Molecular Monitoring of Lymphoma

Jacques J.M. van Dongen, M.D., Ph.D.
Anton W. Langerak, Ph.D.
Tomasz Szczepański, M.D., Ph.D.
Vincent H.J. van der Velden, Ph.D.

The appropriate diagnosis and classification of lymphomas form the basis of clinical patient management, particularly for the choice of treatment protocol. The possibilities for accurate classification of lymphomas have substantially increased over the last decade, particularly by the inclusion of specific immunophenotyping and oncogenetic characteristics. It can be anticipated that the novel developments in the field of gene expression profiling will further improve the classification of lymphomas, with better correlation to outcome.

Despite more accurate classification and better establishment of prognosis in subsets of lymphomas, it is difficult to predict at initial presentation what the treatment effectiveness will be in each individual patient. Treatment effectiveness depends on many factors, such as compliance to the treatment protocol (by the doctor and the patient), intestinal resorption of the drug, efficiency of cytotoxic drug metabolism, condition of the patient (including liver and kidney function), occurrence of infection or other complications, drug resistance of the lymphoma cells, and many other factors as scored in the international prognostic index (IPI).[1]

Over the last decade several large-scale clinical studies have evaluated treatment effectiveness in leukemia patients by measuring the kinetics of disappearance of "minimal residual disease" (MRD) in bone marrow (BM) or peripheral blood (PB) samples during and after treatment.[2] Particularly in acute lymphoblastic leukemia (ALL), MRD diagnostics during the first 3 months of treatment has resulted in the recognition of three MRD-based risk groups, which differ significantly in outcome (6-year event-free survival of 98%, 75%, and 20%).[3,4]

Consequently, also in lymphoma patients, the actual disappearance of malignant cells from PB and BM might be a good surrogate marker to evaluate treatment effectiveness and predict outcome in individual patients. Such MRD monitoring might be particularly helpful in lymphoma types with a high tendency of dissemination to PB and BM, such as small lymphocytic lymphoma, lymphoplasmacytic lymphoma, follicular lymphoma (FL), and mantle cell lymphoma (MCL). In less disseminating lymphomas, such as diffuse large B-cell lymphoma (DLBCL) and anaplastic large-cell lymphoma (ALCL), MRD monitoring in BM or PB might be less informative.

Over the past 15 years, multiple techniques have been evaluated for their potential of detecting MRD with sufficiently high specificity and sensitivity. In practice only flow cytometry and polymerase chain reaction (PCR) techniques appeared to be useful. Flow cytometry uses lymphoma-associated immunophenotypes, oncogene (over)expression (e.g., BCL2 or ALK), and potentially single immunoglobulin (Ig) light-chain (Igκ or Igλ) expression in case of a B-cell lymphoma. PCR techniques exploit rearranged Ig and T-cell receptor (TCR) genes or chromosome aberrations as targets.

Flow cytometry is fast and relatively cheap, but has the disadvantage of a limited sensitivity of one malignant cell in 1000 to 10,000 normal cells (10^{-3} to 10^{-4}) in many types of lymphomas. In some lymphomas the sensitivity is only 10^{-2} to 10^{-3}. This implies that the "dynamic range" of MRD detection in PB and BM is not more than three logs, which limits the possibility to accurately assess the kinetics of tumor load decrease.

PCR–based MRD techniques are more sensitive (10^{-4} to 10^{-6}), but are slower and generally more expensive than flow-cytometric MRD detection, particularly when PCR targets are used, which need to be precisely identified per patient, such as Ig and TCR gene rearrangements or breakpoint fusion regions at the DNA level. Despite these disadvantages most clinical MRD studies in lymphoma patients use PCR techniques for MRD monitoring, because they are applicable in the majority of patients with a high sensitivity.

MRD monitoring potentially has high clinical relevance in curable types of lymphoma, even if only a small subgroup of the patients has a long-term event-free survival. Recognition of this subgroup versus the group of relapsing patients should be the aim of clinical MRD studies, because this would allow treatment stratification in future therapy protocols.

The strategy of MRD monitoring might be different per type of lymphoma: what type of sample is needed for monitoring (PB, BM, or other?); how frequently should the sampling be performed (each week, each month, or other?); and for how long should the MRD monitoring continue (initial treatment phase vs. long-term monitoring, even after withdrawal of treatment?). Logically, early treatment intervention needs detailed MRD information at multiple time points during the first 3 to 6 months of treatment, while long-term MRD monitoring might be relevant for indolent lymphomas with treatment modification in later phases.

This chapter provides the background information of the PCR targets for molecular MRD monitoring (i.e., Ig/TCR gene rearrangements and chromosome aberrations), explains how these targets can be identified, and which real-time quantitative (RQ)-PCR techniques are currently avail-