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EDITORIAL

Flow cytometry analysis of acute promyelocytic leukemia: the power of 'surface hematology'

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An article by Breccia *et al.*[1] published on *Leukemia* highlighted one remarkable aspect of acute promyelocytic leukemia (APL): the development of thrombosis in patients treated with all-*trans* retinoic acid (ATRA) and chemotherapy is significantly associated with specific biological features. These characteristics included CD2 and/or CD15 expression, bcr3 promyelocytic leukemia (PML)/retinoic acid receptor (RAR)α transcript type, *FLT3* gene internal tandem duplications and elevated white blood cell (WBC) count. We were impressed by the strength that flow cytometry studies retain 20 years after the pioneer papers establishing the antigenic features consistently noted to characterize APL.[2,3] Starting from this article, this editorial aims to provide a series of considerations about the impact of flow cytometry on clinical hematology, with particular accent on APL.

The five attributes of modern flow cytometry

Over the years, hematological flow cytometry has revealed a series of attractive features that we can record, bringing to mind the genius of the short prose narrative Italo Calvino and his meditations on the art of writing, as *lightness*, *quickness*, *exactitude*, *visibility* and *multiplicity*. Hematological flow cytometry is *light* because its underlying principle is solid, without any intricate conjecture, and operator involvement is trouble-free. It is *quick*, as first results are available within 20 min from the sample processing and, in an experienced laboratory, acute leukemia diagnosis can be provided within 1 h. Flow cytometry is an *exact* technology, because the analytical process is highly controlled and data are quantitatively expressed. It is *visible*, for the reason that great part of analysis is based upon the pattern recognition and 'eyeballing' of flow cytometry dot, density and contour plots to identify abnormalities. Finally, *multiplicity* is a progressively growing feature of hematocytometry, as polychromatic analysis has reached the level of six colors for routine analysis, eight for minimal residual disease studies and up to 17 in more experimental settings.[4]

The single-cell resolution makes flow cytometry absolutely different from any other tool utilized in diagnostic hematopathology. Jonathan Irish, a young investigator from the Stanford University (CA, USA), defined flow cytometry as a sort of *single-cell proteomics*, owing to its ability to simultaneously detect and quantify many proteins at single-cell level.[5]

The weight of blood cell flow cytometry and the birth of surface hematology

The dialogue between basic flow cytometrists and clinical hematologists has been hampered, during the last years, by a series of misconstructions. It is an old truth that expert cytometrists, theorizing on the state of the art of flow cytometry, tend to talk mainly about or to themselves, and the body of work to which their judgements and speculations must in reality apply is sometimes disregarded. On the other hand, a number of clinical hematologists reveal a dose of skepticism about the extended use of flow cytometry, proposing to reduce its application to the residual spaces in which other technologies use to fail. In an attempt of stimulating basic flow cytometrists to dialogue, at the same time providing some reasoning to doubtful clinicians, we want to forcefully recall that almost all onco-hematological diseases have experienced a profound renovation due to the application of cytometric technologies. All investigators involved in leukemia and lymphoma typing are called daily to provide their substantiated opinion on almost every case, even those cases that seem easily classifiable by conventional cytology. Distinction between immature acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML), immunological subtyping and prediction of karyotypic and biomolecular abnormalities of ALL as well as AML, expression of prognostic markers, detection of molecule potentially exploitable as target for immunotherapy (CD20, CD52, CD33), presence of minimal residual disease, assessment of B-cell and T-cell clonality, detection of multidrug resistance (MDR)-related proteins: this is just an incomplete catalog of the problems a flow cytometrist is daily called to solve.

The most accessible cell structure to be studied by flow cytometry is cell surface. What makes the membrane truly special is the easily detectable presence of a huge amount of different 'peripheral' proteins that are used for various functions such as adhesion, growth factor or chemokine binding, immunoglobulin Fc fragment or complement attachment, enzymatic activity and ion transport. The process of categorizing the antigenic molecules and epitopes associated with human white cells dates back to the early 1980s and, at the present time, established molecules at the surface of leukocytes are upper than 300.[6] Clinical hematology has been enriched with an abundance of new information, as the composition of leukocyte membrane has been almost completely elucidated: this has prompted us to recognize the existence of a new branch of hematology, the *surface hematology*.

APL and the power of surface hematology

APL is a very complex and instructive model for clinical flow cytometrists. We want to focus on seven points to highlight the relevance of flow cytometric studies in the progressive growth of APL awareness:

1. The need for a rapid diagnosis of APL.
2. The search for APL leukemic stem cells.
3. Differentiation potentials of APL cells.
4. Expression of foreign antigens in APL: the case of CD2.
5. Expression of foreign antigens in APL: the case of CD56.
6. APL flow cytometry and maturation therapy.

7. APL flow cytometry and proapoptotic therapy.
8. APL flow cytometry and monoclonal antibody (MoAb) therapy.

The need for a rapid diagnosis of APL

APL is regarded as a hematological emergency and a rapid diagnosis is essential, as it allows the early administration of ATRA, which induces leukemic cell maturation and ameliorates the severe coagulopathy. Although the most rapid technique able to provide a provisional diagnosis of APL is classical morphology, the existence of both false negatives and false positives draws attention to the need for more sensitive and specific methods to screen AML cases before the confirmation of PML/RARα rearrangement.[7] A recently developed technique, the PML protein immunofluorescence,[8,9] is not yet universally used to provide rapidly an unequivocal diagnosis of APL.

The applicability of multicolor flow cytometry for rapid diagnosis of APL has been convincingly described by Alberto Orfao and Elisabeth Paietta.[7,10] APL is characterized by a highly specific immunophenotype, demonstrable by flow cytometry, characterized by consistent absence or very low expression of CD15, CD11a, CD11b, CD11c, CD18 (the β-subunit of CD11a, b, c), CD66b and CD66c. At the same time, the blasts show CD13 heterogeneous expression (broad histogram), whereas CD33 expression is homogeneous (sharp histogram). CD34 and human leukocyte antigen (HLA)-DR are frequently absent. This immunophenotype, in skillful hands, has been reported to show 100% sensitivity and 99% specificity for predicting APL molecular rearrangement.[7,10] Thus, although the final word is doubtlessly pronounced by genotypic analysis, flow cytometry can provide a correct diagnosis of APL within a matter of a few hours and is commonly used as a rapid predictor of karyotypic and biomolecular results.

The search for APL leukemic stem cells

One of the recently proposed questions is 'which is the cancer stem cell in APL?'[11,12] From the arguments reported above, it could seem that APL exhibits a univocal immunophenotype, without exceptions and shades. Actually, flow cytometry enables to detect some divergent behaviors from deep-rooted rules. First of all, the majority of patients operationally classified as CD34 and HLA-DR negative show a small sub-population of leukemic cells (ranging from 1 to 10% of cells) characterized by an apparently non-APL immunophenotype, displaying CD34 and HLA-DR surface expression. This simple observation is the cytometric substantiation of immature progenitor cell involvement in the pathogenesis of APL.

A widely held view is that APL arises at level of myeloid committed progenitors. This acquirement is based upon studies that have used primary patient material to explore the origin of APL. In some reports, the leukemic clone was demonstrated to be restricted to the myeloid lineage with the expression of PML-RARα being frequently detected in the CD34+CD38+ population. By contrast, other experiments demonstrated the presence of the PML-RARα within the majority of cells located in the CD34+CD38− fraction, especially in cases of hypogranular variant APL.[11] Edwards *et al.*[13] demonstrated, by using a fluorescence-activated cell sorter and fluorescence *in situ* hybridization (FISH) approach, the presence of t(15;17) and PML/RARα fusion gene within both primitive CD34+CD38− and committed CD34+CD38+ progenitor cell sub-populations.

Taken together, the presence of both CD34+CD38+ and CD34+CD38− leukemic cells suggests that a significant proportion of APL cases arise in more primitive progenitors than previously considered. These insights have been made possible by the availability of flow cytometry and cell sorting techniques. It is not negligible that analytical flow cytometry, performed in experienced laboratories, is able to unambiguously distinguish small populations of CD34+CD38+ and CD34+CD38− leukemic cells, so giving an immediate representation of the clonal architecture of APL as well as of its cellular hierarchy.

Differentiation potentials of APL cells

An emerging question is if PML/RARα hybrid gene may occur only in progenitor cells whose fate is to differentiate into polymorphonuclear cells or it can appear in the context of lineages different from granulopoiesis. Riccioni *et al.*[14] demonstrated monocytic commitment in APL by cytometric evaluation of *c-fms*. Expression intensity studies showed that *c-fms*^{bright} leukemic blasts preferentially exhibited the capacity for monocytic differentiation as compared to the *c-fms*^{dim} subset. These observations indicate that PML/RARα APL blasts are bipotent for differentiation through both neutrophilic and monocytic lineages. It has also been described as a case of APL showing monocytic differentiation after ATRA therapy,[15] as documented by immunophenotype and FISH studies. We have studied in our laboratory a small series of patients showing an accessory sub-population of leukemic cells characterized by CD14 surface expression. Given its ability to detect small populations of cells by a multidimensional approach, flow cytometry is the most powerful tool able to identify cases with differentiation potential different from granulopoiesis, with high sensitivity and in a single experimental run.

Expression of foreign antigens in APL: the case of CD2

One recurrent argument when discussing about APL surface mosaic is CD2 expression. In order to elucidate the mechanisms underlying CD2 expression in APL, a recently published study investigated the long-range chromatin structure surrounding the CD2 locus in primary APL blasts. The majority of cases examined belonged to the hypogranular variant form of APL and in each case the CD2 locus was found within an open chromatin environment.[16]

The article by Breccia *et al.*[1] published in *Leukemia* deals with the impact of immunophenotype on APL prognosis, with respect to the aptitude to develop thrombotic events. The authors analyzed a large series of APL patients treated with ATRA and idarubicin (the all-*trans* retinoic acid and idarubicin (AIDA) protocol) and compared several clinicobiologic characteristics of patients who developed thrombosis with those who had no thrombosis. Flow cytometry experiments revealed in the former group significant prevalence of CD2 and CD15 expression. No correlation was found with other variables as sex, age, French–American–British subtype, ATRA syndrome or thrombophilic state. These findings indicate that, in APL patients, distinct immunophenotypic behaviors may predict increased risk of developing thrombosis. It is conceivable that CD2+ and CD15+ leukemic promyelocytes are able to produce several changes that can activate the coagulation cascade. Abnormal leukocytes and the coagulation cascade could be capable of forming a mutual network, in which the activation of coagulation could also feed back on leukemic cell activation. The consequence of this could be a systemic procoagulant state. Current research efforts should be aimed to identify the direct participants in the mechanisms of thrombotic events in APL, to define appropriate targets for future drug development.

Expression of foreign antigens in APL: the case of CD56

It has been demonstrated that a positive staining of leukemic promyelocytes for CD56, a neural cell adhesion molecule, is associated with unfavorable prognosis in both AML with t(8;21) and APL.[17] To gain further insights into the prognostic impact of CD56 expression in APL, we studied by flow cytometry a series of 100 consecutive patients uniformly treated with AIDA protocol. We demonstrated the prognostic significance of CD56, providing two novel relevant observations: (i) this marker is an indicator of poorer outcome even in patients receiving the modern state-of-the-art treatment and (ii) the prognostic significance of CD56 expression is retained in the multivariate analysis that include WBC count and is therefore independent of this variable. To explain the worse prognosis of CD56 acute leukemia, different hypotheses have been proposed, including a greater occurrence in these patients of extramedullary involvement and a correlation between CD56 expression and MDR.[17]

APL flow cytometry and maturation therapy

Immunophenotypic studies of surface antigens modulation on APL cells following to ATRA treatment have attracted basic and clinical hematologists for almost a decade. *In vitro* treatment with ATRA dramatically modifies the adhesion phenotype of APL blast cells, promoting a consistently striking upregulation of CD11b, CD11c, CD15, CD65, CD45R0, CD54 and CD38, which is poorly demonstrable in AML. The behavior of CD15s is variable and fully independent from CD15 and CD65 in induction experiments, suggesting a differential enzyme regulation within the selecting ligand system. ATRA is capable of producing a switch from the high- (RA) to the low- (RO) molecular weight isoform of CD45. Of particular interest is the lack of effects on CD11a expression (whose negativity is of pivotal importance in the Paietta diagnostic scheme), which produces an asynchronous phenotype in APL treated by ATRA (CD11a−, CD11b+, CD15+), undetectable on normal maturing myeloid cells.[18]

APL flow cytometry and proapoptotic therapy

Arsenic trioxide (As_2O_3) is an effective drug for APL, acting through a complex mechanism involving apoptosis via elevation of reactive oxygen species, loss of mitochondrial membrane potential and release of cytochrome *c*, activation of the caspase cascade, downregulation of *bcl-2* expression as well as modulation of PML-RARα protein. We tried to define some effects of As_2O_3 at surface membrane level by analyzing different groups of molecules such as β2-integrins, isoforms of CD45 (RA and RO), the CD44 antigen, pan-myeloid antigen CD33 and, finally, the CEA family members (CD66a to CD66e).[19] Our study provided two results that rendered definitive the concept that As_2O_3 had APL specificity. Firstly, as assessed by flow cytometry cell typing, the exposure of both APL and non-APL blasts to As_2O_3 was ineffective in altering the expression of molecules typically related to ATRA-driven differentiation. Secondly, As_2O_3 was able to activate only in APL blast cells a specific process involving the generation of a unique surface antigen mosaic in which the presence of CD66c molecule (NCA-50/90) was predominant.[19] It has been demonstrated that CD66c is associated with tyrosine kinase activity and that its engagement activates a signaling cascade via *Src*-like protein tyrosine kinase *Hck* and *Fgr*, with the activation of *Rac-1*, its substrate PAK and, finally, *Jun*-N-terminal kinase (JNK). As JNK has been recently demonstrated to play a pivotal role in arsenic-dependent apoptosis, it is conceivable that CD66c expression may promote apoptotic processes via the activation of such signaling cascade.[19] As_2O_3-driven expression of CD66c in APL is at the moment an observational finding; further studies are required to elucidate the role of such molecule in APL response to arsenic. In some cases of APL, the p38 mitogen-activated protein kinase signaling cascade may act as a negative feedback regulator for the generation of antileukemic response, and its activation may account for resistance to As_2O_3.[20] The availability of clinically exploitable MoAbs directed to CD66 family opens an interesting *scenario* on the opportunity of promoting the synergistic effects of As_2O_3 and CD66 MoAbs to consistently induce apoptotic death in APL cells, even in resistant cases.

APL flow cytometry and MoAb therapy

Among the innovative therapeutic treatments developed during the recent years, one of the most stimulating is the employment of MoAbs as an effective strategy to cure hematopoietic malignancies. In APL, CD33 antigen has proved a suitable target for immunotherapy. Gemtuzumab ozogamicin, a MoAb direc-

Table 1 Mileposts in the development of acute promyelocytic leukemia flow cytometry

Year	*Feature identified*	*Author*	*Reference*
1986	HLA-DR negativity	San Miguel *et al*.	2
1986	CD2 expression	Sato *et al*.	3
1990	CD9 expression	De Rossi *et al*.	22
1992	CD2 expression and PML gene breakpoints	Claxton *et al*.	23
1993	Low expression of MDR1	Miyachi *et al*.	24
1994	Distinct immunophenotypic profile	Paietta *et al*.	25
1994	CD68 expression	Erber *et al*.	26
1994	ATRA-driven modulation of adhesion molecules	Di Noto *et al*.	18
1996	c-kit/CD117 expression	Di Noto *et al*.	27
1998	Stem cell phenotype in APL	Foley *et al*.	28
1999	CD34, CD15 and CD13 pattern	Orfao *et al*.	7
1999	CD56 and prognosis	Murray *et al*.	29
1999	Stem cell involvement in APL pathogenesis	Edwards *et al*.	13
1999	Arsenic effects on CD66c antigen	Di Noto *et al*.	19
2003	Monocytic commitment in APL	Riccioni *et al*.	14
2004	A surrogate marker profile for rapid diagnosis	Paietta *et al*.	30
2006	Immunophenotypic predictors of thrombosis	Breccia *et al*.	1

Abbreviations: APL, acute promyelocytic leukemia; ATRA, all-*trans* retinoic acid; HLA, human leukocyte antigen; MDR, multidrug resistance; PML, promyelocytic leukemia.

ted against CD33 and conjugated to calicheamicin has been proposed to cure the disease. The engagement of CD33 by gemtuzumab results in internalization of the immunoconjugate and hydrolytic release of calicheamicin, which, in turn, causes irreversible DNA damage and cell death. The success of gemtuzumab therapy is due to two characteristics of APL blasts, both of them detectable by flow cytometry and probably essential for the effectiveness of the strategy: the strong expression of CD33 as well as the weak display of the MDR glycoprotein 170 (Pgp) and of lung resistance-related protein. In particular, the latter feature accounts for the striking sensitivity of APL blasts to calicheamicin.[10,21] Although the expression of CD33 in APL is considered mandatory, MoAb-based therapies imply the respect of a general rule: the presence of molecules to be used as a target for immunotherapy must be demonstrated at single-patient level.

Conclusion

The extraordinary efficacy of flow cytometry analysis of APL has been convincingly documented. Table 1 outlines some of the steps of APL progress of knowledge. This is only a limited example of what we use to call 'the power of surface hematology'.

All the arguments discussed lead us to what we are all interested in: where is APL flow cytometry going? Maybe many answers are possible, but we want to focus on three main goals: (i) a better access to rare cells in the context of minimal residual disease analysis; (ii) the development of studies disclosing the phenotypic mosaics predictive of resistance to ATRA or As_2O_3; (iii) further insights into the expression of a series of resistance associated proteins, with the aim to determine the likelihood of MDR following to chemotherapy.

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References

1 Breccia M, Avvisati G, Latagliata R, Carmosino I, Guarini A, De Propris MS *et al.* Occurrence of thrombotic events in acute promyelocytic leukemia correlates with consistent immunophenotypic and molecular features. *Leukemia* 2006.
2 San Miguel JF, Gonzalez M, Canizo MC, Anta JP, Zola H, Lopez Borrasca A. Surface marker analysis in acute myeloid leukaemia and correlation with FAB classification. *Br J Haematol* 1986; **64**: 547–560.
3 Sato T, Abe R, Kawaguchi M, Uchida T, Kariyone S. Expression of monocytic and T-lymphocytic phenotype in a variant form of acute promyelocytic leukaemia (M3). *Scand J Haematol* 1986; **37**: 151–153.
4 Perfetto SP, Chattopadhyay PK, Roederer M. Seventeen-colour flow cytometry: unravelling the immune system. *Nat Rev Immunol* 2004; **4**: 648–655.
5 Irish JM, Kotecha N, Nolan GP. Mapping normal and cancer cell signalling networks: towards single-cell proteomics. *Nat Rev Cancer* 2006; **6**: 146–155.
6 Zola H, Swart B, Nicholson I, Aasted B, Bensussan A, Boumsell L *et al.* CD molecules 2005: human cell differentiation molecules. *Blood* 2005; **106**: 3123–3126.
7 Orfao A, Chillon MC, Bortoluci AM, Lopez-Berges MC, Garcia-Sanz R, Gonzalez M *et al.* The flow cytometric pattern of CD34, CD15 and CD13 expression in acute myeloblastic leukemia is highly characteristic of the presence of PML-RARalpha gene rearrangements. *Haematologica* 1999; **84**: 405–412.
8 Cullen MJ, Richards SJ, O'Connor SJ, Dickinson H, Sharpe C, Swirsky DM *et al.* Rapid diagnosis of acute promyelocytic leukemia (PML): applicability of flow cytometry and PML protein immunofluorescence. *Cancer Genet Cytogenet* 2004; **148**: 176–177.
9 Falini B, Mason DY. Proteins encoded by genes involved in chromosomal alterations in lymphoma and leukemia: clinical value of their detection by immunocytochemistry. *Blood* 2002; **99**: 409–426.
10 Paietta E. Expression of cell-surface antigens in acute promyelocytic leukaemia. *Best Pract Res Clin Haematol* 2003; **16**: 369–385.
11 Grimwade D, Enver T. Acute promyelocytic leukemia: where does it stem from? *Leukemia* 2004; **18**: 375–384.
12 Puccetti E, Ruthardt M. Acute promyelocytic leukemia: PML/RARalpha and the leukemic stem cell. *Leukemia* 2004; **18**: 1169–1175.
13 Edwards RH, Wasik MA, Finan J, Rodriguez R, Moore J, Kamoun M *et al.* Evidence for early hematopoietic progenitor cell involvement in acute promyelocytic leukemia. *Am J Clin Pathol* 1999; **112**: 819–827.
14 Riccioni R, Saulle E, Militi S, Sposi NM, Gualtiero M, Mauro N *et al.* C-fms expression correlates with monocytic differentiation in PML-RAR alpha+ acute promyelocytic leukemia. *Leukemia* 2003; **17**: 98–113.
15 Naeem M, Harrison K, Barton K, Nand S, Alkan S. A unique case of acute promyelocytic leukemia showing monocytic differentiation after ATRA (all-*trans* retinoic acid) therapy. *Eur J Haematol* 2006; **76**: 164–166.
16 Grimwade D, Outram SV, Flora R, Ings SJ, Pizzey AR, Morilla R *et al.* The T-lineage-affiliated CD2 gene lies within an open chromatin environment in acute promyelocytic leukemia cells. *Cancer Res* 2002; **62**: 4730–4735.
17 Ferrara F, Morabito F, Martino B, Specchia G, Liso V, Nobile F *et al.* CD56 expression is an indicator of poor clinical outcome in patients with acute promyelocytic leukemia treated with simultaneous all-*trans*-retinoic acid and chemotherapy. *J Clin Oncol* 2000; **18**: 1295–1300.
18 Di Noto R, Schiavone EM, Ferrara F, Manzo C, Lo Pardo C, Del Vecchio L. Expression and ATRA-driven modulation of adhesion molecules in acute promyelocytic leukemia. *Leukemia* 1994; **8**: 1900–1905.
19 Di Noto R, Boccuni P, Costantini S, Dello Russo A, Lo Pardo C, Copia C *et al. In vitro* exposure of acute promyelocytic leukemia cells to arsenic trioxide (As_2O_3) induces the solitary expression of CD66c (NCA-50/90), a member of the CEA family. *Tissue Antigens* 1999; **54**: 597–602.
20 Giafis N, Katsoulidis E, Sassano A, Tallman MS, Higgins LS, Nebreda AR *et al.* Role of the p38 mitogen-activated protein kinase pathway in the generation of arsenic trioxide-dependent cellular responses. *Cancer Res* 2006; **66**: 6763–6771.
21 Lo Coco F, Ammatuna E, Noguera N. Treatment of acute promyelocytic leukemia with gemtuzumab ozogamicin. *Clin Adv Hematol Oncol* 2006; **4**: 57–62, 76–77.
22 De Rossi G, Avvisati G, Coluzzi S, Fenu S, Lo Coco F, Lopez M *et al.* Immunological definition of acute promyelocytic leukemia (FAB M3): a study of 39 cases. *Eur J Haematol* 1990; **45**: 168–171.
23 Claxton DF, Reading CL, Nagarajan L, Tsujimoto Y, Andersson BS, Estey E *et al.* Correlation of CD2 expression with PML gene breakpoints in patients with acute promyelocytic leukemia. *Blood* 1992; **80**: 582–586.
24 Miyachi H, Takemura Y, Yonekura S, Komatsuda M, Nagao T, Arimori S *et al.* MDR1 (multidrug resistance) gene expression in adult acute leukemia: correlations with blast phenotype. *Int J Hematol* 1993; **57**: 31–37.
25 Paietta E, Andersen J, Gallagher R, Bennett J, Yunis J, Cassileth P *et al.* The immunophenotype of acute promyelocytic leukemia (APL): an ECOG study. *Leukemia* 1994; **8**: 1108–1112.
26 Erber WN, Asbahr H, Rule SA, Scott CS. Unique immunophenotype of acute promyelocytic leukaemia as defined by CD9 and CD68 antibodies. *Br J Haematol* 1994; **88**: 101–104.

27 Di Noto R, Lo Pardo C, Schiavone EM, Manzo C, Vacca C, Ferrara F *et al.* Stem cell factor receptor (c-kit, CD117) is expressed on blast cells from most immature types of acute myeloid malignancies but is also a characteristic of a subset of acute promyelocytic leukaemia. *Br J Haematol* 1996; **92**: 562–564.
28 Foley R, Soamboonsrup P, Kouroukis T, Leber B, Carter RF, Sunisloe L *et al.* PML/RAR alpha APL with undifferentiated morphology and stem cell immunophenotype. *Leukemia* 1998; **12**: 1492–1493.
29 Murray CK, Estey E, Paietta E, Howard RS, Edenfield WJ, Pierce S *et al.* CD56 expression in acute promyelocytic leukemia: a possible indicator of poor treatment outcome? *J Clin Oncol* 1999; **17**: 293–297.
30 Paietta E, Goloubeva O, Neuberg D, Bennett JM, Gallagher R, Racevskis J *et al.* A surrogate marker profile for PML/RAR alpha expressing acute promyelocytic leukemia and the association of immunophenotypic markers with morphologic and molecular subtypes. *Cytometry B* 2004; **59**: 1–9.