

# METHODS IN MOLECULAR BIOLOGY

*Series Editor*

**John M. Walker**

**School of Life and Medical Sciences**

**University of Hertfordshire**

**Hatfield, Hertfordshire, UK**

For further volumes:

<http://www.springer.com/series/7651>

For over 35 years, biological scientists have come to rely on the research protocols and methodologies in the critically acclaimed *Methods in Molecular Biology* series. The series was the first to introduce the step-by-step protocols approach that has become the standard in all biomedical protocol publishing. Each protocol is provided in readily-reproducible step-by-step fashion, opening with an introductory overview, a list of the materials and reagents needed to complete the experiment, and followed by a detailed procedure that is supported with a helpful notes section offering tips and tricks of the trade as well as troubleshooting advice. These hallmark features were introduced by series editor Dr. John Walker and constitute the key ingredient in each and every volume of the *Methods in Molecular Biology* series. Tested and trusted, comprehensive and reliable, all protocols from the series are indexed in PubMed.

# Leukemia Stem Cells

## Methods and Protocols

Edited by

**César Cobaleda**

*Immune System Development and Function Unit, Centro de Biología Molecular "Severo Ochoa" (CSIC/UAM), Madrid, Spain*

**Isidro Sánchez-García**

*IBMCC, Laboratory 13, CSIC/Universidad de Salamanca, Salamanca, Spain*

*Editors*

César Cobaleda  
Immune System Development  
and Function Unit  
Centro de Biología Molecular  
“Severo Ochoa” (CSIC/UAM)  
Madrid, Spain

Isidro Sánchez-García  
IBMCC, Laboratory 13  
CSIC/Universidad de Salamanca  
Salamanca, Spain

ISSN 1064-3745

Methods in Molecular Biology

ISBN 978-1-0716-0809-8

<https://doi.org/10.1007/978-1-0716-0810-4>

ISSN 1940-6029 (electronic)

ISBN 978-1-0716-0810-4 (eBook)

© Springer Science+Business Media, LLC, part of Springer Nature 2021

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Humana imprint is published by the registered company Springer Science+Business Media, LLC, part of Springer Nature.

The registered company address is: 1 New York Plaza, New York, NY 10004, U.S.A.

---

## Preface

Any new theoretical advance or groundbreaking discovery in science is always faced with initial rejection and opposition, and the cancer stem cell (CSC) theory has not been different. Indeed, even today, more than two decades since the first unambiguous demonstration of their presence in tumors with the advent of flow cytometry and animal transplantations, all the consequences of their existence are far from being taken up to the practice in either the clinic or even in basic or translational cancer research. This inertia of the previous models of cancer, and the reluctance to accept and integrate the CSC theory, could have been understandable in the early years, since (1) the CSC model exposed the reason behind the frustratingly frequent failure of current cancer therapies and (2) cancer studies based on analyzing the tumor mass as a whole lost a large part of their importance.

This reluctance today is indefensible. The existence of CSCs supposes a true change of paradigm in our understanding of cancer, but it will only have a real impact when we properly assimilate its implications and apply this knowledge to both cancer research and cancer treatment. To this aim, the CSC hypothesis should be incorporated from a bottom-up perspective in the design of both basic cancer investigations and therapy development projects. This means that acknowledging the CSC-based nature of tumors should be the starting point for the design of new research and therapeutical approaches. These should replace the top-bottom procedures used still too often today, in which the old paradigms are still assumed, ignoring the fact that they are not useful anymore under the postulates of the CSC theory.

The work that pioneered the demonstration of the CSC hypothesis was performed in the hematopoietic system, and leukemia stem cells (LSCs) were in fact the first CSCs identified. Actually, still today, both normal hematopoietic stem cells (HSCs) and LSCs are the best understood normal/malignant stem cell pair in the human and mouse organisms, and their study keeps leading the way in this field of research, with results that can very often be extrapolated to other tissues and their respective tumors.

The advances in LSC research are being helped by the amazing array of new technological developments that are allowing us to study leukemias with a depth that we could not have imagined just a decade ago. The present volume, part of the *Methods in Molecular Biology* series, aims to provide a comprehensive hands-on manual covering all the techniques involved in the cellular and molecular identification and characterization of both normal hematopoietic and leukemic stem cells, both from human patients and from mouse models of human leukemia. Also, the book covers the most frequently used experimental approaches for the generation of such stem cell-based models of human leukemia.

In order to help the less expert reader to become familiar with the concepts discussed in the protocol chapters, we have also included a review section at the beginning of the book, to provide the reader with quick-reference introductory chapters about the classification and basic biology of human leukemias, the cancer stem cell concept, and about how this concept has important implications for the way we understand, treat, and model leukemia.

Finally, we would like to thank the series editor, Professor John Walker, for giving us the opportunity to work on this project and for his support and guidance during the process. And, last but not least, we would also like to thank all the authors, whose contributions and commitment have made this book possible, for their cooperative effort and understanding throughout the reviewing and editing process.

*Madrid, Spain*  
*Salamanca, Spain*

*César Cobaleda*  
*Isidro Sánchez-García*

---

# Contents

<i>Preface</i> .....	<i>v</i>
<i>Contributors</i> .....	<i>ix</i>

## PART I INTRODUCTORY REVIEWS

1 Introduction and Classification of Leukemias .....	3
<i>Geoffrey Brown</i>	
2 Leukemia Stem Cells: Concept and Implications .....	25
<i>Isidro Sánchez-García and César Cobaleda</i>	
3 Leukemia Stem Cell Drug Discovery .....	39
<i>César Cobaleda and Isidro Sánchez-García</i>	

## PART II PROTOCOLS

4 Analysis and Isolation of Mouse Leukemic Stem Cells .....	51
<i>Fang Dong, Haitao Bai, and Hideo Ema</i>	
5 Mass Cytometry of Hematopoietic Cells .....	65
<i>Astraea Jager, Jolanda Sarno, and Kara L. Davis</i>	
6 PCR Technology to Identify Minimal Residual Disease .....	77
<i>Giovanni Cazzaniga, Simona Songia, and Andrea Biondi</i>	
7 Next-Generation Sequencing Technology to Identify Minimal Residual Disease in Lymphoid Malignancies .....	95
<i>Michaela Kotrova, Nikos Darzentas, Christiane Pott, and Monika Brüggemann</i>	
8 Genomic Inverse PCR for Screening of Preleukemic Cells in Newborns (GIPFEL Technology) .....	113
<i>Daniel Hein, Arndt Borkhardt, and Ute Fischer</i>	
9 Single-Cell Transcriptomic Analysis of Hematopoietic Cells .....	135
<i>Paulina M. Strzelecka, Anna M. Ranzoni, and Ana Cvejic</i>	
10 In-Depth Mass Spectrometry-Based Single-Cell and Nanoscale Proteomics .....	159
<i>Yiran Liang, Thy Truong, Ying Zhu, and Ryan T. Kelly</i>	
11 In Vivo Clonal Analysis of Aged Hematopoietic Stem Cells: Single-Cell Transplantation .....	181
<i>Kyomi J. Igarashi and Ryo Yamamoto</i>	
12 Experimental Competitive Bone Marrow Transplant Assays .....	195
<i>Roxann Hétu-Arbour, Sarah Bouali, and Krista M. Heinonen</i>	
13 Human T-ALL Xenografts .....	215
<i>Patricia Fuentes, María L. Toribio, and Sara González-García</i>	

14	An Experimental and Computational Protocol to Study Cell Proliferation in Human Acute Myeloid Leukemia Xenografts . . . . .	241
	<i>Thalia Vlachou, Marco S. Nobile, Chiara Ronchini, Daniela Besozzi, and Pier Giuseppe Pelicci</i>	
15	Leukemic Stem Cell Culture in Cytokine-Free Medium . . . . .	259
	<i>Xiaolei Liu and Peter S. Klein</i>	
16	Ex Vivo Expansion of Adult Hematopoietic Stem and Progenitor Cells with Valproic Acid . . . . .	267
	<i>Luena Papa, Mansour Djedaini, Manisha Kintali, Christoph Schaniel, and Ronald Hoffman</i>	
17	Isolation, Culture, and Manipulation of Human Cord Blood Progenitors. . . . .	281
	<i>Cristina Prieto, Damia Romero-Moya, and Rosa Montes</i>	
18	Lentiviral Transduction for Optimal LSC/HSC Manipulation. . . . .	299
	<i>Gustavo Mostoslavsky</i>	
19	Characterizing the In Vivo Role of Candidate Leukemia Stem Cell Genes . . . . .	307
	<i>Yu Wei Zhang, Julian Mess, and Nina Cabezas-Wallscheid</i>	
20	Clonal Analysis of Patient-Derived Samples Using Cellular Barcodes . . . . .	317
	<i>Sabrina Jacobs, Leonid V. Bystrykh, and Mirjam E. Belderbos</i>	
21	Arrayed Molecular Barcoding of Leukemic Stem Cells. . . . .	345
	<i>Marion Chapellier and Marcus Järås</i>	
22	In Vivo Generation of Leukemic Stem Cells by HSC Targeting by Transgenesis. . . . .	361
	<i>Carolina Vicente-Dueñas</i>	
23	In Situ Hematopoietic Stem Cell Imaging . . . . .	373
	<i>Aparna Venkatraman, Sarah E. Smith, Sandra Pinho, Meng Zhao, Linheng Li, and Paul Frenette</i>	
24	A Genome Editing System for Therapeutical Targeting of Stem Cells . . . . .	383
	<i>Giacomo Frati and Annarita Miccio</i>	
25	Method for the Generation of Induced Hematopoietic Stem Cells. . . . .	399
	<i>Leonid Olender, Klil Levy, and Roi Gazit</i>	
26	Modeling Leukemia Stem Cells with Patient-Derived Induced Pluripotent Stem Cells. . . . .	411
	<i>André G. Deslauriers, Andriana G. Kotini, and Eirini P. Papapetrou</i>	
27	High-Content Imaging to Phenotype Human Primary and iPSC-Derived Cells. . . . .	423
	<i>Lorenzo Veschini, Heba Sailem, Disha Malani, Vilja Pietiäinen, Ana Stojiljkovic, Erika Wiseman, and Davide Danovi</i>	
28	Bioinformatic Methods to Identify Mutational Signatures in Cancer . . . . .	447
	<i>S. M. Ashiqul Islam and Ludmil B. Alexandrov</i>	
	<i>Index</i> . . . . .	475



---

## Contributors

- LUDMIL B. ALEXANDROV • *Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, USA; Department of Bioengineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA, USA*
- HAITAO BAI • *State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China*
- MIRJAM E. BELDERBOS • *Department of Ageing Biology and Stem Cells, European Research Institute for the Biology of Ageing (ERIBA), University Medical Center Groningen (UMCG), University of Groningen, Groningen, The Netherlands; Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands*
- DANIELA BESOZZI • *Department of Informatics, Systems and Communication, University of Milano-Bicocca, Milan, Italy*
- ANDREA BIONDI • *Department of Medicine and Surgery, University of Milan Bicocca, Monza, Italy; Pediatrics, Ospedale San Gerardo/Fondazione MBBM, Monza, Italy*
- ARNDT BORKHARDT • *Department of Pediatric Oncology, Hematology and Clinical Immunology, University Children's Hospital Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany*
- SARAH BOUALI • *Institut National de la Recherche Scientifique, Laval, QC, Canada*
- GEOFFREY BROWN • *Institute of Clinical Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK; Institute of Immunology and Immunotherapy, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK*
- MONIKA BRÜGGEMANN • *Unit for Hematological Diagnostics, Medical Department II, University Hospital Schleswig-Holstein, Kiel, Germany*
- LEONID V. BYSTRYKH • *Department of Ageing Biology and Stem Cells, European Research Institute for the Biology of Ageing (ERIBA), University Medical Center Groningen (UMCG), University of Groningen, Groningen, The Netherlands*
- NINA CABEZAS-WALLSCHEID • *Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany; Centre for Integrative Biological Signalling Studies (CIBSS), Freiburg, Germany*
- GIOVANNI CAZZANIGA • *Centro Ricerca Tettamanti, Fondazione Tettamanti, Pediatrics, Monza, Italy; Department of Medicine and Surgery, University of Milan Bicocca, Monza, Italy*
- MARION CHAPPELLIER • *Division of Clinical Genetics, Lund University, Lund, Sweden*
- CÉSAR COBALEDA • *Immune System Development and Function Unit, Centro de Biología Molecular "Severo Ochoa" (CSIC/UAM), Madrid, Spain*
- ANA CVEJIC • *Wellcome Trust—Medical Research Council, Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre, Cambridge, UK; Department of Haematology, University of Cambridge, Cambridge, UK; Wellcome Trust Sanger Institute, Cambridge, UK*
- DAVIDE DANОВI • *Stem Cell Hotel, Centre for Stem Cells and Regenerative Medicine, King's College London, London, UK*

- NIKOS DARZENTAS • *Unit for Hematological Diagnostics, Medical Department II, University Hospital Schleswig-Holstein, Kiel, Germany*
- KARA L. DAVIS • *Department of Pediatrics, Bass Center for Childhood Cancer and Blood Disorders, Stanford University, Stanford, CA, USA*
- ANDRÉ G. DESLAURIERS • *Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Biotech Research and Innovation Center, University of Copenhagen, København, Denmark; Center for Hematologic Malignancies, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
- MANSOUR DJEDAINI • *Division of Hematology/Oncology, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
- FANG DONG • *State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China*
- HIDEO EMA • *Department of Regenerative Medicine, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China*
- UTE FISCHER • *Department of Pediatric Oncology, Hematology and Clinical Immunology, University Children's Hospital Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany*
- GIACOMO FRATI • *Imagine Institute, Paris, France*
- PAUL FRENETTE • *Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, Bronx, NY, USA*
- PATRICIA FUENTES • *Interactions with the Environment Program, Immune System Development and Function Unit, Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain*
- ROI GAZIT • *The Shraga Segal Department for Microbiology, Immunology, and Genetics, Faculty of Health Sciences, National Institute for Biotechnology in the Negev, The Ben-Gurion University of the Negev, Beer-Sheva, Israel*
- SARA GONZÁLEZ-GARCÍA • *Interactions with the Environment Program, Immune System Development and Function Unit, Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain*
- DANIEL HEIN • *Department of Pediatric Oncology, Hematology and Clinical Immunology, University Children's Hospital Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany*
- KRISTA M. HEINONEN • *Institut National de la Recherche Scientifique, Laval, QC, Canada*
- ROXANN HÉTU-ARBOUR • *Institut National de la Recherche Scientifique, Laval, QC, Canada*
- RONALD HOFFMAN • *Division of Hematology/Oncology, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
- KYOMI J. IGARASHI • *Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA*
- S. M. ASHIQUL ISLAM • *Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, USA; Department of Bioengineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA, USA*

- SABRINA JACOBS • *Department of Ageing Biology and Stem Cells, European Research Institute for the Biology of Ageing (ERIBA), University Medical Center Groningen (UMCG), University of Groningen, Groningen, The Netherlands*
- ASTRAEA JAGER • *Department of Pediatrics, Bass Center for Childhood Cancer and Blood Disorders, Stanford University, Stanford, CA, USA*
- MARCUS JÄRÅS • *Division of Clinical Genetics, Lund University, Lund, Sweden*
- RYAN T. KELLY • *Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT, USA; Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA, USA*
- MANISHA KINTALI • *Division of Hematology/Oncology, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
- PETER S. KLEIN • *Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
- ANDRIANA G. KOTINI • *Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
- MICHAELA KOTROVA • *Unit for Hematological Diagnostics, Medical Department II, University Hospital Schleswig-Holstein, Kiel, Germany*
- KLIL LEVY • *The Shraga Segal Department for Microbiology, Immunology, and Genetics, Faculty of Health Sciences, National Institute for Biotechnology in the Negev, The Ben-Gurion University of the Negev, Beer-Sheva, Israel*
- YIRAN LIANG • *Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT, USA*
- LINHENG LI • *Stowers Institute for Medical Research, Kansas City, MO, USA*
- XIAOLEI LIU • *Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
- DISHA MALANI • *Institute for Molecular Medicine Finland-FIMM, Helsinki Institute for Life Science-HiLIFE, University of Helsinki, Helsinki, Finland*
- JULIAN MESS • *Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany; Spemann Graduate School for Biology and Medicine (SGBM), Freiburg, Germany; Centre for Integrative Biological Signalling Studies (CIBSS), Freiburg, Germany*
- ANNARITA MICCIO • *Imagine Institute, Paris, France*
- ROSA MONTES • *GENYO Centre for Genomics and Oncological Research, Pfizer-Universidad de Granada—Junta de Andalucía. PTS Granada, Granada, Spain*
- GUSTAVO MOSTOSLAVSKY • *Section of Gastroenterology, Department of Medicine, Center for Regenerative Medicine (CReM), Boston University School of Medicine, Boston, MA, USA*
- MARCO S. NOBILE • *Department of Industrial Engineering and Innovation Sciences, Eindhoven University of Technology, Eindhoven, The Netherlands*
- LEONID OLENDER • *The Shraga Segal Department for Microbiology, Immunology, and Genetics, Faculty of Health Sciences, National Institute for Biotechnology in the Negev, The Ben-Gurion University of the Negev, Beer-Sheva, Israel*
- LUENA PAPA • *Division of Hematology/Oncology, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
- EIRINI P. PAPAPETROU • *Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Black Family Stem Cell Institute, Icahn School of*

- Medicine at Mount Sinai, New York, NY, USA; Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
- PIER GIUSEPPE PELICCI • *Department of Experimental Oncology, IEO, European Institute of Oncology, IRCCS, Milan, Italy*
- VILJA PIETIÄINEN • *Institute for Molecular Medicine Finland-FIMM, Helsinki Institute for Life Science-HiLIFE, University of Helsinki, Helsinki, Finland*
- SANDRA PINHO • *Department of Pharmacology, University of Illinois at Chicago, Chicago, IL, USA*
- CHRISTIANE POTT • *Unit for Hematological Diagnostics, Medical Department II, University Hospital Schleswig-Holstein, Kiel, Germany*
- CRISTINA PRIETO • *VIB Center for Cancer Biology, Leuven, Belgium; KU Leuven Center for Human Genetics, Leuven, Belgium*
- ANNA M. RANZONI • *Wellcome Trust—Medical Research Council, Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre, Cambridge, UK; Department of Haematology, University of Cambridge, Cambridge, UK; Wellcome Trust Sanger Institute, Cambridge, UK*
- DAMIA ROMERO-MOYA • *Department of Anatomy, University of California, San Francisco, CA, USA*
- CHIARA RONCHINI • *Department of Experimental Oncology, IEO, European Institute of Oncology, IRCCS, Milan, Italy*
- HEBA SAILEM • *The Institute of Biomedical Engineering, Oxford, UK*
- ISIDRO SÁNCHEZ-GARCÍA • *Experimental Therapeutics and Translational Oncology Program, Instituto de Biología Molecular y Celular del Cáncer, CSIC/Universidad de Salamanca and Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain*
- JOLANDA SARNO • *Department of Pediatrics, Bass Center for Childhood Cancer and Blood Disorders, Stanford University, Stanford, CA, USA*
- CHRISTOPH SCHANIEL • *Department of Pharmacological Sciences, Mount Sinai Institute for System Biomedicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Department of Cell, Developmental and Regenerative Biology, Black Family Stem Cell Institute, Mount Sinai Institute for System Biomedicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
- SARAH E. SMITH • *Stowers Institute for Medical Research, Kansas City, MO, USA*
- SIMONA SONGIA • *Centro Ricerca Tettamanti, Fondazione Tettamanti, Pediatrics, Monza, Italy*
- ANA STOJILJKOVIC • *Division of Veterinary Anatomy, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
- PAULINA M. STRZELECKA • *Wellcome Trust—Medical Research Council, Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre, Cambridge, UK; Department of Haematology, University of Cambridge, Cambridge, UK; Wellcome Trust Sanger Institute, Cambridge, UK; Department of Haematology, Oncology and Tumor Immunology, Charité-Universitätsmedizin, Berlin, Germany*
- MARÍA L. TORIBIO • *Interactions with the Environment Program, Immune System Development and Function Unit, Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain*
- THY TRUONG • *Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT, USA*
- APARNA VENKATRAMAN • *Stowers Institute for Medical Research, Kansas City, MO, USA*

- LORENZO VESCHINI • *Academic Centre of Reconstructive Science, Faculty of Dentistry, Oral & Craniofacial Sciences, King's College London, London, UK*
- CAROLINA VICENTE-DUEÑAS • *Institute of Biomedical Research of Salamanca (IBSAL), CSIC-Universidad de Salamanca, Salamanca, Spain*
- THALIA VLACHOU • *Department of Experimental Oncology, IEO, European Institute of Oncology, IRCCS, Milan, Italy*
- ERIKA WISEMAN • *Stem Cell Hotel, Centre for Stem Cells and Regenerative Medicine, King's College London, London, UK*
- RYO YAMAMOTO • *Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, USA*
- YU WEI ZHANG • *International Max Planck Research School for Molecular and Cellular Biology (IMPRS-MCB), Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany*
- MENG ZHAO • *Institute of Hematology, Key Laboratory of Stem Cells and Tissue Engineering, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou, China*
- YING ZHU • *Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA, USA*