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Immunophenotypic analysis in 119 patients with acute myeloid leukemia following a previous malignancy: a comparison with the immunophenotype of 231 *de novo* cases

Data regarding the immunophenotypic pattern of 119 cases of acute myeloid leukemia (AML) following a previous malignancy were matched with those of 231 patients with de novo AML in order to identify differences between the 2 groups. We documented the presence of immunophenotypic markers (CD4, CD16, HLA-DR, CD33, CD117) preferentially expressed in de novo AML with respect to AML following a previous malignancy. On the other hand, we demonstrated that there are no differences in antigenic profile between AML following a previous malignancy treated with surgery alone and AML following a previous malignancy treated with chemo- and/or radiotherapy.

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The prognostic relevance of immunophenotype in acute myeloid leukemia (AML) is still controversial[1-10] and, to date, no studies have been performed in patients with secondary AML.

In the present study, we analyzed the immunophenotypic pattern of AML following a previous malignancy in order to investigate: the possible prognostic role of immunophenotype in AML following a previous malignancy, to identify immunophenotypic differences between de novo AML and AML following a previous malignancy and to compare the immunophenotype of patients with AML following a previous malignancy treated with chemo- and/or radiotherapy versus the immunophenotype of AML following a previous malignancy treated with surgery alone. The study population comprised 350 AML patients observed in 5 Divisions of Hematology from July 1992 to June 2000: 119 of the cases of AML followed a previous malignancy whereas 231 of the patients had de novo AML. For each patient clinical and biological characteristics were analyzed: age, sex, WBC count at diagnosis, FAB category, platelet count, hemoglobin level, karyotype, induction treatment, achievement rate and duration of complete remission (CR), and overall survival. Moreover, in the 119 cases of AML following a previous malignancy patients further data were collected: type and date of onset of the previous malignancy, treatment (chemotherapy, radiotherapy, surgery) and outcome of the previous malignancy, and latency between the previous malignancy and AML. Patients with a previous myelodysplastic syndrome not secondary to previous malignancy were excluded from this study. Cytogenetic risk groups were defined as reported elsewhere.[11]

The immunophenotypic pattern of AML following a previous malignancy was compared with that of de novo cases of AML according to age and FAB category (1:2 ratio). The following monoclonal antibodies were used as the first-line panel: CD2, CD3, CD4, CD5, CD7, CD9, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD33, CD34, CD38, CD41, CD45, CD56, CD61, CD117, HLA-DR, MPO. In addition, cells were labeled with antibodies directed against My8. Clinical and biological features of the 119 cases of AML following a previous malignancy are summarized in Table 1. The median latency between the two malignancies was 48 months (range 8-480). All patients were treated for AML, according to the different trials currently in use in the Institutions participating in the study. CR was achieved in 57 patients (48%), 16 patients were resistant (13%), while 38 patients (32%) died during induction chemotherapy. Eight patients (7%) achieved a partial remission (PR).

The expression of informative antigens in the two groups of 350 assessable adult AML patients is presented in Table 2. Patterns of antigen expression in de novo AML and AML following a previous malignancy differed significantly: in particular, CD4

Table 1. Clinical and biological features of patients with sAML.

Patients, no.	119
Age, mean (range)	58 (15-89)
Sex (M/F)	46/73
Primary malignancy:	
Breast	33
Hodgkin's disease	15
Lymphomas	15
Bowel	9
Lung	5
Kidney	5
Gut	4
Uterus	4
Ovary	4
Pharynx-larynx	4
Myelofibrosis	4
Bladder	3
Central nervous system	2
Multiple myeloma	2
Myeloproliferative chronic disease	2
Melanoma	2
Prostate	2
Skin	1
Thyroid	1
Vagina	1
Esophagus	1
Treatment of primary malignancy:	
Surgery	37
Chemotherapy	39
Radiotherapy	15
Combined chemotherapy and radiotherapy	28
FAB:	
M_0	9
M_1	20
M_2	27
M_3	15
M_4	21
M_5	21
M_6	3
M_7	3
Karyotype (on 67 patients):	
Good prognosis	5
Intermediate prognosis	45
Unfavorable prognosis	17
Response to chemotherapy	
Complete remission	57
No response	16
Death in induction	38
Partial remission	8

Table 2. Immunophenotypic pattern in *de novo* AML versus AML following a previous malignancy (PM-AML).

	De novo	*PM-AML*	*p-value*		*De novo*	*PM-AML*	*p-value*
CD2+	28/152 (18%)	6/54 (11%)	0.30	CD20+	2/52 (4%)	2/24 (8%)	0.79
CD3+	8/78 (10%)	7/51 (14%)	0.74	CD22+	1/123 (1%)	2/15 (13%)	0.02
CD4+	11/37 (30%)	2/50 (4%)	0.0025	CD33+	203/213 (95%)	91/106 (86%)	0.006
CD5+	4/33 (12%)	7/35 (20%)	0.58	CD34+	125/215 (56%)	47/96 (49%)	0.16
CD7+	46/201 (23%)	20/75 (27%)	0.62	CD38+	0/0	18/19 (95%)	
CD9+	28/51 (55%)	3/6 (50%)	1	CD41+	3/23 (13%)	1/11 (9%)	1
CD10+	16/142 (11%)	8/72 (11%)	1	CD45+	57/60 (95%)	20/20 (100%)	0.73
CD11b+	11/33 (34%)	14/32 (44%)	0.54	CD56+	23/86 (27%)	8/24 (33%)	0.7
CD11c+	8/12 (647%)	8/23 (35%)	0.15	CD61+	2/50 (4%)	4/20 (20%)	0.09
CD13+	192/218 (88%)	91/112 (84%)	0.13	CD117+	95/125 (76%)	6/13 (46%)	0.04
CD14+	51/179 (28%)	32/79 (40%)	0.07	HLA-DR+	166/202 (82%)	67/98 (68%)	0.01
CD15+	65/123 (53%)	18/35 (51.4%)	1	MPO+	62/82 (75.6%)	19/27 (70.4%)	0.77
CD16+	8/13 (61%)	3/19 (16%)	0.02	MY8+	2/8 (25%)	4/4 (100%)	0.06
CD19+	17/195 (9%)	6/80 (7%)	0.92				

was expressed in 30% of de novo cases of AML compared to in 4% of cases of AML following a previous malignancy (p<0.002) and CD16 could be detected in 61% of the de novo AML compared to in 16% of the AML following a previous malignancy (p<0.02). Similarly, CD33 (95% vs 86%, p<0.006), HLA-DR (82% vs 68%, p<0.01) and CD117 (76% vs 46%, p<0.04) were preferentially expressed by de novo cases compared with AML following a previous malignancy. Conversely, a higher percentage of CD22-expressing cases was found among cases of AML following a previous malignancy compared to de novo AML cases (13% vs 1%, p<0.02). It must be noted that the differences in CD4, CD16 and CD22 expression patterns were based on analysis of a relatively limited number of cases. Of interest, the comparison of antigen expression between patients treated for their previous malignancy by surgery and those treated by chemo and/or radiotherapy showed a significant difference only for CD38 expression (100% vs 34%, p<0.04). No differences were found in the antigenic pattern when we compared patients treated for previous malignancy with alkylating agents with patients who previously received topoisomerase II inhibitors.

Fifty-seven (48%) out of the 119 patients with AML following a previous malignancy achieved CR. None of the antigens

Table 3. Multivariate analysis of parameters influencing outcome in 119 cases of AML following a previous malignancy.

Parameter	*Overall survival (months) p value*	*Disease-free survival (months) p value*
Sex (M Vs F)	0.47	0.86
Age	0.78	0.74
Cytogenetics (favorable Vs unfavorable)	0.0059	0.51
PM (breast Vs other)	0.92	0.60
Therapy (surgery Vs other)	0.38	0.98
FAB (monocytic Vs other)	0.89	0.84
CD34 (positive Vs negative)	0.62	0.86

investigated was found to have prognostic relevance. Similarly, no antigen expression pattern was significantly associated with disease free survival (DFS) or overall survival (OS). However, the DFS and OS were significantly poorer for patients assigned to the unfavorable cytogenetic group category (p<0.05). At multivariate analysis, none of the antigens was significantly associated with the achievement of CR. Furthermore a Cox multivariate analysis that included sex, age, type of previous malignancy, FAB category, type of treatment for previous malignancy (surgery vs chemo- and/or radiotherapy), CD34 expression, and cytogenetic risk category demonstrated that none of them influenced the DFS, while OS was significantly correlated with favorable cytogenetics (Table 3). In our study we observed different antigenic expression in AML following a previous malignancy compared to in cases of de novo AML. Patients with AML following a previous malignancy showed a lower expression of CD117, HLA-DR, CD33 and CD38. In particular, the potential prognostic significance of CD38 expression in AML following chemo/radiotherapy might be of interest and needs to be addressed in a larger cohort of patients.

In our study the immunophenotypic pattern was not significantly correlated with CR rate, DFS or OS; no prognostic roles could be identified for any of the antigens tested. This observation was also strengthened by the multivariate analysis which indicated that cytogenetic risk group was the only significant prognostic factor.

The comparison between the immunophenotypic pattern of AML following a previous malignancy treated with chemo and/or radiotherapy with AML following a previous malignancy treated with surgery alone, usually considered as de novo AML, did not reveal significant immunophenotypic differences. Similarly, no differences in the immunophenotypic pattern were found comparing patients in whom the previous treatment for the first malignancy had been based on alkylating agents with those who had received topoisomerase II inhibitors.

In conclusion, the results of this study indicate that a common immunologic profile between AML following a previous malignancy treated with chemo and/or radiotherapy, and those developing in patients treated only with surgery can be observed. The homogeneous expression of antigens studied in both these AML subgroups seems to confirm the hypothesis that AML arising after another malignancy, independently of the type of treatment, should be considered as secondary AML. Further prospective studies will allow this hypothesis to be proven conclusively.

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Key words: secondary acute myeloid leukemia; immunophenotype.

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Appendix
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Granulocyte colony-stimulating factor reverses cytopenia and may permit cytogenetic responses in patients with chronic myeloid leukemia treated with imatinib mesylate

Imatinib mesylate induces major or complete cytogenetic responses in the majority of patients with chronic myeloid leukemia (CML) in chronic phase. However, 15-40% of patients develop neutropenia and/or thrombocytopenia that makes it necessary to reduce the dosage or to interrupt treatment. Patients with recurrent cytopenias may be less likely to obtain cytogenetic responses. We speculated that low doses of granulocyte colony-stimulating factor (G-CSF) in conjunction with imatinib might offer clinical benefit. Eleven patients with CML in chronic (n=9) or accelerated (n=2) phase who could not tolerate 300 mg/day and had no cytogenetic response after 6 months of imatinib treatment received G-CSF in combination with imatinib. Ten of the 11 patients could then tolerate doses of imatinib equal to or greater than 300 mg/day and 7 patients achieved major (n=6) or complete (n=1) cytogenetic responses. We conclude that G-CSF reverses the hematologic toxicity of imatinib and may thereby increase the proportion of cytogenetic responses.

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Imatinib mesylate (Glivec®) has remarkable activity in the chronic (CP) and accelerated phases (AP) of CML. Kantarjian et al.[1] reported that 41% of patients in CP who had failed to benefit from interferon-α achieved complete cytogenetic remissions after treatment with imatinib. Despite these promising results, 40-60% patients fail to achieve major cytogenetic responses and some CP patients progress to advanced phases of CML while on imatinib. A major problem during imatinib therapy is the development of cytopenias; thus 15%-40% of CP patients and a higher proportion of AP patients[2] develop grade III-IV cytopenias that require dosage reduction to below the accepted therapeutic levels[3,4] or indeed interruption of treatment. The development of cytopenia has been associated with lack of cytogenetic response.[5,6] We speculated that poor tolerance of imatinib associated with cytopenias might be reversed by the use of G-CSF and that this might increase the proportion of cytogenetic responses. Patients with CML in chronic or accelerated phase who failed to achieve cytogenetic responses after 6 months of imatinib therapy and who did not tolerate a dose of 300 mg/day on account of grade III-IV neutropenia and/or thrombocytopenia were eligible for this trial of G-CSF (Filgrastrim®). For patients with iso-

Table 1. Conventional definitions of cytogenetic responses to treatment for chronic myeloid leukemia.

Ph-positive marrow metaphases (%)	*Designation*
0	Complete cytogenetic response (CCR)
1-35	Partial cytogenetic response (PCR)
36-95	Minor cytogenetic response
>95	None

Complete and partial responses are often grouped together as 'Major cytogenetic responses' (MCR).