Leukemia"

17:30 – 17:45 Selected Short talk (10+2)

Alexander Waclawiczek

German Cancer Research Center (DKFZ), Heidelberg, Germany

"Four Leukemic Stem Cell Types mediate Resistance and Response in Acute Myeloid Leukemia"

17:45 – 18:45 Poster session, Cocktail and Networking

PROGRAMME

Friday, 22 November

09:00 – 10:30 **Session 6 - Biology and Treatment of Lymphomas and Myelomas**

Session Chair: Tomàs Navarro

Senior lectures (20+10)

Anna Bigas

Josep Carreras Leukaemia Research Institute (IJC), Hospital del Mar Research Institute, CIBERONC, Catalonia, Spain

"From Stem Cell Biology to Cancer Therapeutics"

Teresa Palomero

Columbia University, New York, USA

"Navigating the Molecular Landscape of Peripheral T-Cell Lymphomas: from Somatic Mutations to Mouse Models and Beyond"

Francesco Bertoni

Institute of Oncology Research, Bellinzona, Switzerland

"Mechanisms of resistance to BCR signaling inhibitors in B-cell lymphomas"

10:30 – 11:00 Selected Short talk (10+2)

Nuno R. dos Santos

i3S - Institute for Research and Innovation in Health - University of Porto, Porto Portugal

"Antibody blockade of the PSGL-1 immune checkpoint enhances T-cell responses to B-cell lymphoma"

Wim Vanden Berghe

University of Antwerp, Antwerp, Belgium

"Resolving epigenetic sensitization mechanisms of ferroptosis signaling to overcome therapy resistance in multiple myeloma"

11:00 – 11:20 Coffee break

11:30 – 11.40 Molecular Oncology Poster Presentation Awards

11:40 – 12.10 Closing Lecture, introduced by Manel Esteller

Ari Melnick

Weill Cornell Medicine, New York, USA

"Epigenetic mechanisms and targeted therapies for B cell lymphoma"

12:10 – 12:15 Final remarks and Farewell

ORGANIZERS

Laura Belver

Josep Carreras Leukaemia Research Institute (IJC), Catalonia, Spain

Dr. Laura Belver (Madrid, Spain, 1983) earned her degrees in Biology (2006) and Biochemistry (2011) from the Autonomous University of Madrid, where she also completed her PhD in Molecular Biology under the supervision of Dr. Almudena Ramiro at the Spanish National Cancer Center (CNIO). Her doctoral research, which explored the role of microRNAs in preventing B-cell-driven autoimmunity (Belver et al., Immunity, 2010), earned her the IV Biogen-Idec Award for Young Scientists and the Outstanding Doctoral Thesis Award from the Autonomous University of Madrid.

In 2012, Dr. Belver joined Dr. Adolfo Ferrando's lab at Columbia University (New York, NY, USA) as a postdoctoral researcher. Her work there centered on molecular mechanisms regulating enhancer function in T-cell acute lymphoblastic leukemia (T-ALL). She was the first to demonstrate the dominant role of oncogenic enhancer accessibility in the development of leukemia (Belver et al., Cancer Discovery, 2019), an achievement recognized at international conferences with several awards, including the Acute Leukemia Forum Young Investigator Award, the European School of Haematology Early Career International Award, and the American Society of Hematology Abstract Achievement Award.

Dr. Belver's work in hemato-oncology spans beyond T-ALL, with significant contributions to other studies on acute lymphoblastic leukemia (Schnell et al., Blood, 2015; Herranz et al., Nature Medicine, 2015; Oshima et al., Nature Cancer, 2020; Antoszewski et al., Blood, 2022), chronic lymphocytic leukemia (Puente et al., Nature, 2015; Fabbri et al., PNAS, 2017), angioimmunoblastic T-cell lymphoma (Cortés et al., Cancer Cell, 2018), diffuse large B-cell lymphoma (Delgado et al., PLoS Genetics, 2021), mantle cell lymphoma (Vilarrasa-Blasi et al., Leukemia, 2022), and multiple myeloma (Gómez-Echarte et al., Haematologica, 2024).

Since 2020, Dr. Belver has led her own research group as a Junior Group Leader at the Josep Carreras Leukemia Research Institute (IJC). Her lab focuses on deciphering the molecular mechanisms underlying various blood cancers and on advancing experimental therapies to improve treatment outcomes for these diseases.

ORGANIZERS

Anna Bigas

Josep Carreras Leukaemia Research Institute (IJC),
Hospital del Mar Research Institute,
CIBERONC,
Catalonia, Spain

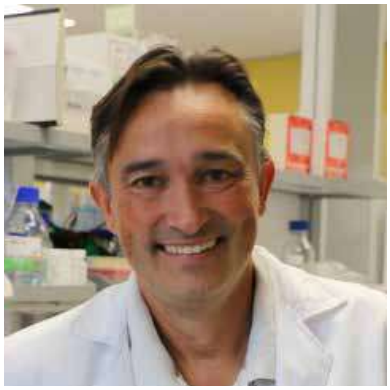
Dr. Anna Bigas holds a PhD in Cell Biology from the University of Barcelona in 1993. Since 2009, she is the coordinator of the Stem Cells and Cancer laboratory in the Hospital del Mar Research Institute (HMRI-IMIM), and since 2020, has a dual affiliation with Josep Carreras Leukemia Research Institute (IJC). Dr. Bigas has a long-standing research interest on hematopoietic stem cells and leukemogenesis. As a postdoctoral fellow at the Fred Hutchinson Cancer Research Center in Seattle (1993-1997), Dr. Bigas made a groundbreaking discovery regarding the role of Notch signaling in hematopoietic differentiation, a significant contribution to the field of hematopoiesis (PNAS 1996, Mol. Cell. Biol 1998).

Since establishing her independent research group in Barcelona, Spain (IRO/IDIBELL, 1998), she has worked to uncover the molecular mechanisms governing stem cell commitment, maintenance, differentiation, and oncogenic transformation, particularly within the hematopoietic system. Through advanced genetic studies, her group has identified key developmental signals necessary for hematopoietic stem cell generation (Development, 2005; EMBO J 2008; JEM 2012, 2013, 2014; Nat Comm 2015; EMBO J 2020; Nat Comm 2024).

Her research has also expanded to investigate oncogenesis and therapy resistance in leukemia and solid cancer. Her studies have primarily focused on the Notch, Wnt, and NF-kB signaling pathways, with applications to T-Acute Lymphoblastic Leukemia, T-ALL (Cancer Cell 2010; Leukemia 2016; Genome Biol 2020; EMBO Mol. Med 2023), cutaneous T-cell lymphoma (Leukemia 2018; Blood Adv 2020), Myelodysplastic and GATA2 syndrome (Haematologica 2023), and intestinal development and cancer (PNAS 2009; Development 2015; Mol Cell 2019).

Dr. Bigas currently serves as the Scientific Director of the National Spanish Cancer Network, CIBERONC (CIBER, Instituto de Salud Carlos III), where she promotes national collaborative cancer research initiatives and is actively involved in European cancer mission projects, including efforts to create a National and European cancer data hub (uncan.eu; Cancer Discov. 2024).

ORGANIZERS



Marcus Buschbeck

Josep Carreras Leukaemia Research Institute (IJC), Catalonia, Spain

Dr. Marcus Buschbeck has been trained in molecular cancer research and chromatin biology at several institutions that include the Max-Planck-Institute of Biochemistry, the University of Oxford and the Center of Genomic Regulation. In 2009 he combined the two fields to start his lab at the IMPPC, a small institute embedded in the biomedical research Can Ruti location in the oustkirts of Barcelona, Spain. By joining the Josep Carreras Institute in Leukaemia Research on the same location at the beginning of 2015 Dr. Buschbeck has also started new lines of research focusing on the hematopoietic stem cell defects known as myelodysplastic syndromes and the blood cancer myeloid leukaemia.

ORGANIZERS

Manel Esteller

Josep Carreras Leukaemia Research Institute (IJC),
CIBERONC
Catalonia, Spain

He graduated in Medicine from the Universitat de Barcelona, where he also obtained his Ph.D. in molecular genetics. Dr. Esteller was a Postdoctoral Fellow and a Research Associate at Johns Hopkins where he studied DNA methylation and human cancer. His work was decisive in establishing promoter hypermethylation of tumor suppressor genes as a common hallmark of cancer.

From October 2001 to September 2008 Manel Esteller was the Leader of the CNIO Cancer Epigenetics Laboratory, where his principal area of research were the alterations in DNA methylation, histone modifications and chromatin in human cancer. Since October 2008 until May 2019, Dr Esteller was the Director of the Cancer Epigenetics and Biology Program (PEBC) in Barcelona and for the period 2019-2024 he guided the Josep Carreras Leukaemia Research Institute (IJC) as Director. Currently he is the Cancer Epigenetics Group Leader at the Josep Carreras Institute, Chairman of Genetics in the School of Medicine of the University of Barcelona, and ICREA Research Professor.

His current research is devoted to the establishment of the epigenome maps in health and disease, and the development of new epigenetic drugs. Author of numerous and highly cited peer-reviewed manuscripts in biomedical sciences, he is also a Member of numerous international scientific societies, Editorial Boards and reviewer for many journals and funding agencies. He has received prestigious recognitions for his scientific achievements among them the World Health Summit Award, the Swiss Bridge Cancer Award and the EACR Cancer Researcher Award Lecture.

Iannis Aifantis

New York University (NYU)
New York, USA

Known for his expertise in the fields of hematopoiesis and acute leukemia, Dr. Aifantis is a Professor and the Chair of the Department of Pathology at NYU School of Medicine. Dr. Aifantis attended the University of Crete in Greece, earned his PhD from the University of Paris V, Rene Descartes and completed his postdoctoral training at Harvard University, Dana Farber Cancer Institute. He started his independent career at University of Chicago in 2013 and joined NYU in 2006.?Throughout his career; he earned many prestigious honors including the 2010 Vilcek Award for Creative Promise and the 2011 McCulloch & Till Award from the International Society?for Hematology and Stem Cell Biology. Moreover, in 2009, he was selected as an Early Career Scientist by the Howard Hughes Medical Institute (HHMI). He is one of the leaders of the fields of hematopoiesis and leukemia, with diverse focus areas that include the study of protein stability, transcriptional/epigenetic regulation and tumor microenvironment. His lab was instrumental in the understanding of the molecular mechanisms of initiation and progression of both acute lymphoid and myeloid leukemia.

Francesco Bertoni

Institute of Oncology Research
Bellinzona, Switzerland

Francesco Bertoni graduated in Medicine and post-graduated in Medical Oncology at the State University of Milan (Italy). He first moved to Switzerland, working with Prof. Emanuele Zucca in the team of Prof. Franco Cavalli in 1995. He then spent periods in London at the Molecular Haematology Unit of the Institute of Child Health in London, in the Department of Oncology of the "Mario Negri" Institute of Pharmacological Research in Milan, Italy, and in the Department of Experimental Haematology of The Barts and The London - Queen Mary's School of Medicine and Dentistry. Since 2003, he has been a group leader and the deputy director at the Institute of Oncology Research in Bellinzona, Switzerland. He is an Adjunct Professor at USI (Universita' della Svizzera italiana), and the current president of the Lymphoma Project Group of the Swiss Group for Clinical Cancer Research (SAKK). He is also the president of the Local Organizing Committee of the International Conference on Malignant Lymphoma (ICML) and a research consultant for the Bellinzona Oncology Southern Switzerland (IOSI).

His interests are anti-lymphoma compounds and lymphoma genomics.

Bertoni is the author/co-author of over 200 original papers, over 50 editorials/invited reviews, 20 book sections, and several abstracts at meetings. Bertoni is the editor-in-chief of Hematological Oncology.

Vincenzo Calvanese

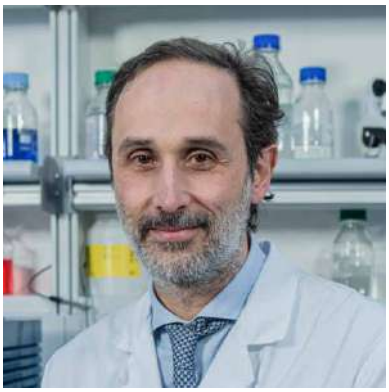
Josep Carreras Leukaemia Research Institute (IJC)
Catalonia, Spain

Dr. Vincenzo Calvanese started working on human stem cells during his PhD at the Spanish National Cancer Research Centre (CNIO) and the Spanish National Biotechnology Centre (CNB), in Madrid, Spain, where he described several epigenetic pathways involved in embryonic stem cell differentiation, directed by Dr. Mario Fraga. After obtaining a PhD in molecular biology from the Autonomous University of Madrid in 2010, he pursued a postdoctoral career in California, USA. Vincenzo's first postdoc experience was aimed of understanding the epigenetic regulation of HIV latency, in Dr. Eric Verdin lab at the Gladstone Institutes in San Francisco. In 2012 he joined Hanna Mikkola's group at Broad Stem Cell Research Center in UCLA, for the study of gene regulation of human hematopoietic stem cells. First as postdoc and later (from 2016) as project scientist in Mikkola's group, he pursued several research lines focused on key areas of human HSC biology. He used a developmental viewpoint, dissecting human HSC generation in vivo and in vitro, as well as a molecular viewpoint, identifying key markers and gene regulation states of HSC stemness. In April 2021, Vincenzo was appointed Junior Group Leader of the Stem Cell Self-Renewal in Hematopoiesis lab at the Laboratory for Molecular Cell Biology, University College London (LMCB-UCL), UK, where he was awarded a Sir Henry Dale research fellowship (Wellcome/Royal Society) to study novel aspects of HSC self-renewal regulation at the crossroad of epigenetics, transcription and metabolism. In 2024, the Calvanese group opens a new location to the Josep Carreras Institute, funded by a Consolidator grant (ERC), an ATRAE grant (Spanish Government) and further support by the Josep Carreras Foundation and the Generalitat de Catalunya, to expand his studies on human HSC and devise new methods to obtain functional cells in vitro to treat leukaemia and other blood diseases.

Jan Cools

KU Leuven, Center for Human Genetics
Belgium

Jan Cools obtained his PhD in 2001 from KU Leuven (Belgium) with a study on chromosomal defects in leukemia. From 2001 to 2003 he continued his research on the genetic causes of leukemia at Harvard Medical School (Boston) where he studied myeloid leukemias and identified the FIP1L1-PDGFRA fusion as a cause of chronic eosinophilic leukemia. After return to Belgium, he was appointed as assistant professor in 2005 and full professor in 2009 at KU Leuven. Since 2008 he is also group leader at VIB, a life sciences institute in Flanders. His team studies the genetic complexity of acute lymphoblastic leukemia and uses that information to develop models of leukemia and novel treatment strategies. He is editor-in-chief of HemaSphere, the new journal of the European Hematology Association (EHA).



Matteo Della Porta

Humanitas Cancer Center
Milan, Italy

He obtained his Medical Degree in Medicine and Surgery with honours at the University of Pavia Medical School, Pavia, Italy in 2000. He completed a postdoctoral fellowship at the University of Pavia under the mentorship of Prof. Mario Cazzola.

From 2008 to 2015, he was an Associate Professor of Clinical Oncology at the University of Pavia.

In 2016, he moved to Humanitas Research Hospital in Milan, where he currently serves as a Full Professor of Hematology and Head of the Leukemia Unit.

His current research interests focus primarily on myeloid neoplasms, particularly in the areas of prognostication, genomics, and the development of artificial intelligence solutions for precision medicine.

Courtney DiNardo

MD Anderson
Houston, USA

Dr. DiNardo is an academic clinical researcher with a primary focus on individualized therapy and precision oncology for myeloid malignancies, including the optimal incorporation of genomics into standard risk assessments and treatment algorithms, the clinical evaluation of targeted therapeutics for molecularly-defined patient subgroups, and designing and executing successful clinical trials.

Dr. DiNardo has served an integral role in several highly influential trials involving IDH1, IDH2 and BCL2 inhibitors, which have led to the FDA approval of three therapies in AML since 2017 (the first-in-class IDH2 inhibitor enasidenib, the IDH1 inhibitor ivosidenib, and the BCL2 inhibitor venetoclax in combination with hypomethylating agents).

Florian H. Heidel

Hannover Medical School (MHH)
Germany

Professor Florian Heidel studied Medicine at the University of Erlangen (Germany) where he also joined the laboratory of Professor Rolf Marschalek to study childhood leukemias harboring KMT2A-fusions. He completed his clinical residency and fellowship in internal medicine, hematology and medical oncology in Mainz under Professor Christoph Huber until 2008. He joined the laboratory of Professor Scott Armstrong at Dana-Farber Cancer Institute, Harvard University, Boston to study regulation of self-renewal in normal and malignant stem cells. In 2015, Prof. Heidel was appointed W2 Professor at the University Hospital Jena and the Leibniz Institute on Aging, FLI, Jena where his research group focused on functional studies of cell signaling, specifically JAK-signaling in models of aging-associated clonal hematopoiesis and myeloid neoplasms.

Since 2023 Professor Florian Heidel is the director and chair of Haematology at the Department of Haematology, Haemostasis, Oncology and Stem Cell Transplantation at Hannover Medical School, Germany. The Department of Haematology, Haemostasis, Oncology and Stem Cell Transplantation at Hannover Medical School, Germany is a center of excellence for acute leukemias and MPN and one of the largest bone marrow transplant centres in Germany.

His research interest is focused on stem cell biology and myeloid neoplasia with a particular interest in myeloproliferative neoplasms (MPN), and he serves as a speaker of the German MPN Study Group.

Prof. Heidel is conducting clinical trials for myeloid neoplasms and heading an experimental research group at Hannover Medical School and the Leibniz Institute on Aging in Jena.

Ross Levine

Memorial Sloan Kettering Institute
New York, USA

Ross Levine is the Senior Vice President, Translational Research, Memorial Hospital at Memorial Sloan Kettering (MSK). He is also an Attending Physician on the Leukemia Service, Department of Medicine, the Laurence Joseph Dineen Chair in Leukemia Research and a Professor of Medicine at Weill Cornell Medical College. Dr. Levine earned his A.B. from Harvard College and a M.D. from Johns Hopkins. Dr. Levine served as a Resident in Internal Medicine at Massachusetts General Hospital and as a Hematology-Oncology Fellow at Dana-Farber Cancer Institute. His laboratory focuses on elucidating the genetic basis of myeloid malignancies, and using this knowledge to improve outcomes for patients with these disorders. Moreover, as a physician scientist, his laboratory has a specific interest in translating this knowledge back to the clinic and in participating in the preclinical and clinical evaluation of targeted therapies for leukemia patients. He has been honored with the Dameshek Prize from the American Society of Hematology, the Boyer Award for Clinical Investigation from Memorial Sloan Kettering Cancer Center, and a NCI Outstanding Investigator R35 Award.

Ari Melnick

Weill Cornell Medicine
New York, USA

Dr. Melnick is a leader in the field of hematologic malignancies and cancer epigenetics. Some of his major findings include the first large-scale epigenomics studies in humans, showing that aberrant epigenetic programming is a hallmark of cancer and that epigenetic diversity is a fundamental determinant of tumor fitness and unfavorable clinical outcomes. This work led him to important mechanistic observations, for example how mutations in IDH1, IDH2 disrupt the epigenome through production of an aberrant oncometabolite, and how mutations in TET2, WT1, IDH1/2 and FLT3 drive leukemogenesis through cooperative reprogramming of the epigenome. He has fundamentally established the role of epigenetic dysfunction in lymphomagenesis, through mechanistic analysis of chromatin modifiers such as EZH2, CREBBP, EP300, KDM1A, KMT2D, HISTH1, BTG1, ARID1A, TBL1XR1 and TET2. He has pioneered the study of nuclear topology mechanisms controlling the humoral immune response and leading to malignant transformation of B-cells. He has developed novel targeted therapies against key lymphoma oncoprotein such as BCL6, SIRT3 and MALT1, and developed the rationale for a number of other targets such as EZH2. Several therapeutic approaches he proposed have been FDA approved or moved to phase III clinical trials. He has authored more than 340 manuscripts, has held many national leadership roles, organized a number of scientific meetings, and invested considerable effort in mentoring trainees and junior faculty, many of whom currently have their own highly distinguished careers in basic and translational research. Dr. Melnick is on the Board of Directors of the Leukemia and Lymphoma Society and the Lymphoma Research Foundation.

Michael Milsom

German Cancer Research Center (DKFZ)
Heidelberg, Germany

Dr. Milsom carried out his PhD. studies at the University of Leeds in the UK, then moved to the Cancer Research UK Manchester Institute in 2001 to work as a Postdoctoral Fellow in the group of Dr. Leslie J. Fairbairn. It was here that Dr. Milsom first became interested in studying hematopoiesis and developed a particular focus on the response of hematopoietic stem cells to chemotherapy. In 2005, Dr. Milsom joined the group of Dr. David A. Williams at Cincinnati Children's Hospital in order to further pursue his work into the role of the DNA damage response in modulating hematopoietic stem and progenitor cell behaviour. This included engineering chemoresistance in stem cells via retroviral gene therapy and the study of bone marrow failure in the inherited bone marrow failure syndrome, Fanconi anemia. Dr. Milsom relocated to Boston Children's Hospital in 2008, again working in the group of David Williams. As well as continuing his studies into the cellular consequences of the defective DNA damage response in Fanconi anemia, he additionally helped devise a novel lentiviral vector with which to treat sickle cell anemia, via targeted knockdown of the transcription factor Bcl11a. In 2010, Dr. Milsom started his own independent research group at the German Cancer Research Center (DKFZ) and Heidelberg Institute for Stem Cell Technology (HI-STEM), in Heidelberg, Germany. In 2017, Dr. Milsom achieved tenure and was promoted to the position of head of the Division of Experimental Hematology. His group continues to focus on the causes and consequences of DNA damage within the hematopoietic stem and progenitor cell department, but have also broadened the focus of their work to encompass other phenomena that have a profound effect upon stem cell aging and malignant transformation, including aberrant programming of the DNA methylome.

Important research outputs include the identification of environmental stress agonists (including inflammation) as a driver of DNA damage in hematopoietic stem cells in vivo and critical mediators of bone marrow failure in the setting of Fanconi anemia1; characterization of differentially methylated regions of the genome which likely govern the execution of lineage-specific gene expression programs during early steps of hematopoietic differentiation2; and the finding that inflammation and infection can drive an irreversible depletion of functional hematopoietic stem cells in vivo, meaning that such challenges can have an cumulative effect over a lifetime and therefore drive the aging process3. Dr. Milsom's group have also contributed to studies which have explored the role of the niche in stem cell aging4, and the nature of mutation acquisition within the stem cell compartment during aging5.

Teresa Palomero

Columbia University
New York, USA

Dr. Teresa Palomero graduated with a degree in Biology from the University of Oviedo, Spain, where she also earned her Ph.D. in Biochemistry and Molecular Biology. She subsequently became a Postdoctoral Fellow at the Dana-Farber Cancer Institute under the mentorship of Dr. Thomas Look. There, she focused on transcription factor oncogenes in T-cell acute lymphoblastic leukemia (T-ALL), employing both zebrafish and human models.

In 2005, Dr. Palomero joined the faculty of the Institute for Cancer Genetics at Columbia University, where her research has concentrated on the genetics and mechanisms of T-cell malignancies. She developed a cutting-edge research program that integrates genomic tools to dissect the oncogenic programs responsible for the transformation of T-cell progenitors in T-ALL (PNAS 2006; PNAS 2009; Nat Med 2009; Nat Med 2012). Her pioneering work includes describing recurrent mutations in PTEN in T-ALL (Nat Med 2007), identifying PHF6 as a novel tumor suppressor gene (Nat Genet 2010) and discovering NT5C2 mutations linked to chemotherapy resistance (Tzoneva et al. Nat Med 2013, Oshima et al., PNAS 2016*; Reglero et al., Cancer Discov, 2022).

Currently, her group primarily studies the mechanisms of transformation in mature T-cell lymphomas, including Peripheral T-cell Lymphoma (PTCL) and Cutaneous T-cell Lymphoma (CTCL). She identified recurrent RHOA G17V and FYN mutations as major drivers of Angioimmunoblastic T-cell Lymphoma (AITL) (Palomero et al. Nat Genet 2014), dissected the mutational landscape of CTCL and Sézary Syndrome (da Silva Almeida et al., Nat Genet 2015*), discovered new activating mutations in VAV1 (Abate et al., PNAS 2017*), and characterized the recurrent FYN-TRAF3IP2 fusion in PTCL (Moon et al., Nature Cancer 2021*).

Her laboratory has also developed some of the first genetically engineered mouse models of AITL (Cortes et al., Cancer Cell 2018*) and PTCL, NOS (Moon et al., Nature Cancer 2021*; Cortes et al., Cell Rep 2022*), which serve as crucial tools for experimental therapeutics targeting these aggressive malignancies.

In addition to her research, Dr. Palomero is a member of numerous international scientific societies and serves on editorial boards and as a reviewer for various journals and funding agencies, both federal and non-profit.

SPEAKERS

Bruno Paiva

Clinica Universidad de Navarra,
Centro de Investigación Médica Aplicada (CIMA)
Spain

Bruno Paiva is Director of the Flow Cytometry Platform and Director of the Monoclonal Gammopathies Research Laboratory, both at the centre for applied medical research (CIMA) University of Navarra, Pamplona, Spain, where he is also a research fellow of the Department of Haematology. He gained his Doctor of Pharmacy degree in in 2007 from the University of Coimbra, Portugal, and his PhD at the Medical School of the University of Salamanca, Spain.

Dr Paiva's main area of expertise is the multidimensional flow cytometry analysis of haematological malignancies. His research focuses on immunogenomics to improve differential diagnosis, risk stratification, and monitoring of patients with monoclonal gammopathies and myeloid malignancies. Dr Paiva's flow cytometry core is the referral laboratory for numerous Hospitals and has been the core of more than 30 national and international clinical trials in multiple myeloma and acute myeloid leukaemia.

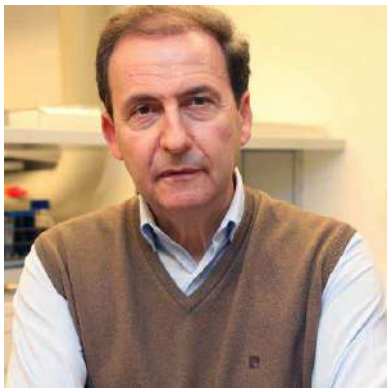
Throughout his 16-year-long research career, Dr Paiva has authored or co-authored more than 200 publications, including more than 30 publications in the most prestigious journal in the haematology sector: Blood. In 2015, Dr Paiva was awarded the Bart Barlogie Young Investigator Award for outstanding research developed in multiple myeloma. In 2022, Dr Paiva was awarded the Brian G.M. Durie Outstanding Achievement Award by the International Myeloma Foundation (IMF) for his contribution to improving the lives of patients with myeloma.

Christoph Plass

German Cancer Research Center (DKFZ)
Heidelberg, Germany

Christoph is the head of the Division of Cancer Epigenomics at the German Cancer Research Center (DKFZ) in Heidelberg. His research focuses on the epigenetic mechanisms driving cancer, with pioneering discoveries in DNA methylation and its role in cancer, particularly in leukemias and solid tumors of the lung, head and neck, and prostate.

Before joining DKFZ, Christoph spent over 15 years in the U.S., where he held prominent academic and research positions at the Roswell Park Cancer Institute and Ohio State University, where he served as a Professor in the Department of Human Cancer Genetics. Christoph earned his PhD in Molecular Genetics from the Medical University of Lübeck, Germany.
Christoph's contributions to science have been widely recognized. Christoph has authored over 400 research articles in prestigious journals, he is Editor-in-Chief of the International Journal of Cancer, and he is also an active member of several renowned societies, including the Mammalian Genome Society, the Society for Neuro-Oncology, the American Association for Cancer Research, and the American Society for Human Genetics.



Alberto Orfao

University of Salamanca, CIBERONC
Spain

Prof. Alberto Órfão, MD PhD, is Full Professor of Immunology (Department of Medicine), vicedirector of the Cancer Research Center of the University of Salamanca (Salamanca, Spain). He has co-authored >830 scientific publications and over 70 patents. He has been awarded with the Gold Medal of the Faculty of Medicine of the University of Coimbra (Coimbra, Portugal) and a Honoris Causa Doctorate from the University of Katowice (Poland), among other >40 recognitions and prizes (e.g., Berend Howen award of the International Society of Laboratory Hematology, Wallace Coulter award of the International Clinical Cytometry Society).

Brigitte Schlegelberger

Hannover Medical School (MHH)
Germany

Brigitte Schlegelberger, MD was head of the Department of Human Genetics at Hannover Medical School and Coordinator of the Subnetwork Familial Leukemia within the European Reference Network PAEDCAN until May last year. Currently, she is member of the advisory board of the ERN GENTURIS (genetic tumor risk syndromes) and member of the scientific advisory board of the Josep Carreras Leukaekemia Research Institute.

Using state-of-the-art techniques her research focuses on the identification of genetic abnormalities in adult and childhood hematological malignancies. She has extensive knowledge in familial leukemia and hereditary cancer. She aims to, not only, translate the increasing genetic knowledge into improved diagnostics, but also to build the basis for the care of affected patients and their families.

Maria L. Toribio

Centro de Biología Molecular "Severo Ochoa"
Madrid, Spain

Maria Luisa Toribio obtained her PhD degree in Biology from the Universidad Complutense de Madrid. She was trained in Immunology in Spain (Hospital Puerta de Hierro, Madrid, and Centro de Biología Molecular Severo Ochoa (CBM). CSIC-Universidad Autónoma de Madrid), France (Institute Pasteur, Paris) and Switzerland (Basel Institute for Immunology, Basel), and was Invited Researcher at the Center for Regenerative Medicine and Stem Cell Institute, Massachusetts General Hospital, Harvard Medical School (Boston, MA). She is CSIC Research Professor since 2003 and Head of the Interaction with the Environment Program (formerly Cell Biology and Immunology Department) at the CBM since 2014. She is an internationally recognized expert in the field of human T-cell development. Her research has contributed to our current understanding of the molecular bases of T-cell development in the human thymus and of the dysregulated pathways leading to T-cell acute lymphoblastic leukemia (T-ALL). The final goal of this research is the identification of novel targets for the development of specific therapies against relapse, the major hurdle of T-ALL. She authors >135 publications in the most prestigious international journals (>5,400 citations, h-index 44) and has received continuous competitive funding from National and International Research Agencies, and several private foundations. She has participated in several National and International Scientific Committees including ANEP, MICINN, ERC Starting Grant, ESF, ANR, SNSF, EHA Research Grants, Children with Cancer UK, Banco de Sabadell Biomedical Research, Fund. BBVA, and ICREA Scientific Evaluation Committee. She is editorial board member of several scientific journals and belongs to the SAB of the IMIM-Hospital del Mar Medical Research Institute, and the Josep Carreras Leukaemia Research Institute.

SPEAKERS

George Vassiliou

Wellcome-MRC Cambridge Stem Cell Institute
Cambridge, UK

George Vassiliou is Professor of Haematological Medicine and co-lead of the Haematological Malignancies Programme at the University of Cambridge, UK. He is also Senior Faculty at the Cambridge Stem Cell Institute and Consultant Haematologist at Cambridge University Hospitals.
His team studies the pre-clinical evolution, molecular pathogenesis and treatment of myeloid malignancies.

Highlights of their work include the co-discovery of the phenomenon of clonal haematopoiesis, the description of its natural history, the mapping of its causes and consequences and the development of multiparameter predictive tools for quantifying individual risk of progression to myeloid neoplasia (MN-Predict). They contributed to our understanding of the pathogenesis of myeloid cancers, including through the development and study of bespoke murine models (including for NPM1, SF3B1, IDH1, DNMT3A and UTX mutations) and identified many novel vulnerabilities of acute myeloid leukaemia through the first genome-wide CRISPR screen in a human cancer, including several for which inhibitors have been developed or entered clinical studies (e.g. METTL3, SRPK1, KAT2A and KAT7). The team also developed the first genomic diagnostic tools for myeloid cancers based on DNA (Karyogene) and RNA (RNAmut) sequencing.

Work in the Vassiliou lab is funded by Cancer Research UK, Wellcome, European Research Council, Leukaemia Lymphoma Society, Rising Tide Foundation for Clinical Cancer Research, Kay Kendall Leukaemia Fund, B lood Cancer UK, EMBO, Astrazeneca and the NIH-OxCam Scholars Program.

A Novel Leukemic Stem Cell Type Linked to Aggressive and Therapy-Resistant Acute Myeloid Leukemia with Monocytic/Dendritic features

Ines Bergerweiss[1], Alexander Waclawiczek[1], Aino-Maija Leppä[1], Dr. Simon Renders[1], Karolin Stumpf[1], Andreas Trumpp[1]

[1]Division of Stem Cells and Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany

Acute myeloid leukemia with monocytic differentiation (Mono-AML) is an AML subtype, characterized by the accumulation of mature, monocyte-like blasts. By combining omics technologies and xenograft models, we have identified a novel subtype of leukemic stem cells (LSCs) termed monocytic/dendritic-LSCs (MoDe-LSCs) in a subset of Mono-AML patients, which lack the conventional LSC markers CD34 and GPR56, but express differentiation markers like CD64 and efficiently engraft in PDX mice. In this aggressive form of AML, we have identified the lysosomal protein LAMP5 as a specific marker on blasts, which resemble monocytic and dendritic cell progenitors (MoDe-AML). MoDe-AML patients exhibit upfront resistance to the combination therapy of Venetoclax and 5-Azacitidine and show poor overall survival regardless of treatment regimen. Genetically, MoDe-LSCs display unique characteristics and are frequently found in AMLs with chromosomal abnormalities such as trisomy 8, KMT2A rearrangements, and complex karyotype. As a next step, we will use Tapestri technology to investigate the clonal evolution and hierarchical acquisition of leukemogenic drivers of MoDe-LSC development, by mapping single-cell copy number and single nucleotide variations, alongside cell surface markers. In summary, this study aims to enhance our understanding of the biological and genetic characteristics of these novel disease-driving MoDe-LSCs and to identify potential targets for this pan-therapy resistant AML subtype.

Activation of Distinct Genomic Regions Identify Subsets of MDS Patients Characterized by Immune Response Activation

Veronica Vallelonga[1], Francesco Gandolfi[1], Matteo Zampini[2], Elena Riva[2], Giulia Maggioni[2], Alberto Termanini[3], Laura Crisafulli[2,4], Francesca Ficara[2,4], Matteo Giovanni Della Porta[2,5], Serena Ghisletti[1,6]

[1]Department of Experimental Oncology, European Institute of Oncology (IEO) IRCCS, Milan, Italy

[2]IRCCS Humanitas Research Hospital, Milan, Italy

[3]Human Technopole, Milan, Italy

[4]Institute for Genetic and Biomedical Research, Milan Unit, CNR, Milan, Italy

[5]Department of Biomedical Sciences, Humanitas University, Milan, Italy

[6]DiSFeB, Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy

Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders with a risk of progression to secondary acute myeloid leukemia (sAML). This study investigates the molecular mechanisms driving MDS progression by characterizing active genomic regulatory regions and their transcriptional impact through H3K27ac ChIP-seq and RNA-seq analysis on CD34+ cells from 363 patients of low-risk MDS (LR-MDS), high-risk MDS (HR-MDS), and sAML. Our data reveal distinct patterns of genomic region activation and transcriptional regulation across different MDS stages. Notably, unsupervised clustering identified a subset of LR- MDS patients exhibiting regulatory and transcriptional signatures similar to HR-MDS and sAML, indicating early molecular events that may predispose patients to disease progression. This subset is characterized by PU.1 genomic occupancy in regions associated with immune and inflammatory response, and exhibits increased SRSF2 mutations, elevated risk of MDS progression, T cell activation and greater susceptibility to infections and cardiovascular events. Overall, this study identifies the molecular factors that predispose low-risk MDS patients to advance to high-risk MDS and eventually develop sAML.

Antibody blockade of the PSGL-1 immune checkpoint enhances T-cell responses to B-cell lymphoma

Nuno R. dos Santos[1], João Pereira[1], Liliana Arede[1], Francisca Ferreira[1], Andreia Matos[1], Dulcineia Pereira[2], Rita Santos[1], Alexandre Carmo[1], Maria José Oliveira[1], José Carlos Machado[1], Delfim Duarte[1]

[1]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

[2]Department of Hematology and Bone Marrow Transplantation, IPO Porto, Porto, Portugal

Despite advancements in cancer immunotherapy, most lymphomas remain unresponsive to checkpoint inhibitors. P-selectin glycoprotein ligand-1 (PSGL-1), recently identified as a promoter of T-cell exhaustion in murine melanoma models, has emerged as a novel immune checkpoint protein and promising immunotherapeutic target. In this study, we investigated the potential of PSGL-1 antibody targeting in B-cell lymphoma. Using allogeneic coculture systems, we demonstrated that targeted antibody interventions against human PSGL-1 enhanced T-cell activation and effector cytokine production in response to lymphoma cells. Moreover, in vitro treatment of primary lymphoma cell suspensions with PSGL-1 antibody resulted in increased activation of autologous lymphoma-infiltrating T cells. Using the A20 syngeneic B-cell lymphoma mouse model, we found that PSGL-1 antibody treatment significantly slowed tumor development and reduced the endpoint tumor burden. This antitumoral effect was accompanied by augmented tumor infiltration of CD4+ and CD8+ T cells and reduced infiltration of regulatory T cells. Finally, anti-PSGL-1 administration enhanced the expansion of CAR T cells previously transferred to mice bearing the aggressive E?-Myc lymphoma cells and improved disease control. These results demonstrate that PSGL-1 antibody blockade bolsters T-cell activity against B-cell lymphoma, suggesting a potential novel immunotherapeutic approach for treating these malignancies.

BCL7A role in lymphomagenesis specifically lies in two functionally distinct amino acids

Pablo Martín López[1], Sergio Castro González[1], Fernando Montenegro Elvira[1], Marta Cuadros Celorrio[1], Pedro Medina Vico[1]

[1]Universidad de Granada

Massive sequencing techniques in the last decade have emphasized the significance of epigenetic factors in tumor development. Notably, the SWI/SNF chromatin remodeling complexes, containing a high prevalence of mutations, play a crucial role. This complex modifies DNA-histone interactions, influencing DNA accessibility for transcriptional regulation. Comprising a catalytic subunit (SMARCA4/2) and associated proteins (BAFS), BCL7A is a recently discovered BAF subunit. Our previous research identified a high mutation frequency within exon 1, with the most prevalent mutation being a splicing alteration (?27 mutation) that results in a truncated, non-functional protein, underscoring the importance of the first exon of BCL7A in its tumor suppressor role. In this study we investigated the role of BCL7A in lymphomagenesis in vivo. Additionally, mutational analyses identified two critical amino acids essential for its tumor suppressor function. Furthermore, we explored the efficacy of SWI/SNF complex inhibitors in germinal center B-cell diffuse large B cell lymphoma (GCB-DLBCL) cell lines and mutant BCL7A contexts.

Chronic lymphocytic leukemia hijacks germinal center B cell transcriptional and epigenetic mechanisms to proliferate

Alba Maiques-Diaz[1], Anna Vidal[1], Liska Caviedes[1], Stella Charalampopoulou[1], Vicente Chapaprieta[1], Victor Jimenez[1], Raúl Fernandez[1], Judith Mateos-Jaimez[1], Dolors Colomer[1,2,3,4], Elias Campo[1,2,3,4], Jose I Martin-Subero[1,3,5]

[1]Fundació de Recerca Clínic Barcelona-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Barcelona, España

[2]Centro de Investigación Biomédica en Red Cáncer (CIBERONC), Madrid, España

[3]Facultad de Medicina, Universidad de Barcelona, Barcelona, España

[4]Hospital Clínic de Barcelona, Barcelona, España

[5]Institución Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, España

Chronic lymphocytic leukemia (CLL) is clinically very heterogeneous, with 25% of cases progressing within five years of diagnosis and requiring treatment, while others remain stable for years. Disease progression is driven by the expansion of clones in response to the lymph node (LN) microenvironment, but the underlying transcriptional and epigenetic rewiring is mostly unknown. In this study, we used an in vitro model of patient-derived CLL cells, to investigate the molecular mechanisms behind CLL proliferation.

We co-cultured peripheral blood (PB) cells from treatment-naive patients with murine stromal cells expressing hCD40 ligand, hIL21 and hBAFF (MM1 cells). After six days, over 80% of CLL cells proliferate (Ki67+, CFSElow), acquiring LN-like markers (CXCR4low, CD86high). Subsequently, we performed RNA-seq and H3K27ac ChIP-seq in 10 independent cases, 5 with a mutated IGHV gene (M-CLL, the clinically indolent subtype), and 5 with unmutated IGHV (U-CLL, the clinically more aggressive subtype), to include the biological and clinical spectrum of the disease. Interestingly, the co-culture induced a homogeneous transcriptional and epigenetic response in both subtypes. Upregulated genes were significantly involved in cell cycle, MYC, mTORC1 signaling, and oxidative phosphorylation, processes known to be operating in clinically aggressive cases. Moreover, H3K27ac ChIP-seq revealed an extensive chromatin reconfiguration with 3,944 regions gaining activation and 1,681 regions losing activation (Deseq2, FDR<10-7). We then characterized the H3K27ac dynamics in comparison with an extended series of PB-derived CLL cases (n=104) and normal B cells at different maturation stages (n=12) (Beekman, 2018), by k- means clustering. We identified that most chromatin activation changes were specific to proliferative CLL cells, with 1,168 CLL-specific peaks, but we also found a large signature of peaks in common with healthy proliferative or germinal center B cells (GCBC), with 800 CLL-GCBC peaks. CLL-specific peaks targeted genes involved in oxidative stress, B-cell receptor, and NFKB signaling (e.g., CCND2, IL10), while CLL-GCBC shared peaks were linked to cell cycle regulation (e.g., E2F2, CDK1) (Goseq analysis).

Finally, we identified transcription factors (TFs) upregulated in CLL cells co-cultured with MM1 cells and in two independent series of paired primary PB-LN CLL samples (n=24, Sun 2023; n=5, unpublished). We focused on TFs whose expression correlated with shorter time to first treatment (based on a multivariate Cox regression analysis of 403 cases from Puente 2015), or whose binding motifs were enriched in the differentially active chromatin regions of proliferative CLL. GFI1, a key TF in hematopoiesis, emerged as significantly upregulated in proliferative cells, highly associated with worse prognosis (Hazard ratio = 3.4, $p < 0.001$) and its binding motif is present in regions that gained H3K27ac upon proliferation (p adj<3.87e-03). GFI1 expression is significantly higher in U-CLL cells and in cases with the IGLV3-21-R110 mutation ($p<0.001$), both linked to an aggressive disease. Interestingly, in mature B cells GFI1 expression is only present at germinal centers. We confirmed that GFI1 protein levels increase in response to proliferative stimuli, including BCR stimulation, in primary CLL cells. CRISPR-Cas9 knock-out of GFI1 in patient-derived cells and cell lines led to reduced proliferation and metabolic activity, while overexpression of GFI1 enhanced the cells proliferative capacity. In summary, these findings suggest that CLL cells hijack GCBC-like mechanisms, such as GFI1 overexpression, to promote an aggressive and proliferative phenotype.

Co-occurring gene mutations alter survival and the genetic landscape in NRAS mutant acute myeloid leukaemia patients

Rachel Devenney[1], Rachelle Irwin[2], Colum Walsh[3], Kyle Matchett[1]

[1]Personalised Medicine Centre, School of Medicine, Ulster University, C-TRIC, Altnagelvin Hospital Campus, Glenshane Road, Derry/Londonderry, BT47 6SB, UK

[2]Centre for Genomic Medicine, Centre for Molecular Biosciences, Ulster University, Coleraine Campus, Cromore Road, Coleraine, BT521SA, Northern Ireland

[3]Department of Cell Biology, Division of Biomedical and Clinical Sciences, Campus US, Linköping University, Linköping, Sweden

Acute myeloid leukaemia (AML) arises from the clonal proliferation of immature myeloid cells due to the sequential acquisition of driver mutations. Patients often harbour a multitude of driver mutations which leads to the development of the disease. NRAS mutations are prevalent in 15% of AML patients and have been reported to have significant impact on survival in vivo when co- occurring with other gene alterations. Consequently, it is necessary to develop a deeper understanding of the implications of co- occurring mutations with mutant NRAS in AML. NRASG12D mutant and wild type cell models were generated using CRISPR-Cas9. An analysis was carried out in silico and in vitro to identify transcriptional and biological differences between NRAS mutant and NRAS wild type. Recurrent co-occurring mutations, and their impact on patient survival was identified utilising three AML patient datasets. Analysis of mRNA sequencing data identified differentially expressed genes and enriched pathways within a cohort of co- mutant patients in comparison to single mutant NRAS. Gene knockdown approaches were employed to investigate the biology of co-occurring mutation candidates in vitro. Survival analysis revealed a trend toward reduced overall survival in mutant NRAS patients compared to patients with wild type NRAS. Further analysis of mutational profiles showed recurrent co-occurring mutations with mutant NRAS including NPM1, SRSF2, FLT3 and epigenetic regulators DNMT3A, TET2, ASXL1. Survival outcomes were analysed in co-mutant patients. ASXL1 significantly reduced overall survival within patient cohorts. Several genes and pathways were found to be dysregulated and enriched within the co-mutant NRAS and ASXL1 cohort in comparison to single mutant NRAS patients. Evidently, co-occurring mutations with NRAS have a role in altering the disease landscape in AML patients. Therefore, there is a clear rationale for developing an understanding of the impact of co-mutations with mutant NRAS.

Deciphering the enhancer landscape of Juvenile Myelomonocytic Leukemia

Noel Gómez-Molina[1,2], Claudia Fiñana[1,2], Sandra Alonso-Moreno[1,2], Elisabeth Serracanta[1,2], Jordi Minguillón[3,4], Kevin Rouault-Pierre[5], Roshani Sinha[6], Emilio Lario[1], Angelika Merkel[1], Francesc Solé[1], Albert Català[7], Antonio Pérez-Martínez[3,4], Alice Bertaina[8], Dominique Bonnet[9], Hélène Cavé[10], Ángel Álvarez-Prado[11], Laura Belver[1,2]

[1]Josep Carreras Leukemia Research Institute (IJC), Badalona, Spain

[2]Catalan Institute of Oncology (ICO), Badalona, Spain

[3]Hospital La Paz Institute for Health Research (IdiPAZ), Madrid, Spain

[4]Spanish National Cancer Center (CNIO), Madrid, Spain

[5]Barts Cancer Institute, London, UK

[6]University of California, San Francisco (UCSF), USA

[7]Sant Joan de Deu Hospital, Barcelona, Spain

[8]Stanford University, Stanford, USA

[9]Francis Crick Institute, London, UK

[10]Robert Debré University Hospital, Paris, France

[11]Ludwig Institute for Cancer Research, Laussane, Switzerland

Juvenile Myelomonocytic Leukemia (JMML) is an aggressive pediatric myeloproliferative neoplasm, clinically characterized by prominent monocytosis and elevated fetal hemoglobin (HbF) levels, being this last feature associated with a worse patient prognosis. A pivotal challenge in JMML research is to better understand the mechanisms driving pathophysiology of the disease, as the coding mutations identified to date are insufficient to explain the complexity of the disease.

Despite the crucial role of enhancers and other non-coding regulatory elements in controlling gene expression, their involvement in JMML remains underexplored. To address this knowledge gap, this study investigates the role of aberrant enhancer function in JMML. First, we characterized the active enhancer profile of JMML cells and compared it to that of healthy hematopoietic stem / progenitor cells (HSPCs), the cell compartment from which JMML originates. This analysis revealed 6,859 aberrantly active and 1,888 aberrantly inactive enhancers in JMML compared to control HSPCs. Importantly, we found that JMML-specific enhancers are enriched with transcription factor binding sites (TFBSs) for key regulators of monocyte differentiation, such as KLF4, NF B, and STAT/IRF. In contrast, JMML aberrantly inactive enhancers are enriched for TFBS of EKLF and NFIA, both of which play a critical role in silencing HbF.

Together, this study provides new insights into the molecular mechanisms underlying central JMML clinical features, revealing a previously overlooked link between aberrant enhancer activity and both monocytosis and elevated HbF levels. Our findings highlight the key role of enhancer dysregulation in JMML and may pave the way for the development of novel therapeutic strategies for this disease.

Decoding genetic and non-genetic mechanisms of relapse and refractoriness in complex karyotype acute myeloid leukemia at single-cell resolution

Frank Y. Huang[1], Alvaro Andrades[2], Aino-Maija Leppä[1], Patrick Hasenfeld[2], Eva Benito[2], Markus Sohn[1], Krzysztof Mrózek[3], Ann-Kathrin Eisfeld[3], Jan O. Korbel[2], Andreas Trumpp[1]

[1]Division of Stem Cells and Cancer, German Cancer Research Center (DKFZ) and DKFZ- ZMBH Alliance, Heidelberg, Germany

[2]European Molecular Biology Laboratory, Genome Biology Unit, Heidelberg, Germany

[3]The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA

AML with complex karyotype (CK-AML), defined by ?3 structural variants (SVs), has a particularly poor prognosis. We have previously developed a single-cell multiomics framework combining haplotype-resolved analysis of SVs and nucleosome occupancy profiling (Strand-seq) with transcriptomic and proteomic profiling (CITE-seq) (Leppä et al., Nat Genet. 2024, in press). Here, we applied this to 39 paired samples from 19 donors obtained at diagnosis and relapse/refractory disease to study how SVs evolve under therapeutic pressure and drive disease progression. We detected SVs at single-cell resolution, ranging from simple deletions and duplications to chromothripsis. We reconstructed clonal phylogenies and identified monoclonal and polyclonal samples, with the latter showing linear or branched growth. Comparing paired samples, we identified distinct patterns of clonal evolution, including selection of major or minor subclones, stable phylogenies or emergence of new subclones. We profiled the same samples using CITE-seq and integrated both datasets via targeted discovery of SVs using Strand-seq-derived breakpoints. This revealed marked differences in cellular differentiation between subclones within samples. Despite the predominantly primitive phenotypes at diagnosis, cellular composition continued to shift towards more stem-like cell types. In summary, we present a comprehensive single-cell analysis of genetic and non-genetic determinants of relapse and refractoriness in CK-AML.

Design and functional characterization of a first-in-class irreversible inhibitor of HOIL-1-interacting protein (HOIP) with selective antitumor activity against B-cell non-Hodgkin lymphoma

Gema Gorjón-de-Pablo[1,2*], Ana Maria Montagut[1*], Marc Armengol[2*], Lucie Combes-Soia[3], Odile Burlet-Schiltz[3], Laia Huguet García[2], Nuría Profitós Pelejà[2], Juliana Carvalho Santos[2], Marcelo Lima Ribeiro[2], Miranda Fernández-Serrano[2], Judith Farrès[4], Pierre G. Lutz[5], Roger Estrada- Tejedor[1], José I. Borrell[1], and Gaël Roué[2]

[1]Grup de Química Farmacèutica, IQS School of Engineering, Ramon Llull University, Barcelona, Spain

[2]Lymphoma Translational Group, Josep Carreras Leukemia Research Institute, Autonomous University of Badalona, Badalona, Spain

[3]Institute of Pharmacology and Structural Biology (IPBS), University of Toulouse, CNRS, UPS, Toulouse, France

[4]Anaxomics Biotech SL, Barcelona, Catalonia, Spain

[5]Toulouse Institute for Infectious and Inflammatory Diseases, CNRS, Inserm, Toulouse, France

Activating single-nucleotide polymorphisms of HOIL-1-interacting protein (HOIP), the catalytic subunit of the linear ubiquitin chain assembly complex (LUBAC), have been shown to promote myeloid differentiation primary response 88 (MYD88)-mediated B- cell lymphomagenesis. In the framework of the EU-funded project, PROTEOblood, we recently demonstrated by coupling Tandem Ubiquitin Binding Entity to mass spectrometry analysis (TUBES-MS), that HOIP expression was positively modulated in diffuse large B-cell lymphoma (DLBCL) 3D spheroids upon the interaction of malignant B cells with tumor-associated macrophages (TAMs). To assess the relevance of targeting HOIP in the activated B-cell-like (ABC) subtype of DLBCL with MYD88 mutation, we used systems biology to build a mathematical model aimed at evaluating the impact of HOIP depletion on DLBCL tumors. From this model, an AI-mediated query demonstrated that HOIP blockade was associated with the suppression of three main pathophysiological motifs in malignant B cells, i.e., cell growth and proliferation, apoptosis evasion and deregulated metabolism. We then undertook a computational study that consisted of a combinatorial substitution of several α-β unsaturated moieties to be used as covalent binding warheads to the catalytic cysteine residue of HOIP. The resulting chemical library was used on subsequent molecular docking to assess the best HOIP binding candidates. Out of the four candidates synthesized from this library, we isolated compound A (Cpd A), a covalent irreversible inhibitor of HOIP with a pyrido[2,3- d]pyrimidine core, which exerted selective antitumor activity in a panel of ABCL- DLBCL cell lines (mean IC50 at 48hours: 90.7± 13.09 μM) while sparing normal B cells. Immunoprecipitation studies demonstrated that Cpd A was able to modulate the interaction between HOIP and the LUBAC component, SHARPIN, leading to the blockade of NF-κB signaling and to the downregulation of several downstream effectors, including CCL3, IL6 and IRF4. Specificity of Cpd A towards HOIP was confirmed by a drug affinity responsive target stability (DARTS) assay based on the immuno-detection of persistent HOIP peptides after Cpd A-mediated blockade of enzymatic proteolysis, and thereafter in a CRISPR-engineered HOIP-knockout ABC-DLBCL model.

Finally, efficacy of the compound was confirmed in vivo in a chicken embryo chorioallantoic membrane (CAM)-derived model of ABCL-DLBCL subjected to a twice weekly dosing with Cpd A, in which the compound achieved a 25% tumor growth inhibition, associated with a 60.5% and 89% reduction in brain and bone marrow infiltration by lymphoma cells, respectively. Altogether, our results confirm that HOIP represents a promising therapeutic target in ABC-DLBCL with activating MYD88 mutation, and that a pyrido[2,3-d]pyrimidine derivative can successfully and specifically block HOIP, resulting in NF-κB disruption and selective antitumor activity in this aggressive subtype of B-cell lymphoma.

Developing a humanised in vivo model to study myeloproliferative neoplasms

Alessandra Ferrelli[1], Syed Mian[1], Marion Piganeau[1], Erika Morsia[2], Matteo Della Porta[3], Claire Harrison[4], Shahram Kordasti[4], Dominique Bonnet[1].

[1]HSC laboratory, The Francis Crick Institute, London, UK

[2]Marche Polytechnic University, Italy

[3]Humanitas Research Hospital, Italy

[4]King's College London, London, UK

MPN are stem and progenitor cell (HSPC) malignancies leading to the overproduction of mutant cells. These include Essential Thrombocythemia (ET), Polycythaemia Vera (PV), and Primary Myelofibrosis (PMF), driven by mutations such as JAK2 (Luque Paz et al, 2023). Research shows mesenchymal stromal cells (MSCs) support fibrosis in PMF (Schneider et al, 2017), though their role in ET and PV remains unclear (Ramos et al, 2017). Additionally, in vivo studies of ET/PV HSPCs face challenges due to low engraftment in xenograft models. We used our 3D scaffold model to develop a humanised MPN model, enabling in vivo functional studies on primary MPN samples and their interaction with the human bone marrow (BM) niche. Using this model, we demonstrated clear engraftment of healthy (HD) HSPCs in scaffolds containing ET or PV MSCs. Additionally, we observed engraftment of ET and PV patient-derived HSPCs in scaffolds pre-seeded with their respective MSCs, providing the first in vivo evidence that ET and PV MSCs sustain both HD and MPN haematopoiesis. Finally, CRISPR editing was performed on HD HSPCs to introduce JAK2 mutation, allowing us to study CRISPR-edited JAK2-mutated cells and their interaction with human stroma. We validated that HD and ET/PV MSCs sustain JAK2-mutated cells in both in vitro and in vivo settings, confirming the results with patient-derived samples. Overall, we offer a novel humanised MPN model and insights into how BM MSCs interact with JAK2 mutant clones.

Developing epigenetic synergistic drug combinations with albendazole in paediatric acute myeloid leukaemia

Sophie Carlin[1], Kyle Matchett[1], Declan McKenna[2], Jonathan Bond[3], Seodhna Lynch[1], Rachel Wilson[4], Alex Thompson[5], Ken Mills[5], Tríona Ní Chonghaile[6]

[1]Personalised Medicine Centre, School of Medicine, Ulster University, C-TRIC, Altnagelvin Hospital Campus, Glenshane Road, Derry/Londonderry, BT47 6SB, UK

[2]School of Biomedical Sciences, Ulster University, Coleraine Campus, Cromore Road, Coleraine

[3]Systems Biology Lab, University College Dublin, Belfield, Dublin

[4]Department of Natural Sciences, University of Bath, Claverton Down, Bath, BA2 7AY, UK

[5]Patrick G Johnston Centre for Cancer Research, Queen's University Belfast, Belfast, BT9 7AE, UK

[6]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, 123 St Stephens Green, Dublin 2, Ireland

Acute myeloid leukaemia (AML) is a leading cause of leukaemia death in children. Advancements in molecular technologies have improved treatments and survival outcomes. However, mortality rates remain high with a five-year OS <70%. Current treatments can cause toxic side effects. Identifying more efficient therapies can help improve this. Drug repurposing and combination can help find better therapies. We previously performed a single agent in-house screen using the Screen-Well® FDA drug library in primary murine cells, representing MLL-AF9-driven paediatric AML and normal karyotype HOXA9-Meis1 driven AML. We identified two anthelmintic agents, albendazole (ABZ) and mebendazole, to have remarkable anti-leukaemia efficacy in vitro and in vivo while having minor effects on normal cells. Due to the novelty of ABZ, we decided to investigate further. High-throughput drug screens and advance bioinformatics will investigate ABZ's ability at reducing AML cell viability as a single agent and in combinations with epigenetic drugs. Top combinations will be taken forward for mechanistic analyses and test preclinical efficacy in patient samples. As a single agent, ABZ successfully reduced AML cell viability. Low doses of ABZ (IC50 <500nM) reduced viability in mouse and human AML cell lines, while having minimal effect on normal cells. ABZ caused G2/M arrest and PARP cleavage, leading to apoptosis, confirmed by flow cytometry and Western blot. In combination, ABZ was shown to have promising synergistic effects with the epigenetic compound 5-AZA. The combination reduced cell viability at lower dosages compared to the drugs as single agents. Future experiments will focus on investigating novel synergistic ABZ-epi combinations. There's an urgency to improve AML treatments. ABZ has shown anti-leukaemia effects with minimal toxicity in normal cells. Current work is focused on evaluating ABZ as a single agent and in combinations with epigenetic drugs to establish novel AML treatments.

Dissecting the role of TE-mediated immune responses following epigenetic treatment in Acute Myeloid Leukemia (AML)

Hale Tunbak[1], Özgen Deniz[1]

[1]Bart's Cancer Institute, Queen Mary University of London

The human genome has been continuously subjected to genome invasions of transposable elements (TEs) throughout evolution, and regulation of these elements, some of which are still mobile, is critical for genomic integrity and normal cellular function of gene expression programs, most of which are under strict epigenetic control. However, epigenetic dysregulations stemming from genome- wide loss of DNA methylation, global changes in histone modification marks and dysregulation of RNA modifications are all hallmarks of cancer and are typically synonymous with TE reactivation. In fact, various studies demonstrate transcriptional activation of TEs in several cancer cell types. Our work aims to dissect the potential impacts of TEs on the host genome in a context which provides an epigenetically relaxed environment for their activation: acute myeloid leukaemia (AML). AML is a highly heterogeneous and aggressive haematological malignancy, characterised by relatively few genetic mutations compared to other cancers. We aim to uncover how epigenetic therapies can be harnessed to help potentiate anti-tumour immune responses against endogenous TEs in AML. Specifically, we focus on DNA hypomethylating agents (5-AZA, Decitabine and DNMT1i) and those targeting histone modifications (including HDACi) to assess their combinatorial effects in promoting anti-tumour immune responses in patients of various genetic backgrounds. Our preliminary findings suggest that these agents result in differential TE and immune response activation in the presence and absence of DNMT3A mutations, the most commonly mutated epigenetic modifier in AML. Our next steps focus on uncovering the mechanism of 'viral mimicry' by interrogating the effects of TE-derived dsRNA and cDNAs on endogenous nucleic sensing pathways.

Embryonic hematopoietic stem and progenitor cells show differential susceptibility to leukemic transformation upon acquisition of JMML-associated KrasG12D mutation

Giulia Quattrini[1], Cristiana Barone[1], Alessandro Muratore[1], Filipa Timóteo-Ferreira[1], Gloria Zambelli[1], Tiphanie Durfort[1], Roberto Orsenigo[2], Cristina D'Orlando[1], Raffaella Meneveri[1], Barbara Vergani[1], Sofia De Marco[1], Cristina Bianchi[1], Silvia Brunelli[1], Emanuele Azzoni[1]

[1]St. Anna Children's Cancer Research Institute (CCRI) Vienna, Austria

[2]School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

[3]Biomedical Research in Melanoma-Animal Models and Cancer Laboratory, Vall d'Hebron Research Institute (VHIR), Vall d'Hebron Hospital Barcelona-UAB, Barcelona, Spain

Juvenile myelomonocytic leukemia (JMML) is a rare pediatric myeloproliferative neoplasm caused by a mutation in the RAS pathway. JMML is clinically heterogeneous and often originates prenatally, but the precise identity of disease propagating cells is still unclear. To investigate the cellular origin of JMML, we generated a preclinical model (Cdh5-CreERT2::KrasLSL- G12D::R26tdTomato transgenic mice) in which we can selectively target the KrasG12D to distinct subset of hematopoietic progenitors emerging from Cdh5+ hemogenic endothelium during embryonic development. Here, we demonstrate that embryonic hematopoietic progenitors show differential susceptibility to JMML-associated mutations. Targeting KrasG12D to erythromyeloid progenitors (EMPs) at E7.5, results in a low-penetrance myeloproliferative disorder with late onset (Fig2A), suggesting that EMPs are not the main cells of origin of JMML. Unexpectedly, targeting KrasG12D to fetal-restricted hematopoietic stem and progenitor cells (HSPCs) at E8.5 or HSCs at E10.5, caused lethal fetal liver (FL) anemia, due to defective erythroid differentiation (Fig1B), more severe if targeted to fetal HSPCs. To circumvent embryonic lethality, we induced KrasG12D in a mosaic-like fashion in HSPCs at E8.5 or HSCs at E10.5, which caused a fully penetrant leukemic phenotype in adult mice with a strong increase in the myelo- monocytic lineage (Fig2B), more aggressive and transplantable (Fig2E,F) when induced the latter. These data suggest that both fetal-restricted HSPCs and HSCs could be involved in the pathogenesis of JMML, and that disease heterogeneity could be partially explained by variability in the cell of origin. We are currently analyzing the molecular and epigenetic changes KrasG12D-linked. Since our preliminary data suggest that patients-derived JMML HSPCs exhibit features of enhanced inflammation, we are investigating its role in disease onset and/or progression using our murine model and humanized mice model (PDX) of JMML.

Endogenous aldehydes are generated during myeloid differentiation and counteracted by glutathione-dependent systems

Marta Valverde[1*], Nati Alquezar-Artieda[1*], Logan Tenney[2], Chris Chang[2], Lucas B. Pontel[1]

[1]Josep Carreras Leukaemia Research Institute, Barcelona, Spain

[2]Princeton University, USA

*Equally contribution

Bone marrow failure, myelodysplasia and acute myeloid leukemia (AML) are severe consequences observed in the inherited human conditions Fanconi anemia (FA) and in the AMeD syndrome that manifest during childhood. These disorders are caused by mutations in the genes coding for the FA DNA repair pathway, or in genes coding for enzymes that metabolize formaldehyde, a reactive aldehyde generated as by-product of cellular metabolism. Indeed, it is thought that the accumulation of endogenous aldehydes underlies the onset of myeloid malignancies in those human conditions; however, the exact role of reactive aldehydes during myeloid differentiation remains unclear.

In this study, we investigated whether endogenous aldehyde production occurs during myeloid differentiation, potentially leading to genomic damage, transformation and/or cell death. By using a selective chromatin formaldehyde-probe (RFAP2-nuc) and a lipid peroxide sensor (C11-BODIPY) in cellular models of myeloid differentiation, we detected a significant peak of endogenous formaldehyde, and a surge of lipid peroxidation that can produce reactive aldehydes from polyunsaturated fatty acids. Both formaldehyde accumulation and lipid peroxidation are counteracted by systems that depend on glutathione (GSH) as a cofactor. Accordingly, we found that GSH synthesis and cystine import through SLC7A11 – a source of cysteine for GSH synthesis- are required during myeloid differentiation to limit lipid peroxidation and cell death by ferroptosis. Interestingly, AML tumors showing high expression of GSH synthesis and SLC7A11 present a more aggressive phenotype, suggesting that GSH and cystine import can benefit leukemic cells, likely by preventing the accumulation of reactive aldehydes.

In sum, we propose that protection against endogenous reactive aldehydes is necessary for a successful differentiation of myeloid progenitors, while it contributes to cancer growth during disease progression.

Epigenetic dissection of transposable elements in KMT2A-rearranged paediatric AML

Eve Leddy[1], William Villiers[2], Richard Dillon[2], Özgen Deniz[3]

[1]Centre for Haemato-Oncology, Barts Cancer Institute, QMUL, London, EC1M 6BQ, UK

[2]Department of Medical and Molecular Genetics, Kings College London, London, UK

[3]Centre for Haemato-Oncology, Barts Cancer Institute, QMUL, London, EC1M 6BQ, UK

KMT2A-rearrangements (KMT2A-r) are the most common cytogenetic aberrations seen in paediatric acute myeloid leukaemia (pAML), identified in up to 15% of cases in children and 60% in infants (1). These fusions are associated with poorer prognosis than other subtypes, involving higher relapse rates and lower overall survival (2). KMT2A-r is linked to dysregulated epigenetic reading and modifying activities, altering the chromatin landscape. Improved understanding of how KMT2A-r remodels chromatin to promote oncogenesis in pAML could provide new therapeutic targets for this poor-risk patient group. In this study, we looked at the non- coding transposable element (TE) portion of the genome as it has not been investigated in pAML before. TEs are an understudied reservoir of potential cis-regulatory sequences, and are usually epigenetically repressed in healthy somatic cells. We have previously shown that epigenetic silencing of adult AML-specific TE families, such as LTR2B, slows proliferation and affects nearby gene expression indicative of their enhancer activity in AML (3). Using epigenetic profiling of 123 pAML patients from the MyeChild cohort we demonstrate that in KMT2A-r pAML patients, a family of TEs plays a similar role to LTR2B in adult AML. This family is enriched for chromatin accessibility (ATAC-seq), as well as the enhancer-associated histone modifications H3K27ac and H3K4me1 (CUT&RUN). Further work will involve functionally validating these TEs as enhancers using CRISPR-mediated epigenetic silencing and activation of this TE family in KMT2A-r pAML cell lines and patient samples. This is in addition to integration with RNA-seq and methyl-seq data to gain a fuller picture of how this TE family is regulated and may be promoting pro-leukaemic transcriptional programmes. The final aim of this project will be identifying ways to therapeutically target the pro-leukaemic-TE axis in pAML which can be translated to the clinic.

Exploring Leukemic Stem Cell–Niche Interactions in a Bone Marrow Organoid Model

Karolin Stumpf[1], Alexander Waclawiczek[1], Aino-Maija Leppä[1], Simon Renders[1], Ines Bergerweiss[1], Frank Huang[1], Abdullah Khan[2], Bethan Psaila[2], Andreas Trumpp[1]

[1]German Cancer Research Center

[2]MRC Weatherall Institute of Molecular Medicine

Changes in the bone marrow (BM) microenvironment in acute myeloid leukemia (AML) support the persistence of leukemic stem cells (LSCs), driving disease progression and relapse. To date, systems to study malignant hematopoiesis in the context of its niche, such as PDX models and in vitro co-cultures, are limited by the engraftment potential of primary cells and the represented niche complexity. Here, we employ a human iPSC-derived bone marrow organoid (BMO) system, first described by Psaila group (Khan et al., 2023). This system yields vascularized 3D structures containing stromal and hematopoietic cell types, enabling the investigation of LSC-niche interactions. Based on a projection of LSC transcriptomes of 138 AML patients onto a healthy BM reference map, we identified two predominant LSC types resembling healthy hematopoietic lineage trajectories: Lymphoid-Myeloid Primed Progenitor and Megakaryocyte/Erythroid Progenitor LSCs. These are not only associated with distinct genetics but also with therapy-specific responses. BMOs support the engraftment of both LSC types via a semi-active CXCL12/CXCR4-dependent process. While the cellular composition of BMOs remains largely unchanged upon LSC engraftment, some patient LSCs unable to grow in PDX produce engraftment in BMOs. Overall, these data validate the BMO system as a valuable alternative to PDX models, making it a powerful tool to study niche interactions and screen new therapeutics in the setting of perturbed hematopoiesis.

Four Distinct Leukemic Stem Cell Types Mediate Response and Resistance in Acute Myeloid Leukemia

Alexander Waclawiczek[1], Aino-Maija Leppä[1], Simon Renders[1], Karolin Stumpf[1], Ines Bergerweis[1], Carsten Müller-Tidow[2], Andreas Trumpp[1]

[1]German Cancer Research Center (DKFZ)

[2]University Hospital Heidelberg

While Leukemic Stem Cells (LSCs) are known to initiate and drive AML progression, their role in treatment response remains unclear. In a study of over 150 AML patients, we reveal significant patient-to-patient variability, showing that different therapy outcomes correspond to distinct LSC types. By mapping the LSC transcriptomes of 138 AML patients to a reference atlas of healthy adult hematopoiesis, we identify four LSC types. Three subtypes of canonical LSCs resemble different hematopoietic stem and progenitor cell (HSPC)-like states: Lymphoid-Myeloid Primed Progenitors (LMPP-LSCs) are the most common, followed by Megakaryocyte-Erythrocyte Progenitors (MEP-LSCs) in 34% of patients, and Hematopoietic Stem Cells (HSC-LSCs) in 12%. The LSC type influences the differentiation and lineage direction of LSCs and their progeny and determined BCL-2 family expression, inflammatory properties, metabolism and cell cycle of LSCs. Additionally, xenograft assays from over 100 patient samples reveal a fourth, non-canonical LSC type, distinct from classical HSPC-like states. This type expresses markers of early monocyte/dendritic cell progenitors and generates LAMP5+ monocytic/dendritic precursor blasts, linked to an aggressive AML subtype, with the highest self-renewal capacity observed. Given this LSC type's more differentiated state, AML could be targeted via surface markers without harming healthy HSPCs which is a critical limitation of current approaches. Lastly, chemo-based and BCL-2 inhibitor Venetoclax- based therapy responses are associated with specific LSC types, which can evolve under treatment pressure. Longitudinal analyses show that Venetoclax selects for BCL-xL-dependent LSCs with megakaryocytic lineage features. Inhibition of BCL-xL can block LSC plasticity towards the MEP-lineage and enhance blast clearance in a PDX model. In conclusion, LSC differentiation states, shaped by both genetic and non-genetic factors, and lineage plasticity drive treatment resistance.

Greedy, iterative tissue engineering approximates cell types dynamically by dissecting chromatin profiles

Luis Montano-Gutierrez[1], Sophie Müller[1], Ana Kutschat[1], Davide Seruggia[1], Florian Halbritter[1]

[1]St. Anna Children's Cancer Research Institute (CCRI) Vienna, Austria

A prime goal of regenerative biology is to craft cells in vitro that are indistinguishable from the target cells to be replaced in vivo. However, the search space for ingredient combinations, concentrations, and application times can become exponentially large. Moreover, optimisation can be imprecise when only assessing phenotype via few markers. Greedy machine learning algorithms overcome similar search problems step by step by testing multiple alternatives in parallel and iteratively choosing the solution closest to the target at each step. Here, we applied greedy principles to optimise differentiation of hematopoietic stem cells into erythroblasts in two sequential rounds. At each round we phenotyped 8 alternative treatments at the chromatin level (ATAC-seq) and compared them to a hematopoietic cell type chromatin reference. After comparing 72 treatments, we found that the treatment that produced the closest cells to erythroblasts after round 2 stemmed from the best treatment at round 1, proving the principle that greedy optimisation can be a viable strategy. Additionally, we found time-sensitive contributions of some components to the erythroid phenotype. Finally, we identified chromatin regions that were incompletely reprogrammed after in-vitro differentiation, and suggested chemical perturbations that could close these gaps. In future, our methodology can help craft notoriously difficult cell identities in vitro, such as B cells.

HDAC7 induction combined with standard-of-care chemotherapy provides a therapeutic advantage in t(4;11) infant B-ALL

Oriol de Barrios[1], Mar Gusi-Vives[1], Olga Collazo[1], Ainara Meler[1], Ingrid Ocón-Gabarró[1], Paola A Romecín[2], Alba Martínez-Moreno[2], Juan Ramón Tejedor[3,4], Mario F. Fraga[3,4], Pauline Schneider[5], Giovanni Cazzaniga[6], Rolf Marschalek[7], Ronald W Stam[5], Clara Bueno[2], Pablo Menéndez[2,8,9,10,11], Maribel Parra[1]

[1]Lymphocyte Development and Disease Group, Josep Carreras Leukaemia Research Institute, Badalona, Spain

[2]Josep Carreras Leukaemia Research Institute, School of Medicine, University of Barcelona, Barcelona, Spain

[3]Nanomaterials and Nanotechnology Research Center (CINN-CSIC), Health Research Institute of Asturias (ISPA), Institute of Oncology of Asturias (IUOPA), 33012, Oviedo, Spain

[4]CIBER-ER-ISCIII, Madrid. Spain

[5]Princess Maxima Center for Paediatric Oncology, Utrecht, The Netherlands

[6]Centro Ricerca Tettamanti, Department of Pediatrics, University of Milano-Bicocca, Monza, Italy

[7]Institute of Pharmaceutical Biology/DCAL, Goethe-University, Frankfurt, Germany

[8]Red Española de Terapias Avanzadas (TERAV), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

[9]Department of Biomedicine, School of Medicine, University of Barcelona, Barcelona, Spain

[10]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona. Spain

[11]Centro de Investigación Biomédica en Red (CIBERONC), ISCIII, Madrid, Spain

Infants younger than one year diagnosed of pro-B acute lymphoblastic leukemia (pro-B-ALL) and t(4;11) chromosomal rearrangement represent a subgroup of patients with adverse outcome, mainly unresponsive to standard therapy. Moreover, immune escape mechanisms, that often involve the loss of B cell features, lead to CD19-directed immunotherapy failure.

Our research has demonstrated the essential role of B lymphocyte factor HDAC7 in this malignancy. These infants present very low levels of this biomarker, whose induction doubles patient survival. In this sense, after identifying its repression mechanism, we have identified a combinatorial therapy (including Menin inhibitor MI-538 and Chidamide) that triggers HDAC7 expression in t(4;11) pro-B-ALL cells. This treatment shifts transcriptomic profile of leukemic pro-B cells towards a more differentiated and less malignant B-cell state.

In addition, our proposed HDAC7-inducing therapy has demonstrated to reduce proliferation and colony formation in vitro. Remarkably, the generation of stable cell lines with impaired HDAC7 induction has proofed that this effect depends, at least partially, on HDAC7 expression. Additionally, the incorporation of MI-538 and Chidamide to conventional chemotherapy significantly reduces bone marrow engraftment of primary t(4;11) pro-B-ALL cells in immunodeficient mice, while delaying relapse onset. Finally, ex vivo culturing of t(4;11) patient-derived leukemic cells has reported that our newly developed therapy increases glucocorticoid sensitivity.

The ex vivo culture of t(4;11) pro-B-ALL cells revealed the presence of a subpopulation with low CD19 expression, more likely to escape from CAR-T immunotherapy. Interestingly, HDAC7-inducing therapy shifts this CD19low cells into CD19high population, thus preventing them from acquiring a myeloid phenotype as a potential immune escape mechanism.

In summary, we propose HDAC7 induction as a new strategy to enhance patient's therapy response and, ultimately, to improve overall survival. Since infants are normally excluded from clinical trials as vulnerable population, this treatment opens a new field for personalized medicine in infant leukemia.

Identifying Novel Senescence Biomarkers Induced by CHOP Therapy in Non-Hodgkin Lymphoma and Their Potential as Therapeutic Targets

J. Balibrea-Rull[1], D. Olivares[1], C. Guardia1, E. Castillo[1], T. Navarro[1], E. Mereu[1], V. Rodilla[1]

[1]Cancer Heterogeneity and Hierarchies, Josep Carreras Leukaemia Research Institute (IJC), Badalona, Spain

Introduction. Non-Hodgkin lymphoma (NHL) constitutes 90% of all lymphomas, and despite its heterogeneity, the standard treatment remains being R-CHOP, a combination of chemotherapy; cyclophosphamide, doxorubicin and vincristine (CHO); with rituximab (anti- CD20 monoclonal antibody). However, relapse affects 30-40% of patients, underscoring the need to understand treatment resistance mechanisms.Senescence, recognized as a cancer hallmark, is induced by chemotherapy, termed therapy-induced senescence (TIS). Although it halts cell cycle progression, senescent cells (SCs) may promote tumorigenesis through traits like resistance to apoptosis and the senescence-associated secretory phenotype (SASP). Identifying senescence targets is crucial due to its role in cancer, and the lack of reliable biomarkers caused by its heterogeneity.

Understanding TIS in NHL and identifying effective targets to eradicate SCs could improve patient outcomes. Our study aims to discover biomarkers and senescence targets in NHL, developing methods to selectively eliminate SCs, thereby restraining tumor progression and preventing relapse.

Materials and Methods. We characterized TIS in NHL cells using three cell lines representative of different NHL subtypes, treated with the chemotherapeutic regimen CHO. We assessed senescence induction via RT-qPCR for classical senescence markers and SA-βgal staining. Through a comprehensive transcriptomic analysis, we aimed to pinpoint common differentially expressed candidates as potential senescence targets. Validation of these candidates was primarily conducted using immunocytochemistry, Western blot, and flow cytometry. In vivo studies are anticipated to utilize xenograft models of NHL.

Results and Discussion. Our initial findings from TIS characterization in NHL cells showed distinct senescence induction across the cell lines, evidenced by SA-βgal staining and upregulation of senescence markers. Subsequent analyses revealed a set of differentially expressed potential targets, which we further validated in vitro. Moreover, we have evaluated different strategies to inhibit these senescence markers and their consequences. With the idea of stratifying patients that could benefit from these novel regimens, we have correlated the expression of certain genes in peripheral blood in response to CHO.

Conclusion. Integrating transcriptomic data and validation studies, we strive to identify and evaluate senescence targets, potentially offering new therapeutic avenues to disrupt pro- tumorigenic pathways and enhance treatment responses in NHL.

Integrative single-cell multi-omics of CD19-CARpos and CARneg T cells suggest drivers of immunotherapy response in B-cell neoplasias

Aina Rill-Hinarejos[1,3,15], Mercedes Guerrero-Murillo[1,2,3,15], Juan L. Trincado[1,15], Alex Bataller[4], Valentín Ortiz-Maldonado[2,4], Daniel Benítez-Ribas[5,6], Marta Español-Rego[5,6], E Azucena González-Navarro[5,6], Nuria Martínez-Cibrián[4], Doménica Marchese[7], Lourdes Martín-Martín[8], Alejandro Martín García-Sancho[9], Susana Rives[10,11], Holger Heyn[7], Manel Juan[2,5,6], Álvaro Urbano-Ispizúa[1,2,4,6], Julio Delgado[2,4,6,12], Alberto Orfao[8,12], Clara Bueno[1,2,12*], Pablo Menendez[1,2,12,13,14,20*], Elisabetta Mereu[1*]

[1]Josep Carreras Leukemia Research Institute, Barcelona, Spain

[2]Spanish Network for Advanced Therapies, RICORS-TERAV, ISCIII, Spain

[3]PhD programme in Biomedicine, University of Barcelona, Barcelona, Spain

[4]Department of Hematology, Hospital Clinic de Barcelona, Barcelona, Spain

[5]Department of Immunology and immunotherapy, Hospital Clinic de Barcelona, Barcelona, Spain

[6]Institut d'Investigacions Biome ` diques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

[7]CNAG-CRG, Centre for Genomic Regulation, Barcelona Institute of Science and Technology (BIST), Barcelona, Spain

[8]Cancer Research Centre (IBMCC, USAL-CSIC), Institute for Biomedical Research of Salamanca (IBSAL) and Department of Medicine and Cytometry Service (NUCLEUS Research Support Platform), University of Salamanca (USAL), Salamanca, Spain

[9]Department of Hematology, University Hospital of Salamanca (HUS/IBSAL), CIBERONC, University of Salamanca and Cancer Research Institute of Salamanca-IBMCC (USAL-CSIC), Salamanca, Spain

[10]Pediatric Oncology and Hematology Department, Hospital Sant Joan de De u de Barcelona, Barcelona, Spain

[11]Institut de Recerca Sant Joan de De u, Barcelona, Spain

[12]Spanish Collaborative Cancer Network, CIBERONC, ISCIII, Spain

[13]Department of Biomedicine, University of Barcelona, Barcelona, Spain

[14]Institucio Catalana de Recerca i Estudis Avanc ats (ICREA), Barcelona, Spain

[15]These authors contributed equally

*Correspondence

Chimeric Antigen Receptor T-cell (CAR T-cell) therapies have revolutionized the treatment of relapsed/refractory (r/r) hematological cancers, extending patient survival and advancing immunotherapy. Despite these advancements, many patients relapse within a year post-treatment, primarily due to the quality and state of the input cells. To bridge the knowledge gap regarding the distinct roles and behaviors of transduced (CAR+) and endogenous (CAR-) T-cells, and their synergistic functions in achieving long-term remission, we conducted a comprehensive comparison of scRNA/TCR-seq profiles of CAR+ and CAR- T-cells from infusion products and their peak expansion in five r/r B-ALL patients treated with CAR T-cell therapy (Varni-cel). Key findings include: Higher CD4:CD8 ratios at infusion correlate with better clinical outcomes, highlighting the role of CD4 T-cells in orchestrating immune responses. Higher exhaustion scores in CAR-T products are linked to poorer treatment responses. Patients with significant in vivo expansion of CAR+ γδ T cells demonstrated better clinical outcomes.

These results were validated in larger cohorts, including 18 B-ALL patients treated with varni-cell and 58 B-cell lymphoma patients treated with lisa-cel or axi-cel. Our study underscores the importance of integrating scRNA-seq and scTCR-seq for monitoring CAR T-cell dynamics, providing insights into the clonal kinetics and heterogeneity of T-cell responses. The expansion of CD8+ cytotoxic T-cells with low proliferation and the emergence of γδ T-cells post-infusion suggest their critical roles in tumor control, advocating for their preservation during CAR T-cell engineering. Our data offer a deeper understanding of T-cell responses post-CAR T-cell therapy and potential drivers of immunotherapy success.

Lactate promotes fumarate accumulation and fibrotic transformation in myelofibrosis tumor microenvironment

Sebastiano Giallongo[1], Lucia Longhitano[2], Enrico La Spina[2], Jessica Ferrigno[2], Deborah Calvo[3], Angela Maria Amorini[2], Francesco Bellia[2], Francesco Di Raimondo[4], Alessandra Romano[4], Giuseppe Palumbo[5], Cesarina Giallongo[5]

[1]Faculty of Medicine and Surgery, University of Enna "Kore", 94100 Enna, Italy

[2]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy

[3]Division of Hematology, AOU "Policlinico-Vittorio Emanuele", 95123 Catania, Italy

[4]Department of General Surgery and Medical-Surgical Specialties, University of Catania, Catania, Italy

[5]Department of Medical and Surgical Sciences and Advanced Technologies "G.F. Ingrassia", Catania, Italy

Myelofibrosis (MF) is a myeloproliferative neoplasm characterized by ineffective hematopoiesis, bone marrow fibrosis and splenomegaly, eventually progressing to acute myeloid leukemia. The malignant clone, harboring JAK2V617F mutation, increase glycolytic activity, leading to lactate release within the tumor microenvironment (TME). However, the impact of this metabolite on the various TME compartments remains unclear. In line with this, we detected a higher concentration of lactate in MF sera compared to age-matched healthy donors. Therefore, we evaluated lactate effect on TME by exposing peripheral blood mononucleated cells to MF sera. We observed an increased percentage of regulatory T cells and myeloid-derived suppressor cells, which can be reversed blocking lactate-selective channel monocarboxylate transporter 1 (MCT1) by AZD3965. During MF pathogenesis, mesenchymal stromal cells (MSCs) undergo phenotypic and epigenetic reprogramming, increasing the release of pro-inflammatory cytokines as part of senescence-associated secretory phenotype and heterochromatin content. To investigate the role of lactate in MSC reprogramming, we exposed healthy MSCs to the metabolite. Our results demonstrated collagen accumulation and increased metalloproteinase release a senescent phenotype. Corroborating this, we observed the same outcomes following MF sera exposure, which were reverted by MCT1 inhibition. Interestingly, we detected lactate-induced fumarate accumulation in stromal cells, eventually mirrored in MF patients' sera. Moreover, fumarate exposure promoted collagen accumulation and senescence in MSCs, as well as enhanced DNA and histone methylation. Finally, we established a TPOhigh zebrafish model recapitulating MF hallmarks. Our data indicated increased lactate accumulation, leading to collagen deposition within the zebrafish whole kidney marrow, thereby demonstrating lactate as a crucial oncometabolite in the onset of MF and TME pro-fibrotic establishment.

Leukemia aggressiveness is driven by chromatin remodeling and expression changes of core regulators

Gracia Bonilla[1], Alexander Morris[1], Sharmistha Kundu[1], Anthony Ducasse[1], Nathan Jeffries[2], Kashish Chetal[1], Emma Yvanovich[2], Rana Barghout[2], David Scadden[2], Michael Mansour[3], Robert Kingston[1], David Sykes[2], Francois Mercier[4], Ruslan Sadreyev[1]

[1]Department of Molecular Biology, Massachusetts General Hospital, Boston MA, USA

[2]Center for Regenerative Medicine, Massachusetts General Hospital, Boston MA, USA

[3]Division of Infectious Diseases, Massachusetts General Hospital, Boston MA, USA

[4]Department of Medicine, McGill University, Montreal, Quebec, Canada

Molecular mechanisms driving clonal aggressiveness in leukemia are not fully understood. We tracked and analyzed MLL- rearranged leukemic clones independently evolving towards higher aggressiveness. More aggressive subclones lost their growth differential ex vivo but restored it upon secondary transplantation, suggesting molecular memory of aggressiveness. Development of aggressiveness was associated with clone-specific gradual modulation of chromatin states and expression levels across the genome, with a surprising preferential trend of reversing the earlier changes between normal and leukemic progenitors. To focus on the core aggressiveness program, we identified genes with consistent changes of expression and chromatin marks that were maintained in vivo and ex vivo in both clones. Overexpressing selected core genes (Smad1 as aggressiveness driver, Irx5 and Plag1 as suppressors) affected leukemic progenitor growth in the predicted way and had convergent downstream effects on central transcription factors and repressive epigenetic modifiers, suggesting a broader regulatory network of leukemic aggressiveness. Reference: Bonilla G, Morris A, Kundu S, Ducasse A, Jeffries NE, Chetal K, Yvanovich EE, Barghout R, Scadden D, Mansour MK, Kingston RE, Sykes DB, Mercier FE, Sadreyev RI (2024) Leukemia aggressiveness is driven by chromatin remodeling and expression changes of core regulators. BioRxiv doi: https://doi.org/10.1101/2024.02.29.582846

Leveraging Machine Learning to reverse immunosuppression after CAR T cell therapy

A. Buratin[1*], E. Mereu[1*]

[1]Josep Carreras Leukaemia Research Institute (IJC), Badalona, Spain

*Corresponding Authors

Chimeric Antigen Receptor (CAR) T-cell therapy has revolutionized the treatment of hematologic malignancies, but overcoming therapeutic resistance and disease progression remains a key challenge. In this study, we harness single-cell multi-omics to improve CAR T-cell therapy outcomes for multiple myeloma (MM), introducing a machine learning (ML) approach to prioritize the cell types most responsive to therapy.

We performed an extensive single-cell T cell receptor (TCR) and RNA sequencing analysis on CD3+ T cells from 11 MM patients treated with CAR T therapy targeting B-cell maturation antigen (BCMA). Samples were collected longitudinally from the infusion product (IP) through to the peak of CAR T-cell expansion. Our analysis confirms previous findings of a higher CD4:CD8 ratio in CAR-positive T cells within the IP, prompting further exploration of the immune system dynamics underlying therapy response.

Using an interpretable ML algorithm, we identified the cell populations driving therapeutic resistance. Classifier predictions were compared with response labels, and cell types were prioritized based on the area under the receiver operating characteristic curve in cross-validation analyses. A Lasso model selected key genes differentiating favorable and poor responses, achieving 0.8 accuracy in classifying cells based on their sensitivity to anti-BCMA CAR T therapy.

By generating predictive scores for each cell based on gene expression, we highlighted clonally expanded CD8+ T cell subtypes associated with poor therapeutic outcomes. These findings provide a foundation for understanding the cellular heterogeneity that influences therapy efficacy.

Our work demonstrates the power of single-cell analysis for elucidating the complexities of immunotherapy and constructing predictive models. By targeting specific cellular phenotypes and pathways identified through this approach, we aim to develop combinatorial treatment strategies that promote more durable remissions for CAR T-cell therapy patients.

Low input capture Hi-C (liCHi-C) identifies promoter-enhancer interactions at high-resolution

Laureano Tomás-Daza[#1,2], Llorenç Rovirosa[#1], Paula López-Martí[1,2], Andrea Nieto-Aliseda[1], François Serra[1], Ainoa Planas-Riverola[1], Oscar Molina[1], Rebecca McDonald[3], Cedric Ghevaert[3,4], Esther Cuatrecasas[5], Dolors Costa[6,7,8], Mireia Camós[9,10,11], Clara Bueno[1], Pablo Menéndez[1,12], Alfonso Valencia[2,12], Biola M Javierre[1,13]

[1]Josep Carreras Leukaemia Research Institute, Badalona, Barcelona, Spain

[2]Barcelona Supercomputing Center, Barcelona, Barcelona, Spain

[3]Wellcome-MRC Cambridge Stem Cell Institute, Cambridge, UK

[4]NHS Blood and Transplant, Cambridge, UK

[5]Pediatric Institute of Rare Diseases, Sant Joan de Déu Hospital, Esplugues de Llobregat, Barcelona, Spain

[6]Hospital Clinic, Barcelona, Spain

[7]Institute of Biomedical Research August Pi i Sunyer, Barcelona, Spain

[8]Cancer Network Biomedical Research Center, Barcelona, Spain

[9]Sant Joan de Déu Research Institute, Esplugues de Llobregat, Barcelona, Spain

[10]Sant Joan de Déu Hospital, Esplugues de Llobregat, Barcelona, Spain

[11]Center for Biomedical Research in the Rare Diseases Network (CIBERER), Carlos III Health Institute, Madrid, Spain

[12]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

[13]Institute for Health Science Research Germans Trias i Pujol, Badalona, Barcelona, Spain

[#]Contributed equally

Long-range interactions between regulatory elements and promoters are key in gene transcriptional control; however, their study requires large amounts of starting material, which is not compatible with clinical scenarios nor the study of rare cell populations. Here we introduce low input capture Hi-C (liCHi-C) as a cost-effective, flexible method to map and robustly compare promoter interactomes at high resolution. As proof of its broad applicability, we implement liCHi-C to study normal and malignant human hematopoietic hierarchy in clinical samples. We demonstrate that the dynamic promoter architecture identifies developmental trajectories and orchestrates transcriptional transitions during cell-state commitment. Moreover, liCHi-C enables the identification of disease-relevant cell types, genes and pathways potentially deregulated by non-coding alterations at distal regulatory elements. Finally, we show that liCHi-C can be harnessed to uncover genome-wide structural variants, resolve their breakpoints and infer their pathogenic effects. Collectively, our optimized liCHi-C method expands the study of 3D chromatin organization to unique, low-abundance cell populations, and offers an opportunity to uncover factors and regulatory networks involved in disease pathogenesis.

MDS to AML transformation: aiming to delineate the distinct roles of STAT3 and STAT5 target gene networks

Eirini Sofia Fasouli[1], Sofia Barda1, Konstantina Giavi[1], Charikleia Makri[1], Leonid Bystrykh[2], Anthi Bouchla[3], Emmanouil Athanasiadis[1], Giannis Vatsellas[1], Vasiliki Pappa[3] and Eleni Katsantoni[1]

[1]Basic Research Center, Biomedical Research Foundation, Academy of Athens, Athens, Greece

[2]UMCG / University of Groningen, Groningen, The Netherlands

[3]Second Department of Internal Medicine and Research Institute, Hematology Unit, National and Kapodistrian University of Athens, Medical School, University General Hospital "Attikon", Athens, Greece

Accumulation of mutations in hematopoietic stem cells leads to hematologic malignancies such as Myelodysplastic Syndrome (MDS), a heterogeneous group of disorders, characterized by hematopoietic dysfunction and impaired differentiation. MDS commonly progresses to Acute Myeloid Leukemia (AML), an aggressive type of leukemia. STAT3 and STAT5 are key regulators of several cellular processes and have been strongly associated with hematopoiesis. Abnormal STAT3 and STAT5 signaling have been implicated in various hematologic malignancies, including MDS and AML, rendering them appealing therapeutic targets. Hence, to elucidate the role of STAT3 and STAT5 in MDS to AML transformation, the direct and indirect transcriptional networks of each factor have been identified. RNA-seq results from STAT3, STAT5A, or STAT5B KDs combined with the differential binding of each factor in MDS and AML cell lines (explored through CUT&Tag) have revealed distinct roles for the three factors in each state, including pathways involved in the cell cycle and apoptosis. Higher chromatin accessibility in MDS, compared to AML, has unveiled potential changes in accessibility for STAT3 and STAT5 binding sites between MDS and AML, indicating plausible leukemia-promoting changes in AML. Also, a possible interplay between STAT3 and STAT5 activation in AML has been uncovered, describing a functional overlap in AML, for the regulation of shared targets essential for important cellular functions, such as leukemic metabolism. Furthermore, a potential cross-talk of STAT5 factors with IKAROS family members has been linked to AML transformation, as differential regulation has been detected between MDS and AML conditions after the KD of STAT5A and STAT5B. In conclusion, our results show a distinct function between the two STAT5 factors (STAT5A and STAT5B) in MDS and AML conditions and delineate the role of STAT3, STAT5A and STAT5B factors and their target gene networks in leukemic transformation, thus providing novel targets for therapeutic management of MDS and AML.

Modulating immune cell fate and inflammation through CRISPR-mediated DNA methylation editing

Gemma Valcárcel[1], Anna V. López-Rubio[1,3], Aleksey Lazarenkov[1,3], Clara Berenguer[1], Josep Calafell-Segura[2], Javier Rodríguez-Ubreva[2], Esteban Ballestar[2] and José Luis Sardina[1]

[1]Epigenetic Control of Haematopoiesis Group. Josep Carreras Leukaemia Research Institute (IJC), Campus Can Ruti, 08916 Badalona, Spain

[2]Epigenetics and Immune Disease Group. Josep Carreras Leukaemia Research Institute (IJC), Campus Can Ruti, 08916 Badalona, Spain

[3]Equal contribution

Immune cell differentiation and activation are associated with widespread DNA methylation changes; however, the causal relationship between these changes and their impact in shaping cell fate decisions still needs to be fully elucidated. Here, we conducted a genome-wide analysis to investigate the relationship between DNA methylation and gene expression at gene regulatory regions in human immune cells. By utilizing CRISPR/dCas9-TET1 and -DNMT3A epigenome editing tools, we successfully established a cause-and-effect relationship between the DNA methylation levels of the promoter of the Interleukin1-receptor antagonist (IL1RN) gene and its expression. Most importantly, we observed that modifying the DNA methylation status of the IL1RN promoter is sufficient to alter human myeloid cell fate and change the cellular response to inflammatory stimuli, resulting in pro-inflammatory cytokine release and a distinctive capacity to support cancer growth. Collectively, our findings demonstrate the potential of targeting specific DNA methylation events to directly modulate immune and inflammatory responses, providing a proof-of-principle for intervening in a broad range of inflammation-related diseases.

Multi-technique approach for the cytogenetic and genomic characterization of the MDS-L cell line

Julia Mestre[1,2], Lorea Chaparro[1], Isabel Granada[3], Mar Mallo[1], Emili Cid[4], Oriol Calvete[1], Ruth M. Risueño[5,6], Daniel T. Starczynowski[7], Francesc Sole[1,2]

[1]MDS Research Group, Josep Carreras Leukaemia Research Institute, ICO-Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, 08916 Badalona, Spain

2Facultat de Biociències, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

3Hematology Department, Hospital Germans Trias i Pujol, Institut Català d'Oncologia, Josep Carreras Leukaemia Research Institute, Barcelona, Spain

4Laboratory of Immunohematology and Glycobiology, Josep Carreras Leukaemia Research Institute (IJC), Campus Can Ruti, Camí de les Escoles, Badalona, Barcelona, 08916, Spain

5Leukos Biotech, 08021 Barcelona, Spain

6Faculty of Education, University of Atlántico Medio, 35017 Las Palmas, Spain

7Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Among all the leukemia cell lines described in the literature, the MDS-L cell line has been proven to be the sole line established from a patient during the myelodysplastic syndrome phase of the disease. However, few studies have been published regarding its genomic complexity, limiting its applicability in the development of in vitro models of myelodysplastic syndromes pathology. To better characterize the chromosomal alterations of the MDS-L, we combined conventional G banding and molecular cytogenetic techniques with the novel Optical Genome Mapping (OGM) technology. In addition, the mutational landscape was defined by targeted next generation sequencing. Two karyotypically distinct cell populations were detected by G-banding technique, both showing a complex karyotype. G-banding and OGM studies allowed the identification of new structural alterations not previously described, namely der(1)t(1;7)(q11;q11.2), del(1)(q11), der(4)t(4;5)(p16;q11.2), i(5)(p10), der(6)t(6;15)(p21.3;q15), i(8)(q10), der(9)t(9;10)(q34;p11.21), der(19)t(6;19)(p13;p22) and i(22)(q10). Multiple copy number variants and loss of heterozygosity regions were identified by OGM and SNP microarrays techniques. Chromosome breakpoints were clearly defined by OGM, allowing the identification of gene disruption events. Moreover, M-FISH technique helped to validate the translocations, determine the origin of the extra material seen by karyotype and identify cryptic rearrangements. Finally, next generation sequencing studies allowed the detection of mutations in CEBPA, NRAS, TET2 and TP53 genes linked to the myelodysplastic syndromes pathology. Our multi-technique approach has successfully enabled the precise definition of the MDS-L cell line genomics complexity, exposing also the applicability of each technique to identify different genetic alterations.

Mutant CEBPA promotes tolerance to inflammatory stress through deficient AP-1 activation

Maria Cadefau-Fabregat[1,2], Gerard Martínez-Cebrián[1], Lucía Lorenzi[1], José Manuel Castelló-García[1], Alejandro Vaquero[1], Sergi Cuartero[1,2]

[1]Josep Carreras Leukaemia Research Institute (IJC), Badalona, Spain

[2]Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain

The CEBPA transcription factor is frequently mutated in acute myeloid leukemia (AML). Mutations in the CEBPA gene, which are typically biallelic, result in the production of a shorter isoform known as p30. Both the canonical 42-kDa isoform (p42) and the AML-associated p30 isoform bind chromatin and activate transcription, but the specific transcriptional programs controlled by each protein and how they are linked to a selective advantage in AML is not well understood. Here, we show that cells expressing the AML-associated p30 have reduced baseline inflammatory gene expression and display altered dynamics of transcriptional induction in response to LPS, consequently impacting cytokine secretion. This confers p30-expressing cells an increased resistance to the adverse effects of prolonged exposure to inflammatory signals. Mechanistically, we show that these differences primarily arise from the differential regulation of AP-1 family proteins. In addition, we find that the impaired function of the AP-1 member ATF4 in p30-expressing cells alters their response to ER stress. Collectively, these findings uncover a novel link between mutant CEBPA, inflammation and the stress response, which may explain the unusual frequency of CEBPA mutations in AML.

Myelodysplastic syndrome patients with mutations in cohesin subunit STAG2 show an altered transcriptomic signature indicative of an reduced inflammatory response both at bulk and single cell resolution

Shubhra Ashish Bhattacharya[1,2*], Eve Dias[1,2*], David Corujo[1], René Winkler[1], Jeannine Diesch[1], Maki Sakuma[3,5], Vanesa Valero[1], Roberto Malinverni[1], Hind Medyouf[7], Lionel Adès[6], Emmanuelle Clappier[6], Wencke Walter[3], Torsten Haferlach[3], Marcus Buschbeck[1,4]

[1]Josep Carreras Leukaemia Research Institute (IJC), Badalona, Spain

[2]PhD Programme in Cell Biology, Autonomous University of Barcelona, Spain

[3]Munich Leukemia Laboratory, Munich, Germany

[4]Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain

[5]Technical University Munich, Graduate Center of Medicine and Health, Munich, Germany

[6]Saint-Louis Research Institute, Université Paris-Cité, France

[7]Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Frankfurt am Main, Germany

*Equal contributors

Loss of function mutations in the cohesin complex subunit- stromal antigen 2 (STAG2) occurs frequently in myelodysplastic syndromes (MDS). These mutations are associated with an increased risk of transformation to secondary Acute Myeloid Leukemia (sAML) with a worse prognosis. The cohesin complex has an essential function in the three-dimensional organization of chromatin and gene regulation and by studying the transcriptomic changes at the bulk and single cell resolution we can identify specific gene and pathway deregulated in STAG2 mutated MDS which can be functionally characterized in cell line models mimicking STAG2 loss.

Whole transcriptome sequencing data from bone marrow samples of a cohort of 753 MDS patients including 48 samples with STAG2 mutations (STAG2mut) was used to identify gene signature specific to STAG2. The changes in cell population were estimated in the bulk transcriptome using gene signature-based enrichment method and further precisely characterized in single cell RNAseq data (scRNA) from bone marrow of MDS patients (STAG2mut n = 5; STAG2wt n = 4). The AML cell line OCI-AML5 mimicking STAG2 loss (OCI-AML5ΔSTAG2) was generated using CRISPR-Cas9 system and RNA-sequencing was performed to further characterize it.

scRNA data was clustered and annotated followed by manual curation and refinement of cell labels. A pseudo-bulk analysis of STAG2mut monocytes compared to STAG2wt monocytes revealed downregulation of class I and II HLA molecules in STAG2mut monocytes. This was confirmed in OCI-AML5ΔSTAG2 which showed downregulation of HLA class II molecules. Pathway enrichment analysis of STAG2mut monocytes showed lower enrichment for key immunological pathways such as inflammatory response and TNF signaling via NFKb. These results point towards cell-type specific transcriptomic changes in STAG2mut patients. Additionally, bulk transcriptomic data of STAG2mut patients showed downregulation of genes involved in innate immunity responses such as FFAR2, CCL7, CCR1 among others alongside the downregulation of pathways such as TNF signaling via NFKb and inflammatory response.

Deconvolution analysis of bulk transcriptome indicated a differentiation block as demonstrated by an enrichment of HSC and GMP transcriptomic signature in STAG2mut patients, this result was strengthened by the cytomorphology data of STAG2mut patients which showed a higher relative count of immature cell population such as myeloblasts and promyelocytes. OCI-AML5ΔSTAG2 was also suggestive of altered differentiation observed by a change in colony morphology. Additionally, transcriptomic analysis of the cell line model showed downregulation of genes involved in cell-to-cell adhesion and migration. In line with this, we observed STAG2 knockout cells in the co-culture system were less sensitive to differentiation signals secreted by HS27, a mesenchymal stromal cell line.

Overall, combining observations from bone marrow from STAG2mut patients and cell model with STAG2 loss, we have identified distinct transcriptional alterations and differentiation behavior. This will allow us to characterize further pathways through which STAG2 loss modifies cell behavior leading to disease progression.

NFAT5 counters long-term IFN-I responses in hematopoietic stem cells to preserve reconstitution potential

Cristina Lopez-Rodriguez[1], Laia Traveset[1], Victor Cerdan Porqueras[1], Hector Huerga Encabo[1], Jose Aramburu[1]

[1]Department of Medicine and Life Sciences, Universitat Pompeu Fabra

Hematopoietic stem cells (HSCs) readily recover from acute stress, but persistent stress can reduce their viability and long-term potential. We report that nuclear factor of activated T cells 5 (NFAT5), a transcription modulator of inflammatory responses, protects the HSC pool under stress. NFAT5 limits HSC differentiation to multipotent progenitors (MPPs) after bone marrow transplantation and ablation with ionizing radiation or chemotherapy. NFAT5-deficient HSCs fail to support long-term reconstitution of hematopoietic progenitors and mature blood cells after serial transplant, and competitive transplant assays shows that these defects are HSC- intrinsic. NFAT5-deficient HSCs exhibit enhanced expression of type I interferon (IFN-I) response genes after transplant, and suppressing IFN-I-receptor prevents their exacerbated differentiation and cell death after reconstitution and improves long-term regeneration. Blockade of IFN-I receptor also prevented the overdifferentiation of NFAT5-deficient HSCs after bone marrow ablation. These findings show that long-term IFN-I responses to different hematopoietic stressors drive HSCs towards more differentiated progenitors, and that NFAT5 has an HSC-intrinsic role limiting IFN-I responses to preserve reconstitution potential. Our identification of cell-intrinsic mechanisms that strengthen the resistance of HSCs to stress could help to devise approaches to protect long-term stemness during the treatment of hematopoietic malignancies.

p53, a master remodeler of the spatio-temporal chromatin architecture

François Serra[#1], Andrea Nieto-Aliseda[#1], Lucía Fanlo-Escudero[#1], Llorenç Rovirosa[1], Mónica Cabrera-Pasadas[1,2], Aleksey Lazarenkov[1], Blanca Urmeneta[1], Alvaro Alcalde-Merino[1], Emanuele M Nola[1], Andrei L Okorokov[3], Peter Fraser[4], Mariona Graupera[1,5,6], Sandra D Castillo[1], Jose L Sardina[1], Alfonso Valencia[2,5], Biola M Javierre[1,7]

[1]Josep Carreras Leukaemia Research Institute, Barcelona, Spain

[2]Barcelona Supercomputing Center, Barcelona, Spain

[3]Wolfson Institute for Biomedical Research, University College London, London, UK

[4]Department of Biological Science, Florida State University, Tallahassee, FL, USA

[5]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

[6]CIBERONC, Instituto de Salud Carlos III, Madrid, Spain

[7]Institute for Health Science Research Germans Trias i Pujol, Barcelona, Spain

[#]Contributed equally

Activation of the p53 tumor suppressor triggers a transcriptional program to control cellular response to stress. However, the molecular mechanisms by which p53 controls gene transcription are not completely understood. Here, we uncover the critical role of spatio-temporal genome architecture in this process. We demonstrate that p53 drives direct and indirect changes in genome compartments, topologically associating domains, and DNA loops prior to one hour of its activation, which escort the p53 transcriptional program. Focusing on p53-bound enhancers, we report 340 genes directly regulated by p53 over a median distance of 116 kb, with 74% of these genes not previously identified. Finally, we showcase that p53 controls transcription of distal genes through newly formed and pre-existing enhancer-promoter loops in a cohesin dependent manner. Collectively, our findings demonstrate a previously unappreciated architectural role of p53 as regulator at distinct topological layers and provide a reliable set of new p53 direct target genes that may help designs of cancer therapies.

Pre-existing stem cell heterogeneity dictates clonal responses to acquisition of cancer driver mutations

Indranil Singh[1], Daniel Fernandez Perez[1], Pedro Sánchez[1], Alejo E. Rodriguez[2]

[1]Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Catalonia, Spain

[2]ICREA, Catalan Institution for Research and Advanced Studies Barcelona, Catalonia, Spain

Cancer cells display wide phenotypic variation even across patients with the same mutations. Differences in the cell of origin provide a potential explanation, but these assays have traditionally relied on surface markers, lacking the clonal resolution to distinguish heterogeneous subsets of stem and progenitor cells. To address this challenge, we developed STRACK, an unbiased framework to longitudinally trace clonal gene expression and expansion dynamics before and after the acquisition of cancer mutations. We studied two different leukemia driver mutations, Dnmt3a-R882H and Npm1cA, and found that the response to both mutations was highly variable across different stem cell states. Specifically, a subset of differentiation-biased stem cells, which normally become outcompeted with time, can efficiently expand with both mutations. Npm1c mutations surprisingly reversed the intrinsic bias of the clone-of-origin, with stem-biased clones giving rise to more mature malignant states. We propose a clonal "reaction norm", in which pre-existing clonal states dictate different cancer phenotypic potential.

Recurrent Splicing Mutations in Diffuse Large B-Cell Lymphoma Mediated by Aberrant Somatic Hypermutation

Maria Benitez-Cantos[1], Carlos Cano[1], Marta Cuadros[1], Pedro P. Medina[1]

[1]GenyO

[2]Universidad de Granada

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma. An important mutagenic process in DLBCL is aberrant somatic hypermutation (aSHM) by cytidine deaminase (AID) activation, which occurs preferentially at RCH/TW sequence motifs near transcription start sites. The splice sequences are highly conserved, rich in RCH/TW motifs, and recurrently mutated in DLBCL. Therefore, we hypothesize that aSHM may cause recurrent splice mutations in DLBCL. Aims: This work aims to test whether AID mutates splice sequences more preferentially over other genomic regions in DLBCL and determine these mutations' role in lymphomagenesis. Results: Somatic mutations from 3 DLBCL cohorts and 12 other tumor types were reannotated to study the enrichment in splice mutations in lymphoid malignancies with AID activity. Single base substitutions in RCH or TW contexts proximal to a TSS (<3kb) were analyzed to follow an aSHM pattern. In a meta-cohort of > 1,800 DLBCLs, we found that 77.5% of splicing mutations in 29 recurrently mutated genes followed aberrant somatic hypermutation patterns. Comparing sequencing data from mouse models with (Ung-/-Msh2-/-) and without AID activity (Aicda-/-) showed that the splice donor sequences were the top genomic feature enriched in AID-induced mutations ($p < 0.0001$). Finally, we determined that most AID-related splice site mutations are clonal within a sample, denoting that aSHM may cause early loss-of-function events in lymphomagenesis.

Resolving epigenetic sensitization mechanisms of ferroptosis lipidome-kinome signaling to overcome therapy resistance in multiple myeloma

Wim Vanden Berghe[1], Amber Driesen[1], Emilie Logie[1], Maarten Dhaenens[2], Jerome Moreaux[3], Tom Vanden Berghe[4], Luliana Vintea[4], Gerben Menschaert[5]

[1]Cell Death Signaling Epigenetics lab, University Antwerp

[2]Laboratory of Pharmaceutical Biotechnology, Proteomics and Mass Spectrometry, UGent

[3]University of Montpellier 1, UFR de Médecine, Montpellier, France

[4]Cell Death Signaling Ferroptosis lab, University Antwerp

[5]Biobix, Laboratory of Bioinformatics and Computational Genomics, UGent

Treatment failure and remission remain a major challenge multiple myeloma (MM) treatment, which until today remains incurable. Therefore, compounds inducing ferroptosis, a form of iron and lipid peroxidation-regulated cell death, are appealing alternative treatment strategies. Both ferroptosis and the epigenetic machinery are heavily influenced by oxidative stress and iron metabolism changes. In this study, we found that different multiple myeloma cell types respond to ferroptosis induction and epigenetic reprogramming. LC-MS/MS analysis revealed the formation of non-heme iron-histone complexes and altered expression of novel histone ferrolipidation as well as DNA repair and cellular senescence modifications. In line with this observation, EPIC BeadChip measurements of significant DNA methylation changes in ferroptotic myeloma cells demonstrated an enrichment of CpG probes located in genes associated with cell cycle progression and senescence. Finally, by phosphopeptidome based (Pamchip) kinase activity profiling, we further identified a ferroptosis specific kinome signature associated with cytotoxic lipid peroxidation distinct from apoptosis cell death signaling. Overall, our data show that ferroptotic cell death is associated with a lipidome-kinome-epigenome stress signaling response that might advance the therapeutic applicability of ferroptotic drug compounds to overcome MM therapy resistance.

SMARCA4 mutations as drivers for T-cell acute lymphoblastic leukemia (T-ALL) relapse

Violeta García-Hernández[1], María Maqueda[1], Alba Vidal[1], Jessica González[1], Arnau Iglesias[1], Lluís Espinosa[1], Anna Bigas[1]

1Cancer Research Program, Hospital del Mar Research Institute, Barcelona, Spain. CIBERONC (Instituto de Salud Carlos III), Spain

Resistance to chemotherapy is the major driver of leukemia relapse with dismal prognosis affecting adults and children. We have recently identified relapse-specific mutations in T-ALL patients that affect the chromatin remodeler SMARCA4, among other chromatin modifiers (Sentís 2020). We concluded that the relapse clone was already present at diagnosis at a very low frequency, indicating that this clone resisted the treatment. Hence, we hypothesize that dysregulation of chromatin remodeling is the driving force of relapse in some T-ALL patients. To investigate the contribution of SMARCA4 to T-ALL evolution, we engineered relapse- associated SMARCA4 mutations in T-ALL cell lines by CRISPR/Cas9. In vitro assays indicate that SMARCA4 disruption confers multidrug resistance and competitive advantage to therapy in T-ALL cells. By ATAC and RNAseq analysis of SMARCA4-disturbed cells prior and after short-term chemotherapy, we observed that SMARCA4 mutations create a chromatin and transcriptome landscape less responsive to treatment, with basal changes globally retained. We have now performed single-nuclei multiomics to infer affected enhancers and gene regulatory networks, estimate transcriptional variation and explore its involvement in tumor plasticity. Our next goal will be to apply this knowledge to in vivo and patient-derived models. This study will provide putative therapeutic targets in T-ALL and may provide new premises for epigenetically dysregulated cancers.

Succinate receptor 1 restricts hematopoiesis and prevents acute myeloid leukemia progression

Vincent Cuminetti[1], Sra. Joanna Konieczny[2], Emeline Boet[3], Aurora Bernal[2], Marc Ferré[1], Manuel J. Gomez[4], Franco Grimolizzi[2], Nuria Vilaplana-Lopera[5], Alicia Villatoro[2], Carlos Torroja[4], Hagar Taman[6], Ruth H. Paulssen[6], Giovanna Giovinazzo[7], Thomas Vogl[8], Catoline A. Heckman[9], Anders Vik[10], Paloma García[5], Dra. Fátima Sánchez-Cabo[4], Dr. Jean-Emmanuel Sarry[3], Dra. Lorena Arranz[1]

[1]Stem Cells, Ageing and Cancer Research Group, Centre of Embryology and Healthy Development, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, 0373 Oslo, Norway

[2]Stem Cells, Ageing and Cancer Research Group, Department of Medical Biology, Faculty of Health Sciences, UiT – The Arctic University of Norway, MH2 building level 10, 9019 Tromsø, Norway

[3]Centre de Recherches en Cancérologie de Toulouse, Université de Toulouse, Inserm U1037, CNRS U5077, Toulouse, France

[4]Bioinformatics Unit, Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), 28029 Madrid, Spain

[5]Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom

[6]Genomics Support Centre Tromsø, Faculty of Health Sciences, UiT – The Arctic University of Norway, MH building level 9, 9019 Tromsø, Norway

[7]Pluripotent Cell Technology Unit, CNIC, 28029 Madrid, Spain

[8]Institute of Immunology, University of Münster, 48149 Münster, Germany

[9]Institute for Molecular Medicine Finland, FIMM, Helsinki Institute of Life Science, iCAN Digital Precision Cancer Medicine Flagship, University of Helsinki, 00014 Helsinki, Finland

[10]Department of Hematology, University Hospital of North Norway, 9019 Tromsø, Norway

Hematopoietic stem cell (HSC) quiescence is fine-tuned by the hypoxic niche of the bone marrow. Succinate accumulates under hypoxia and regulates several functions like hypoxia response through hypoxia-inducible factor-1, resulting in IL-1? production in macrophages. Additional effects of succinate include histone succinylation and histone and DNA methylation. Further, Succinate- receptor 1 (Sucnr1) signaling may be pro- or anti-inflammatory, and cancer-derived succinate promotes macrophage polarization and cancer metastasis via Sucnr1. Despite these intriguing roles for the Sucnr1 in inflammation and cancer, few studies have explored its role in hematopoiesis under health or malignancy. Deletion of Sucnr1 in vivo results in an expansion of hematopoietic stem and progenitor cells (HSPC) and hematopoiesis. Our data show that activation of Sucnr1 counterbalances the stimulatory effect of succinate at least partially through a direct effect on HSPC and preserves their transcriptional programs via control of S100a8/S100a9. In acute myeloid leukemia (AML) patients, we found that low SUCNR1 represents a marker for reduced survival. Consistent with low SUCNR1 expression, we found high S100A8 and S100A9 expression in M4-M5 AML patients and in single-cell RNA-sequencing cluster analyses from persistent cells after cytarabine treatment in AML patient-derived xenografts, correlating with a residual disease signature. Succinic acid, which displays both Sucnr1-dependent and independent effects, promoted disease progression in low Sucnr1-expressing mouse models of pre-leukemic myelopoiesis or AML and in AML xenografts. Cis-epoxy succinate, a strong Sucnr1 agonist, sensitized AML cell lines to cytarabine. In conclusion, Sucnr1 signaling is a regulatory pathway that counterbalances intracellular succinate effects and restricts hematopoiesis via control of S100a8/S100a9. Its dysregulation emerges as contributor to AML that may open new therapeutic alternatives for patients.

Targeting Fatty Acid Oxidation Pathways to Overcome Proteasome Inhibitor Resistance in Multiple Myeloma

Fatma Necmiye Kaci[1], Alessio Lepore[1], Aqeelah Mohammad[1], Abdullah Almarzouq[1], Isabel Craghill[1], Christoph Driessen[2], Lenka Besse[2], Concetta Bubici[3], Salvatore Papa[1]

[1]Leeds Institute of Medical Research, Faculty of Medicine and Health, University of Leeds, St. James' University Hospital, Leeds, UK

[2]Cantonal Hospital St Gallen, Clinic for Oncology and Hematology, St. Gallen, Switzerland

[3]Centre for Genome Engineering and Maintenance, Department of Life Sciences, College of Health, Medicine and Life Sciences, Brunel University London, Uxbridge, UK

Multiple myeloma (MM) is a blood cancer that affects plasma cells; despite recent significant advancements in therapy, the disease remains incurable with only a 53% 5-year survival rate. Given this grim reality, there is an urgent need to discover new vulnerabilities in MM and explore innovative therapeutic approaches. In this study, we investigated the possible anti-myeloma efficacy of a recently developed chemical inhibitor known as DIAPO (drug-inducing apoptosis). DIAPO has demonstrated the ability to trigger apoptosis in both treatment-naïve multiple myeloma cells and cells that have developed resistance to commonly used proteasome inhibitors such as bortezomib and carfilzomib. We used both in vitro and in vivo MM xenografts models to assess various cellular processes including cell viability, apoptosis, and cellular metabolism (specifically oxygen consumption rates and fatty acid oxidation). Mechanistically, we discovered that DIAPO inhibits crucial survival signalling pathways in proteasome inhibitor-resistant cells, particularly by suppressing the expression of key regulators of fatty acid oxidation. Our findings suggest that DIAPO could be a promising treatment for patients with multiple myeloma, particularly for those who have developed resistance to current standard therapies and provide a strong rationale for extending the preclinical and clinical testing of DIAPO to improve patient outcome in MM.

Targeting Polycomb for treatment of KMT2A-rearranged acute myeloid leukemia

Andreas Lennartsson[1]

[1]Karolinska Institutet, Sweden

KMT2A-rearrangements (KMT2A-r) are the most common cytogenetic aberrations seen in paediatric acute myeloid leukaemia (pAML), identified in up to 15% of cases in children and 60% in infants (1). These fusions are associated with poorer prognosis than other subtypes, involving higher relapse rates and lower overall survival (2). KMT2A-r is linked to dysregulated epigenetic reading and modifying activities, altering the chromatin landscape. Improved understanding of how KMT2A-r remodels chromatin to promote oncogenesis in pAML could provide new therapeutic targets for this poor-risk patient group. In this study, we looked at the non- coding transposable element (TE) portion of the genome as it has not been investigated in pAML before. TEs are an understudied reservoir of potential cis-regulatory sequences, and are usually epigenetically repressed in healthy somatic cells. We have previously shown that epigenetic silencing of adult AML-specific TE families, such as LTR2B, slows proliferation and affects nearby gene expression indicative of their enhancer activity in AML (3). Using epigenetic profiling of 123 pAML patients from the MyeChild cohort we demonstrate that in KMT2A-r pAML patients, a family of TEs plays a similar role to LTR2B in adult AML. This family is enriched for chromatin accessibility (ATAC-seq), as well as the enhancer-associated histone modifications H3K27ac and H3K4me1 (CUT&RUN). Further work will involve functionally validating these TEs as enhancers using CRISPR-mediated epigenetic silencing and activation of this TE family in KMT2A-r pAML cell lines and patient samples. This is in addition to integration with RNA-seq and methyl-seq data to gain a fuller picture of how this TE family is regulated and may be promoting pro-leukaemic transcriptional programmes. The final aim of this project will be identifying ways to therapeutically target the pro-leukaemic-TE axis in pAML which can be translated to the clinic.

The dark side of stemness – residual hematopoietic stem cells in pediatric B-cell acute lymphoblastic leukemia

Jadwiga Filipek-Gorzala[1], Patrycja Kwiecinska[1], Kacper Kowalski[1], Izabella Skulimowska[1], Sr. Paweł Kozuch[2], Kinga Gawlińska[2], Monika Gońka[2], Jan Morys[2], Sylwester Mosiołek[2], Alicja Józkowicz[2], Karolina Bukowska-Strakova[3], Katarzyna Pawlińska-Wasikowska[4], Szymon Skocze?[4], Maciej Siedlar[3], Walentyna Balwierz[4], Agata Szade[2], Krzysztof Szade[1]

[1]Laboratory of Stem Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University

[2]Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University

[3]Department of Clinical Immunology, Institute of Pediatrics, Jagiellonian University Medical College

[4]Department of Pediatric Oncology and Hematology, Institute of Pediatrics, Jagiellonian University Medical College

[5]King's College London, London, UK

Hematopoietic stem cells (HSCs) produce all blood cells throughout the life of an organism. However, long lifespan predisposes them to accumulate mutations. While somatic mutations in HSCs are well-evidenced in adult leukemia, the role of HSCs in development of pediatric leukemias remains unclear. The aim of the study is to characterize genetics, transcriptome, and functional properties of HSCs in pediatric B-cell acute lymphoblastic leukemia (B-ALL). HSC pool was quantified using flow cytometry. Whole exome sequencing (exome-seq) was performed to define the patient-specific mutational landscape. DNA was isolated from single HSC-derived colonies and specific target regions were then amplified using PCR, followed by sequencing. Bone marrow samples were enriched in phenotypic HSCs by cell sorting and single-cell RNA sequencing (scRNA-seq) was performed. Genotyping of Transcriptomes (GoT) analysis was conducted when possible. At diagnosis, most patients had detectable HSCs. However, a subset of patients lacked HSCs but exhibited an expanded progenitor pool. Exome-seq identified somatic mutations in both epigenetic and signaling genes. Variant allele frequency analysis suggested somatic evolution but did not reveal distinct mutation patterns. scRNA- seq analysis identified a subpopulation of HSCs that expressed B-lineage-specific genes. Non-leukemic B-progenitors were rare and separated from leukemic blasts. Targeted DNA sequencing and GoT analyses revealed the presence of preleukemic mutations in some HSCs. Functionally, HSCs exhibited heterogeneity in single-cell colony assay. To sum up, our findings reveal alterations in residual HSCs in pediatric B-ALL patients and suggest their involvement in leukemia development. While HSCs maintained multipotent differentiation potential, we identified the subpopulation that expressed genes associated with B-cell lineage commitment. Additionally, our research uncovered preleukemic mutations within HSCs in certain cases.

Therapy-induced senescence favors tumor spreading in non-Hodgkin lymphoma

Guardia C[1], Balibrea J[1], Castillo-Agea E[1], Olivares D[1], Sydorenko M[1], Ortega-Alvarez D[1], Vinuesa E[1], Rodilla V[1]

(1) Cancer Heterogeneity and Hierarchies Group, Josep Carreras Leukaemia Research Institute (IJC). Badalona, Spain

The prognosis for non-Hodgkin lymphoma (NHL) patients varies significantly, with many experiencing relapses within 2–3 years following standard chemotherapy. Chemotherapeutic agents, being genotoxic, target proliferating cells indiscriminately and induce senescence in healthy tissues, leading to long-term side effects akin to age-related conditions. Research on cancer survivors who received chemotherapy during childhood highlights persistent sequelae such as organ dysfunction, cognitive impairment, and secondary malignancies. Despite well- documented cytotoxic effects, the role of therapy-induced senescence (TIS) in NHL progression is not fully understood. To investigate how senescent cells affect NHL progression, we employed single-cell RNA sequencing (scRNA-seq) and functional assays in immunocompetent mice. Our results indicate that the CHOP chemotherapy regimen, a standard treatment for NHL, induces canonical senescence markers and senescence-associated secretory phenotype (SASP) factors in healthy murine tissues. This senescent microenvironment facilitates lymphoma dissemination, particularly to the liver, a common site for extranodal involvement. RNA sequencing and principal component analysis (PCA) revealed significant alterations in the liver gene expression profile, with Cdkn1a (p21) being one of the most upregulated genes in CHOP- treated mice. Furthermore, CHOP treatment increased the expression of pro-inflammatory pathways such as IL-6-JAK-STAT and NF-κB, suggesting that TIS promotes lymphoma spread. Single-cell transcriptomics identified endothelial cells as major contributors to TIS, showing substantial upregulation of Cdkn1a (p21), which was further confirmed by increased SA-β- galactosidase activity in human endothelial cell cultures. Importantly, senescence in endothelial cells was associated with significant neutrophil infiltration, as evidenced by elevated levels of neutrophil-related markers, including s100a8, s100a9, and Mpo, at both RNA and protein levels. Notably, high expression of S100A8/S100A9 correlated with worse prognosis in publicly available RNA-seq databases of diffuse large B-cell lymphoma (DLBCL) patients. In conclusion, our findings suggest that chemotherapy primes tissue microenvironment to enhance lymphoma dissemination, with senescent endothelial cells and subsequent neutrophil infiltration playing crucial roles. While senescence disrupts immune responses through both cell-autonomous mechanisms and the SASP, its specific impact on neutrophil recruitment is still underexplored. Senescent endothelial cells may create a microenvironment that promotes neutrophil activation and recruitment, thereby amplifying inflammatory responses and facilitating lymphoma spread. Targeting senescent endothelial cells and/or modulating neutrophil recruitment may offer novel therapeutic strategies to mitigate chemotherapy-induced side effects and limit NHL progression.

Uncovering TET2 chromatin targets at the leukemic onset

Aleksey Lazarenkov[1], Anna V. López-Rubio[1], Clara Berenguer[1], Gemma Valcárcel[1], Mireia Obiols[1], Gregoire Stik[1] and José L. Sardina[1].

[1]Josep Carreras Leukaemia Research Institute (IJC)

Epigenetic modifications, including DNA methylation, have been shown to play a prominent role in influencing cell identity in hematopoiesis. TET enzymes oxidize 5mC residues to 5hmC, favoring DNA demethylation and transcriptional activation. Among them, TET2 is mainly found in the myeloid lineage, and its gene is frequently mutated in a broad range of myeloid malignancies. TET2 loss-of-function is associated with enhanced proliferation of blood progenitors and heightened resistance to inflammation-related apoptosis, leading to clonal expansion of TET2-mutated cells. However, the molecular mechanisms underlying such profound phenotypes are yet to be fully uncovered.

In this study, we extensively profiled TET2 genome occupancy in a highly controllable, rapid, and uniform cellular model of myeloid commitment. We crossed our data with publicly available datasets containing information about chromatin accessibility (by ATAC-seq), configuration (by HiC-seq), and state (by TT-seq and ChIP-seq for histone marks) during myeloid establishment. As a result, we discovered the role of TET2 in activating cell fate commitment programs. We identified subsets of TET2-bound regulatory regions that get demethylated and activated upon myeloid commitment, as well as uncovered novel TET2 implications in long-range chromatin remodeling. Furthermore, we profiled the DNA methylation and expression events affected by TET2 loss of function, and along with the TET2 chromatin occupancy data, we used them to identify TET2 bona fide chromatin targets during myeloid establishment. To gain insight into the potential involvement of these novel TET2 targets in a leukemic context, we then crossed our data with publicly available methylation data in TET2-mutated AML patients (LAML-TCGA). We observed that 13 genes were both detected as TET2 targets in our system and hypermethylated in AML patients, highlighting their potential involvement in the molecular mechanisms underlying TET2 mutations. Among them, we identified AGO2, which enhancer gets demethylated by TET2 during myeloid commitment, ultimately resulting in transcriptional modulation through direct enhancer-promoter contact.

Altogether, our unbiased chromatin target identification offers a unique opportunity to investigate the relevance of DNA methylation events during myeloid differentiation. We have shown the importance of TET2 in targeting gene regulatory elements of crucial myeloid genes that get abnormally hypermethylated and downregulated upon TET2 loss of function, as seen in TET2-mutated AML patients. This underscores the need to investigate our bona fide TET2 targets, such as AGO2, for potential new therapeutic approaches to address myeloid malignancies in patients.

Understanding DNA methylation regulation in hematopoiesis at single-cell resolution

Michael Scherer[1], Indranil Singh[2], Martina Braun[3], Alejo Rodriguez-Fracticelli[2], Lars Velten[3]

[1]German Cancer Research Center

[2]Institute for Research in Biomedicine

[3]Centre for Genomic Regulation

DNA methylation has important roles in establishing cell type identity in hematopoiesis. In addition, the regulatory machinery of DNA methylation is heavily altered in hematological conditions and malignancies. During this project, we explored the role of DNA methylation in hematopoiesis at the single-cell level. Through our single-cell methods scTAM-seq, we were able to trace both the cellular differentiation state and clonal identity through the DNA methylation state of the cell. Hematopoietic clones as identified through our developed method – EPI-clone - showed excellent overlap with ground-truth clonal markers. Using the unique combination of cell state and clonal information, we were for able to functionally characterize hematopoietic stem cell clones. We showed that during murine aging the number of blood-producing clones decreases, while there is an increase of clones harboring almost exclusively immature cells. In humans, we found that clones identified through EPI-clone carry numerous markers of clonal hematopoiesis including loss of Y chromosome and somatic mutations. EPI-clone enables the functional characterization of hematopoietic stem cell clones in native development both in mice and humans. ScTAM-seq and its extension EPI-clone enable the high-throughput characterization of DNA methylation patterns in healthy and malignant hematopoiesis. We envision to apply our single-cell method to trace DNA methylation alterations in acute myeloid leukemia.

Unraveling tumor intrinsic responses: IFN-gamma predicts clinical immunotherapy efficacy and exclusively triggers apoptosis in solid tumors while sparing hematologic malignancies

Víctor Sánchez Miñarro[1*], Alex Martínez-Sabadell[1*], Aleix Franquesa-Sánchez[1*], Manel Esteller[1,2,3,4,#] and Enrique J. Arenas[1,#]

[1]Cancer Epigenetics Group, Josep Carreras Leukemia Research Institute (IJC), Badalona, Barcelona, Catalonia, Spain

[2]Bioinformatic Unit, Josep Carreras Leukemia Research Institute (IJC), Badalona, Barcelona, Catalonia, Spain

[3]Centro de Investigacion Biomedica en Red Cancer (CIBERONC), Madrid, Spain

[4]Institucio Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Catalonia, Spain

[5]Physiological Sciences Department, School of Medicine and Health Sciences, University of Barcelona (UB), Barcelona, Catalonia , Spain

*Equal contributors

#Corresponding Authors

Over the past lustrum, tumour intrinsic interferon gamma (IFNɣ) has emerged as a pivotal factor in the realm of cancer immunotherapy resistance for solid tumors, being determinant for optimal cancer immunotherapy response to both antigen presentation dependent immunotherapies such as immune checkpoint blockade and T cell redirection therapies like chimeric antigen receptor (CAR) T cells and bispecific antibodies (bsAbs) whose recognition do not rely on the expression of the major histocompatibility complex I (MHC-I). However, the requirement of tumor IFNɣ for the efficient killing of CAR T cells and bsAbs in hematologic tumors, which has shown remarkable clinical efficacy, remains uncertain, yielding contradictory results. Furthermore, these studies employed genetically modified knock-out (KO) models with complete absence of the pathway, overlooking its potential role in tumors that acquire resistance through transcriptional downmodulation. In addition, it has been demonstrated distinct molecular mechanisms between complete IFNɣ non-responders and partial responders, both of whom exhibit resistance. Furthermore, the tumor antigen's biology, the intrinsic differences between bsAbs and CAR T cells, and the precise anti-tumor mechanism of action exerted by IFNɣ has been disregarded. In this piece of work, using hematologic cancer cells naturally expressing CD19 or engineered to express HER2, and solid cancer cells expressing HER2 or engineered to express CD19, and bsAbs and CAR T cells targeting these antigens, we demonstrate that a transcriptional impairment of tumor IFNɣ signaling employing shRNAs targeting the IFNGR1 gene leads to resistance to bsAbs and CAR T cells exclusively in solid tumors. In addition, we show that IFNɣ induces apoptosis in solid but not in hematologic cancer cells, and we show that hematologic cancer cells exhibit a higher basal intrinsic IFNɣ signaling which result in a suboptimal response to IFNɣ and subsequent submaximal upregulation of downstream effector targets such as FAS, ICAM-1, PD-L1 and HLAs, which can explain their lack of response to the pro-apoptotic effects of IFNɣ. Importantly, by analyzing publicly available transcriptomic data from hundreds of cancer cell lines of various origins, we confirmed that hematologic cancers express significantly higher levels of IFNɣ-related genes, particularly IRF1, the master regulator of the IFNɣ signaling pathway. Furthermore, an analysis of tumor fractions from scRNA-seq datasets of patients treated with various cancer immunotherapies--specifically immune checkpoint blockade (anti-PD1) for solid tumors and CAR T cells or BsAbs targeting CD19 for leuakemia patients— demonstrates that a tumor IFNɣ signature predicts clinical efficacy exclusively in solid tumors while sparing leakemia cancers. These findings underscore the clinical need to develop tailored treatments that are able to activate tumor IFNɣ signaling to maximize cancer immunotherapy efficacy specifically in solid tumors, thereby minimizing unnecessary toxicities in hematologic cancers.

WITH THE SUPPORT OF