Hematology, in general, and the study of haematological malignancies in particular, is one of the areas of medicine in which the application of flow cytometry has undergone a major development in the last decade. This is related to the fact that flow cytometry requires single cell suspensions, which are easily obtained from peripheral blood (PB) samples and bone marrow (BM) aspirates. Moreover, single cell suspensions can also be prepared from lymph node biopsies and fine needle aspirates, BM biopsies and biopsies from other lymphoid tissues. In addition, since the late seventies’, haematopoietic cells have been used as a source of antigens to develop monoclonal antibody (Mab) reagents, leading to the availability of an increasingly high number of reagents directed against haematopoietic cell markers. These unique features, together with the continuous advances in laser technology, optics, fluorochrome chemistry, bead technology, informatics and the production of Mab, have reshaped the way flow cytometry is used in haematology and have expanded its applications. Accordingly, at present, flow cytometry is the method of choice for immunophenotypic characterization of haematological malignancies at diagnosis and for the immunophenotypic monitoring of minimal residual disease, during and after therapy. In addition, it is also a primary laboratory diagnostic tool in patients suspected of having paroxysmal nocturnal haemoglobinuria, systemic mastocytosis and primary thrombocytopenias such as Glanzmann disease and Bernard-Soulier syndrome, for the detection of anti-platelet antibodies and the quality control of both leukocyte contamination in transfusion products and of CD34+ haematopoietic stem and precursor cells and CD3+ T-lymphocytes in transplant cell products. The increased diagnostic use of flow cytometry is certainly related to its relative simplicity, high sensitivity and specificity and the possibility of providing clinically useful results in a short period of time.

In this paper we review the currently most promising applications of flow cytometry in the diagnosis of haematological malignancies (Table 1), particularly those related to the multiparameter immunophenotypic identification, enumeration and characterization of leukaemic cells.

Immunophenotyping of haematological malignancies

Immunophenotyping of leukaemias and other haematological malignancies has become one of the most relevant clinical applications of flow cytometry. Initially, its utility was mainly focused on the further characterization of leukaemic cells and classification of the disease, once diagnosis of leukaemia/lymphoma had already been established. Antigen expression was commonly evaluated with relatively restricted panels of single, unconjugated Mab reagents, using mononuclear cell-enriched samples which contained high percentages of neoplastic cells. A clear example of such use is the establishment of the lymphoid versus myeloid origin of blast cells in acute leukaemia and the subclassification of both T-cell and B-cell precursor (BCP) acute lymphoblastic leukaemia (ALL) according to the expression of maturation-associated antigens, as proposed in 1995 in the EGIL classification: pro-T/TI (CD7+ and CyCD3+, other T-cell markers-negative), pre-T/TII (CD7+, CyCD3+, sCD3, CD1a and CD2 and/or CD8 and/or CD5), common-T/TIII (CD7+, CyCD3+, CD1a), mature-T/TIV (CD7+, sCD3, CD1a) and pro-B/BI (CD19+, CyCD79a, Ig, CD10), common-B/BI (CD19+, CyCD79a, CD10, Ig), pre-B/III (CD19+, CyCD79a, CyIgM, slg) and mature-B/BIV (CD19+, CyCD79a, slg).
As a consequence, the pan-

In the more mature neo-

and CD38, alone or in combination

A new era started, in which flow cytometric

Based on these strategies, immuno-

This was mainly

CD19 and CD7 were also

Table 1. Clinical applications of flow cytometric immuno-

<table>
<thead>
<tr>
<th>Type of medical indication</th>
<th>Disease category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic screening</td>
<td>B- and T-cell chronic lymphoproliferative disorders (CLPD)</td>
</tr>
<tr>
<td></td>
<td>Myelodysplastic syndromes (MDS)</td>
</tr>
<tr>
<td></td>
<td>Plasma cell dyscrasias (PCD)</td>
</tr>
<tr>
<td></td>
<td>Paroxysmal nocturnal haemoglobinuria</td>
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<tr>
<td></td>
<td>Systemic mastocytosis</td>
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</tbody>
</table>
| Immunophenotypic classifica-

Screening for genetic abnormalities

<table>
<thead>
<tr>
<th>Screening for genetic abnormalities</th>
<th>Disease category</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Acute myeloblastic leukaemia</td>
</tr>
<tr>
<td></td>
<td>Acute lymphoblastic leukaemias (childhood and adult)</td>
</tr>
<tr>
<td></td>
<td>B-cell chronic lymphoproliferative disorders</td>
</tr>
<tr>
<td>Others</td>
<td>Prognostic stratification (e.g. CLPD, MDS)</td>
</tr>
<tr>
<td></td>
<td>Staging and evaluation of disease extension (e.g. CLPD)</td>
</tr>
<tr>
<td></td>
<td>Minimal residual disease detection (e.g.: AL, CLPD, PCD)</td>
</tr>
<tr>
<td></td>
<td>Prediction/monitoring of response to therapy (e.g.: CLPD, AML)</td>
</tr>
</tbody>
</table>

* For further information please see references 2, 3 and 4.

sis. Among others, CD38 and, more recently intracel-

ular ZAP70 expression, were shown to be related to a

worse clinical outcome in B-cell chronic lympho-

cytic leukaemia (B-CLL). As a consequence, the pan-

els of Mab used for leukaemia immunophenotyping at
diagnosis, were extended.11

In parallel, the number of good-quality Mab reagents increased as did the number of different flu-
orochrome-conjugated Mab reagents started to be simultaneously used in benchtop flow cytometers. These developments have progressively pushed the applications of multicolor stainings. Once the reactivity for three, four or more fluorochrome-conjugated Mab reagents started to be simultaneously assessed, strategies for the precise identification of normal leukocyte sub-

populations in haematological samples started to be

built-up, thereby decreasing the need for using pre-

enrichment steps for mononuclear cells. At the same
time, the concept of gating markers developed.15

Accordingly, stainings with one or more common

markers in all multicolor combinations of Mab in a

Emerging applications of multiparameter flow cytometric

immunophenotyping in the diagnosis of haematological

malignancies

For many years now, efforts have been made to

identify tumour-specific antigens in leukaemia and

lymphoma cells. This has led to the identification of
tumour-specific antigens such as the idiotypic pro-
tein in mature neoplastic B-cells and plasma cells or

fusion proteins resulting from specific chromosomal
translocations (e.g.: BCR/ABL, TEL/AML1, PML/RARα, among others) particularly in acute

leukaemias. However, from a practical point of view,

most of these tumour-specific antigens have not been

introduced in routine diagnostics for the specific

identification of neoplastic cells. This was mainly
due to: i) the lack of high quality reagents for the

identification of these tumour-specific markers, ii)

their relatively low expression levels in neoplastic
cells and iii) their complexity, due to the occurrence
of different genomic breakpoints or individual vari-

ability in case of idiotypic proteins. In contrast, the

identification of altered expression patterns of one or

more normal proteins in leukaemic cells – leu-

kaemia/lymphoma-associated aberrant phenotypes –

has proven to have a much higher and easier applica-

bility in discriminating leukaemic cells from normal

cells. Accordingly, previous studies indicate that in

virtually all patients with T-ALL (>99%) BCP-ALL

(>95%), B-CLPD (>95%), plasma cell dyscrasias

(10%) and most AML (>80%), neoplastic cells dis-
play aberrant phenotypes which allow their discrimination from normal leukocytes in blood and BM (Table 2).\textsuperscript{1,14} The exact aberrant phenotypes displayed by leukaemic cells usually differ between distinct disease groups. In addition, they may also vary between different patients within a disease category and even between different neoplastic cell populations within a single patient.\textsuperscript{14} In general, a diagnostic category, lymphoid-lineage malignancies show common and more stable aberrant phenotypes than AML or other myeloid neoplasias.\textsuperscript{1,14} As an example, patients suffering from a typical B-cell chronic lymphocytic leukaemia (B-CLL) consistently (>95% of the cases) show dim co-expression of CD22, CD20 and/or CD81 among CD5+/CD23- B-cells,\textsuperscript{12,17} while overexpression of BCL2 in neoplastic B-cells with a follicular immunophenotype (CD10+, CD38+) represents a hallmark of follicular B-cell non-Hodgkin's lymphoma (NHL) with t(14;18).\textsuperscript{12} Based on these observations, unique multicolor combinations of Mab (e.g. Bcl2/CD10/CD38/CD20 for t(14;18) follicular B-cell NHL) can be built for the identification of malignant cells in patients suffering from a specific haematological malignancy.\textsuperscript{1,14} In contrast, in AML, the most common aberrant phenotype (e.g. CD33\textsuperscript{high}, HLADR\textsuperscript{-}, CD34\textsuperscript{-}, CD15\textsuperscript{-}, CD14\textsuperscript{-}, CD11b\textsuperscript{-} neutrophil lineage cells) may be present in less than one third of all cases (Table 2).\textsuperscript{14} However, a more detailed analysis of the aberrant phenotypes identified in AML, as well as in BCP-ALL, shows that a high concordance exists between specific aberrant phenotypes and genetic lesions that define diagnostic subcategories of both ALL and AML according to the WHO classification.\textsuperscript{6}

<table>
<thead>
<tr>
<th>Group</th>
<th>Disease</th>
<th>% Expression of aberrant Phenotype</th>
<th>Most frequently expressed aberrant phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute leukaemias</td>
<td>B-cell precursor acute lymphoblastic leukaemia (ALL)</td>
<td>&gt;95%</td>
<td>Asynchronous antigen expression: TdT\textsuperscript{+}/CD10\textsuperscript{-}/CD38\textsuperscript{-}/CD34\textsuperscript{-}, The exact aberrant phenotypes displayed by leukaemic cells usually differ between distinct disease groups. In addition, they may also vary between different patients within a disease category and even between different neoplastic cell populations within a single patient.\textsuperscript{14} In general, a diagnostic category, lymphoid-lineage malignancies show common and more stable aberrant phenotypes than AML or other myeloid neoplasias.\textsuperscript{1,14} As an example, patients suffering from a typical B-cell chronic lymphocytic leukaemia (B-CLL) consistently (&gt;95% of the cases) show dim co-expression of CD22, CD20 and/or CD81 among CD5+/CD23- B-cells,\textsuperscript{12,17} while overexpression of BCL2 in neoplastic B-cells with a follicular immunophenotype (CD10+, CD38+) represents a hallmark of follicular B-cell non-Hodgkin's lymphoma (NHL) with t(14;18).\textsuperscript{12} Based on these observations, unique multicolor combinations of Mab (e.g. Bcl2/CD10/CD38/CD20 for t(14;18) follicular B-cell NHL) can be built for the identification of malignant cells in patients suffering from a specific haematological malignancy.\textsuperscript{1,14} In contrast, in AML, the most common aberrant phenotype (e.g. CD33\textsuperscript{high}, HLADR\textsuperscript{-}, CD34\textsuperscript{-}, CD15\textsuperscript{-}, CD14\textsuperscript{-}, CD11b\textsuperscript{-} neutrophil lineage cells) may be present in less than one third of all cases (Table 2).\textsuperscript{14} However, a more detailed analysis of the aberrant phenotypes identified in AML, as well as in BCP-ALL, shows that a high concordance exists between specific aberrant phenotypes and genetic lesions that define diagnostic subcategories of both ALL and AML according to the WHO classification.\textsuperscript{6}</td>
</tr>
</tbody>
</table>
Diagnostic screening of haematological malignancies using multiparameter flow cytometric immunophenotyping

Recent reports indicate that multiparameter flow cytometric immunophenotyping is a fast, sensitive and specific approach in routine diagnostic screening for the presence of neoplastic lymphoid cells in peripheral blood (PB), bone marrow (BM) and other haematological and non-haematological samples. Accordingly, we have recently shown that the use of a single four-colour, seven-Mab tube (CD8-sIgκ/CD56-sIgλ/CD4-CD19/CD3) for the screening of absolute lymphocytosis, allows the identification of B-cell neoplasias with an extremely high sensitivity and specificity when compared to conventional cytomorphology, immunophenotyping and molecular diagnostic approaches.10 Similarly, once this approach is applied for evaluation of fine-needle aspirates from lymph nodes, a high diagnostic efficiency was also observed (Orfao et al., unpublished observations). Recently, it has also been shown that multiparameter flow cytometric immunophenotyping also has high diagnostic value for the identification of leptomeningeal involvement in B-NHL patients with cytomorphologically occult disease, allowing the identification of small numbers of neoplastic cells in spinal fluid (down to 1 cell/3 μl).11,19 In parallel, the use of Mab panels directed against different members of the TCRβ and TCRγ/δ families is of great utility for the diagnosis of T-cell clonality in both PB and lymph node samples, particularly once combined with antibodies for the identification of cells expressing leukaemia/lymphoma-associated phenotypes.20,21

Immunophenotypic classification of haematological malignancies

Once the presence of leukaemic cells in a sample is suspected and demonstrated, immunophenotyping allows their precise characterization and enumeration. For many years, specific attention was paid to the evaluation of the similarities between leukaemic and normal haematopoietic cells.8 Based on this information, in most haematological malignancies, leukaemic cells could be assigned to the B- and T- lymphoid vs. myeloid lineages and they could be classified according to their maturation stage (for example CD34+ and/or TdT leukaemic cells were classified as immature, while cells expressing sIg, sCD3/TCR in the absence of CD34, were more likely classified as mature).8,9 This information was of great help for the diagnostic classification of haematological malignancies. Clear examples of such utility were the phenotypic diagnosis of lineage involvement in acute leukaemias, the identification of biphenotypic acute leukaemias and the phenotypic classification of ALL.8

In recent years, the availability of more detailed and complete knowledge about the normal phenotypic patterns of different haematopoietic lineages and maturation-associated cell compartments,22 has contributed to the identification of new subtypes of acute non-lymphoblastic leukaemias (ANLL). As an example, at present it is well-established that in a small subset of ANLL, blast cells show immunophenotypic features which are characteristic of normal plasmaclamydoid dendritic cells (pDC), including a strong reactivity for both HLADR and CD123, positivity for CD4 and CD36, in the absence of other highly-specific neutrophil and monocytic lineage-associated intracellular markers (e.g.: CyMPO, CyLisozyme). However, in contrast with their normal counterpart, neoplastic pDC typically show coexpression of CD56 and NG2 (7.1) together with abnormally low CD45 levels in the absence of CD34 expression.24 Consequently, the introduction of new combinations of markers for detection of early commitment of neoplastic cells to specific haematopoietic cell lineages, has proven to be of value for a more sensitive subclassification of AML, according to the myeloid lineages involved.24 As an example, the combination of CD64 and CD36 expression appears to be more sensitive than CD14 alone for the identification of commitment of myeloid blast cells towards the monocytic lineage.24 Similarly, cytoplasmic expression of tryptase has been associated with specific subtypes of AML in which a variable degree of involvement of the basophil and/or mast cell lineages exist.25

In addition to the similarities observed between neoplastic and normal haematopoietic cells, aberrant patterns of protein expression have been identified in most leukaemic cells, which have proven to reflect underlying cytogenetic abnormalities.6,12,14,26-32 Consequently, the detailed characterization of such aberrant phenotypes, not only allows discrimination between normal and neoplastic cells, but it is also increasingly used for a more accurate classification of the disease and for identification of cases carrying specific genetic abnormalities in which rapid, cost-effective confirmatory molecular studies must be performed allowing rapid initiation of therapy; this applies both to acute leukaemias and B-CLPD.6,12,14,26-32 For example, blast cells in acute promyelocytic leukaemia patients carrying the t(15;17) show commitment into the neutrophil lineage (CMPO+, CD33+, CD13+) with a maturation arrest at the promyelocytic stage (HLADR+/dim, CD15+hematogeous, CD33+strong, CD117+‘, CD11b) but they lack the typical strong expression of CD15 of t(15;17)-negative normal (reactive) promyelocytes.24,25 In turn, in adults with BCRABL+ BCP-ALL, blast cells show an immature
BCP phenotype (CD34<sup>strong+</sup>, CD10<sup>dim+</sup>, CD13<sup>-/dim</sup>) but with abnormally low and heterogeneous expression of CD38,24 while children with TELAML1 typically show a common/BII BCP-ALL phenotype associated with an heterogeneous CD54 expression, in the absence of reactivity for CD20,26-34 in turn, among cases with MLL gene rearrangements a pro-B/BI phenotype with reactivity for NG2 (7.1) and the CD15 and CD65 myeloid-associated markers, is frequently observed.30,32 Table 3 provides a list of those aberrant phenotypes frequently associated with different genetic subgroups of acute leukemias.

**Immunophenotypic characterization of myelodysplastic syndromes**

For many years, the use of single stainings of mononuclear cell fractions from BM samples, has limited the utility of immunophenotyping in the diagnosis of myelodysplastic syndromes (MDS). Nevertheless, early studies already identified abnormal patterns of antigen expression in the BM of MDS patients.24,36,38 For example, decreased expression of CD35, CD11b, CD15, CD11a, CD54 and CD116 together with abnormally high percentages of CD33<sup>+</sup>, CD87<sup>+</sup>, CD14<sup>+</sup>, CD44<sup>-</sup> and CD64<sup>+</sup>, were reported on PB neutrophils in a variable proportion of MDS patients (between 18% and 80% of the cases).24 Similarly, an increased expression of precursor and early myeloid markers such as CD117, HLADR, CD34, CD33 and CD13, together with a lower reactivity for more mature antigens (e.g.: CD11b, CD11c, CD16 and NAT-9), have also been described in the BM of MDS patients.24 More recent studies show that multiparameter flow cytometric characterization of specific BM subpopulations according to their lineage and maturation stage, are of great clinical utility for the diagnosis of MDS patients in whom inconclusive morphological and cytogenetic features are found as well as for their prognostic stratification.24,36,38-40

However, it should be noted that most abnormalities are related to the leukocyte compartment whereas only small percentages of cases with abnormal antigen expression were detected in the erythroid compartment (e.g.: altered reactivity for CD36, CD71, glycophorin A and/or CD45). Remarkably, information about the antigen expression patterns of megakaryocytic precursors in MDS, is scanty.

The most common patterns of altered antigen expression include: 1) an increased number of myeloblasts; 2) abnormal sideward light scatter (SSC) for neutrophil and monocyctic lineage cells; 3) abnormal expression patterns of CD13/CD16 or CD11b/CD16, together with coexpression of CD56 and other lymphoid-associated markers in BM neutrophil-lineage cells; 4) coexpression of CD56 on monocytes; 5) abnormal expression patterns of CD71, glycophorin A and/or CD45 on nucleated red cells; 6) increased numbers of megakaryocytic cells, and; 7) an altered myeloid/lymphoid cell ratio.24,38-40

**Other applications of flow cytometry in the diagnosis and management of patients with haematological malignancies**

Apart from immunophenotyping for diagnosis and classification of acute leukemias, B-CLPD and MDS, the specific identification and enumeration of leukemic cells based on their aberrant phenotype is currently used for disease staging and for monitoring minimal residual disease (MRD) levels during and after therapy. Accordingly, detection of minimal numbers of neoplastic cells infiltrating PB, BM and the central nervous system (CNS), currently represents one of the most widely used applications of flow cytometry to evaluate the extent of the disease in both B- and T-cell lymphomas and, to a lower extend, also in ALL patients suspected of CNS involvement.12,16-18 Furthermore, evaluation of MRD during and after therapy, has proven to be of great help for predicting impending relapses in acute leukemia patients14 and for early evaluation of the efficacy of different treatment modalities in mature lymphoid neoplasias.1 Additionally, antigen expression patterns might also be used for predicting...
response to tumour cell-associated antigen-directed therapies and they may point out potential new drug targets for inducing cell differentiation or apoptosis pathways or for inhibiting cell proliferation.

In addition to the above listed applications of immunophenotyping in the diagnosis of haematological malignancies, staining for one or more antigens can also be combined with analysis of DNA content.

Moreover, the analysis of cell cycle distribution of neoplastic cells has also proven to be relevant for prognostic stratification of NHL and MM, where an increased proliferation is usually associated with high-grade disease and a worse clinical outcome, respectively.

Despite the fact that immunophenotypic approaches can also be combined with assessment of drug resistance at both the antigenic and functional levels, the diagnostic and prognostic relevance of these assays at diagnosis, remains to be established.

**New flow cytometry tools for the immunophenotypic diagnosis of haematological malignancies**

In general, it is expected that the application of flow cytometry in the diagnosis of haematological neoplasias will go on expanding. Apart from the development, identification and evaluation of new antigenic markers, future studies shall also take

<table>
<thead>
<tr>
<th>Tools</th>
<th>Current availability</th>
<th>Further requirements</th>
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<tbody>
<tr>
<td>Multicolor flow cytometry</td>
<td>4 to 17 colours</td>
<td>Required number of colors to be determined</td>
</tr>
<tr>
<td>Compatible fluorochromes</td>
<td>Up to 17 colours</td>
<td>Development of new high-quality fluorochromes (e.g. quantum dots)</td>
</tr>
<tr>
<td>New monoclonal antibody reagents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early myeloid markers</td>
<td>CD36/CD64&lt;sup&gt;a&lt;/sup&gt; IREM1&lt;sup&gt;a&lt;/sup&gt; Kell&lt;sup&gt;a&lt;/sup&gt; EoP&lt;sup&gt;a&lt;/sup&gt; vonWillebrand factor&lt;sup&gt;a&lt;/sup&gt; CD203c&lt;sup&gt;a&lt;/sup&gt; Tryptase&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Adapt and standardize existing reagents for their use in flow cytometry</td>
</tr>
<tr>
<td>Fusion proteins</td>
<td>PML-RARα&lt;sup&gt;c&lt;/sup&gt; TEL-AML-1&lt;sup&gt;c&lt;/sup&gt; E2A-PBX1&lt;sup&gt;c&lt;/sup&gt; BTK&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Improve the quality of the performance of some of the existing reagents</td>
</tr>
<tr>
<td>Mutated/dysregulated proteins</td>
<td>Bcl-2&lt;sup&gt;a&lt;/sup&gt; p53&lt;sup&gt;a&lt;/sup&gt; Rb&lt;sup&gt;a&lt;/sup&gt; Bcl-6&lt;sup&gt;a&lt;/sup&gt; Bcl-10&lt;sup&gt;a&lt;/sup&gt; Cyclin D1&lt;sup&gt;a&lt;/sup&gt; C-myc&lt;sup&gt;a&lt;/sup&gt; MLL E2A&lt;sup&gt;a&lt;/sup&gt; FoxP1&lt;sup&gt;a&lt;/sup&gt; RAG1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Develop new reagents for the detection of genetic markers (e.g. BCR/ABL fusion protein, mutated CD117, mutated JAK2, RAG2, etc.)</td>
</tr>
<tr>
<td>Other markers</td>
<td>ZAP70&lt;sup&gt;a&lt;/sup&gt; AKT&lt;sup&gt;c&lt;/sup&gt; BLIMP-1&lt;sup&gt;c&lt;/sup&gt; Pax-5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Develop new reagents for the detection of oncogenes, tumor suppressor genes and proteins involved in signal transduction pathways</td>
</tr>
<tr>
<td>Software tools</td>
<td>Limited software programs for multicolor analysis</td>
<td>Advanced automated multicolor software tools</td>
</tr>
</tbody>
</table>

<sup>a</sup>Good results; <sup>b</sup>Poor results in patient samples; <sup>c</sup>Not available for flow cytometry or further testing needed
advantage of recent improvements in instrumenta-
tion, bead technology, multicolor stainings and mul-
tiparameter analyses (Table 4). Accordingly, the
development of new flow cytometers with increasing
multicolor capabilities together with the avail-
ability of a greater number of high quality, compati-
ble fluorochromes, has pushed the use of 8- and
more colors in routine diagnostic laboratories in the
last two years. In parallel, the development of high-
ly-sensitive four-way, high-speed sorting instruments
has facilitated routine purification of neoplastic cells
from patient samples for further genetic and molecu-
ar diagnostic studies, which is of particular relevance
once minimal disease levels are detected (e.g. BM
aspirate samples from patients with monoclonal gammapathies).

As in the past, production, identification and eval-
uation of new Mab reagents also represents an area
with a major impact on the future development of
clinical flow cytometry in haematology. The avail-
ability and evaluation of new markers for the identi-
fication of early commitment of haematopoietic cells
into the monocytic (e.g.: CD36 in combination with
CD64), erythroid (e.g.: Kell gp), megakaryocytic (e.g.:
von Willebrand factor), basophil/mast cell (e.g.:
CD203c, tryptase), eosinophil (e.g.: EoPO) and den-
dritic cell lineages, will certainly contribute to
improve the classification of ANLL. Similarly, the
development of high quality monoclonal antibody
reagents directed against transcription factors would
also be of great utility to dissect the pathways
involved in leukemogenesis. Even more interesting,
are those efforts recently made to produce pairs of
Mab directed against fusion proteins derived from
gene rearrangements occurring in specific chromoso-
mal translocations (e.g.: BCR-ABL protein) and that
could be used for the identification of diagnostic
genetic markers by flow cytometry both in cell
lysates and in single cell suspensions. In this area,
the parallel development of multiplexed-bead arrays
for the quantitative evaluation of soluble proteins in
both serum and cell lysates, will certainly facilitate
the rapid introduction of newly developed antibody
reagents for the identification of fusion proteins,
mutated proteins and/or phosphorylated proteins,
and as well as for the quantitative evaluation of proteins
cleaved from the surface of neoplastic cells (e.g.: β2-
microglobulin in plasma cell disorders).

Despite the great clinical utility of flow cytometric
immunophenotyping of haematological malignan-
cies and the promising technological advances which
have occurred in the past few years, standardization
of technical procedures as well as of data analysis,
interpretation and reporting still remains a major
challenge. In line with this, different, automated and
semi-automated sample preparation devices have
been produced and commercialised in the last
decade. In addition, recent reports show that new
software tools based on vector quantization
approaches can be produced and applied to the auto-
mated analysis of flow cytometry data files. In line
with this, we have recently shown that the screening
for mature B-cell neoplasias in patients displaying an
absolute lymphocytosis in PB, could be fully auto-
mated, providing an extremely high specificity and
sensitivity in the diagnosis of B-cell malignancies.

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