Review of Contemporary Philosophy
ISSN: 1841-5261, e-ISSN: 2471-089X
Vol 22 (1), 2023
Pp 4614 - 4626

Applications of Flow Cytometry in The Assessment of Minimal Residual Disease in Hematologic Malignancies: A Comprehensive Review

[1]-Sadiyah Abdu Yahya Jedaibi,[2]- Mohammed Khalifah Alkhalifah,[3]- Hussain Mohammed Ghalib Alsayed ,[4]- Ali Mohammed Ahmed Alqassmi,[5]-Yousef Obaidullah Al-Harbi,[6]- Mohammed Ali Mousa Harbi,[7]- Abdullah Omar Almehmadi ,[8]-Riyad Yahya Thubab,[9]-Ahmed Bakri Hassan Somili,[10]-Jawad Hussain Jawad Al Lowimi,[11]-Turki Saleh Mosleh Albishri ,[12] -Ahmed Yousef Ahmed Oraybi ,[13]- Bader Saad Farhan Aljohani,[14]- Ahmed Mohammed Albjees,[15]- Mohammed Omer Mustafa Nehari

[1] KSA, Ministry Of Health, Hospital Sabya
[2] KSA, Ministry Of Health, King Fahad Specialist Hospital
[3] Princess Nourah Bint Abdurahman University-King Abdullah Bin Abdulaziz University Hospital
[4] Jazan University Hospital, Jazan University
[5] KSA, Ministry Of Health, Al Salam Hospital In Medina
[6] King Khalid University Hospital Riyadh
[7] KSA, Ministry Of Health, ALSALAM Hospital
[8] KSA, Ministry Of Health, King Fahad Central Hospital In Jazan
[9] KSA, Ministry Of Health, GENERAL SAMTAH HOSPITAL
[10]KSA, Ministry Of Health, AL OMRAN GENERAL HOSPITAL
[11]KSA, Ministry Of Health, Altalaa PHC
[12]KSA, Ministry Of Health, Health Programs , Vectors Disease Department
[13]KSA, Ministry Of Health, King Khaled Hospital Tabuk Health Cluster
[14]KSA, Ministry Of Health, Alahssa Health Cluster
[15]KSA, Ministry Of Health, GENERAL SABYA HOSPITAL

Abstract

Background: Flow cytometry (FC) has revolutionized the diagnosis and monitoring of hematologic malignancies, particularly in the detection of minimal residual disease (MRD). The ability to identify rare leukemic cells at low levels provides critical prognostic insights that inform therapeutic strategies.

Methods: This review synthesizes recent advancements in flow cytometric techniques, including multiparametric flow cytometry (MFC) and next-generation flow cytometry (NGF), in the context of MRD assessment. The paper evaluates the integration of immunophenotypic and molecular diagnostics for the characterization of tumor clones in various hematologic disorders.

Results: Recent studies demonstrate that MFC can achieve sensitivity comparable to molecular methods, detecting MRD levels as low as 0.001%. The implementation of standardized protocols and advanced software tools enhances the reliability of MRD assessments. Notably, the identification of leukemia-associated immunophenotypes (LAIPs) and the application of the “different-from-normal” (DfN) approach significantly improve the specificity of MRD detection.

Conclusion: Flow cytometry serves as a vital tool in the monitoring of MRD in hematologic malignancies, facilitating timely therapeutic interventions and improving patient outcomes. Continued advancements in technology and methodology are essential for overcoming existing challenges and enhancing the precision of MRD evaluations across diverse clinical settings.

Keywords: Flow cytometry, minimal residual disease, hematologic malignancies, multiparametric analysis, leukemia.

Received: 16 October 2023 **Revised**: 29 November 2023 **Accepted**: 13 December 2023

1. Introduction

Since the final decades of the previous century, the advancement of cost-effective and sensitive techniques for identifying rare leukemic cells, still discernible at minimal levels in either the bone marrow (BM) or peripheral blood (PB) of hematologic patients during post-treatment monitoring, has significantly enhanced our capacity to quantify the burden of resistant or recurrent disease, surpassing the limits of morphological remission. The concept of "Minimal, or Measurable, Residual Disease" (MRD) quickly became a crucial prognostic factor, playing an essential role in therapeutic decision-making. Currently, MRD monitoring is strongly advised in several contexts of hematologic malignancies, serving not only as a significant independent prognostic factor post-diagnosis but also as the optimal instrument for directing risk-based therapy approaches. This strategy aims to target remaining neoplastic cells before evident clinical recurrence, with the possibility to cure patients by eliminating resistant cancer stem cells [1,2].

Two complementary diagnostic methodologies are presently employed to conduct a comprehensive genomic and phenotypic characterization of tumor clones at diagnosis, and subsequently, during the clinical remission phase, to reliably deliver precise minimal residual disease quantifications, predicated on the meticulous identification of tumor-specific aberrant characteristics in each instance [3,4]. Molecular diagnostics strives to characterize the tumor genome by identifying unique genomic abnormalities of neoplastic clones, while flow cytometry concurrently assesses tumor phenotypes, emphasizing distinct antigenic patterns associated with neoplasia (Figure 1). Molecular diagnostics is now transitioning from qualitative PCR and real-time quantitative PCR (RQ-PCR) to improved PCR techniques, such as droplet digital PCR, and whole genome sequencing technologies, known as next-generation sequencing (NGS). Immunophenotypic studies have seen significant advancements in both technical and interpretive dimensions, transitioning from basic (4-color) flow cytometry to multidimensional cell analyses (≥6–8 colors), often referred to as “multiparametric flow cytometry” (MFC). Currently, MFC-based MRD detection relies on the concurrent identification of multiple phenotypic markers (typically 6–8 antigens) and the ability to analyze large cell populations within a few minutes, thereby achieving detection limits comparable to those of the most sensitive molecular techniques. Next-generation flow cytometry (NGF) signifies a significant advancement in high-throughput flow techniques, enabling the rapid acquisition of several million cells (>10^7) and achieving a sensitivity comparable to molecular methods (10^−6). The MFC-MRD analysis may use advanced software tools for automated gating of significant populations (APS) and aided analysis of maturation routes, ensuring reliable and repeatable findings with up to 18 colors. Notwithstanding advancements in technology and computing, certain competencies remain essential for conducting reliable and precise MRD evaluations using the MFC technique, hence limiting the applicability of MFC-MRD analysis to a limited number of highly trained facilities [5].

Over the last two decades, significant advancements in cell capture and multiparametric analysis have been made to address the traditional constraints of MFC regarding repeatability and comparability. Thorough guidelines for the immunophenotypic study of hematolymphoid neoplasms were first established by the Clinical Cytometry Society at the 2006 Bethesda International Consensus Conference [1]. In 2012, the EuroFlow collaboration, focused on enhancing standardization and directing the advancement of MFC-based diagnostics, introduced innovative consensus procedures that redefined operational requirements for MFC applications in the diagnostic evaluation of hematologic disorders [2].

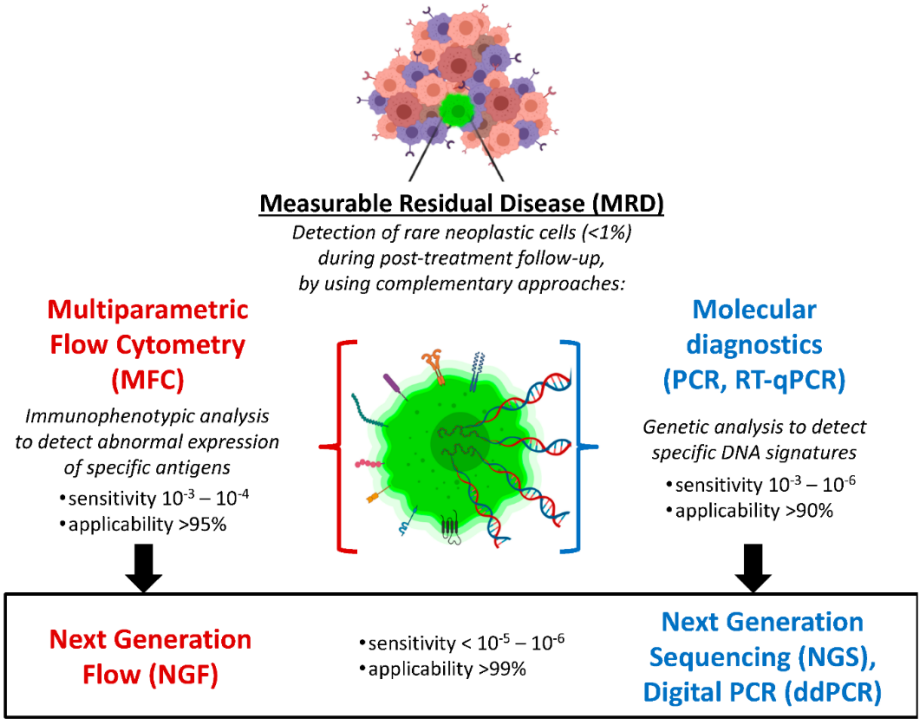

Figure 1. Complementary immunophenotypic and molecular methodologies for hematologic minimal residual disease monitoring.

2. Acute Myeloid Leukemia (AML)

Acute myeloid leukemia (AML) is a diverse neoplasm marked by the perilous proliferation of bone marrow myeloid progenitors, often necessitating prompt therapeutic escalation, contingent upon the recurrence risk for each patient after first induction therapy. The 2017 European LeukemiaNet (ELN) guidelines for acute myeloid leukemia (AML) included minimal residual disease (MRD) evaluation as a routine practice, with MRD status becoming essential for determining the depth of treatment response, as per the new criteria "Complete Remission with/without MRD." [3] In 2018, the ELN MRD Working Party delineated five rationales for conducting either immunophenotypic or molecular MRD diagnostics in AML: (i) to furnish an objective framework for determining a more profound remission status, (ii) to enhance outcome prognostication and guide post-remission therapy, (iii) to detect imminent relapse and facilitate early intervention, (iv) to enable more rigorous post-transplant monitoring, and (v) to serve as a surrogate endpoint to expedite drug evaluation and approval [4].

Per ELN recommendations, MFC-MRD panels should encompass a fundamental array of surface markers, specifically: early progenitor-associated markers, myeloid lineage-associated markers, along with additional differentiation markers, maintaining a core set that includes CD33, CD34, CD117, CD13, CD7 , and CD15, while consistently employing CD45 gating and forward scatter/sideward scatter (FSc/SSc) plots. When necessary, a "monocytic tube" must be included, comprising: CD4, CD11b, HLA-DR, CD14, CD34, CD33, CD45, and CD64 [4]. While not yet fully validated, contemporary experimental configurations using ≥8 colors (i.e., contextually examining a minimum of 8 immunophenotypic characteristics on acquired cells) are anticipated to significantly enhance the specificity of MFC-MRD tests. In post-acquisition multiparametric analysis, the identification of the residual leukemic population can be enhanced through two complementary methods: the first involves assessing patient-specific "leukemia-associated immunophenotypes" (LAIPs) at diagnosis and consistently tracking these original LAIPs during post-therapy follow-up; the second, termed the "different-from-normal (DfN) approach," entails a broader evaluation of abnormal differentiation and maturation patterns that may arise during bone marrow monitoring [4]. This advanced multiparametric analysis can effectively complement traditional LAIP-focused studies by aiding in the identification of changes in the aberrant phenotype of leukemic cells,

known as "immunophenotype shifts," which involve the gain or loss of antigens due to clonal selection events, the prognostic significance of which remains largely uncertain.

LAIPs, distinguished by lineage infidelity or asynchronous expression of differentiation markers, serve as notable "DfN phenotypic signatures" and may sometimes fulfill a specialized diagnostic function. Consequently, the ELN MRD Working Party advised the implementation of a combined "LAIP-based DfN approach," whereby particular LAIP monitoring is effectively included in a comprehensive immunophenotypic profile of bone marrow cells. It is recommended to do a comprehensive examination of the aforementioned indicators at both diagnosis and follow-up, ideally using the same set of tubes and combinations of antibody/fluorochrome [4]. Notably, comprehensive MFC-MRD monitoring may reveal specific phenotypic shifts that could facilitate the utilization of novel targeted therapies developed for other malignancies, as evidenced by the clinical case of a CD19-positive AML relapse successfully treated with DLI infusions in conjunction with anti-CD19 blinatumomab, consistent with the application of such bispecific T-cell engagers (BiTE) in patients with mixed phenotype acute leukemias.

Regarding the pre-analytical phase, proper collection of bone marrow samples is essential to ensure accurate estimation of minimal residual disease burden. To achieve this essential step, ELN guidelines recommend utilizing the initial pull of bone marrow aspirate and obtaining the minimal volume possible (preferably 3–5 mL) to prevent the prevalent issue of bone marrow contamination with peripheral blood, as dilution may adversely affect the measured percentages of leukemic cells. Due to the limited BM volumes available for MFC-MRD analysis, it is advisable to optimize staining panels to minimize the number of tubes used, while also recognizing that a high acquisition target (preferably 1 million CD45+ leukocytes per tube) is necessary to maximize the efficacy of MFC-MRD diagnostics. It is advisable to first assess the degree of bone marrow sample contamination with peripheral blood by determining the proportion of mature neutrophils present in each instance during post-acquisition analysis. The ELN recommendations presently do not suggest the use of PB samples for MFC-MRD evaluation, especially when low MRD values are anticipated [4]. Recent research indicates that PB MFC-MRD may serve as a biomarker for imminent AML recurrence [7], implying that some MFC-MRD applications might soon be corroborated using PB samples, as previously shown for molecular MRD monitoring.

Concerning clinical decision-making, ELN recommendations advocate for a cut-off of 0.1% (10−3) to define “positive” (≥0.1%) vs “negative” (<0.1%) MFC-MRD results, since this threshold has proven significant in the majority of MFC-MRD-guided clinical trials conducted to date. Nevertheless, although not being officially validated, it is essential to consider whether, among MFC-MRD negative individuals, MFC-MRD is undetectable (usually <0.001%) or quantifiable (range from 0.001% to 0.1%) using existing methodologies. In contrast to molecular MRD [8], there is less evidence on the ideal timing for MFC-MRD assessment during AML follow-up: further assessments following key consolidation time points (i.e., post-induction and post-consolidation therapy) are often dictated by the treatment plan. It may be prudent to do MFC-MRD every three months, at least throughout the first two years of follow-up. Additional research is necessary (i) to examine the predictive significance of MFC-MRD log10 alterations (similar to molecular MRD), and (ii) to elucidate the clinical implications of undetectable MFC-MRD, perhaps identifying individuals with an excellent prognosis. Currently, molecular MRD monitoring is viable solely in the presence of specific translocations or mutations, while awaiting clinical validation of promising NGS applications. Consequently, MFC-MRD diagnostics remain the exclusive method for monitoring the majority of AML patients, particularly those in the intermediate-risk category, which is generally defined by the lack of genetic or molecular abnormalities [9].

MFC-MRD monitoring may be expanded beyond the fundamental phenotypic indicators to include the examination of other pertinent antigens, which have shown potential use for diagnostic clarification, prognostic evaluation, and therapeutic applications. A comprehensive MFC-MRD study identified 22 markers aberrantly expressed in AML patients (specifically, CD18, CD9, CD32, CD25, CD366, CD44, CD47, CD54, CD59, CD86, CD64, CD68, CD96, CD93, CD97, CD123, CD99, CD200, CD52, CD300a/c, CX3CR1, and CD371), enabling precise differentiation between normal and leukemic stem cell (LSC) populations among hematopoietic progenitors, thereby providing a highly sensitive and specific tool for MRD monitoring

(capable of detecting one leukemic cell among over 105 normal BM cells) [10]. Currently, CD123 and CD133 are the most promising markers for monitoring leukemic stem cells (LSCs), supported by a framework that includes CD34, CD38, CD117, CD33, CD90, and CD45, and are also emerging as appealing targets for innovative immunotherapies [11]. The CD123 antigen (IL-3 receptor alpha chain) has garnered significant interest, having recently been established as the primary diagnostic marker for blastic plasmacytoid dendritic cell neoplasm (BPDCN). Its expression has been documented in various malignancies, including acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), myeloproliferative neoplasms, systemic mastocytosis, acute lymphoblastic leukemia (ALL), Hodgkin lymphoma, and hairy cell leukemia (HCL) [12,13].

In acute myeloid leukemia (AML), CD123 was strongly expressed by leukemic stem cells (LSCs) and more differentiated leukemic blasts but missing in normal counterparts. It correlated with the presence of FLT3/ITD-positive clones, serving as a negative prognostic marker at diagnosis [14-16]. Various targeted therapies aimed at targeting CD123-positive cells are now undergoing clinical trials. Talacotuzumab (JNJ-56022473), a humanized anti-CD123 monoclonal antibody, demonstrated ADCC-mediated cytotoxicity and direct inhibitory effects on IL-3-dependent tumor proliferation in AML xenograft murine models; however, it has not yet exhibited a significant therapeutic benefit, either as a monotherapy or in conjunction with decitabine, potentially due to insufficient cell-mediated cytotoxicity in patients with advanced disease [17-19]. The therapeutic efficacy may be enhanced by the advancement of anti-CD123 antibody-drug conjugate (ADC) strategies, akin to the FDA-approved anti-CD33 gemtuzumab.

Consistent with this notion, SL401 (tagraxofusp), a recombinant protein including IL-3 coupled to diphtheria toxin (licensed in 2018 for BPDCN), has shown excellent outcomes in a cohort of CD123+ AML/MDS patients [20]. Flotetuzumab (MGD006), a humanized bispecific dual-affinity re-targeting (DART) antibody that targets CD123 on leukemic cells and CD3ε on T cells, has shown promising efficacy in adult patients with relapsed/refractory acute myeloid leukemia (AML) [21]. Additionally, the CD133 antigen is prominently expressed by early hematopoietic progenitors and serves as a universal marker for leukemic stem cells, perhaps indicating a negative prognostic importance [11]. CD133 is a recognized primary marker of cancer stem cells across various tumor types and has been identified as a promising target for innovative chimeric antigen receptor (CAR) T cell therapies in solid tumors, including hepatocellular, pancreatic, and gastrointestinal carcinomas, as well as glioblastomas. This indicates the potential to explore this strategy in the context of leukemic disorders [22,23]. Moreover, C-type lectin-like molecule 1 (CLL-1), which has recently been identified as strongly expressed in acute myeloid leukemia (AML) blasts and generally lacking in hematopoietic progenitors, has been effectively targeted by either bispecific T-cell engagers (BiTEs) or chimeric antigen receptor T cells (CAR-T) in preclinical AML models [24,25].

3. Chronic Myeloid Leukemia (CML)

In chronic myeloid leukemia (CML), characterized by BCR-ABL1 translocations (the Philadelphia chromosome), minimal residual disease (MRD) is traditionally assessed using RQ-PCR, which has been extensively standardized across global laboratories, and its prognostic significance has been thoroughly incorporated into the therapeutic management based on tyrosine kinase inhibitors (TKIs). Nonetheless, MFC-MRD diagnostics may be important in identifying and monitoring the CML-LSC population (immunophenotypically characterized by the co-expression of CD34, CD26, CD56, CD93, CD123, and absence of CD38), whose elimination is the paramount difficulty in CML therapy [26-29]. A flow cytometry-based immunobead assay, distinct from immunophenotypic methods, for detecting BCR-ABL1 fusion proteins in cell lysates has been reported. The protein-based method known as “cytometric bead array (CBA)” is quick and straightforward, although it has poor sensitivity (10−2), restricting its use in clinical practice [30-32]. Additionally, a novel experimental approach, integrating a molecular technique known as "in situ proximity ligation assay" with flow cytometry as the readout (PLA-flow), demonstrated commendable sensitivity (10−4) and a strong correlation with BCR-ABL1 RQ-PCR, enabling precise identification of leukemic cells containing BCR-ABL1 fusion proteins while concurrently evaluating the expression of significant surface markers [33].

4. Acute Lymphoblastic Leukemia (ALL)

In acute lymphoblastic leukemia (ALL), marked by the aggressive proliferation of bone marrow lymphoblasts of either B-cell (B-ALL) or T-cell (T-ALL) lineage, minimal residual disease (MRD) serves as a critical prognostic indicator. It is extensively utilized to delineate varying relapse risks and to inform clinical decisions, particularly regarding treatment intensification through hematopoietic stem cell transplantation (HSCT), applicable to both adult and pediatric populations. Seminal studies in juvenile acute lymphoblastic leukemia (ALL) have shown the primary clinical use of minimal residual disease (MRD) detection with multiparameter flow cytometry (MFC) in hematologic patients [34,35]. Currently, RQ-PCR of Ig/TCR gene rearrangements and specific gene fusions is the most validated method for MRD monitoring in ALL; however, LAIP detection via MFC is frequently recommended to enhance the molecular approach, as MFC offers quicker, precise results and more comprehensive biological insights [36-44].

In B-ALL, the primary issue for MFC-MRD assessment is to phenotypically differentiate normal, regenerative B-cell precursors (BCPs, referred to as hematogones, which are particularly prevalent in the bone marrow during post-treatment follow-up) from leukemic B lymphoblast populations (i.e., neoplastic BCPs) [46-48]. Pathologic clones are often recognized by atypical expression patterns of conventional B-cell precursor markers, namely CD34, CD19, CD10, and CD20, and sometimes by the presence of unique aberrant antigens such as CD7, CD33, and CD58. A comprehensive array of primary BCP markers (CD34, CD19, CD10, CD20, together with CD38 and CD45) forms the foundation of the 8-color B-ALL MRD tubes recommended by key clinical studies, demonstrating substantial agreement between MFC and RQ-PCR methodologies for B-ALL MRD assessment [49,50]. In addition to the six common antigens, supplementary significant markers were typically included in the optimized tubes, although other B-ALL diagnostic markers, including CD79a, TdT, and CD24, were usually deemed less informative for MRD evaluation. Mature B-cell markers, especially surface immunoglobulins, are generally not advised for minimal residual disease surveillance, since they fail to distinguish between normal and leukemic B-cell precursors.

A recent multicentric EuroFlow study [36] has shown that a fully standardized bulk lysis protocol utilizing two stepwise designed 8-color tubes (incorporating the BCP backbone panel along with CD81, and either CD66c/CD123 or CD73/CD304, combined in the PE fluorescence channel of each tube) enables highly sensitive MRD measurements (up to 10−5, comparable to the PCR method, with >90% concordance) in nearly all B-ALL patients (>98%), provided that a substantial number of events are acquired (>4 million BM cells). In recent years, the increasing clinical application of innovative immunotherapies targeting CD19 (such as blinatumomab and CAR-T cells) and CD22 (inotuzumab ozogamicin) has prompted the expansion of MFC-MRD analysis to include CD22 and CD24, which are expressed in early B-cell precursors (even before CD19). This extension is crucial for monitoring B-ALL clones that exhibit persistent downregulation of CD19, a common occurrence following targeted therapies, as well as for identifying CD22+ cases that qualify for inotuzumab ozogamicin. Consequently, the MFC-MRD laboratory should be consistently apprised of the immunotherapies used in each instance to effectively establish patient-specific MRD panels [36,51].

Clinical studies on B-ALL prognostic variables indicate that MFC-MRD is classified as “negative” (full MRD remission) when below 0.01%, while values between 0.01% and 1% are deemed “positive” (MRD persistence/recurrence). Nonetheless, the use of the NGF methodology has shown that very low MRD levels (10−5 or below) may be detected, hence achieving the sensitivity threshold of molecular techniques. In this perspective, the term “undetectable MRD” should exclusively denote the total absence of neoplastic cells, reflecting optimal method sensitivity, whereas detectable but non-quantifiable (<0.01%) MRD levels (technically termed “negative”) may signify a significant “grey area” of concern. The most clinically significant MFC-MRD time points are: (i) early in induction treatment (usually on day +15) and (ii) after induction/consolidation therapy, both of which reliably distinguish between patients with very good outcomes (demonstrating rapid tumor clearance) and those with poor outcomes due to persistent MRD, respectively [41,52,53]. Early prognostic stratifications are crucial for determining the therapeutic approach to allogeneic HSCT transplantation. Conversely, long-term monitoring in B-ALL remains insufficiently standardized; the time and clinical applications of MFC-MRD follow-up are ambiguous and

often dictated by the therapy approach used. MRD reappearance is generally identified when values of ≥0.01% are observed and confirmed in a later sample; however, evidence of low-level MFC-MRD recurrence (<0.01%) may also be considered [54].

In comparison to AML, the need for representative bone marrow samples in B-ALL is analogous, if not more critical. However, the dual analytical method (LAIP- and DfN-based) is not absolutely essential, since B-ALL is fundamentally a less diverse condition. In this context, an extensive DfN-based analysis that encompasses myeloid and T-cell antigens may enhance the detection of aberrant clones experiencing immunophenotypic alterations, which could have significant biological and therapeutic implications, especially in patients diagnosed with mixed phenotype acute leukemia (MPAL) [55].

In the context of MRD monitoring, MFC-based analyses may enhance the prognostic assessment of protective tumor-specific T-cell responses, either naturally present in the bone marrow or arising post-therapeutic T-cell infusions, as conceptually illustrated in Philadelphia chromosome-positive (Ph+) B-ALL patients, which exhibit inverse correlations between cytotoxic Bcr-Abl-specific/WT-1-specific T lymphocytes and MRD trends [56-58].

In the context of T-ALL, the analytical characteristics and therapeutic uses of MFC-MRD have shown several parallels with the B-ALL framework [59]. In T-ALL, a special LAIP study is designed to identify malignant lymphoblasts with aberrant expression of T-cell progenitor markers. The identification of immature T-cell precursors in either bone marrow or peripheral blood is regarded as an aberrant result, with peripheral blood with minimal residual disease potentially indicating an increased chance of extramedullary recurrence [60,61]. T-ALL backbone panels are designed to assess the asynchronous expression of traditional T-cell maturation markers, including membrane/cytoplasmic CD3, CD5, CD7, CD2, CD4, CD8, CD34, and CD45, alongside the aberrant expression of thymic antigens like CD99 and CD1a, in addition to TdT, CD10, CD38, and CD56 [50,62,63]. Recent studies have repeatedly shown that the MFC-MRD test, conducted shortly after induction treatment utilizing 6–8 color panels with a threshold of 0.01%, serves as a reliable prognostic indicator in both adults and children with T-ALL [64-66]. Additional research is required to determine whether individuals with early undetected MFC-MRD qualify for decreased-intensity treatment, and if innovative NGF strategies may enhance MRD identification and clinical management in T-ALL patients [67,68].

5. Conclusions

The substantial predictive importance of MRD detection has significantly influenced the treatment of hematologic patients. MFC-MRD diagnostics provides a unique chance to phenotypically identify resistant or recurring neoplastic cells since they manifest in minimal quantities in the bone marrow and perhaps in the peripheral blood. Currently, standardized minimal residual disease (MRD) flow cytometry (MFC) serves as a crucial strategic instrument, supplementing molecular MRD evaluation, for risk-adapted therapeutic decision-making in various hematologic contexts. Currently, MRD-driven therapy intensification is a cornerstone in the treatment of acute leukemias, however, the use of this "pre-emptive" approach in chronic hematologic neoplasms requires more research. Specifically, it is necessary to evaluate the extent to which the identification of positive minimal residual disease (MRD) can prompt timely therapeutic adjustments, or if stable low-level disease may be regarded as an adequate therapeutic outcome for certain patient subsets (e.g., frail patients) with chronic lymphocytic leukemia (CLL) or multiple myeloma (MM).

The MFC-MRD technique is crucial for examining the expression levels and clonal distributions of certain surface antigens that may be targeted by innovative immunotherapies, such as monoclonal antibodies and CAR-T cells. In comparison to molecular MRD techniques, MFC-based MRD detection is now somewhat less sensitive, although it is quicker and relevant to the majority of patients, offering a beneficial cost-effectiveness profile in routine clinical practice. Nevertheless, high-throughput "next-generation" methodologies are anticipated to address existing biological and technical challenges associated with both MFC and molecular MRD techniques, offering exceptional sensitivity (<10−6) and applicability (>99%), along with the comprehensive phenotypic and genetic characterization of the neoplastic clone (Figure 1). Nevertheless, the regular use of MFC-MRD is sometimes impeded by technical difficulties and interpretive

intricacies, thus it remains prudent to consolidate such investigations in proficient labs. Flow cytometry continues to depend significantly on the proficiency of operators, who must consider pre-analytical biases and variability in devices, fluorochromes, analysis software, and specific antigens. The gradual use of automated cytometric assessments using specialist software is expected to enhance the uniformity of complicated multiparametric analyses.

Advancements in MFC-MRD measurement are presenting new challenges concerning the appropriate clinical application of data on exceedingly low MRD levels. Variations across different hematologic contexts, the biological implications of these MRD levels, the associated relapse kinetics, and the optimal clinical management remain largely uncharted. It is plausible that some pertinent immunological variables may be directly involved in "pre-clinical" tumor growth, consistent with the prevailing idea that almost all neoplasms are regulated by the immune system and must evade host immunity to proliferate. In the context of MRD detection, the MFC-based technique may also facilitate the examination of the frequencies and functioning of tumor-specific T lymphocytes, which are increasingly recognized as a crucial protective factor against neoplastic proliferation in both hematologic and solid malignancies.

Additional collaborative efforts within international networks are necessary to enhance the standardization of MFC-based clinical laboratory protocols, more effectively integrate cytometric and molecular MRD monitoring in the clinical management of hematologic malignancies, and fully leverage the capabilities of MFC diagnostics to formulate and direct innovative therapeutic strategies. The integration of advanced MRD diagnostics, antitumor T cell monitoring, and innovative targeted therapies may enable the identification and eradication of both dormant cancer stem cells and neoplastic subclones evading host immunosurveillance, thereby advancing personalized medicine for hematologic patients.

References

[1] Wood, B.L.; Arroz, M.; Barnett, D.; DiGiuseppe, J.; Greig, B.; Kussick, S.J.; Oldaker, T.; Shenkin, M.; Stone, E.; Wallace, P. 2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry: Optimal Reagents and Reporting for the Flow Cytometric Diagnosis of Hematopoietic Neoplasia. Cytometry 2007, 72B, S14–S22.

[2] Van Dongen, J.J.M.; Lhermitte, L.; Böttcher, S.; Almeida, J.; van der Velden, V.H.J.; Flores-Montero, J.; Rawstron, A.; Asnafi, V.; Lécrevisse, Q.; Lucio, P.; et al. EuroFlow Antibody Panels for Standardized N-Dimensional Flow Cytometric Immunophenotyping of Normal, Reactive and Malignant Leukocytes. Leukemia 2012, 26, 1908–1975.

[3] Döhner, H.; Estey, E.; Grimwade, D.; Amadori, S.; Appelbaum, F.R.; Büchner, T.; Dombret, H.; Ebert, B.L.; Fenaux, P.; Larson, R.A.; et al. Diagnosis and Management of AML in Adults: 2017 ELN Recommendations from an International Expert Panel. Blood 2017, 129, 424–447.

[4] Schuurhuis, G.J.; Heuser, M.; Freeman, S.; Béné, M.-C.; Buccisano, F.; Cloos, J.; Grimwade, D.; Haferlach, T.; Hills, R.K.; Hourigan, C.S.; et al. Minimal/Measurable Residual Disease in AML: A Consensus Document from the European LeukemiaNet MRD Working Party. Blood 2018, 131, 1275–1291.

[5] Plesa, A.; Labussière-Wallet, H.; Hayette, S.; Salles, G.; Thomas, X.; Sujobert, P. Efficiency of Blinatumomab in a t(8;21) Acute Myeloid Leukemia Expressing CD19. Haematologica 2019, 104, e487–e488.

[6] El Chaer, F.; Ali, O.M.; Sausville, E.A.; Law, J.Y.; Lee, S.T.; Duong, V.H.; Baer, M.R.; Koka, R.; Singh, Z.N.; Wong, J.; et al. Treatment of CD19-Positive Mixed Phenotype Acute Leukemia with Blinatumomab. Am. J. Hematol. 2019, 94, E7–E8.

[7] Zeijlemaker, W.; Kelder, A.; Oussoren-Brockhoff, Y.J.M.; Scholten, W.J.; Snel, A.N.; Veldhuizen, D.; Cloos, J.; Ossenkoppele, G.J.; Schuurhuis, G.J. Peripheral Blood Minimal Residual Disease May Replace Bone Marrow Minimal Residual Disease as an Immunophenotypic Biomarker for Impending Relapse in Acute Myeloid Leukemia. Leukemia 2016, 30, 708–715.

[8] Dillon, R.; Potter, N.; Freeman, S.; Russell, N. How We Use Molecular Minimal Residual Disease (MRD) Testing in Acute Myeloid Leukaemia (AML). Br. J. Haematol. 2021, 193, 231–244.

[9] Ivey, A.; Hills, R.K.; Simpson, M.A.; Jovanovic, J.V.; Gilkes, A.; Grech, A.; Patel, Y.; Bhudia, N.; Farah, H.; Mason, J.; et al. Assessment of Minimal Residual Disease in Standard-Risk AML. N. Engl. J. Med. 2016, 374, 422–433.

[10] Coustan-Smith, E.; Song, G.; Shurtleff, S.; Yeoh, A.E.-J.; Chng, W.J.; Chen, S.P.; Rubnitz, J.E.; Pui, C.-H.; Downing, J.R.; Campana, D. Universal Monitoring of Minimal Residual Disease in Acute Myeloid Leukemia. JCI Insight 2018, 3, e98561.

[11] Kandeel, E.Z.; El Sharkawy, N.; Hanafi, M.; Samra, M.; Kamel, A. Tracing Leukemia Stem Cells and Their Influence on Clinical Course of Adult Acute Myeloid Leukemia. Clin. Lymphoma Myeloma Leuk. 2020, 20, 383–393.

[12] Testa, U.; Pelosi, E.; Castelli, G. CD123 as a Therapeutic Target in the Treatment of Hematological Malignancies. Cancers 2019, 11, 1358.

[13] Nasillo, V.; Riva, G.; Paolini, A.; Forghieri, F.; Roncati, L.; Lusenti, B.; Maccaferri, M.; Messerotti, A.; Pioli, V.; Gilioli, A.; et al. Inflammatory Microenvironment and Specific T Cells in Myeloproliferative Neoplasms: Immunopathogenesis and Novel Immunotherapies. Int. J. Mol. Sci. 2021, 22, 1906.

[14] Jordan, C.T.; Upchurch, D.; Szilvassy, S.J.; Guzman, M.L.; Howard, D.S.; Pettigrew, A.L.; Meyerrose, T.; Rossi, R.; Grimes, B.; Rizzieri, D.A.; et al. The Interleukin-3 Receptor Alpha Chain is a Unique Marker for Human Acute Myelogenous Leukemia Stem Cells. Leukemia 2000, 14, 1777–1784.

[15] Testa, U.; Riccioni, R.; Militi, S.; Coccia, E.; Stellacci, E.; Samoggia, P.; Latagliata, R.; Mariani, G.; Rossini, A.; Battistini, A.; et al. Elevated Expression of IL-3Rα in Acute Myelogenous Leukemia is Associated with Enhanced Blast Proliferation, Increased Cellularity, and Poor Prognosis. Blood 2002, 100, 2980–2988.

[16] Al-Mawali, A.; Gillis, D.; Lewis, I. Immunoprofiling of Leukemic Stem Cells CD34+/CD38−/CD123+ Delineate FLT3/ITD-Positive Clones. J. Hematol. Oncol. 2016, 9, 61.

[17] Montesinos, P.; Roboz, G.J.; Bulabois, C.-E.; Subklewe, M.; Platzbecker, U.; Ofran, Y.; Papayannidis, C.; Wierzbowska, A.; Shin, H.J.; Doronin, V.; et al. Safety and Efficacy of Talacotuzumab plus Decitabine or Decitabine Alone in Patients with Acute Myeloid Leukemia not Eligible for Chemotherapy: Results from a Multicenter, Randomized, Phase 2/3 Study. Leukemia 2021, 35, 62–74.

[18] Pemmaraju, N.; Lane, A.A.; Sweet, K.L.; Stein, A.S.; Vasu, S.; Blum, W.; Rizzieri, D.A.; Wang, E.S.; Duvic, M.; Sloan, J.M.; et al. Tagraxofusp in Blastic Plasmacytoid Dendritic-Cell Neoplasm. N. Engl. J. Med. 2019, 380, 1628–1637.

[19] Mani, R.; Goswami, S.; Gopalakrishnan, B.; Ramaswamy, R.; Wasmuth, R.; Tran, M.; Mo, X.; Gordon, A.; Bucci, D.; Lucas, D.M.; et al. The Interleukin-3 Receptor CD123 Targeted SL-401 Mediates Potent Cytotoxic Activity against CD34+CD123+ Cells from Acute Myeloid Leukemia/Myelodysplastic Syndrome Patients and Healthy Donors. Haematologica 2018, 103, 1288–1297.

[20] Lane, A.A. Targeting CD123 in AML. Clin. Lymphoma Myeloma Leuk. 2020, 20 (Suppl. 1), S67–S68.

[21] Uy, G.L.; Aldoss, I.; Foster, M.C.; Sayre, P.H.; Wieduwilt, M.J.; Advani, A.S.; Godwin, J.E.; Arellano, M.L.; Sweet, K.L.; Emadi, A.; et al. Flotetuzumab as Salvage Immunotherapy for Refractory Acute Myeloid Leukemia. Blood 2021, 137, 751–762.

[22] Alhabbab, R.Y. Targeting Cancer Stem Cells by Genetically Engineered Chimeric Antigen Receptor T Cells. Front. Genet. 2020, 11, 312.

[23] Bueno, C.; Velasco-Hernandez, T.; Gutiérrez-Agüera, F.; Zanetti, S.R.; Baroni, M.L.; Sánchez-Martínez, D.; Molina, O.; Closa, A.; Agraz-Doblás, A.; Marín, P.; et al. CD133-Directed CAR T-Cells for MLL Leukemia: On-Target, off-Tumor Myeloablative Toxicity. Leukemia 2019, 33, 2090–2125.

[24] Ma, H.; Padmanabhan, I.S.; Parmar, S.; Gong, Y. Targeting CLL-1 for Acute Myeloid Leukemia Therapy. J. Hematol. Oncol. 2019, 12, 41.

[25] Ngai, L.L.; Ma, C.Y.; Maguire, O.; Do, A.D.; Robert, A.; Logan, A.C.; Griffiths, E.A.; Nemeth, M.J.; Green, C.; Pourmohamad, T.; et al. Bimodal Expression of Potential Drug Target CLL-1 (CLEC12A) on CD34+ Blasts of AML Patients. Eur. J. Haematol. 2021.

[26] Carter, B.Z.; Mak, D.H.; Cortes, J.; Andreeff, M. The Elusive Chronic Myeloid Leukemia Stem Cell: Does it Matter and How Do We Eliminate it? Semin. Hematol. 2010, 47, 362–370.

[27] Janssen, J.J.W.M.; Deenik, W.; Smolders, K.G.M.; van Kuijk, B.J.; Pouwels, W.; Kelder, A.; Cornelissen, J.J.; Schuurhuis, G.J.; Ossenkoppele, G.J. Residual Normal Stem Cells Can Be Detected in Newly Diagnosed Chronic Myeloid Leukemia Patients by a New Flow Cytometric Approach and Predict for Optimal Response to Imatinib. Leukemia 2012, 26, 977–984.

[28] Raspadori, D.; Pacelli, P.; Sicuranza, A.; Abruzzese, E.; Iurlo, A.; Cattaneo, D.; Gozzini, A.; Galimberti, S.; Baratè, C.; Pregno, P.; et al. Flow Cytometry Assessment of CD26+ Leukemic Stem Cells in Peripheral Blood: A Simple and Rapid New Diagnostic Tool for Chronic Myeloid Leukemia. Cytometry 2019, 96, 294–299.

[29] Herrmann, H.; Sadovnik, I.; Eisenwort, G.; Rülicke, T.; Blatt, K.; Herndlhofer, S.; Willmann, M.; Stefanzl, G.; Baumgartner, S.; Greiner, G.; et al. Delineation of Target Expression Profiles in CD34+/CD38− and CD34+/CD38+ Stem and Progenitor Cells in AML and CML. Blood Adv. 2020, 4, 5118–5132.

[30] Weerkamp, F.; Dekking, E.; Ng, Y.Y.; van der Velden, V.H.J.; Wai, H.; Böttcher, S.; Brüggemann, M.; van der Sluijs, A.J.; Koning, A.; Boeckx, N.; et al. Flow Cytometric Immunobead Assay for the Detection of BCR-ABL Fusion Proteins in Leukemia Patients. Leukemia 2009, 23, 1106–1117.

[31] Raponi, S.; De Propris, M.S.; Wai, H.; Intoppa, S.; Elia, L.; Diverio, D.; Vitale, A.; Foa, R.; Guarini, A. An Accurate and Rapid Flow Cytometric Diagnosis of BCR-ABL Positive Acute Lymphoblastic Leukemia. Haematologica 2009, 94, 1767–1770.

[32] Yujie, W.; Yu, Z.; Sixuan, Q.; Li, W.; Peng, L.; Zeng, G.; Sujiang, Z.; Jianyong, L. Detection of BCR-ABL Fusion Proteins in Patients with Leukemia Using a Cytometric Bead Array. Leuk. Lymphoma 2012, 53, 451–455.

[33] Löf, L.; Arngården, L.; Olsson-Strömberg, U.; Siart, B.; Jansson, M.; Dahlin, J.S.; Thörn, I.; Christiansson, L.; Hermansson, M.; Larsson, A.; et al. Flow Cytometric Measurement of Blood Cells with BCR-ABL1 Fusion Protein in Chronic Myeloid Leukemia. Sci. Rep. 2017, 7, 623.

[34] Brüggemann, M.; Raff, T.; Kneba, M. Has MRD Monitoring Superseded Other Prognostic Factors in Adult ALL? Blood 2012, 120, 4470–4481.

[35] Berry, D.A.; Zhou, S.; Higley, H.; Mukundan, L.; Fu, S.; Reaman, G.H.; Wood, B.L.; Kelloff, G.J.; Jessup, J.M.; Radich, J.P. Association of Minimal Residual Disease With Clinical Outcome in Pediatric and Adult Acute Lymphoblastic Leukemia: A Meta-Analysis. JAMA Oncol. 2017, 3, e170580.

[36] Theunissen, P.; Mejstrikova, E.; Sedek, L.; van der Sluijs-Gelling, A.J.; Gaipa, G.; Bartels, M.; Sobral da Costa, E.; Kotrová, M.; Novakova, M.; Sonneveld, E.; et al. Standardized Flow Cytometry for Highly Sensitive MRD Measurements in B-Cell Acute Lymphoblastic Leukemia. Blood 2017, 129, 347–357.

[37] Gupta, S.; Devidas, M.; Loh, M.L.; Raetz, E.A.; Chen, S.; Wang, C.; Brown, P.; Carroll, A.J.; Heerema, N.A.; Gastier-Foster, J.M.; et al. Flow-Cytometric vs. -Morphologic Assessment of Remission in Childhood Acute Lymphoblastic Leukemia: A Report from the Children's Oncology Group (COG). Leukemia 2018, 32, 1370–1379.

[38] Burns, M.A.; Place, A.E.; Stevenson, K.E.; Gutiérrez, A.; Forrest, S.; Pikman, Y.; Vrooman, L.M.; Harris, M.H.; Hunt, S.K.; O'Brien, J.E.; et al. Identification of Prognostic Factors in Childhood T-Cell Acute Lymphoblastic Leukemia: Results from DFCI ALL Consortium Protocols 05-001 and 11-001. Pediatr. Blood Cancer 2021, 68, e28719.

[39] Ciudad, J.; San Miguel, J.F.; López-Berges, M.C.; Vidriales, B.; Valverde, B.; Ocqueteau, M.; Mateos, G.; Caballero, M.D.; Hernández, J.; Moro, M.J.; et al. Prognostic Value of Immunophenotypic Detection of Minimal Residual Disease in Acute Lymphoblastic Leukemia. J. Clin. Oncol. 1998, 16, 3774–3781.

[40] Malec, M.; Björklund, E.; Söderhäll, S.; Mazur, J.; Sjögren, A.M.; Pisa, P.; Björkholm, M.; Porwit-MacDonald, A. Flow Cytometry and Allele-Specific Oligonucleotide PCR Are Equally Effective in Detection of Minimal Residual Disease in ALL. Leukemia 2001, 15, 716–727.

[41] Vidriales, M.-B.; Pérez, J.J.; López-Berges, M.C.; Gutiérrez, N.; Ciudad, J.; Lucio, P.; Vazquez, L.; García-Sanz, R.; del Cañizo, M.C.; Fernández-Calvo, J.; et al. Minimal Residual Disease in Adolescents (Older than 14 Years) and Adult Acute Lymphoblastic Leukemias: Early Immunophenotypic Evaluation Has High Clinical Value. Blood 2003, 101, 4695–4700.

[42] Björklund, E.; Mazur, J.; Söderhäll, S.; Porwit-MacDonald, A. Flow Cytometric Follow-up of Minimal Residual Disease in Bone Marrow Gives Prognostic Information in Children with Acute Lymphoblastic Leukemia. Leukemia 2003, 17, 138–148.

[43] Dworzak, M.N.; Gaipa, G.; Ratei, R.; Veltroni, M.; Schumich, A.; Maglia, O.; Karawajew, L.; Benetello, A.; Pötschger, U.; Husak, Z.; et al. Standardization of Flow Cytometric Minimal Residual Disease Evaluation in Acute Lymphoblastic Leukemia: Multicentric Assessment Is Feasible. Cytometry B Clin. Cytom. 2008, 74, 331–340.

[44] Robillard, N.; Cavé, H.; Méchinaud, F.; Guidal, C.; Garnache-Ottou, F.; Rohrlich, P.S.; Avet-Loiseau, H.; Garand, R. Four-Color Flow Cytometry Bypasses Limitations of IG/TCR Polymerase Chain Reaction for Minimal Residual Disease Detection in Certain Subsets of Children with Acute Lymphoblastic Leukemia. Haematologica 2005, 90, 1516–1523.

[45] Garand, R.; Beldjord, K.; Cavé, H.; Fossat, C.; Arnoux, I.; Asnafi, V.; Bertrand, Y.; Boulland, M.-L.; Brouzes, C.; Clappier, E.; et al. Flow Cytometry and IG/TCR Quantitative PCR for Minimal Residual Disease Quantitation in Acute Lymphoblastic Leukemia: A French Multicenter Prospective Study on Behalf of the FRALLE, EORTC and GRAALL. Leukemia 2013, 27, 370–376.

[46] Denys, B.; van der Sluijs-Gelling, A.J.; Homburg, C.; van der Schoot, C.E.; de Haas, V.; Philippé, J.; Pieters, R.; van Dongen, J.J.M.; van der Velden, V.H.J. Improved Flow Cytometric Detection of Minimal Residual Disease in Childhood Acute Lymphoblastic Leukemia. Leukemia 2013, 27, 635–641.

[47] Modvig, S.; Hallböök, H.; Madsen, H.O.; Siitonen, S.; Rosthøj, S.; Tierens, A.; Juvonen, V.; Osnes, L.T.N.; Vålerhaugen, H.; Hultdin, M.; et al. Value of Flow Cytometry for MRD-Based Relapse Prediction in B-Cell Precursor ALL in a Multicenter Setting. Leukemia 2021, 35, 1894–1906.

[48] Theunissen, P.M.J.; Sedek, L.; De Haas, V.; Szczepanski, T.; Van Der Sluijs, A.; Mejstrikova, E.; Nováková, M.; Kalina, T.; Lecrevisse, Q.; Orfao, A.; et al. Detailed Immunophenotyping of B-Cell Precursors in Regenerating Bone Marrow of Acute Lymphoblastic Leukaemia Patients: Implications for Minimal Residual Disease Detection. Br. J. Haematol. 2017, 178, 257–266.

[49] Wells, D.A.; Sale, G.E.; Shulman, H.M.; Myerson, D.; Bryant, E.M.; Gooley, T.; Loken, M.R. Multidimensional Flow Cytometry of Marrow Can Differentiate Leukemic From Normal Lymphoblasts and Myeloblasts After Chemotherapy and Bone Marrow Transplantation. Am. J. Clin. Pathol. 1998, 110, 84–94.

[50] DiGiuseppe, J.A.; Wood, B.L. Applications of Flow Cytometric Immunophenotyping in the Diagnosis and Posttreatment Monitoring of B and T Lymphoblastic Leukemia/Lymphoma. Cytometry B Clin. Cytom. 2019, 96, 256–265.

[51] Liu, Z.; Li, Y.; Shi, C. Monitoring Minimal/Measurable Residual Disease in B-Cell Acute Lymphoblastic Leukemia by Flow Cytometry during Targeted Therapy. Int. J. Hematol. 2021, 113, 337–343.

[52] Basso, G.; Veltroni, M.; Valsecchi, M.G.; Dworzak, M.N.; Ratei, R.; Silvestri, D.; Benetello, A.; Buldini, B.; Maglia, O.; Masera, G.; et al. Risk of Relapse of Childhood Acute Lymphoblastic Leukemia Is Predicted by Flow Cytometric Measurement of Residual Disease on Day 15 Bone Marrow. J. Clin. Oncol. 2009, 27, 5168–5174.

[53] Eveillard, M.; Robillard, N.; Arnoux, I.; Garand, R.; Rialland, F.; Thomas, C.; Strullu, M.; Michel, G.; Béné, M.C.; Fossat, C.; et al. Major Impact of an Early Bone Marrow Checkpoint (Day 21) for Minimal Residual Disease in Flow Cytometry in Childhood Acute Lymphoblastic Leukemia. Hematol. Oncol. 2017, 35, 237–243.

[54] Szczepański, T. Why and How to Quantify Minimal Residual Disease in Acute Lymphoblastic Leukemia? Leukemia 2007, 21, 622–626.

[55] Oberley, M.J.; Raikar, S.S.; Wertheim, G.B.; Malvar, J.; Sposto, R.; Rabin, K.R.; Punia, J.N.; Seif, A.E.; Cahen, V.C.; Schore, R.J.; et al. Significance of Minimal Residual Disease in Pediatric Mixed Phenotype Acute Leukemia: A Multicenter Cohort Study. Leukemia 2020, 34, 1741–1750.

[56] Rezvani, K.; Yong, A.S.M.; Savani, B.N.; Mielke, S.; Keyvanfar, K.; Gostick, E.; Price, D.A.; Douek, D.C.; Barrett, A.J. Graft-versus-Leukemia Effects Associated with Detectable Wilms Tumor-1 Specific T

Lymphocytes after Allogeneic Stem-Cell Transplantation for Acute Lymphoblastic Leukemia. Blood 2007, 110, 1924–1932.

[57] Riva, G.; Luppi, M.; Barozzi, P.; Quadrelli, C.; Basso, S.; Vallerini, D.; Zanetti, E.; Morselli, M.; Forghieri, F.; Maccaferri, M.; et al. Emergence of BCR-ABL-Specific Cytotoxic T Cells in the Bone Marrow of Patients with Ph+ Acute Lymphoblastic Leukemia during Long-Term Imatinib Mesylate Treatment. Blood 2010, 115, 1512–1518.

[58] Riva, G.; Luppi, M.; Quadrelli, C.; Barozzi, P.; Basso, S.; Vallerini, D.; Zanetti, E.; Morselli, M.; Forghieri, F.; Maccaferri, M.; et al. BCR-ABL-Specific Cytotoxic T Cells in the Bone Marrow of Patients with Ph(+) Acute Lymphoblastic Leukemia during Second-Generation Tyrosine-Kinase Inhibitor Therapy. Blood Cancer J. 2011, 1, e30.

[59] Krampera, M.; Vitale, A.; Vincenzi, C.; Perbellini, O.; Guarini, A.; Annino, L.; Todeschini, G.; Camera, A.; Fabbiano, F.; Fioritoni, G.; et al. Outcome Prediction by Immunophenotypic Minimal Residual Disease Detection in Adult T-Cell Acute Lymphoblastic Leukaemia. Br. J. Haematol. 2003, 120, 74–79.

[60] van der Velden, V.H.J.; Jacobs, D.C.H.; Wijkhuijs, A.J.M.; Comans-Bitter, W.M.; Willemse, M.J.; Hählen, K.; Kamps, W.A.; van Wering, E.R.; van Dongen, J.J.M. Minimal Residual Disease Levels in Bone Marrow and Peripheral Blood Are Comparable in Children with T Cell Acute Lymphoblastic Leukemia (ALL), but Not in Precursor-B-ALL. Leukemia 2002, 16, 1432–1436.

[61] Keegan, A.; Charest, K.; Schmidt, R.; Briggs, D.; Deangelo, D.J.; Li, B.; Morgan, E.A.; Pozdnyakova, O. Flow Cytometric Minimal Residual Disease Assessment of Peripheral Blood in Acute Lymphoblastic Leukaemia Patients Has Potential for Early Detection of Relapsed Extramedullary Disease. J. Clin. Pathol. 2018, 71, 653–658.

[62] Dworzak, M.N.; Fröschl, G.; Printz, D.; Zen, L.D.; Gaipa, G.; Ratei, R.; Basso, G.; Biondi, A.; Ludwig, W.-D.; Gadner, H. CD99 Expression in T-Lineage ALL: Implications for Flow Cytometric Detection of Minimal Residual Disease. Leukemia 2004, 18, 703–708.

[63] Tembhare, P.R.; Sriram, H.; Khanka, T.; Chatterjee, G.; Panda, D.; Ghogale, S.; Badrinath, Y.; Deshpande, N.; Patkar, N.V.; Narula, G.; et al. Flow Cytometric Evaluation of CD38 Expression Levels in the Newly Diagnosed T-Cell Acute Lymphoblastic Leukemia and the Effect of Chemotherapy on Its Expression in Measurable Residual Disease, Refractory Disease, and Relapsed Disease: An Implication for Anti-CD38 Immunotherapy. J. Immunother. Cancer 2020, 8.

[64] Vega-García, N.; Perez-Jaume, S.; Esperanza-Cebollada, E.; Vicente-Garcés, C.; Torrebadell, M.; Jiménez-Velasco, A.; Ortega, M.; Llop, M.; Abad, L.; Vagace, J.M.; et al. Measurable Residual Disease Assessed by Flow-Cytometry is a Stable Prognostic Factor for Pediatric T-Cell Acute Lymphoblastic Leukemia in Consecutive SEHOP Protocols Whereas the Impact of Oncogenetics Depends on Treatment. Front. Pediatr. 2020, 8, 614521.

[65] Tembhare, P.R.; Narula, G.; Khanka, T.; Ghogale, S.; Chatterjee, G.; Patkar, N.V.; Prasad, M.; Badrinath, Y.; Deshpande, N.; Gudapati, P.; et al. Post-Induction Measurable Residual Disease Using Multicolor Flow Cytometry is Strongly Predictive of Inferior Clinical Outcome in the Real-Life Management of Childhood T-Cell Acute Lymphoblastic Leukemia: A Study of 256 Patients. Front. Oncol. 2020, 10, 577.

[66] Wang, H.; Zhou, Y.; Huang, X.; Zhang, Y.; Qian, J.; Li, J.; Li, C.; Li, X.; Lou, Y.; Zhu, Q.; et al. Minimal Residual Disease Level Determined by Flow Cytometry Provides Reliable Risk Stratification in Adults with T-cell acute Lymphoblastic Leukaemia. Br. J. Haematol. 2021, 193, 1096–1104.

[67] Modvig, S.; Madsen, H.O.; Siitonen, S.M.; Rosthøj, S.; Tierens, A.; Juvonen, V.; Osnes, L.T.N.; Vålerhaugen, H.; Hultdin, M.; Thörn, I.; et al. Minimal Residual Disease Quantification by Flow Cytometry Provides Reliable Risk Stratification in T-cell acute Lymphoblastic Leukemia. Leukemia 2019, 33, 1324–1336.

[68] Tembhare, P.R.; Chatterjee, G.; Khanka, T.; Ghogale, S.; Badrinath, Y.; Deshpande, N.; Panda, D.; Patkar, N.V.; Narula, G.; Girase, K.; et al. Eleven-Marker 10-Color Flow Cytometric Assessment of Measurable Residual Disease for T-Cell Acute Lymphoblastic Leukemia Using an Approach of Exclusion. Cytometry B Clin. Cytom. 2020, 100, 421–433.

تطبيقات تقنية تحليل التدفق في تقييم المرض المتبقي الأدنى في الأورام الدموية: مراجعة شاملة

الملخص

الخلفية :أحدثت تقنية تحليل التدفق (FC) ثورة في تشخيص ومراقبة الأورام الدموية، وخاصة في الكشف عن المرض المتبقي الأدنى .(MRD) إن القدرة على تحديد خلايا اللوكيميا النادرة بمستويات منخفضة توفر رؤى تنبؤية حاسمة تؤثر على استراتيجيات العلاج.

الطرق :تقوم هذه المراجعة بتجميع التقدمات الحديثة في تقنيات تحليل التدفق، بما في ذلك تحليل التدفق متعدد المعلمات (MFC) وتحليل التدفق من الجيل التالي(NGF) ، في سياق تقييم المرض المتبقي الأدنى. تقيم الورقة دمج التشخيص المناعي الجزيئي والتشخيصات الجزيئية لتوصيف نسخ الورم في مختلف الاضطرابات الدموية.

النتائج :تُظهر الدراسات الحديثة أن تقنية تحليل التدفق متعدد المعلمات يمكن أن تحقق حساسية مماثلة للطرق الجزيئية، حيث يمكنها الكشف عن مستويات المرض المتبقي الأدنى تصل إلى 0.001%. تعزز تنفيذ البروتوكولات الموحدة وأدوات البرمجيات المتقدمة موثوقية تقييمات المرض المتبقي الأدنى. ومن الجدير بالذكر أن تحديد الأنماط المناعية المرتبطة باللوكيميا (LAIPs) وتطبيق نهج "المختلف عن الطبيعي (DfN) "يحسن بشكل كبير من دقة الكشف عن المرض المتبقي الأدنى.

الخاتمة :تعتبر تقنية تحليل التدفق أداة حيوية في مراقبة المرض المتبقي الأدنى في الأورام الدموية، مما يسهل التدخلات العلاجية في الوقت المناسب ويحسن نتائج المرضى. إن التقدم المستمر في التكنولوجيا والمنهجيات أمر ضروري لتجاوز التحديات الحالية وتعزيز دقة تقييمات المرض المتبقي الأدنى عبر بيئات سريرية متنوعة.

الكلمات المفتاحية :تحليل التدفق، المرض المتبقي الأدنى، الأورام الدموية، التحليل متعدد المعلمات، اللوكيميا.