

implications? We found A91V in 12% of a cohort of Caucasian children with ALL. This prevalence exceeded that of 3.9% observed in our fully comparable control population from the same geographic area. Since the patients with ALL were not selected for any additional criteria, including HLH-like clinical features, this finding suggests that the single amino acid change A91V in the perforin is significantly associated with the risk of developing childhood ALL. The presenting features of children with A91V and ALL were not different from those of the remaining ALL patients. In conclusion, we suggest that impaired function of the cytotoxic machinery, as induced by the A91V transition, may result in different clinical manifestations and may predispose to ALL, the most common type of childhood cancer.

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## Reversal of bone marrow angiogenesis in chronic lymphocytic leukemia following fludarabine therapy

**We evaluated bone marrow (BM) angiogenesis in 12 responding patients with Binet stage B chronic lymphocytic leukemia who received either chlorambucil or fludarabine. Microvessel density (MVD) was compared between pre-treatment marrow samples and those obtained after at least 4 cycles of chemotherapy. BM angiogenesis decreases in all patients but one, although subset analysis revealed the decrease of BM angiogenesis was significant only in patients who received fludarabine. Even if based on a small number of patients, these results point out the potential *in vivo* anti-angiogenic activity of fludarabine.**

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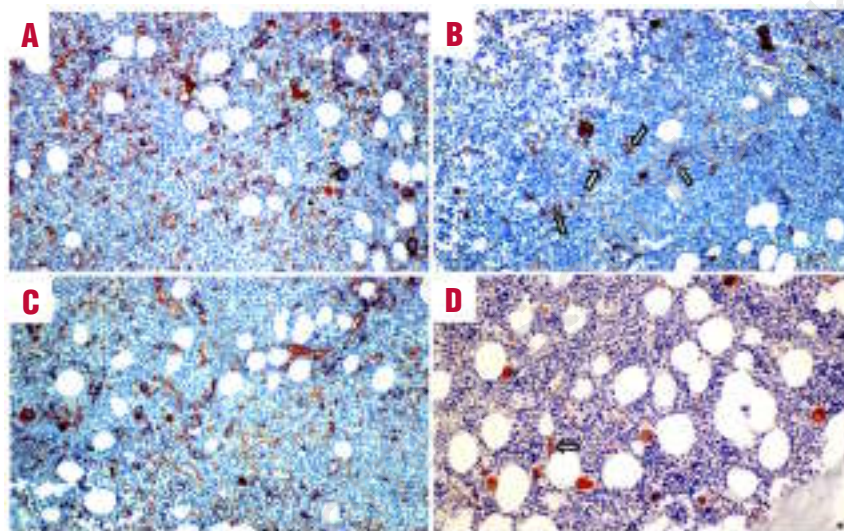
Research over the past two decades has unravelled the importance of abnormal, increased angiogenesis in the development and spread of both solid and hematologic tumors.<sup>1-6</sup> In the context of hematologic malignancies, increased bone marrow (BM) angiogenesis has been demonstrated in multiple myeloma, chronic myeloid leukemia, acute myeloid or lymphocytic leukemia, chronic lymphocytic leukemia (CLL) as well as myelodysplastic syndromes and has been found to have prognostic value.<sup>1-6</sup> However, changes in BM angiogenesis after therapy have not been systematically evaluated.

With this in mind we analyzed the effect of chemotherapy on BM angiogenesis in responder B-cell CLL patients. The study included 12 symptomatic Binet stage B patients (median age 54 year; range, 28-67; M,7; F,5) who received intermittent chlorambucil (5 patients) or fludarabine (7 patients) as up-front therapy. Paraffin-embedded BM biopsy blocks were used to prepare slides for microvessel density (MVD) determination according to previously described methods.<sup>6</sup> BM evaluations were performed at the time of diagnosis and after at least 4 courses of chemotherapy (median, 8; range 4-12). According to the criteria proposed by the National Cancer Institute (NCI)<sup>7</sup> 7 patients were considered in complete remission (CR) and 5 in good partial remission (G-PR). Minimal residual disease (MRD), assessed in flow cytometry as the percentage of CD19<sup>+</sup>/CD5<sup>+</sup> BM cells, closely reflected the type of therapy: chlorambucil, 21.8% (range, 11-29.9%); fludarabine, 5.2% (range, 0.1-11%;  $p=0.006$ ; Mann-Whitney test). BM MVD decreased in all but one patient (Table 1). Specifically, the median microvessel area was  $2.616 \text{ mm}^2 \times 10^{-2}$  (range, 0.545-4.126) before therapy and  $0.644 \text{ mm}^2 \times 10^{-2}$  (range, 0.383-1.914) after therapy ( $p=0.003$ ; Mann-Whitney test). Finally, we wondered whether different therapies could affect the amount of MVD reduction. Separate comparisons carried out in patients treated with chlorambucil and fludarabine revealed a significant decrease of MVD only in patients who received the latter treatment ( $p=0.04$ ). Interestingly, no correlation was found between the amount of MVD reduction related to the therapy and MRD as defined by the percentage of BM CD5<sup>+</sup>/CD19<sup>+</sup> cells (Spearman  $r = -0.042$ ;  $p=0.907$ ).

**Table 1.** Clinico-hematologic characteristics of patients at the start of therapy and changes of BM microvessel area at the response evaluation.

Patient	Age/ Sex	Clinical Stage (Binet/Rai)	BM histology	b-2M (mg/L)	Therapy (number of cycles)	Response (NCI criteria)	CD5/CD19 <sup>+</sup> cells in BM at the time of response evaluation	BM Microvessel area at the start of therapy (mm <sup>2</sup> ×10 <sup>2</sup> )	BM Microvessel area at the time of response evaluation (mm <sup>2</sup> ×10 <sup>2</sup> )	Time to retreatment (months)
G.M.	61/F	B/II	Interstitial (I)	5.7	CLB (12)	CR	22.6%	2.747	1.112	(+50)
A.L.	62/F	B/II	Interstitial (I)	3.1	CLB (12)	CR	21.0%	0.545	0.609	(+45)
E.L.	65/M	B/II	Mixed (I+N)	5.0	CLB (6)	CR	11.0%	3.939	0.415	Still CR (+86)
A.D.	55/F	B/II	Nodular (N)	3.0	CLB (9)	G-PR	29.9%	0.977	0.644	(+12)
A.T.	48/M	B/II	Interstitial (I)	3.3	CLB (10)	G-PR	ND	4.128	0.915	(+9)
C.D.	44/M	B/II	Interstitial (I)	4.0	Fludara (6)	CR	0.2	0.655	0.383	Still CR (+33)
A.N.	67/M	B/II	Mixed (I+N)	3.1	Fludara (9)	CR	0.1	2.484	0.551	(+9)
B.C.	62/M	B/II	Interstitial (I)	4.3	Fludara (8)	G-PR	5.5	2.846	0.657	(+57)
M.M.	54/M	B/II	Diffuse (D)	3.9	Fludara (8)	G-PR	1.0	2.882	1.914	(+14)
D.T.	36/M	B/II	Interstitial (I)	4.3	Fludara (4)	CR	11.0	0.729	0.414	(+60)
M.R.	28/F	B/II	Interstitial (I)	3.6	Fludara (4)	G-PR	9.1	3.110	0.862	(+24)
C.M.	52/F	B/II	Nodular (N)	3.3	Fludara (4)	CR	5.2	1.519	0.645	Still CR (+39)

CR: complete remission; G-PR: good partial remission; ND: not done; CLB: chlorambucil.



**Figure 1.** Staining of B-CLL bone marrow with FVIII-RA. (A-B) Patient whose BM microvessel area and number were evaluated before (A) and after (B) therapy with chlorambucil. (C-D) Patient evaluated before (C) and after (D) therapy with fludarabine.

The effect of chemotherapy on BM angiogenesis has been evaluated in patients with multiple myeloma treated with either conventional or high-dose therapy. In these studies no changes of BM angiogenesis could be observed.<sup>8-9</sup> Unlike the lack of resolution of angiogenesis reported with these therapies, a significant decrease in MVD was reported in 81 patients with multiple myeloma who responded to treatment with thalidomide.<sup>10</sup> Furthermore, imatinib was reported to have anti-angiogenic capacity, normalizing BM vascularity, in 82 patients with chronic myeloid leukemia. In that study a significant anti-angiogenic effect was also observed after therapy with hydroxyurea. In contrast hematologic response obtained with interferon did not reflect changes of BM angiogenesis.<sup>11</sup>

In the present study we demonstrate that BM angiogenesis decreases significantly in CLL patients who obtain a response to fludarabine therapy. These results,

although based on a limited number of patients, lend support to the hypothesis that angiogenesis is a relevant target of therapy in CLL and provide insight into the role of fludarabine as a potential anti-angiogenic agent. This is at least in part corroborated by the apparent absence of correlation between minimal residual disease and degree of BM angiogenesis reduction.

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**Malignant Lymphomas**

**Early and late infectious consequences of adding rituximab to fludarabine and cyclophosphamide in patients with indolent lymphoid malignancies**

**Whether the addition of rituximab to fludarabine and cyclophosphamide (FC) increases early or late infection risk remains poorly defined. This retrospective analysis of 160 patients treated with FC±rituximab found no evidence of increased infection among patients receiving FC+rituximab, providing some evidence of safety for the continued exploration of this regimen.**

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The combination of fludarabine and cyclophosphamide (FC) is highly effective against indolent lymphoid malignancies, but its use is associated with infectious toxicity related to transient myelosuppression and prolonged T-cell depletion.<sup>1</sup> The anti-CD20 antibody rituximab demonstrates significant *in vitro* synergy with fludarabine,<sup>2</sup> and in combination with FC (FCR) is associated with improved outcomes in patients with chronic lymphocytic leukemia (CLL) and indolent non-Hodgkin's lymphomas.<sup>3,4</sup> However, rituximab also effectively depletes peripheral B cells and causes variable suppression of serum immunoglobulins,<sup>5</sup> and may increase the frequency of severe neutropenia when administered with

**Table 1.** Baseline characteristics of the FC and FCR cohorts. FC: fludarabine and cyclophosphamide; FCR: FC and rituximab.

Baseline characteristics	FC cohort (N=63)	FCR cohort (n=97)	p value
Age-median (range)	60 (36-80)	60 (30-89)	0.96
Male sex	42 (67%)	62 (64%)	0.62
No previous treatment	11 (17%)	24 (25%)	0.33
Relapsed or refractory disease	52 (83%)	73 (75%)	0.33
Pretreated patients	n=52	n=73	
Previous fludarabine use	17 (33%)	26 (36%)	0.85
Fludarabine refractory	1 (2%)	6 (8%)	0.24
Previous stem cell transplantation	4 (8%)	3 (4%)	0.45
Number of prior therapies median (range)	2 (1-10)	2 (1-9)	0.40
Chronic lymphocytic leukemia	21 (33%)	42 (43%)	0.32
Polyclonal lymphocytic leukemia	5 (8%)	1 (1%)	0.03
Follicular lymphoma	22 (35%)	32 (33%)	0.74
Mantle cell lymphoma	3 (5%)	8 (8%)	0.53
Marginal zone lymphoma	3 (5%)	3 (3%)	0.68
Waldenström's macroglobulinemia	9 (14%)	9 (9%)	0.32
"Low-grade" lymphoma, not specified	0 (0%)	2 (2%)	0.52
Months from diagnosis -median (range)	48 (0-324)	38 (0-196)	0.81
ECOG Performance Status -median (range)	1 (0-3)	1 (0-3)	0.26
Elevated serum LDH	11/61 (18%)	16/66 (24%)	0.74
International Prognostic Index -median (range)	2 (0-4)	2 (0-5)	0.51
Grade 3+ infection past 12 months	7 (11%)	17 (18%)	0.37
Asplenic	6 (9%)	6 (6%)	0.54
Median baseline ANC ( $\times 10^9/L$ ) (range)	3.5 (0-26)	2.9 (0.6-11)	0.11
Median baseline IgG (g/L) (range)	5 (2-17)	7 (1-20)	0.12
Infection prophylaxis during therapy (% pts) <sup>a</sup>	17 (27%)	27 (28%)	0.95
PCP (bactrim/dapsone)	4 (6%)	14 (14%)	0.20
Antiviral (aciclovir/valaciclovir)	1 (2%)	10 (10%)	0.05
Antifungal (fluconazole/itraconazole)	5 (8%)	31 (32%)	<0.01
G-CSF use			
Median number of cycles (range)	4 (1-6)	4 (1-6)	0.35
Total evaluable cycles	214	355	

<sup>a</sup>Patients who received hematopoietic growth factor or prophylactic antimicrobial agents for one or more cycles of chemotherapy were considered to have had infection prophylaxis for the purposes of comparative analyses.

chemotherapy.<sup>6</sup> Therefore, there is significant concern surrounding a possible increased risk of infection with addition of rituximab to FC, particularly in pretreated and older patients with pre-existing risk factors for severe infections.<sup>7</sup> In order to explore this issue, we retrospectively analyzed infectious episodes during chemotherapy and in the first 12 months of remission among consecutive patients treated with FC (n=63; fludarabine 25 mg/m<sup>2</sup> i.v. days 1-3, cyclophosphamide 250 mg/m<sup>2</sup> i.v. days 1-3, repeated 4 weekly) or FCR (n=97; FC + ritux-