Myeloid Leukemia
Preface

Recurring, nonrandom cytogenetic abnormalities are common in hematological malignancies, and their recognition has paved the way for the identification and therapeutic exploitation of the clonal molecular lesions that are uniquely associated with specific subtypes of myeloid leukemia. Appreciation of the prognostic importance of these cytogenetic and molecular genetic abnormalities has provided the major thrust for the emergence of new genetically based leukemia classifications.

*Myeloid Leukemia: Methods and Protocols* is devoted to a review of those laboratory techniques that are most likely to assist laboratory scientists and hematologists in the investigation and management of patients with myeloid malignancies. Scientists will benefit from the provision of a wide range of protocols that are documented in sufficient detail to enable their rapid implementation in a routine molecular hematology laboratory. Clinicians will also be rewarded by a concise description of the relevance of the assays, combined with recommendations for how each assay should be integrated into the overall management plan.

The early chapters deal with generally applicable techniques for molecular biology, cytogenetics, and fluorescence in situ hybridization. A comprehensive overview of the expanding field of real-time quantitative polymerase chain reaction is also included, so as to provide a background for the large proportion of chapters that utilize variations of this technique. The remainder of *Myeloid Leukemia: Methods and Protocols* is primarily directed toward acute myeloid leukemia (AML), with particular attention to the molecular lesions that enable prognostic stratification and facilitate monitoring for minimal residual disease. In particular, qualitative and quantitative methodologies for assessment of core binding factor leukemias and acute promyelocytic leukemia are presented. In addition to gene rearrangements, other prognostically relevant molecular lesions, such as FLT3 mutations and WT-1 overexpression, are covered. The rapidly developing field of oligonucleotide microarrays is addressed with a detailed methodological description and a review of the complex range of applicable statistical tools. This is then supplemented by another chapter that deals with a novel monoclonal antibody microarray approach for the diagnosis and classification of AML.

In addition, chapters addressing important molecular aspects of chronic myeloid leukemia, polycythemia rubra vera, essential thrombocythemia, hypereosinophilia, and myelodysplasia have also been included. Finally, a
comprehensive review of methods for the assessment of hemopoietic chimerism in the setting of nonmyeloablative stem cell transplantation has been provided, because transplantation is an important component of the overall management of patients with acute and chronic myeloid malignancies.

Although the topics covered do not include all of the molecular abnormalities associated with myeloid malignancies, they broadly encompass those assays of immediate clinical relevance, and provide helpful strategies that are adaptable now and in the future for other molecular lesions that might be considered equally relevant.

The contributions of the many authors writing in *Myeloid Leukemia: Methods and Protocols* are gratefully acknowledged; they have generously shared the techniques that have proved so successful in their own laboratories. As with other volumes in the Methods in Molecular Medicine series, a collection of invaluable Notes accompanies each chapter to highlight specific technical issues, with the aim of facilitating the rapid establishment of the assays in the reader’s own facility. Finally, we would also like to acknowledge the helpful assistance of Amy Nixon in coordinating the assembly of this volume.

*Harry Iland*
*Mark Hertzberg*
*Paula Marlton*
Contents

Preface .......................................................................................................................... v
Contributors .............................................................................................................. ix

1 Isolation of RNA and DNA From Leukocytes and cDNA Synthesis
   Joop H. Jansen and Bert A. van der Reijden ......................................................... 1
2 Cytogenetic and FISH Techniques in Myeloid Malignancies
   Lynda J. Campbell .................................................................................................. 13
3 Overview of Real-Time RT-PCR Strategies for Quantification
   of Gene Rearrangements in the Myeloid Malignancies
   Christophe Picard, Monique Silvy, and Jean Gabert ........................................ 27
4 Diagnosis and Monitoring of Chronic Myeloid Leukemia
   by Qualitative and Quantitative RT-PCR
   Susan Branford and Timothy Hughes ............................................................... 69
5 Detection of BCR-ABL Mutations and Resistance
   to Imatinib Mesylate
   Susan Branford and Timothy Hughes ............................................................... 93
6 Deletion of the Derivative Chromosome 9
   in Chronic Myeloid Leukemia
   Lynda J. Campbell ............................................................................................... 107
7 Diagnosis and Monitoring of PML-RARA-Positive Acute
   Promyelocytic Leukemia by Qualitative RT-PCR
   Vincenzo Rossi, Laura Levati, and Andrea Biondi .......................................... 115
8 Diagnosis and Monitoring of PML-RARα-Positive Acute
   Promyelocytic Leukemia by Quantitative RT-PCR
   Elisa Mokany, Alison V. Todd, Caroline J. Fuery,
   and Tanya L. Applegate .................................................................................... 127
9 Diagnosis and Monitoring of AML1-MTG8 (ETO)-Positive
   Acute Myeloid Leukemia by Qualitative
   and Real-Time Quantitative RT-PCR
   Khalid Tobal and John A. Liu Yin ..................................................................... 149
10 Diagnosis and Monitoring of CBFB-MYH11-Positive Acute Myeloid
    Leukemia by Qualitative and Quantitative RT-PCR
    Bert A. van der Reijden and Joop H. Jansen ................................................ 163
11 Detection of the FIP1L1-PDGFRα Fusion in Idiopathic Hypereosinophilic Syndrome and Chronic Eosinophilic Leukemia
   Jan Cools, Elizabeth H. Stover, and D. Gary Gilliland ..................... 177
12 FLT3 Mutations in Acute Myeloid Leukemia
   Hitoshi Kiyoi and Tomoki Naoe ................................................... 189
13 WT-1 Overexpression in Acute Myeloid Leukemia and Myelodysplastic Syndromes
   Daniela Cilloni, Enrico Gottardi, and Giuseppe Saglio .................... 199
14 Classification of AML by DNA-Oligonucleotide Microarrays
   Alexander Kohlmann, Wolfgang Kern, Wolfgang Hiddemann, and Torsten Haferlach .......................................................... 213
15 Classification of AML Using a Monoclonal Antibody Microarray
   Richard I. Christopherson, Kerryn Stoner, Nicole Barber, Larissa Belov, Adrian Woolfson, Mike Scott, Linda Bendall, and Stephen P. Mulligan ........................................................... 241
16 Methods for the Detection of the JAK2 V617F Mutation in Human Myeloproliferative Disorders
   Peter J. Campbell, Linda M. Scott, E. Joanna Baxter, Anthony J. Bench, Anthony R. Green, and Wendy N. Erber ....... 253
17 Overexpression of PRV-1 Gene in Polycythemia Rubra Vera and Essential Thrombocythemia
   Maurizio Martini, Luciana Teofili, and Luigi M. Larocca .................... 265
18 Chimerism Analysis Following Nonmyeloablative Stem Cell Transplantation
   Thomas Lion and Franz Watzinger .................................................. 275
Index ............................................................................................................ 297
Contributors

TANYA L. APPLEGATE • Johnson & Johnson Research Pty. Limited, NSW, Australia

NICOLE BARBER • School of Molecular and Microbial Biosciences, University of Sydney, NSW, Australia

E. JOANNA BAXTER • Department of Haematology, University of Cambridge, Cambridge, UK

LARISSA BELOV • School of Molecular and Microbial Biosciences, University of Sydney, NSW, Australia

ANTHONY J. BENCH • Department of Haematology, Addenbrooke’s Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

LINDA BENDALL • Westmead Institute for Cancer Research, Westmead Millennium Institute, Westmead, NSW, Australia

ANDREA BIONDI • Centro Ricerca M. Tettamanti, Clinica Pediatrica Università di Milano Bicocca and Dipartimento di Medicina Clinica, Prevenzione e Biotecnologie Sanitarie, Ospedale San Gerardo, Monza, Italy

SUSAN BRANFORD • Division of Molecular Pathology, Institute of Medical and Veterinary Science, South Australia, Australia

LYNDA J. CAMPBELL • Cytogenetics Department, St. Vincent’s Hospital, Victoria, Australia

PETER J. CAMPBELL • Department of Haematology, University of Cambridge, Cambridge, UK

RICHARD I. CHRISTOPHERSON • School of Molecular and Microbial Biosciences, University of Sydney, NSW, Australia

DANIELA CILLONI • Department of Clinical and Biological Sciences, University of Turin, Turin, Italy

JAN COOLS • Department of Human Genetics, Flanders Interuniversity Institute for Biotechnology (VIB), University of Leuven, Leuven, Belgium

WENDY N. ERBER • Department of Haematology, Addenbrooke’s Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

CAROLINE J. FUERY • Johnson & Johnson Research Pty. Limited, NSW, Australia

JEAN GABERT • Department of Biochemistry & Molecular Biology, Hôpital Universitaire Nord (AP-HM), ERT-MÉDIA-IFR Jean Roche-Université de la Méditerranée, Marseille, France
D. GARY GILLILAND • Brigham and Women’s Hospital, Harvard Medical School, and Howard Hughes Medical Institute, Harvard University, Boston, Massachusetts

ENRICO GOTTARDI • Department of Clinical and Biological Sciences, University of Turin, Turin, Italy

ANTHONY R. GREEN • Department of Haematology, University of Cambridge, and Department of Haematology, Addenbrooke’s Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

TORSTEN HAERLACH • Laboratory for Leukemia Diagnostics, Department of Internal Medicine III, Ludwig-Maximilians-University, Munich, Germany

WOLFGANG HIDDEMANN • Laboratory for Leukemia Diagnostics, Department of Internal Medicine III, Ludwig-Maximilians-University, Munich, Germany

TIMOTHY HUGHES • Division of Hematology, Institute of Medical and Veterinary Science, South Australia, Australia

JOOP H. JANSEN • Central Hematology Laboratory, University Medical Center St. Raboud, The Netherlands

WOLFGANG KERN • Laboratory for Leukemia Diagnostics, Department of Internal Medicine III, Ludwig-Maximilians-University, Munich, Germany

HITOSHI KIYOI • Department of Infectious Diseases, Nagoya University School of Medicine, Nagoya, Japan

ALEXANDER KOHLMANN • Laboratory for Leukemia Diagnostics, Department of Internal Medicine III, Ludwig-Maximilians-University, Munich, Germany

LUIGI M. LAROCCA • Instituto di Anatomia Patologica and Ematologia, Università Cattolica del Sacro Cuore, Roma, Italy

LAURA LEVATI • Centro Ricerca M. Tettamanti, Clinica Pediatrica Università di Milano Bicocca and Dipartimento di Medicina Clinica, Prevenzione e Biotecnologie Sanitarie, Ospedale San Gerardo, Monza, Italy

THOMAS LION • Children’s Cancer Research Institute (CCRI), Vienna, Austria

MAURIZIO MARTINI • Instituto di Anatomia Patologica and Ematologia, Università Cattolica del Sacro Cuore, Roma, Italy

ELISA MOKANY • Johnson & Johnson Research Pty. Limited, NSW, Australia

STEPHEN P. MULLIGAN • School of Molecular and Microbial Biosciences, University of Sydney, NSW, Australia

TOMOKI NAOE • Department of Hematology, Nagoya University Graduate School of Medicine, Nagoya, Japan
Contributors

CHRISTOPHE PICARD • Department of Biochemistry & Molecular Biology, Hôpital Universitaire Nord (AP-HM), ERT-MEIDIA-IFR Jean Roche-Université de la Méditerranée, Marseille, France

VINCENZO ROSSI • Centro Ricerca M. Tettamanti, Clinica Pediatrica Università di Milano Bicocca and Dipartimento di Medicina Clinica, Prevenzione e Biotecnologie Sanitarie, Ospedale San Gerardo, Monza, Italy

GIUSEPPE SAGLIO • Department of Clinical and Biological Sciences, University of Turin, Turin, Italy

LINDA M. SCOTT • Department of Haematology, University of Cambridge, Cambridge, UK

MIKE SCOTT • Department of Haematology, Addenbrookes NHS Trust, Cambridge, UK

MONIQUE SILVY • Department of Biochemistry & Molecular Biology, Hôpital Universitaire Nord (AP-HM), ERT-MEIDIA-IFR Jean Roche-Université de la Méditerranée, Marseille, France

KERRYN STONER • Department of Haematology, Addenbrookes NHS Trust, Cambridge, UK

ELIZABETH H. STOVER • Brigham and Women’s Hospital, Harvard Medical School, Boston, MA

LUCIANA TEOFILI • Instituti di Anatomia Patologica and Ematologia, Università Cattolica del Sacro Cuore, Roma, Italy

KHALID TOBAL • The Rayne Institute, King’s College Hospital, London, United Kingdom

ALISON V. TODD • Johnson & Johnson Research Pty. Limited, NSW, Australia

BERT A. VAN DER REIJDEN • Central Hematology Laboratory, University Medical Center St. Raboud, Nijmegen, The Netherlands

FRANZ WATZINGER • Children’s Cancer Research Institute (CCRI), Vienna, Austria

ADRIAN WOOLFSON • University of Cambridge School of Clinical Medicine, Addenbrooke’s Hospital, Cambridge, United Kingdom

JOHN A. LIU YIN • Hematology Department, University of Manchester, Manchester, UK