Flow Cytometry

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KEYWORDS

• Waldenström macroglobulinemia • Flow cytometry • Immunophenotyping • B cells • Plasma cells

KEY POINTS

- The Waldenström's clone (B cells and plasma cells) harbors unique immunophenotypic characteristics that differ from other gammopathies and immunoglobulin M (IgM)–secreting lymphomas.
- IgM monoclonal gammopathy of undetermined significance and smoldering WM display clonal B cells with the phenotypic signature of the malignant WM clone.
- Multiparameter flow cytometry (MFC) immunophenotyping identifies high-risk smoldering WM and symptomatic WM patients with inferior outcomes.
- Response assessment by MFC is highly predictive of progression-free and overall survival.

INTRODUCTION

The World Health Organization defines Waldenström macroglobulinemia (WM) as a lymphoplasmacytic lymphoma (LPL) associated with a monoclonal immunoglobulin M (IgM) protein, and bone marrow (BM) infiltration by small lymphocytes that may exhibit plasma cell (PC) differentiation.[1] Because LPL infrequently involves the lymph nodes or other extramedullary sites, demonstration of BM infiltration (eg, through trephine biopsy) is therefore essential for the diagnosis of WM.[2] Many patients who fulfill the criteria for WM do not require immediate therapy because they are detected before developing disease-related symptoms.[3] These patients are classified as smoldering WM, a clinically recognized entity with a cumulative probability of progression to symptomatic WM, amyloidosis, or lymphoma of 65% at 10 years.[4] Smoldering WM

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patients have a greater risk of progression to full-blown disease than IgM monoclonal gammopathy of undetermined significance (MGUS) cases (18% at 10 years).[5] There is some controversy to distinguish between smoldering WM and IgM MGUS; some groups use the amount of serum concentration of the M-protein and BM infiltration, whereas a consensus panel defined BM disease involvement on histologic examination as the main criterion.[2,6] It is plausible to assume that similarly to multiple myeloma (MM), most (if not all) WM patients have eventually gone through the benign stages of IgM MGUS and smoldering WM before developing clinical symptoms.[7] Therefore, the availability of objective criteria for the differential diagnosis between these conditions as well as more accurate estimation of their risk of progression is important to individualize monitoring of patients with premalignant conditions.

Multiparameter flow cytometry (MFC) immunophenotyping has been a mainstay in the diagnosis and monitoring of most hematologic malignancies.[8–12] Together, with the patient's clinical history, analytical results, and morphologic assessment of blood and marrow smears, MFC is also part of the initial diagnostic workup, mainly because of its ability to typically provide conclusive results within a few hours. It should be noted that MFC immunophenotyping provides accurate assessment of the expression of multiple markers and their fluorescence intensity in thousands of individual cells, and allows clear discrimination between aberrant and both normal and reactive cells, even when they are present at low or very low frequencies in a sample.[13] These are unique features of MFC because conventional morphologic approaches fail to distinguish clonal from normal cells, and molecular approaches mainly focus on the detection of specific genetic markers in whole BM and not in single cells.[13] Thus, a growing number of studies have applied MFC immunophenotyping to the study of IgM MGUS or WM (**Fig. 1**), and its utility will be reviewed here.

THE IMMUNOPHENOTYPIC ATLAS OF THE NORMAL B-CELL DEVELOPMENT

The origin of B-cell malignancies is associated with three main stages of B-cell differentiation:

i. B-lymphoblastic leukemias/lymphomas arise during the development of naive B cells from hematopoietic stem cells in the BM;
ii. B-cell lymphoproliferative disorders have been associated with antigen-dependent differentiation of naive B cells into memory B cells in secondary lymphoid tissues (lymph nodes and mucosa-associated lymphoid tissues), BM, and spleen; and

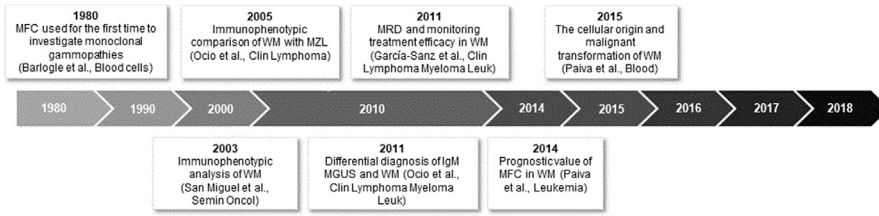

Fig. 1. Time axis highlighting the most important discoveries concerning MFC and its use in WM. (*Modified from* Jelinek T, Bezdekova R, Zatopkova M, et al. Current applications of multiparameter flow cytometry in plasma cell disorders. Blood Cancer J 2017;7(10):e617; with permission.)