Leukemia and Lymphoma: Detection of Minimal Residual Disease is being published at a time when the detection of microscopically unobservable disease in the leukemias and lymphomas is leaving its adolescent stage and entering the early stages of maturity. Detection of disease at levels almost four orders of magnitude below detection by light microscopy has now been accomplished and many of the methods have been shown to provide reproducible results. The groundwork has thus been laid for future construction of a methodology superior to the present methods of outcome prediction. Results of this nature will undoubtedly be necessary to justify the expense of future large-scale clinical trials using the various minimal residual disease (MRD) techniques that have been developed during the past decade. This places the burden of proof on those clinical investigators, statisticians, and basic scientists who are convinced that such measurements have an important role in producing future advances in treatment outcome.

As editors we have chosen contributors who have been successful in applying their chosen technique to the particular diseases that are their interests. We have especially tried to select those authors who were responsible for developments in the technique they used to make residual disease measurements. For this reason, we have encouraged them to give their insights into the methodology used in their research. As a result the reader will find several different descriptions of similar laboratory and analysis techniques, each of which we hope will prove helpful. We have taken advantage of the expertise of Professor Ludwig and Dr. Ratei to include a separate chapter on flow cytometry techniques. This addition should facilitate understanding the chapters on the use of flow cytometry to detect residual disease in the lymphoid and myeloid leukemias. In addition, one of us (DAJ) has written a rigorous mathematical description of an approach to the analysis of the predictive capabilities of an MRD detection system that accompanies a clinical trial. There is also a section of Editor’s Notes at the end of this volume that contains comments on portions of each chapter. We hope that these comments will be found helpful to readers.

The chapter by Dr. Dario Campana describes the use of a patient-specific immunophenotype to identify residual leukemia in patients who are in remission. He carefully describes the different categories of leukemia-associated immunophenotypes and shows how they are used to follow patients in remission. His research has shown that he can detect one leukemia cell among ten thousand normal marrow cells.

Dr. Geoffrey Neale, a veteran contributor to the study of Childhood Acute Lymphocytic Leukemia using polymerase chain reaction (PCR)-based methods, describes the original methods for the detection of residual disease using
PCR. He then progresses to a discussion of the various methods of quantitation of the level of disease. He completes his chapter with a description of real-time quantitative PCR (RQ-PCR). Throughout the chapter he gives the outcome of clinical studies of MRD in patients during remission.

Following Dr. Johnston’s chapter on the analysis of MRD studies, we both have written a brief discussion on the evaluation of these techniques when used in clinical trials. In particular we address the question of assessing the predictive capability of a study that follows patients during and after therapy. We use simple arithmetic methods of performing these evaluations.

The predictive properties of semiquantitative PCR measurements of MRD prior to and after allogeneic stem cell transplantation of children with ALL are described by Moppet et al., who outline their experience with the technique in this setting. This chapter provides the reader with many possible routes for future MRD studies of allogeneic transplantation.

Drs. Foroni, Mortuza, and Hoffbrand give a detailed summary of the high sensitivity monitoring of adult patients with ALL. They review the approaches used to detect residual leukemia during remission. They define high and low risk groups according to the measured response to therapy and also contrast MRD in adult and childhood ALL.

Professor San Miguel and his colleagues present an extensive discussion of the immunophenotypes observed in patients with Acute Myeloid Leukemia (AML) and the frequent occurrence of asynchronous antigen expression in this disease. This chapter gives an excellent presentation of the methods of confirming the detection sensitivity of the flow cytometry–based assay. The results of clinical studies using this method to identify risk groups are presented.

The chapter presented by Drs. Marcucci and Caligiuri discusses the non-random chromosomal abnormalities in AML that lead to chimeric fusion genes thought to be leukemia-specific. Reverse transcription PCR (RT-PCR) is capable of detecting these transcripts with high sensitivity and thereby allows monitoring of the leukemia during remission. The authors review the results of clinical studies that detect these fusion transcripts and the use of their data in stratifying patients during remission according to the risk of relapse. They also discuss the importance of high sensitivity, as well as the associated hidden risks.

Dr. Lo Coco and Ms. Diverio discuss detection of MRD in Acute Promyeloctic Leukemia (APL), a subtype of AML that is associated with a specific chromosomal translocation, the t(15;17). The detection of the reverse transcription product of this fusion gene using RT-PCR has become an important aspect of both the diagnosis and monitoring during remission of this leukemia. The authors present a comprehensive review of the laboratory and clinical aspects of this endeavor.
The detection of MRD in Chronic Myelogenous Leukemia (CML) is described in the chapter of Drs. Cross and Hochhaus. Their discussion begins with the molecular genetics of the CML-associated t(9;22) chromosomal translocation and the BCR-ABL fusion gene. This is followed immediately by a section on the methods used to detect CML cells and leads to the application of RT-PCR to detect the BCR-ABL fusion transcript. The presentation of qualitative RT-PCR to detect the presence of the leukemia-associated transcript is closely followed by a thorough discussion of the methods for quantitation of the number of BCR-ABL transcripts. The chapter concludes with a critical discussion of the results of BCR-ABL detection in patients with CML. The problems associated with defining molecular relapse is presented in addition to a brief discussion about the presence of the BCR-ABL transcript in normal individuals.

Drs. Krackhardt and Gribben describe the detection of the t(14;8), t(11;14), t(8;14), t(2;5), t(11;18), and the antigen receptor gene rearrangements. They then present quantitation strategies based upon competitive PCR. They then apply the PCR technique to detection of these translocations in bone marrow and peripheral blood of patients with Non-Hodgkin’s Lymphoma who have been treated with autologous bone marrow stem cell transplantation. The issue of whether or not “molecular complete remission” is the goal of therapy is presented in an unambiguous manner.

Tsimberidou et al. summarize the literature regarding the use of real-time and conventional PCR in patients with Non-Hodgkin’s Lymphoma and t(14;18). They then describe real-time PCR, as they apply it in their laboratory, to specimens from patients with follicular lymphoma. They studied peripheral blood and bone marrow in these patients, as well as peripheral blood from normal donors. Their technique employs the simultaneous amplification of an internal beta actin sequence for quantitation and comparison.

Drs. Lee and Cabanillas describe the application of PCR to monitoring follicular lymphoma in patients with all stages of disease during remission. They use PCR results to define molecular nonresponders and develop a multivariate analysis of these patients. There was a high complete remission rate for patients who were molecular responders and a low rate for the nonresponders. They extend this work to patients treated with bone marrow transplantation.

Throughout this book there are several issues that reappear frequently. They represent uncertainties about MRD that must be resolved before these assays achieve status as a reliable tool for clinical decision-making. Since these issues are essentially of equal importance, the following is not in any particular order of impact: (1) Some investigators have observed persistent low levels of detectable disease in patients who remain in clinical remission.
This observation is closely coupled to the question of the optimal (most cost effective?) detection sensitivity. It also raises the question of interference by normal background. (2) The capability of detecting disease at submicroscopic levels has given rise to the real possibility of new definitions of the clinical terms remission and relapse.

These new definitions might allow improvements in treatment outcomes if they were properly established. For example, a reliable definition of molecular remission applied in cases where it was found persistently could lead to decreased treatment morbidity. There are two possible benefits of a reliable definition of molecular relapse. First, the signal for molecular relapse would, optimally, appear when the disease level is quite low and, at this level, the disease may be sensitive to many innovative therapeutic interventions. Second, prior to clinical relapse the patient is probably better able to tolerate intensive therapy than after clinical relapse. The actual implementation of these new criteria would provide an entrée to a completely new area of clinical investigation that could be very beneficial to patient care. (3) Many authors have noted that standardization is necessary. This standardization must include not only laboratory methods, but also the statistical methods used to analyze the data. This is an absolute requirement for the comparison of data from different institutions. (4) Finally, the emergence of the RQ-PCR technique as the method of choice is quite apparent in these chapters and in the recent literature. It seems probable that this development will facilitate the standardization of detection and quantitation techniques. We make these observations here so that the reader may keep them in mind as he/she reads the following chapters. If this book is to have any impact, it will hopefully inspire its readers to find solutions to the problems that now face the field.

The editors would like to thank the authors for the variety of their excellent chapters. More important, we would like to thank them and the many others working with MRD for the lively and impassioned discussions of MRD and their willingness to share their technical methods and ideas for the direction, use, and application of MRD. This has advanced the techniques and applications far beyond what we could have done working individually.

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