bjh research paper

CD90/Thy-1 is preferentially expressed on blast cells of high risk acute myeloid leukaemias*

Francesco Buccisano,[1] Francesca Maria Rossi,[2] Adriano Venditti,[1] Giovanni Del Poeta,[1] Maria Christina Cox,[1] Elisabetta Abbruzzese,[1] Maurizio Rupolo,[3] Massimiliano Berretta,[3] Massimo Degan,[3] Stefania Russo,[3] Anna Tamburini,[1] Luca Maurillo,[1] Maria Ilaria Del Principe,[1] Massimiliano Postorino,[1] Sergio Amadori[1] and Valter Gattei[2]

[1]*Chair of Hematology, University Tor Vergata and Ospedale S. Eugenio, Rome,* [2]*Clinical and Experimental Hematology Research Unit, and* [3]*Medical Oncology, Centro di Riferimento Oncologico, IRCCS, Aviano (PN), Italy*

 British Journal of Haematology, **125,** 203–212

Received 8 October 2003; accepted for publication 4 January 2004

Correspondence: Francesco Buccisano, Cattedra di Ematologia, Università di Roma 'Tor Vergata', Policlinico Tor Vergata, V.le Oxford 81 – 00133, Rome, Italy. E-mail: francesco.buccisano@uniroma2.it

*Supported in part by: MURST, Programmi di Ricerca di Interesse Nazionale 2000; the Associazione Italiana per la Ricerca sul Cancro (AIRC) and the Ministero della Salute, Rome, Italy.

Summary

Different transformation mechanisms have been proposed for elderly acute myeloid leukaemia (AML) and secondary AML (sAML) when compared with *de novo* AML or AML of younger patients. However, little is known regarding differences in the immunophenotypic profile of blast cells in these diseases. We systematically analysed, by flow cytometry, 148 patients affected by *de novo* (100 cases) or sAML (48 cases). By defining a cut-off level of 20% of $CD34^+$ cells co-expressing CD90, the frequency of $CD90^+$ cases was higher in sAML (40%) *versus de novo* AML (6%, $P < 0{\cdot}001$), elderly AML (>60 years) (24%) *versus* AML of younger patients (10%, $P = 0{\cdot}010$) and poor- *versus* good-risk karyotypes (according to the Medical Research Council classification, $P < 0{\cdot}001$). The correlation between CD90 expression, sAML and unfavourable karyotypes was confirmed by analysing the subset of $CD34^+$ AML cases alone (91/148). Consistently, univariate analysis showed that expression of CD90 was statistically relevant in predicting a shorter survival in $CD90^+$ AML patients ($P = 0{\cdot}042$). Our results, demonstrating CD90 expression in AML with unfavourable clinical and biological features, suggest an origin of these diseases from a CD90-expressing haemopoietic progenitor and indicate the use of CD90 as an additional marker of prognostic value in AML.

Keywords: CD90/Thy1, secondary AML, poor-risk cytogenetics.

The acute myeloid leukaemias (AML) represent a heterogeneous group of clonal bone marrow (BM) disorders arising as the result of progressive genetic damage occurring in haemopoietic progenitor cells (Fialkow *et al*, 1989, 1991). These genetic lesions produce a complete dysregulation of the cell-growth mechanism with the accumulation of blast cells in BM and peripheral blood (PB), eventually overcoming normal haemopoiesis (Fialkow *et al*, 1989, 1991).

Distinct subgroups of AML, characterized by completely different clinical courses, have been identified (Head, 1996; Leith *et al*, 1997). In particular, the prognosis of AML has been reported to be age dependent, with chemoresistance being observed more frequently in older patients (Head, 1996; Leith *et al*, 1997). This is supposedly based on differences in the underlying genetic defects of AML in elderly when compared with younger patients. For example, balanced chromosomal translocations without gain or loss of genetic material are reported to be more frequent in younger AML patients, whereas unbalanced abnormalities involving complete or partial losses of chromosomes or more complex karyotype aberrations are more often detected in elderly AML patients (Mauritzson *et al*, 1999; Mrozek *et al*, 2000; Moorman *et al*, 2001; Schoch *et al*, 2001). Therefore, at least two genetically defined subgroups of AML may be identified, strikingly differing in their age-specific incidences. In this regard, a common mechanism of transformation has been proposed for elderly AML, AML with complex karyotypes and secondary AML (sAML), which implies that multiple genetic hits that

occur over a lifetime, may drive an early myeloid stem cell towards dysplastic growth dysregulation and eventually overt leukaemia (Johansson *et al*, 1996; DePinho, 2000; Pedersen-Bjergaard *et al*, 2002).

Although sAML, AML of the elderly or with aberrant karyotypes can be viewed as biologically related entities that are distinct from *de novo* AMLs or AML of younger patients, little information is currently available regarding the putative differences in the immunophenotypic profile of blast cells in these diseases (Rosenfeld & Kantarjian, 2001).

The CD90/Thy-1 antigen is a 25–35 kDa, glycosylphosphatidylinositol (GPI)-linked surface protein expressed on primitive haemopoietic stem cells in normal BM, cord blood and fetal liver cells. Although its function has not been completely unveiled, it is likely that CD90, like other adhesion molecules, participates in haemopoietic cell adhesion and recognition (Low & Kincade, 1985). Furthermore, the blockage of CD90 by specific antibodies (anti-Thy-1 antibody 5E10) inhibits the *in vitro* expansion of human $CD34^+/CD90^+$ cord blood cells (Mayani & Lansdorp, 1994). In normal BM, about 5–25% of $CD34^+$ cells co-express CD90 (Lansdorp *et al*, 1990; Craig *et al*, 1993; Humeau *et al*, 1996), this subset of progenitors contains cells with long-term culture-initiating activity and is capable of reconstituting haematopoiesis both in animal models and in human beings (Baum *et al*, 1992; Sumikuma *et al*, 2002). Although this data indicate CD90 as a marker of haemopoietic precursors with stem cell properties, the expression of this molecule in AML blasts has been poorly investigated (Holden *et al*, 1995; Campos & Guyotat, 1996; Blair *et al*, 1997; Inaba *et al*, 1997; Kozii *et al*, 1997; Wuchter *et al*, 2001) and no correlation with clinical and biological features has yet been proposed.

In the present study, by analysing the expression of CD90 in a wide panel of AML cases, we demonstrated that CD90 expression was more frequently expressed on the blast cells of specific subsets, i.e. sAML, AML with complex karyotypes and *de novo* AML of elderly. A significantly lower survival was consistently documented in $CD90^+$ AML patients. Overall, this data suggest that CD90 is preferentially expressed on AMLs with distinguishable clinical and biological features, raising the hypothesis that a CD90-expressing haemopoietic progenitor may be the target of the initial genetic lesion in these particular subsets of disease (Nilsson *et al*, 2000, 2002).

Patients and methods

Patients

This study includes 148 consecutive patients affected by AML, observed at the Chair of Haematology of the University 'Tor Vergata' of Rome and at the Division of Medical Oncology of the Centro di Riferimento Oncologico of Aviano. AML cases were classified according to the revised French–American–British (FAB) and World Health Organization (WHO) classifications (Bennet *et al*, 1982; Vardiman *et al*, 2002). FAB assignment was integrated by the presence of an immunophenotypic profile of blast cells consistent with FAB subgrouping, as described (Gattei *et al*, 1997). The sAML subgroup included AML evolving from an antecedent myelodysplastic-myeloproliferative disease lasting more than 6 months before the onset of overt AML, and AML with a marrow dysplasia consistent with the reported criteria (Vardiman *et al*, 2002). In all cases, AML classification was made at least by two independent observers who were unaware of the CD90 expression results. Data on response to chemotherapy were available for 125 of 148 AML patients. In the case of non-M3 FAB subtypes, patients undergoing intensive chemotherapy (75/116) received an induction regimen combining daunorubycin (50 mg/m^2), or mitoxantrone (7–12 mg/m^2) or idarubicin (10 mg/m^2) for 3 d, in association with cytosine arabinoside (100 mg/m^2 continuous infusion, days 7–10) and etoposide (100 mg/m^2 for 5 d). Postremission therapies included cytosine arabinoside-containing regimens according to large European trials (Zittoun *et al*, 1999). Patients affected by acute promyelocytic leukaemia (FAB-M3, nine cases) were treated according to published protocols (Mandelli *et al*, 1997).

Immunophenotype

Anticoagulated BM and PB samples were collected before therapy. Approval for this study was obtained from the institutional review board. Informed consent was provided according to the declaration of Helsinki. Surface antigens were analysed by two- or three-colour immunofluorescence by combining phycoerithrin (PE)-, fluorescein isothyocyanate (FITC)- and peridinium chlorophyll protein-conjugated monoclonal antibodies (mAbs). Sources and specificities of the mAbs used for immunophenotype and lineage assessment of AML blasts (recognizing the following antigens: CD33, CD13, CD14, CD19, CD20, CD34, CD15, CD7, CD3, CD4, CD8, CD2, CD5, CD10, CD19, CD22, CD20, CD11b, CD11c, HLA-DR, CD56, CD61, CD62, CD41, CD42) have been reported in detail previously (Gattei *et al*, 1997; Venditti *et al*, 1998a). The FITC-conjugated anti-CD90/Thy-1 5E10 mAb, purchased from Pharmingen (Becton-Dickinson, San Jose, CA, USA), was employed in combination with PE-labelled anti-CD34 mAbs. Immunophenotypes were carried out on PB and/or BM samples, which were either directly analysed at time of collection or, following separation by centrifugation on a Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient, frozen as mononucleated living cells in liquid nitrogen until use. AML samples that contained >90% blast cells were selected for molecular studies (see below) and were further purified by immunomagnetic depletion of residual T and B cells, as described (Aldinucci *et al*, 2002). Irrelevant isotype-matched antibodies (Becton-Dickinson) were used to determine background fluorescence. Viable, antibody-labelled blast cells were identified according to their forward and side scatter or CD45 expression and side scatter, electronically gated and

analysed on a FACScalibur flow cytometer (Becton-Dickinson), by means of the CellQuest software (Becton-Dickinson) collecting at least 10 000 events. Expression data for CD90 in AMLs were reported as the percentage of CD34 cells co-expressing the CD90 antigen. In particular, in keeping with previous studies (Holden *et al*, 1995; Inaba *et al*, 1997, 1998; Kozii *et al*, 1997), a sample was classified $CD90^+$ when at least 20% of CD34 cells displayed a fluorescence intensity for CD90 >98–99% of the same cell population stained with an isotype-matched control immunoglobulin.

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA (1 μg) from selected AML samples (14 cases), and primary BM stromal cells utilized as CD90 positive control (Craig *et al*, 1993; Mayani & Lansdorp, 1994; Gattei *et al*, 1996), were extracted by the guanidium thiocyanate method and reverse transcribed in a 20-μl reaction mix as described. cDNA (2 μl) was amplified with 25 pmol of primers specific for CD90 (sense, 5′-AAC GGC CTG CCT AGT GGA C-3′, region 131–149; antisense, 5′-CCA GAG GTG TGC GGA GAG-3′, region 744–727), using the following PCR conditions: 4 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 59°C and 1·5 min at 72°C, and a final extension of 5 min at 72°C. All cDNAs were checked for first strand synthesis as reported (Degan *et al*, 2000). Ten millilitre of amplified product were run in ethidium bromide-stained 1·5% agarose gels.

Immunoprecipitation with anti-CD90 5E10 mAbs and Western blotting

Cell lysates (500 μg to 1 mg) from purified AML blasts, the SD-1 ($CD90^+$) and the KG-1A ($CD90^-$) cell lines were prepared and precleared exactly as described (Craig *et al*, 1993). Similarly, for immunoprecipitation, unlabelled anti-CD90 5E10 mAbs at the final concentration of 5 μg/ml were added to the cleared cell lysates and immunoprecipitated with protein A-Sepharose beads (Craig *et al*, 1993). After the beads were washed with buffers of increasing salt concentrations (Craig *et al*, 1993), the samples were boiled, subjected to 4–20% sodium dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS–PAGE) in non-denaturating conditions and the proteins revealed by Western blotting, utilizing the anti-CD90 5E10 mAb in conjunction with horseradish peroxidase conjugated anti-mouse IgG (Amersham Life Science, Amersham, UK) in a standard enhanced chemiluminescence system (ECL-Plus, Amersham, UK).

Cytogenetics

Procedures for cytogenetic evaluation have been described in detail previously (Stasi *et al*, 1993). Briefly, following a methotrexate cell synchronization and a direct preparation, chromosomes were examined after Giemsa staining. At least 20 metaphases were analysed in patients designated as having a normal karyotype. Karyotypes were interpreted using International System for Cytogenetic Nomenclature criteria (Mitelman, 1995). The detection of a minimum of two metaphases with an identical rearrangement or extra chromosome was regarded as evidence of the existence of an abnormal clone (Mitelman, 1995). For correlation studies between CD90 expression and karyotypes, patients were stratified according to Medical Research Council (MRC) classification into favourable, intermediate or adverse cytogenetic risk groups (Grimwade *et al*, 1998).

Statistical analysis

The association between the expression of CD90 and clinical data at diagnosis, i.e. age, sex, FAB subtype, white blood counts, percentage of BM blasts and cytogenetic class was studied by analysis of variance. For non-normal distributed values, differences were calculated by means of standard deviations of the *t*-test. The relationship between dichotomic variables was assessed by two-sided chi-square test (Armitage & Berry, 1987). The Kaplan–Meier method (Kaplan & Meier, 1958) was used for estimation of overall survival (OS). OS was calculated from the date of diagnosis to the date of death whatever the cause or last follow-up. For comparison of survival of two or more groups, the log-rank test was applied. To evaluate the simultaneous impact of different variables on duration of OS, a multivariate analysis was performed using the Cox regression model. Only variables for which univariate analysis had shown a significant association were considered in the multivariate analysis. Among them, expression of multidrug resistance 1 (MDR1)-associated glycoprotein p170 and of Bcl-2 and Bax oncoproteins, whose prognostic impact on AML has been already described (Del Poeta *et al*, 1996, 1999, 2003), were also considered. In our AML series, data on the expression of MDR1-associated glycoprotein p170 and of Bcl-2 and Bax oncoproteins, investigated as reported in detail elsewhere (Del Poeta *et al*, 1996, 1999, 2003), were available in 78 and 70 of 148 cases respectively. In all cases, a *P*-value of 0·05 or less was considered statistically significant.

Results

Table I summarizes the clinical features of AML patients analysed for CD90 expression by flow cytometry. Among 148 AML patients, all FAB subtypes were represented and cytogenetic profiles were available for 68 of 148 patients. CD90 was exclusively expressed by the $CD34^+$ cell fraction (Fig 1A and B). In particular, CD90 antigen was usually expressed on $CD34^{bright}$ cells (Fig 1A, panel A), although, in a minority of cases, the $CD34^{dim}$ blast fraction alone was predominantly stained with the anti-CD90 mAb 5E10 (Fig 1A, panels B and C). When lysates of blast cells from an AML case expressing CD90 antigens at a high cell density (see Fig 1A, panel A) were checked for immunoprecipitation with the

Table I. Clinical characteristics of patients.

	AML
Number of patients	148
Secondary AML*	48 (32)
Age, years, median (range)	64 (12–89)
Male/female	62/86
FAB subtypes	
M0	10 (8)
M1	35 (26)
M2	28 (21)
M3	9 (7)
M4	22 (17)
M5	24 (18)
M6	3 (2)
M7	2 (1)
Cytogenetic-risk groups†	
Good/favourable	12 (18)
Intermediate	43 (63)
Poor/adverse	13 (19)

AML, acute myeloid leukaemia; FAB, French–American–British classification.

Values are given as *n* (%).

*Defined according to WHO criteria (Vardiman *et al*, 2002), and including 15 cases formerly classified as refractory anaemia with excess of blasts in transformation.

†Patients were stratified according to the Medical Research Council classification of cytogenetic risk, into 'favourable' [cases with t(8;21), t(15;17), or inv(16), irrespective of the presence of additional cytogenetic abnormalities], 'adverse' [cases lacking these favourable risk aberrations in which complex cytogenetic changes (five or more unrelated abnormalities), −5, del(5q), −7, or 3q abnormalities] and 'intermediate' [cases with normal karyotype, 11q23 abnormalities, trisomy 8 (+8), or other chromosomal changes not encompassed by the favourable or adverse risk groups] risk groups.

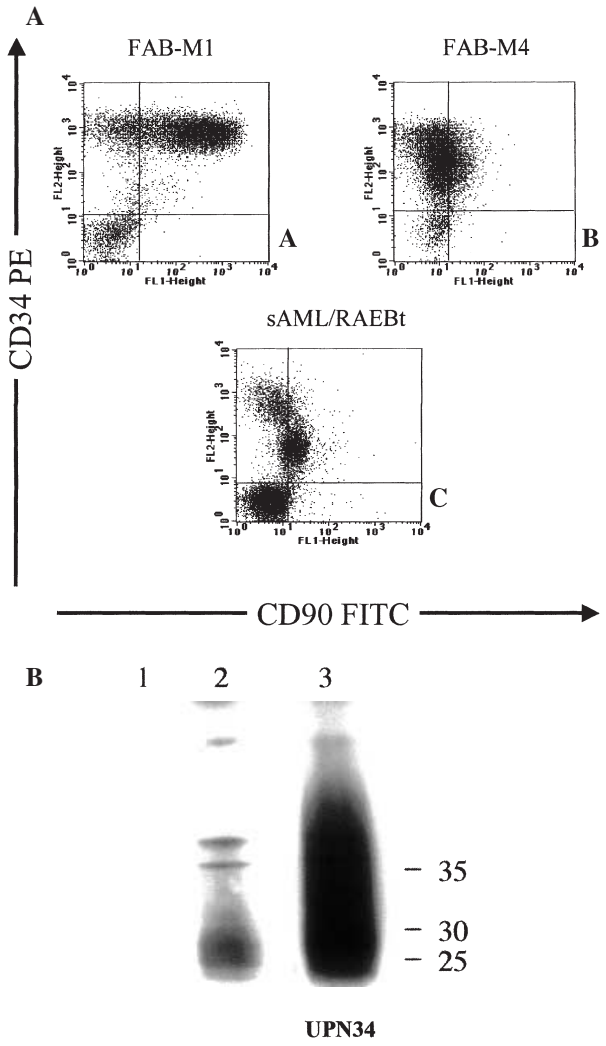

Fig 1. CD90 and CD34 expression in representative MDS and AML cases. (A) Cells from MDS and AML cases were double-stained with anti-CD90-FITC (*x*-axis, green fluorescence) and anti-CD34-PE (*y*-axis, red fluorescence) mAbs and analysed by flow cytometry, collecting at least 10 000 events. Dot-plots represent expression patterns obtained by analysing cells contained within an SSC^{low}/$FSC^{intermediate/high}$ electronic gate. FAB classification for each case is reported above the corresponding dot-plot. Each dot-plot shows a representative pattern for CD90/CD34 expression, as detailed in the text. (B) Immunoprecipitation and Western blotting: cell lysates (about 500 μg) from the KG-1A ($CD90^-$) cell line (lane 1), the SD-1 ($CD90^+$) cell line (lane 2) and purified AML blasts from the AML case reported in A (upper left panel) were immunoprecipitated with anti-CD90 5E10 mAbs and protein A-Sepharose beads. Samples were then subjected to 4–20% SDS–PAGE in non-denaturating conditions and proteins revealed by Western blotting, utilizing the same mAb in conjunction with horseradish peroxidase-conjugated anti-mouse IgG in a standard enhanced chemiluminescence system.

anti-CD90 mAb 5E10, a broad band with a major component at about 25 kDa, similar to that previously described (Craig *et al*, 1993) and stronger than that expressed by the $CD90^+$ cell line SD-1, was observed (Fig 1B).

By establishing a cut-off level at 20% of CD34 cells co-expressing CD90, the overall frequency of $CD90^+$ cases was 17% (25/148). A summary of the characteristics of $CD90^+$ AML cases is reported in Table II. In a more complete flow-cytometric profile, the 25 cases of $CD90^+$ AML expressed, along with CD34 (25/25), the other progenitor markers CD117 (21/25) and HLA-DR (22/25), as well as the myeloid antigens CD13 (24/25) and CD33 (22/25) in almost all cases. Other phenotypic markers were either mainly negative (CD7) or variably expressed (CD14, CD15) by $CD90^+$ AML blasts.

CD90 expression was also assessed at the mRNA level by RT-PCR analysis of purified AML blasts of 14 cases. As shown in Fig 2, the presence of an amplified product of the expected size (614 bp) was consistent with flow cytometric data. In particular, a specific band was detected in four AML cases that were $CD90^+$ by flow cytometry, and in an additional case (UPN33) in which the percentage of CD34 cells co-expressing CD90 was slightly below the borderline values (15%, not shown). Conversely, cDNAs from nine AML cases classified as $CD90^-$ by flow cytometry displayed either no CD90-specific transcripts (7/9 cases) or very faint amplification bands (2/9 cases, Fig 2).

Table II. Characteristics of CD90$^+$ MDS and AML patients.

UPN	Age (years)	WHO	FAB	BM blasts (%)	% CD34$^+$ cells*	% CD90$^+$ cells†	Karyotype	Cytogenetic risk‡
UPN32	74	*De novo* AML	M0	80	32	53	t(9;22)	Adverse
UPN34	67	*De novo* AML	M1	65	88	74	–	–
UPN35	77	*De novo* AML	M2	45	67	21	–	–
UPN36	61	*De novo* AML	M2	50	76	45	−5, −10, −15, −7, t(3;5)	Adverse
UPN37	62	*De novo* AML	M4	94	25	26	–	–
UPN38	78	*De novo* AML	M5	89	39	39	–	–
UPN18	70	sAML	RAEBt	25	65	26	–	–
UPN19	78	sAML	RAEBt	23	28	49	46 xy	Intermediate
UPN20	53	sAML	RAEBt	24	92	44	–	–
UPN21	47	sAML	RAEBt	27	83	38	–	–
UPN22	72	sAML	RAEBt	25	88	46	–	–
UPN23	65	sAML	RAEBt	28	37	27	–	–
UPN24	62	sAML	RAEBt	21	79	35	–	–
UPN25	82	sAML	RAEBt	21	55	77	–	–
UPN39	68	sAML	M0	87	50	50	−7	Adverse
UPN40	74	sAML	M1	81	45	45	del(2)(p23p24)	Intermediate
UPN41	74	sAML	M1	90	54	30	−7	Adverse
UPN45	53	sAML	M2	61	45	65	–	–
UPN46	82	sAML	M2	45	25	23	–	–
UPN47	67	sAML	M4	55	95	92	46 xy	Intermediate
UPN49	18	sAML	M4	43	37	37	–	–
UPN42	70	sAML§	M1	89	96	31	–	–
UPN43	69	sAML§	M1	92	93	35	t(9;22)	Adverse
UPN44	28	sAML§	M1	84	75	58	t(9;22)	Adverse
UPN50	62	sAML§	M7	79	75	40	–	–

UPN, unique patient number; WHO, World Health Organization; FAB, French–American–British classification; BM, bone marrow; MDS, myelodysplastic syndromes; AML, acute myeloid leukaemia; RA, refractory anaemia; RAEB, RA with excess blasts; RAEBt, RAEB in transformation; sAML, secondary AML.

*Figures refer to the percentage of CD34$^+$ cells within the blast gate, defined according to forward and side scatter or CD45 expression and side scatter.

†Figures refer to percentage of CD34$^+$ cells that co-expressed the CD90$^+$ antigen, as evaluated by flow cytometry using anti-CD90-FITC mAb in conjunction with anti-CD34-PE mAb.

‡For AML the cytogenetic risk was classified according to the MRC risk classification into favourable, intermediate and adverse risk, as in Table I.

§Secondary AML, evolved from a previous myeloproliferative disorder.

When comparing the expression of CD90 in *de novo* AML (100 patients) *versus* sAML [48 patients, defined according to WHO criteria (Vardiman *et al*, 2002)], a significantly higher frequency ($P < 0{\cdot}001$) of CD90$^+$ cases was found in the sAML group (40% vs. 6%; Table III). Furthermore, by dividing all AML cases according to a cut-off of 60 years of age, the frequency of CD90$^+$ cases was significantly higher ($P = 0{\cdot}010$) in the group of patients who were older than 60 years, when compared with patients aged <60 years (24% vs. 8%; Table III). By splitting the groups of secondary or *de novo* AML according to the age of patients, the highest frequency of CD90$^+$ cases was confirmed in the sAML group, without any impact of age (Fig 3), whereas, in the *de novo* AML group, CD90 was exclusively expressed in elderly patients ($P = 0{\cdot}011$, Fig 3). The higher frequency of CD90 expression in sAML when compared with *de novo* AML also holds true by considering the group of CD34$^+$ AML (91/148 cases) alone (44% vs. 12%; $P < 0{\cdot}001$, data not shown). Cytogenetics were informative in 68 of 148 patients, including nine of 25 CD90$^+$ cases (Tables I and II). Distribution of the prognostic classes according to MRC classification (Grimwade *et al*, 1998) showed a significant correlation ($P < 0{\cdot}001$) between CD90 expression and unfavourable karyotypes (Table III). In particular, six CD90$^+$ AML accounted for the 50% of AML cases with unfavourable cytogenetics t(9;22), chromosome 5/7 abnormalities or complex karyotype, while only three of 56 AMLs bearing all the other karyotypes were CD90$^+$ ($P < 0{\cdot}001$, not shown). Statistical significance for MRC cytogenetic classification was also maintained by separately analysing the group of 38 CD34$^+$ AML cases with informative cytogenetics ($P = 0{\cdot}024$, not shown).

Clinical data on treatment and survival were available for 125 of 148 AML patients, 84 of whom underwent intensive chemotherapy. As shown in Fig 4A, CD90 expression identified a cohort of patients that showed a significantly shorter survival in univariate analysis ($P = 0{\cdot}042$). Similarly,

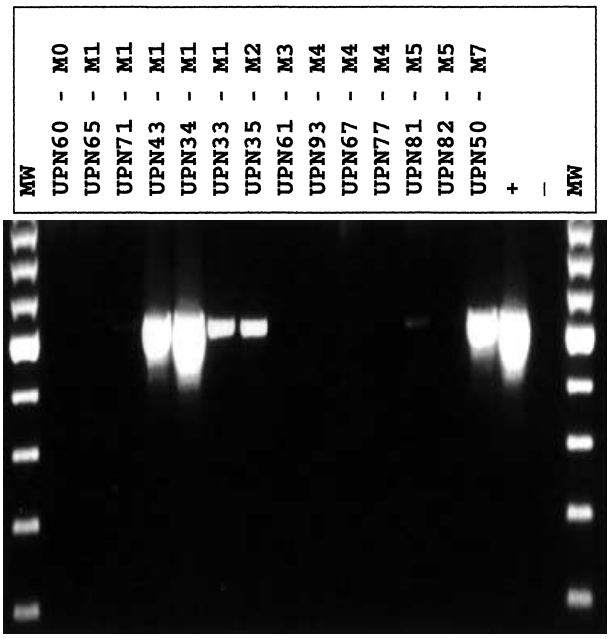

Fig 2. Expression of CD90 mRNA by AML blasts. Total RNA was isolated from purified AML blasts, reverse transcribed and amplified with primers pair specific for CD90. Amplified products were resolved on ethidium bromide-stained agarose gel and visualized by ultraviolet light. AML cases are identified by their unique patient number (UPN) and FAB assignment. The SD-1 and KG-1A cell lines were utilized as positive (+) and negative (−) controls respectively. The band displaying a stronger intensity in molecular weight marker (MW) lanes correspond to 600 bp.

Table III. Correlation between CD90 expression and clinical features in AML cases.

	$CD90^+$ cases/total (%)	*P*-values
De novo AML	6/100 (6)	<0·001
sAML	19/48 (40)	
>60 years	20/84 (24)	0·010
<60 years	5/64 (8)	
MRC classification,* *n* (%)		
Favourable	0/12 (0)	<0·001
Intermediate	3/43 (7)	
Adverse	6/13 (46)	

AML, acute myeloid leukaemia; sAML, secondary AML; MRC, Medical Research Council.

*Patients stratified according to the MRC classification of cytogenetic risk, into favourable, intermediate and adverse risk groups, as in Table I.

expression of other phenotypic markers, including CD34 and MDR1-associated glycoprotein p170 (Del Poeta *et al*, 1996, 1999) along with a Bax/Bcl-2 expression ratio below the cut-off value of 0·3 (Del Poeta *et al*, 2003), age above 60 years and the presence of unfavourable karyotypes according to the MRC classification, showed a bad prognostic impact in our AML series, when separately analysed (Fig 4B and Table IV).

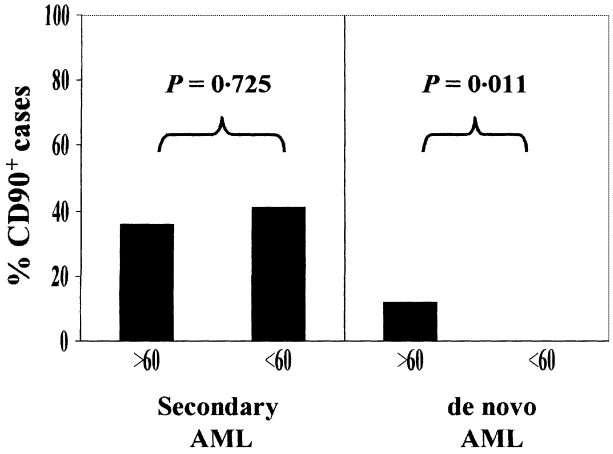

Fig 3. Differential expression of CD90 by age in secondary and *de novo* AML cases. Expression of CD90 was investigated by flow cytometry in 48 secondary and 100 *de novo* AML cases, splitting each group according to the age of patients (> or < of 60 years of age). By defining a cut-off value of 20% $CD34^+$ cells co-expressing CD90, the frequency of $CD90^+$ cases was higher in secondary when compared with *de novo* AMLs, without any impact of age ($P = 0·725$). Within the group of patients affected by *de novo* AMLs, all $CD90^+$ cases clustered in the group of patients older than 60 years of age ($P = 0·011$).

According to a multivariate analysis of the data, however, an independent predictive value on AML survival was confirmed only for the expression of MDR1-associated glycoprotein p170 and a Bax/Bcl-2 expression ratio of ≤0·3 (Table IV).

Discussion

Currently available data regarding the expression of CD90 in haemopoietic disorders, including AML or myelodysplastic syndromes (MDS), refers either to studies on relatively small heterogeneous series of patients (Holden *et al*, 1995; Inaba *et al*, 1997; Kozii *et al*, 1997) or, when carried out on larger cohorts of patients, usually does not compare CD90 expression in the various subsets of myeloid malignancies (Campos & Guyotat, 1996; Wuchter *et al*, 2001).

In this study, we systematically analysed, by flow cytometry, 148 patients affected by *de novo* (100 cases) or secondary AML (48 cases). In keeping with some previous studies (Holden *et al*, 1995; Inaba *et al*, 1997; Kozii *et al*, 1997), we found that CD90 was always expressed in the $CD34^+$ cell fraction. For this reason, CD90 expression data were reported as the percentage of $CD34^+$ cells. Most of the above reported studies also identified a sample as $CD90^+$ when as few as 5–10% of cells were stained over their respective controls (Holden *et al*, 1995; Campos & Guyotat, 1996; Kozii *et al*, 1997). Conversely, in other reports (Inaba *et al*, 1997, 1998), the standard cut-off of 20% of positive cells was maintained to distinguish between $CD90^+$ and $CD90^-$ cases. According to these latter studies, we choose to qualify a sample as $CD90^+$ when at least 20% of $CD34^+$ cells displayed fluorescence intensity for CD90 >98–99% of the same cell population stained with an

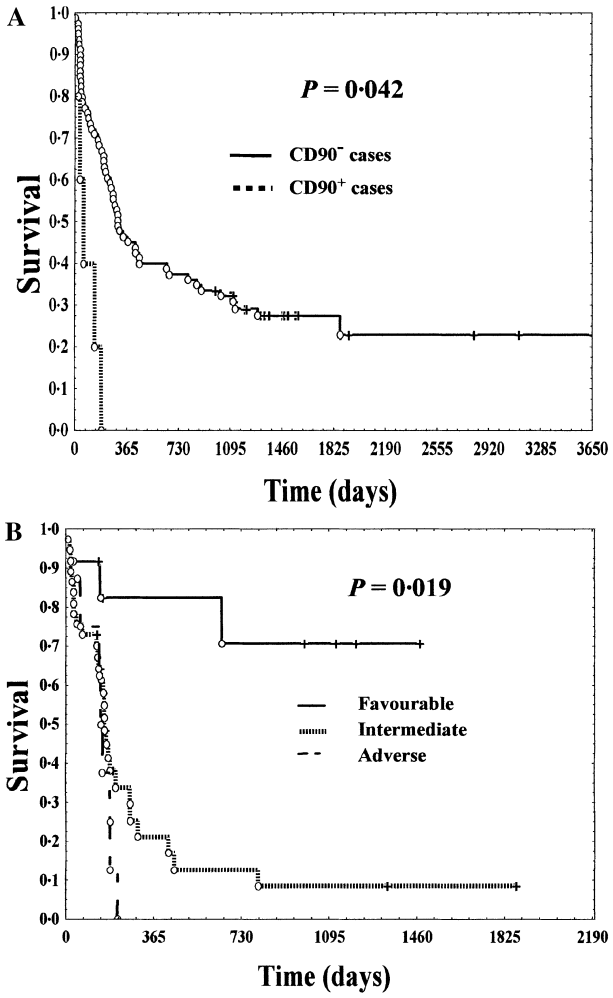

Fig 4. Impact of CD90 expression and karyotype on survival in AML. Survival data were analysed on 84 AML patients undergoing intensive chemotherapy. (A) In univariate analysis, CD90 expression identified a cohort of patients showing a significantly shorter survival ($P = 0{\cdot}042$). (B) Differences in survival by comparing the various subgroups with favourable, intermediate or adverse prognostic cytogenetic profiles according to the MRC classification of cytogenetic risk were statistically significant ($P < 0{\cdot}001$). Median follow-up was 48 months (range 4–128 months).

isotype-matched control immunoglobulin. Consistently, RT-PCR assays performed by amplifying cDNAs from purified AML blasts revealed high amounts of specific CD90 transcripts in those samples classified as $CD90^+$ by flow cytometry. The specific binding of the 5E10 mAb to CD90 was confirmed by Western blot analysis of cell lysates from an AML sample expressing CD90 antigens at a high intensity in flow cytometry.

By using the standard 20% cut-off value in our cohort of 148 AML patients, 25 cases (17%) were classified as $CD90^+$. This observation enabled our data to be compared with results from other studies (Holden *et al*, 1995; Inaba *et al*, 1997; Wuchter *et al*, 2001), in which the percentage of $CD90^+$ cases, when higher than in our series (Campos & Guyotat, 1996; Kozii *et al*, 1997), was usually due to a lower reference value, e.g. 5% of

Table IV. Univariate and multivariate analysis of variables affecting survival in 148 acute myeloid leukaemia patients.

	Univariate	Multivariate
p170	0·007	0·002
Bax/Bcl ratio	0·007	0·006
CD90	0·042	NS
CD34	0·030	NS
Age >60 years	0·007	NS
Karyotype (MRC)	<0·001	NS

MRC, Medical Research Council; NS, not significant.
The Kaplan–Meier method was used to estimate survival; for the comparison in two or more groups, the log-rank test was applied. For multivariate analysis proportional Cox regression was employed. For all these tests $P \leq 0{\cdot}05$ was considered significant.

positive cells, chosen as cut-off between $CD90^+$ and $CD90^-$ cases.

By analysing the characteristics of $CD90^+$ AML cases of our series, some observations emerged. First, we demonstrated that the percentage of $CD90^+$ cases was greater in sAML, i.e. AML evolved from a previous MDS or myeloproliferative disorder (Vardiman *et al*, 2002), than in *de novo* AMLs. Secondly, the frequency of $CD90^+$ cases was significantly higher in the group of patients older than 60 years of age. Thirdly, by splitting the groups of sAML and *de novo* AML according to age, we confirmed the higher frequency of $CD90^+$ cases in the sAML group, regardless of age, and demonstrated that, among *de novo* AMLs, all the $CD90^+$ cases clustered in the cohort of elderly patients. These findings seemed to be in contrast with Campos and Guyotat (1996) who, in a series 50 AMLs, did not find any correlation between CD90 expression and other clinical findings, including cytogenetics, age or preceding myelodysplasia. These divergent results could be explained by considering the study by Campos and Guyotat (1996) were carried out in a smaller group of patients and, again, used a different cut-off (5% of positive cells) to discriminate between $CD90^+$ and $CD90^-$ cases (Campos & Guyotat, 1996). However, analysis of our data according to the same criteria (Campos & Guyotat, 1996) substantially yielded the same statistically significant results.

The correlation between CD90 expression and cytogenetics is another finding of our study. In fact, among 68 AML patients with informative cytogenetics, CD90 expression strongly correlated with unfavourable karyotypes, classified according to the MRC classification (Grimwade *et al*, 1998). In particular, almost all $CD90^+$ AML cases fell within the group of AMLs carrying high-risk karyotypes, such as t(9;22), chromosome 5/7 abnormalities and complex karyotypes. Conversely, CD90 expression was never found in AML cases with favourable karyotypes (Grimwade *et al*, 1998). These results are in agreement with the presence of the bcr-abl translocation in the $CD90^+$ cell fraction of chronic myeloid leukaemia (Brendel *et al*, 1999), as well as with the notion that CD90 expression in pre-B acute lymphoblastic leukaemia was higher

in cases with karyotypic abnormalities, including t(9;22), than in those with normal karyotypes (Takahashi *et al*, 1998). Correlation between CD90 expression and unfavourable cytogenetics is in agreement with the significantly lower incidence of high or intermediate-risk karyotypes in *de novo* AMLs in patients under 55 years of age, as previously observed (Venditti *et al*, 1998b) Furthermore, CD90 expression identified a cohort of patients with a lower survival, these differences resembling those found by comparing the various subgroups with favourable, intermediate or unfavourable karyotypes (Bloomfield *et al*, 1998).

It is generally accepted that chemoresistance still represents a major problem in the management of AML (Del Poeta *et al*, 1996, 1999, 2003). It has been demonstrated that both a low ratio between pro-apoptotic (Bax) and anti-apoptotic proteins (Bcl-2) (Del Poeta *et al*, 2003), and the high level of expression of the p170 glycoprotein efflux pump system (Del Poeta *et al*, 1996, 1999) has a negative prognostic impact in AML. Interestingly, when considering those $CD90^+$ AMLs in which both MDR1 phenotype and Bax/Bcl-2 expression were analysed (7/25), all cases expressed either a low Bax/Bcl-2 ratio or high MDR1 levels.

Taken together, our data indicated that the expression of CD90 was significantly higher in those AML subtypes usually characterized by a poor prognosis, such as sAML, elderly AML, *de novo* AML with unfavourable cytogenetics or unfavourable drug-resistance profile. This corroborates the notion indicating these specific AML subsets as almost overlapping entities sharing several clinical and biological features, including a worse prognosis (Hann *et al*, 1997; Leith *et al*, 1997; Rosenfeld & Kantarjian, 2001), a phenotype of blast cells more often expressing CD34 (Masuya *et al*, 1993; Rosenfeld & Kantarjian, 2001) and, as shown here, CD90, as well as a pathogenesis characterized by progressive and multiple genetic damage, eventually leading to a complete dysregulation of cell growth and differentiation (Heaney & Golde, 1999). The close similarities of these diseases with MDS prompted us to investigate the expression of CD90 by the CD34-expressing cell fraction of MDS. Preliminary studies on a series of 28 MDS patients (seven with refractory anaemia; 21 with refractory anaemia with excessive blasts) revealed a higher frequency of $CD90^+$ cases when compared with the overall AML group (61% vs. 17%, $P < 0{\cdot}001$), but not to the sAML subset (61% vs. 40%, $P = 0{\cdot}75$; F. Buccisano and V. Gattei, unpublished observations).

In this context, our findings corroborate the hypothesis that these diseases may arise as the result of transformation of an earlier myeloid progenitor with a $CD34^+/CD90^+$ phenotype (Mayani & Lansdorp, 1994; Haase *et al*, 1997; Saitoh *et al*, 1998; Nilsson *et al*, 2000, 2002). In this regard, the observation that the same correlations found by analysing the whole AML series also holds true by considering the homogeneous group of $CD34^+$ AML alone, gives further evidence of a role of CD90 in distinguishing patients with particular 'stem cell features'. The different patterns of CD34/CD90 expression, identified in this study by flow cytometry, could imply the involvement of different subsets of progenitors expressing CD34 and/or CD90 at various intensities. Whether the $CD90^+/CD34^{bright}$ or $CD90^+/CD34^{dim}$ blast cell subsets actually represent the phenotypic counterparts of progenitors with distinctive proliferative or differentiation potential needs to be investigated by specific *in vitro* assays on sorted fractions (Pinto *et al*, 1992; Blair *et al*, 1997; Haase *et al*, 1997; Saitoh *et al*, 1998; Nilsson *et al*, 2000, 2002).

Acknowledgments

The authors wish to thank Prof. A. Colombatti (Experimental Oncology 2, CRO, Aviano) for helpful discussion and Dr A. Pinto (Istituto Nazionale dei Tumori 'Fondazione Pascale', Naples, Italy) for his encouragement and support during the preliminary phases of this study.

References

Aldinucci, D., Poletto, D., Nanni, P., Degan, M., Rupolo, M., Pinto, A. & Gattei, V. (2002) CD40L induces proliferation, self-renewal, rescue from apoptosis, and production of cytokines by CD40-expressing AML blasts. *Experimental Hematology*, **30,** 1283–1292.

Armitage, P. & Berry, G. (1987) *Statistical Methods in Medical Research*. Blackwell Scientific, London, UK.

Baum, C.M., Weissman, I.L., Tsukamoto, A.S., Buckle, A.M. & Peault, B. (1992) Isolation of a candidate human hematopoietic stem-cell population. *Proceedings of the National Academy of Sciences of the United States of America*, **89,** 2804–2808.

Bennet, J.M., Catovsky, D., Daniel, M.T., Flandrin, G., Galton, D.A., Gralnick, H.R. & Sultan, C. (1982) The French–American–British (FAB) co-operative group. Proposal for the classification of the myelodysplastic syndromes. *British Journal of Haematology*, **51,** 189–199.

Blair, A., Hogge, D.E., Ailles, L.E., Lansdorp, P.M. & Sutherland, H.J. (1997) Lack of expression of Thy-1 (CD90) on acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo. *Blood*, **89,** 3104–3111.

Bloomfield, C.D., Lawrence, D., Byrd, J.C., Carroll, A., Pettenati, M.J., Tantravahi, R., Patil, S.R., Davey, F.R., Berg, D.T., Schiffer, C.A., Arthur, D.C. & Mayer, R.J. (1998) Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. *Cancer Research*, **58,** 4173–4179.

Brendel, C., Mohr, B., Schimmelpfennig, C., Muller, J., Bornhauser, M., Schmidt, M., Ritter, M., Ehninger, G. & Neubauer, A. (1999) Detection of cytogenetic aberrations both in CD90 (Thy-1)-positive and (Thy-1)-negative stem cell (CD34) subfractions of patients with acute and chronic myeloid leukemias. *Leukemia*, **13,** 1770–1775.

Campos, L. & Guyotat, D. (1996) Expression of Thy-1 antigen (CDw90) on adult acute leukemia blast cells (letter). *Blood*, **87,** 413–414.

Craig, W., Kay, R., Cutler, R.L. & Lansdorp, P.M. (1993) Expression of Thy-1 on human hematopoietic progenitor cells. *Journal of Experimental Medicine*, **177,** 1331–1342.

Degan, M., Mazzocco, F.T., Di Francia, R., Rossi, F.M., Pinto, A. & Gattei, V. (2000) Normalizing complementary DNA by quantitative

reverse transcriptase-polymerase chain reaction of beta2-microglobulin: molecular monitoring of minimal residual disease in acute promyelocytic leukemia. *Diagnostic and Molecular Pathology*, **9,** 98–109.

Del Poeta, G., Stasi, R., Aronica, G., Venditti, A., Cox, M.C., Bruno, A., Buccisano, F., Masi, M., Tribalto, M., Amadori, S. & Papa, G. (1996) Clinical relevance of P-glycoprotein expression in de novo acute myeloid leukemia. *Blood*, **87,** 1997–2004.

Del Poeta, G., Venditti, A., Stasi, R., Aronica, G., Cox, M.C., Buccisano, F., Tamburini, A., Bruno, A., Maurillo, L., Battaglia, A., Suppo, G., Epiceno, A.M., Del Moro, B., Masi, M., Amadori, S. & Papa, G. (1999) P-glycoprotein and terminal transferase expression identify prognostic subsets within cytogenetic risk classes in acute myeloid leukemia. *Leukemia Research*, **23,** 451–465.

Del Poeta, G., Venditti, A., Del Principe, M.I., Maurillo, L., Buccisano, F., Tamburini, A., Cox, M.C., Franchi, A., Bruno, A., Mazzone, C., Panetta, P., Suppo, G., Masi, M. & Amadori, S. (2003) Amount of spontaneous apoptosis detected by Bax/Bcl-2 ratio predicts outcome in acute myeloid leukemia (AML). *Blood*, **101,** 2125–2131.

DePinho, R.A. (2000) The age of cancer. *Nature*, **408,** 248–254.

Fialkow, P.J., Raskind, W.R., Singer, J.W., Dow, L.W., Najfeld, V. & Veith, R. (1989) Clonal development of the acute leukemias. *Bone Marrow Transplantation*, **4**(Suppl. 1)**,** 76–78.

Fialkow, P.J., Janssen, J.W. & Bartram, C.R. (1991) Clonal remissions in acute nonlymphocytic leukemia: evidence for a multistep pathogenesis of the malignancy. *Blood*, **77,** 1415–1417.

Gattei, V., Aldinucci, D., Quinn, J.M., Degan, M., Cozzi, M., Perin, V., Iuliis, A.D., Juzbasic, S., Improta, S., Athanasou, N.A., Ashman, L.K. & Pinto, A. (1996) Human osteoclasts and preosteoclast cells (FLG 29.1) express functional c-kit receptors and interact with osteoblast and stromal cells via membrane-bound stem cell factor. *Cell Growth and Differentiation*, **7,** 753–763.

Gattei, V., Degan, M., Gloghini, A., De Iuliis, A., Improta, S., Rossi, F.M., Aldinucci, D., Perin, V., Serraino, D., Babare, R., Zagonel, V., Gruss, H.J., Carbone, A. & Pinto, A. (1997) CD30 ligand is frequently expressed in human hematopoietic malignancies of myeloid and lymphoid origin. *Blood*, **89,** 2048–2059.

Grimwade, D., Walker, H., Oliver, F., Wheatley, K., Harrison, C., Harrison, G., Rees, J., Hann, I., Stevens, R., Burnett, A. & Goldstone, A. (1998) The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood*, **92,** 2322–2333.

Haase, D., Feuring-Buske, M., Schafer, C., Schafer, C., Schoch, C., Troff, C., Gahn, B., Hiddemann, W. & Wormann, B. (1997) Cytogenetic analysis of CD34+ subpopulations in AML and MDS characterized by the expression of CD38 and CD117. *Leukemia*, **5,** 674–679.

Hann, I.M., Stevens, R.F., Goldstone, A.H., Rees, J.K., Wheatley, K., Gray, R.G. & Burnett, A.K. (1997) Randomized comparison of DAT versus ADE as induction chemotherapy in children and younger adults with acute myeloid leukemia. Results of the Medical Research Council's 10th AML trial (MRC AML10). Adult and Childhood Leukaemia Working Parties of the Medical Research Council. *Blood*, **89,** 2311–2318.

Head, D.R. (1996) Revised classification of acute myeloid leukemia. *Leukemia*, **10,** 1826–1831.

Heaney, M.L. & Golde, D.W. (1999) Myelodysplasia. *New England Journal of Medicine*, **340,** 1649–1660.

Holden, J.T., Geller, R.B., Farhi, D.C., Holland, H.K., Stempora, L.L., Phillips, C.N. & Bray, R.A. (1995) Characterization of Thy-1 (CDw90) expression in CD34+ acute leukemia. *Blood*, **86,** 60–65.

Humeau, L., Bardin, F., Maroc, C., Alario, T., Galindo, R., Mannoni, P. & Chabannon, C. (1996) Phenotypic, molecular, and functional characterization of human peripheral blood CD34+/THY1+ cells. *Blood*, **87,** 949–955.

Inaba, T., Shimazaki, C., Sumikuma, T., Takahashi, R., Hirai, H., Kikuta, T., Sudo, Y., Yamagata, N., Ashihara, E., Goto, H., Fujita, N., Yoshimura, M. & Nakagawa, M. (1997) Flow cytometric analysis of Thy-1 expression in CD34-positive acute leukemia. *International Journal of Hematology*, **66,** 315–323.

Inaba, T., Shimazaki, C., Sumikuma, T., Shimura, K., Takahashi, R., Hirai, H., Ashihara, E., Sudo, Y., Murakami, S., Haruyama, H., Fujita, N., Yoshimura, M. & Nakagawa, M. (1998) Flow cytometric analysis of Thy-1 expression in myelodysplastic syndrome. *Internal Journal of Hematology*, **68,** 403–410.

Johansson, B., Mertens, F. & Mitelman, F. (1996) Primary vs. secondary neoplasia-associated chromosomal abnormalities-balanced rearrangements vs. genomic imbalances? *Genes, Chromosomes and Cancer*, **16,** 155–163.

Kaplan, E.L. & Meier, P. (1958) Non parametric estimations from incomplete observations. *Journal of American Statistical Association*, **53,** 457–461.

Kozii, R., Wilson, J., Persichetti, J., Phelps, V., Ball, S.E. & Ball, E.D. (1997) Thy-1 expression on blast cells from adult patients with acute myeloid leukemia. *Leukemia Research*, **21,** 381–385.

Lansdorp, P.M., Sutherland, H.J. & Eaves, C.J. (1990) Selective expression of CD45 isoforms on functional subpopulations of CD34+ haemopoietic cells from human bone marrow. *Journal of Experimental Medicine*, **172,** 363–366.

Leith, C.P., Kopecky, K.J., Godwin, J., McConnell, T., Slovak, M.L., Chen, I.M., Head, D.R., Appelbaum, F.R. & Willman, C.L. (1997) Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. *Blood*, **89,** 3323–3329.

Low, M.G. & Kincade, P.W. (1985) Phosphatydilinositol is the membrane anchoring domain of the Thy-1 glycoprotein. *Nature*, **318,** 62–64.

Mandelli, F., Diverio, D., Avvisati, G., Luciano, A., Barbui, T., Bernasconi, C., Broccia, G., Cerri, R., Falda, M., Fioritoni, G., Leoni, F., Liso, V., Petti, M.C., Rodeghiero, F., Saglio, G., Vegna, M.L., Visani, G., Jehn, U., Willemze, R., Muus, P., Pelicci, P.G., Biondi, A. & Lo Coco, F. (1997) Molecular remission in PML/RAR alpha-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. *Blood*, **90,** 1014–1021.

Masuya, M., Kita, K., Shimizu, N., Ohishi, K., Katayama, N., Sekine, T., Otsuji, A., Miwa, H. & Shirakawa, S. (1993) Biologic characteristics of acute leukemia after myelodysplastic syndrome. *Blood*, **81,** 3388–3394.

Mauritzson, N., Johansson, B., Albin, M., Billstrom, R., Ahlgren, T., Mikoczy, Z., Nilsson, P.G., Hagmar, L. & Mitelman, F. (1999) A single-center population-based consecutive series of 1500 cytogenetically investigated adult hematological malignancies: karyotypic features in relation to morphology, age and gender. *European Journal of Haematology*, **62,** 95–102.

Mayani, H. & Lansdorp, P.M. (1994) Thy-1 expression is linked to functional properties of primitive hematopoietic progenitor cells from human umbilical cord blood. *Blood*, **99,** 1909–1912.

Mitelman, F. (ed.) (1995) *ISCN: An International System for Human Cytogenetic Nomenclature*. Karger, Basel, Switzerland.

Moorman, A.V., Roman, E., Willett, E.V., Dovey, G.J., Cartwright, R.A. & Morgan, G.J. (2001) Karyotype and age in acute myeloid leukemia. Are they linked? *Cancer Genetics and Cytogenetics*, **126,** 155–161.

Mrozek, K., Heinonen, K. & Bloomfield, C.D. (2000) Prognostic value of cytogenetic findings in adults with acute myeloid leukemia. *Internal Journal of Hematology*, **72,** 261–271.

Nilsson, L., Astrand-Grundstrom, I., Arvidsson, I., Jacobsson, B., Hellström-Lindberg, E., Hast, R. & Jacobsen, S.E.W. (2000) Isolation an characterization of hematopoietic progenitor/stem cells in 5q-deleted myelodysplastic syndromes. Evidence for involvement at the hematopoietic stem cell level. *Blood*, **96,** 2012–2021.

Nilsson, L., Åstrand-Grundström, I., Anderson, K., Arvidsson, I., Hokland, P., Bryder, D., Kjeldsen, L., Johansson, B., Hellström-Lindberg, E., Hast, R. & Jacobsen, S.E.W. (2002) Involvement and functional impairment of the $CD34^{+}$ $CD38^{-}$ Thy-1^{+} hematopoietic stem cell pool in myelodysplastic syndromes with trisomy 8. *Blood*, **100,** 259–267.

Pedersen-Bjergaard, J., Andersen, M.K., Christiansen, D.H. & Nerlov, C. (2002) Genetic pathways in therapy-related myelodysplasia and acute myeloid leukemia. *Blood*, **99,** 1909–1912.

Pinto, A., Aldinucci, D., Gattei, V., Zagonel, V., Tortora, G., Budillon, A. & Cho-Chung, Y.S. (1992) Inhibition of the self-renewal capacity of blast progenitors from acute myeloblastic leukemia patients by site-selective 8-chloroadenosine 3′,5′-cyclic monophosphate. *Proceedings of the National Academy of Sciences of the United States of America*, **89,** 8884–8888.

Rosenfeld, C. & Kantarjian, H. (2001) Is myelodysplastic related acute myelogenous leukemia a distinct entity from de novo acute myelogenous leukemia? Potential for targeted therapies. *Leukemia & Lymphoma*, **41,** 493–500.

Saitoh, K., Miura, I., Takahashi, N. & Miura, A.B. (1998) Fluorescence in situ hybridisation of progenitor cells obtained by fluorescence activated cell sorting for the detection of cells affected by chromosome abnormality trisomy 8 in patients with myelodysplastic syndromes. *Blood*, **92,** 2886–2892.

Schoch, C., Haferlach, T., Haase, D., Fonatsch, C., Loffler, H., Schlegelberger, B., Staib, P., Sauerland, M.C., Heinecke, A., Buchner, T., Hiddemann, W. & German AML Cooperative Study Group (2001) Patients with de novo acute myeloid leukaemia and complex karyotype aberrations show a poor prognosis despite intensive treatment: a study of 90 patients. *British Journal of Haematology*, **112,** 118–126.

Stasi, R., Del Poeta, G., Masi, M., Tribalto, M., Venditti, A., Papa, G., Nicoletti, B., Vernole, P., Tedeschi, B., Delaroche, I., Mingarelli, R. & Dallapiccola, B. (1993) Incidence of chromosome abnormalities and clinical significance of karyotype in de novo acute myeloid leukaemia. *Cancer Genetics and Cytogenetics*, **67,** 28–34.

Sumikuma, T., Shimazaki, C., Inaba, T., Ochiai, N., Okano, A., Hatsuse, M., Ashihara, E. & Nakagawa, M. (2002) CD34+/CD90+ cells infused best predict late haematopoietic reconstitution following autologous peripheral blood stem cell transplantation. *British Journal of Haematology*, **117,** 238–244.

Takahashi, T., Mizutani, M., Miwa, H., Katayama, N., Nishii, K., Shikami, M., Yamaguchi, M., Shiku, H., Kamada, N. & Kita, K. (1998) Frequent expression of human Thy-1 antigen on pre-B cell acute lymphoblastic leukemia with t(9;22). *International Journal of Hematology*, **67,** 369–378.

Vardiman, J.W., Harris, N.L. & Brunning, R.D. (2002) The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*, **100,** 2292–2302.

Venditti, A., Del Poeta, G., Buccisano, F., Tamburini, A., Cox-Froncillo, M.C., Aronica, G., Bruno, A., Del Moro, B., Epiceno, A.M., Battaglia, A., Forte, L., Postorino, M., Cordero, V., Santinelli, S. & Amadori, S. (1998a) Prognostic relevance of the expression of Tdt and CD7 in 335 cases of acute myeloid leukemia. *Leukemia*, **12,** 1056–1063.

Venditti, A., Del Poeta, G., Buccisano, F., Tamburini, A., Cox-Froncillo, M.C., Bruno, A., Del Moro, B., Epiceno, A.M., Masi, M., Amadori, S. & Stasi, R. (1998b) Biological features of acute myeloid leukemia in the elderly (letter). *Blood*, **92,** 697–699.

Wuchter, C., Ratei, R., Spahn, G., Schoch, C., Harbott, J., Schnittger, S., Haferlach, T., Creutzig, U., Sperling, C., Karawajew, L. & Ludwig, W.D. (2001) Impact of CD133 (AC133) and CD90 expression analysis for acute leukemia immunophenotyping. *Haematologica*, **86,** 154–161.

Zittoun, R., Suciu, S., De Witte, L., Resegotti, L., Liso, V., Willemze, R., Belhabri, A., Amadori, S., Giustolisi, R., Labor, B., Fioritoni, G., Rossi Ferrini, P., Leone, G., Jehn, U., Keating, S., Fazi, P., Dardenne, M., Solbu, G. & Mandelli, F. (1999) Comparison of three intercalating agents in induction and consolidation in acute myelogenous leukemia (AML) followed by autologous or allogeneic transplantation: preliminary results of the EORTC-GIMEMA AML-10 randomized trial (abstract). *Blood*, **94**(Suppl. 1)**,** 658.