

Acute leukemia in polycythemia vera: an analysis of 1638 patients enrolled in a prospective observational study

Guido Finazzi, Vanesa Caruso, Roberto Marchioli, Giovanni Capnist, Teodoro Chisesi, Carlo Finelli, Luigi Gugliotta, Raffaele Landolfi, Jack Kutti, Heinz Gisslinger, Raphael Marilus, Carlo Patrono, Enrico Maria Pogliani, Maria Luigia Randi, Ana Villegas, Gianni Tognoni, and Tiziano Barbui, for the ECLAP Investigators

Progression to acute myeloid leukemia/myelodysplastic syndromes (AML/MDS) is a possible evolution of polycythemia vera (PV), but whether some patients are at increased natural risk for this complication and how much the contribution of pharmacologic cytoreduction can affect the natural course of the disease remain uncertain. The European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) prospective project included 1638 patients with PV. AML/MDS was diag-

nosed in 22 patients after a median of 2.5 years from recruitment in the study and a median of 8.4 years from the diagnosis of PV. Variables associated with progression to AML/MDS were assessed using different models of multivariate analysis. Older age was confirmed as the main independent risk factor (hazard ratio [HR], 4.30; 95% confidence interval [95% CI], 1.16-15.94; $P = .0294$), whereas overall disease duration failed to reach statistical significance (more than 10 years: HR,

1.91; 95% CI, 0.64-5.69; $P = .2466$). Exposure to P32, busulphan, and pipobroman (HR, 5.46; 95% CI, 1.84-16.25; $P = .0023$), but not to hydroxyurea (HU) alone (HR, 0.86; 95% CI, 0.26-2.88; $P = .8021$), had an independent role in producing an excess risk for progression to AML/MDS compared with treatment with phlebotomy or interferon. (Blood. 2005;105:2664-2670)

© 2005 by The American Society of Hematology

Introduction

The clinical course of polycythemia vera (PV) is characterized by significant thrombohemorrhagic complications and variable risk for disease transformation into myeloid metaplasia with myelofibrosis (MMM) or acute myeloid leukemia/myelodysplastic syndromes (AML/MDS). The incidence of AML/MDS ranges from 5% to 15% after 10 years of disease with progressive increase of risk over time.¹⁻¹²

Although AML/MDS appears to be a major contributor to the death of patients with PV, there is no generally applicable way to predict which patient is likely to acquire this fatal complication. Initial splenic enlargement, marked leukocytosis, myelofibrotic features, and abnormal karyotype are reported to be associated with the risk for AML/MDS, but they could not be consistently established as useful predictive factors.¹³⁻¹⁷ The use of chlorambucil or P32 is consistently associated with increased risk for leukemic transformation.^{1,3,4} Although hydroxyurea (HU) and pipobroman seem to be less leukemogenic than other agents, their effect on the occurrence of AML/MDS remains a controversial issue,⁵⁻¹² and clearcut evidence supporting which treatment has the best benefit-risk ratio is lacking. The relative rarity of the disease, its long-term course, the late development of malignant complications, and the unwillingness to randomize PV patients to potentially leukemogenic drugs have been major obstacles to the launching of large, well-designed, comparative trials, which are the most appropriate strategy to adequately address such open issues.

The prospective, observational database of the large cohort of the European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) study, in which 1638 PV patients were monitored using the same methodology used for a clinical trial, is a unique opportunity for a comprehensive reassessment of the risk for AML/MDS associated with clinical and laboratory patients' characteristics and with cytoreductive strategies adopted in clinical practice.

Patients, materials, and methods

Patient procedures

ECLAP study procedures were planned to reflect the routine care of patients with PV without increasing the burden of clinical work at participating centers.¹⁸ During the study, the investigators were asked to register all patients with new and old diagnoses of PV made according to the criteria established by the Polycythemia Vera Study Group (PVSG) or by Pearson and Messinezy,^{19,20} regardless of age, therapy, or duration of disease. Patients were treated with phlebotomy, cytoreductive agents, or both according to the clinical practice in use in each center. Treatment strategies had to be in compliance with the recommendation to maintain the hematocrit value at 0.45 or less and the platelet count at $400 \times 10^9/L$ or less. Data regarding clinical outcomes, treatments, and laboratory values

From the Consorzio Mario Negri Sud, Santa Maria Imbaro; Policlinico S Orsola, Bologna; Ospedale S Gerardo, Monza; University of Padua, Dip. Scienze Mediche e Chirurgiche, Padova; Ospedale Umberto I, Venezia-Mestre; Catholic University School of Medicine, Rome; Ospedali Riuniti, Bergamo; Ospedale di Reggio Emilia; Ospedale S Giovanni e Paolo, Venezia; University of Rome "La Sapienza," Italy; Sahlgrenska Hospital, Göteborg, Sweden; Department of Hematology and Blood Coagulation, University of Vienna, Austria; Tel-Aviv Souraski Medical Center, Israel; and Hospital Universitario S Carlos, Madrid, Spain.

Submitted September 3, 2004; accepted November 19, 2004. Prepublished online as *Blood* First Edition Paper, December 7, 2004; DOI 10.1182/blood-2004-09-3426.

A complete list of the ECLAP Investigators appears in the Appendix.

The ECLAP Project was supported by the European Union, BIOMED 2 Program (contract ERBBMH4CT961433).

Reprints: R. Marchioli, ECLAP Co-ordinating Centre, Consorzio Mario Negri Sud, Via Nazionale, 6630 Santa Maria Imbaro, Italy; e-mail marchioli@negrisud.it.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2005 by The American Society of Hematology

were collected with the same methodology followed for the nested trial on low-dose aspirin and were specifically recorded at follow-up visits at 12, 24, 36, 48, and 60 months. An ad hoc committee of expert clinicians ensured the validation of causes of death and of nonfatal major events. We examined the rate of AML/MDS diagnoses and classifications that were established using the French-American-British (FAB) Cooperative Group criteria,^{21,22} and subsequently we analyzed possible risk factors for the occurrence of AML/MDS. According to the Declaration of Helsinki and the requirements of international good clinical practice rules, informed consent was obtained from all patients included in the study.

Statistical methods

The following variables at registration were evaluated: age, sex, disease duration, splenomegaly, packed cell volume (PCV) level, platelet count, white blood cell (WBC) count, cholesterol values, and treatments. The presence of thrombocytosis (platelet count greater than $400 \times 10^9/L$) and leukocytosis (WBC count greater than $12 \times 10^9/L$) at diagnosis of PV was also included in the analysis. Disease duration was defined as the years between the diagnosis of PV and the time of registration.

Continuous variables have been assessed as such and then categorized according to quartiles or median values. Because the findings did not materially change, we report here the results of the analysis using clinically meaningful cutoffs for age, disease duration, and hematologic parameters, or, when appropriate, by collapsing categories with similar levels of risk. Cytoreductive interventions were also categorized according to their expected leukemogenic potential: (1) no treatment, phlebotomy alone, or interferon alone or with phlebotomy; (2) HU as the only cytoreductive drug; and (3) other cytoreductive agents, alone or in combination.

Differences in proportions were ascertained by means of χ^2 analysis for proportions and *t* test for continuous variables. Separate multivariate age- and sex-adjusted Cox proportional hazard models were fitted to evaluate each variable. Factors found to be associated with increased risk for leukemia were further analyzed by fitting a multivariate Cox proportional hazard model adjusted for all the potential predictors together. To assess possible effect modifications caused by the interaction among age, sex, disease duration, and various clinical variables, we included in the multivariate models interaction terms and carried out separate analyses for different subgroups of patients. Because the results did not materially change with the testing of possible interactions among the variables, we present here only the results for the final multivariate model. Cox proportional hazards event-free survival curves adjusted for covariates were plotted using the corrected group prognosis method.²³

All *P* values were 2-sided. All analyses were performed using the SAS statistical package (SAS Institute, Cary, NC).

Results

Patients

Patients received diagnoses of PV between 1964 and 2001 and were registered in the study between 1997 and 2001. Median time from diagnosis to registration was 3.5 years.

Median age of the entire cohort was 62.1 years at diagnosis of PV and 67.3 years at registration. Four percent of patients were younger than 40, 24.8% were between 40 and 59, 30% were between 60 and 70, and 41.1% were older than 70 years of age. PV was diagnosed in 62% of patients within 5 years of registration, whereas it was diagnosed in 14.4% of patients more than 10 years earlier.

At the time of study closure, patients had been followed up for a median of 2.8 years (4393 person-years; maximum follow-up, 5.3 years). Twenty-one cases of AML and one case of MDS (refractory anemia with excess of blasts in transformation [RAEB-t]) with rapid progression to AML (1.3%) were diagnosed after a median of 2.5 years (range, 0.5-4.1 years) from the registration and 8.4 years

(range, 2.2-19.8 years) from the diagnosis of PV. Only 3 cases of AML were preceded by a spent phase with myelofibrosis. All AML/MDS cases were fatal, and no patients survived more than 6 months after the diagnosis of AML/MDS.

The principal characteristics of the patients at registration are shown in Table 1. At the time of diagnosis of PV, patients with AML/MDS were more likely than PV patients without AML/MDS to have had WBC counts exceeding $12 \times 10^9/L$ (59.1% vs 40.2%; *P* = .0733). At recruitment, patients with AML/MDS were more likely to have been older (71 vs 65 years; *P* = .0036), to have had longer disease duration (7.0 vs 3.5 years; *P* = .0067), to have experienced previous hemorrhagic event (22.7% vs 7.9%; *P* = .0116), and to have had lower total blood cholesterol level (157 vs 188 mg/dL; *P* = .0157). Patients with AML/MDS were more likely to have been treated with more than 1 cytoreductive drug (18.2% vs 3.3%; *P* = .0002) and to have experienced more frequent use of P32 (13.6% vs 2.5%; *P* = .0014), busulphan (18.2% vs 3.5%; *P* = .0003), and pipobroman (18.2% vs 6.3%; *P* = .0246).

Multivariate analysis

Table 2 shows the results of multivariate analysis. Compared with patients younger than 60, those older than 70 were at higher risk for AML/MDS (HR, 4.30; 95% CI, 1.16-15.94; *P* = .0294), whereas the association between overall disease duration and risk for AML/MDS did not reach statistical significance. Women were at higher risk (HR, 2.93; 95% CI, 1.18-7.26; *P* = .0205) than men. A barely not statistically significant association was found between WBC count greater than $12 \times 10^9/L$ and probability of AML/MDS (HR, 2.55; 95% CI, 0.94-6.91; *P* = .065). Low blood cholesterol levels at recruitment were strongly associated with evolution to AML/MDS (HR, 6.58; 95% CI, 2.08-20.86; *P* = .0014).

Phlebotomy, hydroxyurea, and interferon were not associated with AML/MDS, whereas pipobroman, busulfan, and P32 were significantly associated with a 4- to 8-fold increased risk for AML/MDS. Compared with patients treated with phlebotomy or interferon, patients receiving hydroxyurea as the only cytoreductive drug had no increased risk for AML/MDS, whereas those treated with pipobroman, busulfan, chlorambucil, or P32, alone or in combination with other drugs, were at significantly higher risk for AML/MDS (HR, 5.46; 95% CI, 1.84-16.25; *P* = .0023). When we evaluated patients receiving HU plus alkylating agents or P32, the risk for AL was significantly increased (HR, 7.58; 95% CI, 1.85-31.00; *P* = .0048) compared with patients treated with phlebotomy or interferon. In patients who received "only" alkylating agents or P32, the risk for AL was 4-fold higher (HR, 4.69; 95% CI, 1.42-15.46; *P* = .0110). Leukemia-free survival times during follow-up for these 4 groups of treatment are shown in Figure 1.

Discussion

In our study, we registered 1638 patients with diagnoses of PV. New patients and patients with the disorder for more than 20 years were included and then prospectively followed up for a median of 2.8 years. This is the largest and most up-to-date prospective cohort in PV, and it offers a unique opportunity to reassess the factors that influence the natural history of the disease.

One of the principal strengths of the ECLAP project is the use of the same rigorous prospective design with centralized monitoring and validation of events as that used for the nested ECLAP trial.¹⁸ The inclusion of patients with old diagnoses of PV and the large

Table 1. Characteristics of patients

	Patients with AML/MDS, N = 22, 1.34%	Patients without AML/MDS, N = 1616, 98.66%	P
Sex (male)	8 (36.36)	934 (57.80)	.0434
Mean age at registration, y (SD)	70.90 (7.90)	65.33 (12.71)	.0036
Median time from diagnosis of PV to registration, y (range)	7.0 (0.68-18.48)	3.45 (0-39.53)	.0067
Hematologic parameters at diagnosis of PV (%)			
Clinically palpable splenomegaly	11 (50.00)	651 (40.28)	.3563
Platelet count > 400 × 10 ⁹ /L	14 (63.64)	985 (60.95)	.7977
WBC count > 12 × 10 ⁹ /L	13 (59.09)	650 (40.22)	.0733
Clinical and laboratory parameters at registration			
Splenomegaly (%)	12 (54.55)	720 (44.55)	.3492
PCV, L/L (SD)	0.45 (0.06)	0.47 (0.06)	.1051
Platelet count, × 10 ⁹ /L (SD)	319 (177)	400 (208)	.0695
WBC count, × 10 ⁹ /L (SD)	12.70 (7.42)	10.89 (8.64)	.3490
Cholesterol level, mg/dL (SD)	157 (58)	188 (45)	.0157
Treatment at registration (%)			
Phlebotomy	13 (59.09)	1027 (63.55)	.6660
P32	3 (13.64)	41 (2.54)	.0014
Hydroxyurea	10 (45.45)	783 (48.45)	.7798
Busulphan	4 (18.18)	57 (3.53)	.0003
Chlorambucil	0 (0.00)	5 (0.31)	.7939
Pipobroman	4 (18.18)	102 (6.31)	.0246
Interferon	1 (4.55)	63 (3.90)	.8764
Groups of treatment at registration (%)			
No treatment, phlebotomy only, or interferon only	5 (22.73)	664 (41.09)	.0818
Hydroxyurea as only cytoreductive drug	6 (27.27)	736 (45.54)	.0873
Any other cytoreductive drug, alone or in combination	11 (50.00)	216 (13.37)	< .0001
More than 1 cytoreductive drug	4 (18.18)	54 (3.34)	.0002
Event at follow-up (%)			
Thrombosis	5 (22.73)	226 (13.99)	.2419
Hemorrhage	4 (18.18)	117 (7.24)	.0513

sample size allowed us to observe incident cases of AML/MDS in patients who had already been exposed to cytoreductive agents, giving the opportunity to estimate the risk for AML/MDS during different stages of the disease and to partially overcome the limitations of a 3-year follow-up study. We observed 22 patients with AML/MDS, and this figure, though limited, is not far from the figures observed in the largest trials and observational studies available in PV (Table 3).²⁴⁻²⁸

The relationship between advanced age and AML/MDS has been already reported.²⁹ It is not surprising that a relationship also exists between advanced age and progression to AML/MDS in patients with PV. A more intriguing finding is the higher risk for progression to AML/MDS in women. Previous studies on AML/MDS and on progression to AML/MDS in patients with PV found a similar incidence in men and women.^{16,29} In further analysis (data not shown), we observed that this increased risk could not be attributed to the greater number of elderly women; within all age groups women were always at higher risk for AML/MDS AL than men, and this risk was more noticeable among patients older than 70. In addition, the proportions of use of alkylating agents and the median times of disease duration and follow-up were similar in men and women. Thus, the increased risk for leukemia in women with PV is unexplained and remains to be confirmed in further studies.

Although the group of patients with progression to AML/MDS had a longer evolution of disease than did patients without progression (8.4 vs 6.4 years from disease diagnosis to the last follow-up information), disease duration did not reach the formal statistical significance level. This result may be influenced by the fact that for subjects who were older at diagnosis—and who had the highest incidence of AML/MDS—the time from diagnosis to

progression was shorter than it was in patients whose disease was diagnosed when they were younger (data not shown). This finding seems to confirm advanced age as a central independent risk factor and suggests at least 2 explanations for such increased risk for AML/MDS in older patients. The first is that a more aggressive form of PV may affect older persons. The second is that the expected lower life expectancy of older patients may allow the use of more aggressive cytoreductive strategies whose leukemogenic effect is supposed to appear in the long term. However, the increased life expectancy of Western populations observed in recent years and the possibility that malignancy may develop in elderly patients after shorter periods of exposure to drugs might explain such excess of AML/MDS.

Because the choice of therapeutic strategy was left to the judgment of patients' physicians and the only inclusion criteria regarding treatment was the maintenance of recommended levels of PCV, the ECLAP study constitutes a suitable framework for studying the association between current therapeutic strategies and development of AML/MDS. The difficulties in conducting long-term, large-scale studies, according to proper methodology (ie, randomized clinical trials), of a chronic disease such as PV, which is influenced by multiple, even unknown, factors, justify the limited number of comparative studies published to date and the substantial differences in the incidence of AL in studies with comparable times of follow-up and with evaluations of similar therapeutic strategies (Table 3). Similarly, although higher incidences of AL have been reported with the use of old strategies, such as alkylating agents and radiation, scarcely used today, the issue of leukemogenicity of drugs frequently used (such as HU and pipobroman) is still controversial.

Table 2. Multivariate analysis

	Patients with AML/MDS N = 22	Patients without AML/MDS N = 1616	HR (95% CI) [P]
Age at registration, y (%)			
Younger than 60	3 (13.64)	470 (29.08)	1.00
60-69	5 (22.73)	487 (30.14)	1.86 (0.42-8.32) [.4167]
70 and older	14 (63.64)	659 (40.78)	4.30 (1.16-15.94) [.0294]
Sex (% females)	14 (63.64)	682 (42.20)	2.93 (1.18-7.26) [.0205]
Time from diagnosis to registration, y (%)			
0-5	9 (40.91)	1006 (62.25)	1.00
6-10	6 (27.27)	381 (23.58)	1.27 (0.43-3.73) [.6700]
More than 10	7 (31.82)	229 (14.17)	1.91 (0.64-5.69) [.2466]
Presence of splenomegaly* (%)	15 (68.18)	934 (57.80)	0.91 (0.35-2.40) [.8491]
Laboratory parameters at registration (%)			
PCV, L/L			
0.45 or less	10 (47.62)	546 (38.72)	1.00
More than 0.45	11 (52.38)	864 (61.28)	1.22 (0.49-3.04) [.6693]
Platelet count, × 10 ⁹ /L			
400 or less	15 (68.18)	921 (57.60)	1.00
More than 400	7 (31.82)	678 (42.40)	0.45 (0.17-1.18) [.1027]
WBC count, × 10 ⁹ /L			
12 or less	12 (60.00)	1165 (73.92)	1.00
More than 12	8 (40.00)	411 (26.08)	2.55 (0.94-6.91) [.0650]
Cholesterol level, mg/dL			
More than 150	6 (46.15)	811 (82.59)	1.00
150 or less	7 (53.85)	171 (17.41)	6.58 (2.08-20.86) [.0014]
Treatment at registration (%)			
Phlebotomy	13 (59.09)	1027 (63.55)	0.91 (0.37-2.21) [.8261]
Hydroxyurea	10 (45.45)	783 (48.45)	1.09 (0.42-2.80) [.8654]
Interferon	1 (4.55)	63 (3.90)	1.24 (0.16-9.80) [.8397]
Busulphan	4 (18.18)	57 (3.53)	8.64 (2.44-30.60) [.0008]
Pipobroman	4 (18.18)	102 (6.31)	4.32 (1.27-14.68) [.0191]
P32	3 (13.64)	41 (2.54)	8.96 (2.13-37.58) [.0027]
Chlorambucil	0 (0.00)	5 (0.31)	NA
Treatment at registration, grouped (%)			
No treatment, phlebotomy only, interferon only†	5 (22.73)	664 (41.09)	1.00
Hydroxyurea as only cytoreductive drug	6 (27.27)	736 (45.54)	0.86 (0.26-2.88) [.8021]
Any other cytoreductive drug, alone or in combination	11 (50.00)	216 (13.37)	5.46 (1.84-16.25) [.0023]

Analysis was adjusted for sex, age, disease duration, treatment at recruitment, presence of splenomegaly, cholesterol level (2 categories), and hematologic parameters at baseline (2 categories).

*At diagnosis or at registration.

†Forty patients received interferon, alone or in combination with phlebotomy.

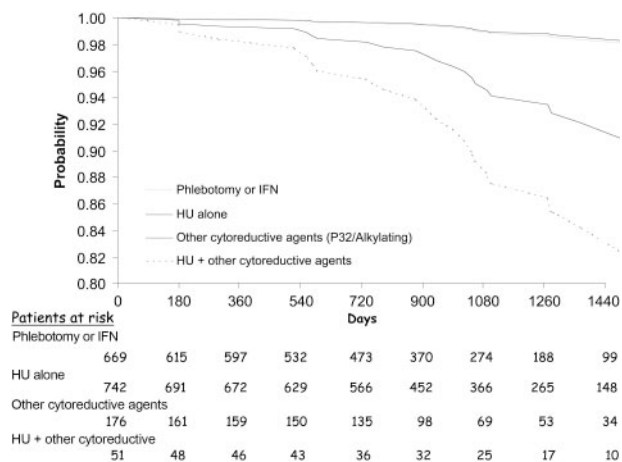


Figure 1. Leukemia-free survival during follow-up according to the treatment received at registration. Gray line indicates phlebotomy or interferon (IFN); thin line, HU alone; bold line, other cytoreductive agents; and broken line, HU + other cytoreductive agents.

In our study we considered patients undergoing phlebotomy or interferon therapy as the only cytoreductive agent as a reference category that could likely represent the natural risk for PV patients to progress to AML/MDS. The incidence rate of AML/MDS for this group was similar to that found in patients treated with HU as the only pharmacologic treatment (0.29 per 100 persons per year), whereas this rate was 1.8 per 100 persons per year in those receiving at least one alkylating agent or P32 at recruitment. Such findings were confirmed by the results of multivariate analysis, showing that the risk for AML/MDS in patients treated with HU alone was not higher than that of the reference category, whereas this risk was significantly higher in patients treated with pipobroman, busulphan, and P32 alone or in combination with HU. These findings are in agreement with those of randomized clinical trials suggesting an elevated incidence of AML/MDS in patients treated with alkylating agents¹ or with radiation^{3,19} or when cytoreductive drugs were used in combination (eg, P32 or busulphan and HU) to achieve more adequate control of hematocrit values.^{4,28,30}

Prospective observational studies have shown incidence rates of AML/MDS ranging between 0.5% and 1.0% per year in patients

Table 3. Principal randomized clinical trials and observational studies evaluating occurrence of AML/MDS in PV patients

Study/year of publication	Strategies	N	Median follow-up, y	Cases of AL	Estimated incidence of AL, %	Estimated annual incidence rate
Randomized clinical trials						
PVSG-01, 1981 ^{1,19}	Phlebotomy	134	8.6	2	1.5	0.17
	Chlorambucil	141		19	13	1.5
	P32	156		15	9.6	1.1
EORTC, 1981 ²	P32	140	8	2	1.4	0.17
	Busulphan	145		3	2.0	0.25
FPSG, 1997 (older than 65) ⁴	P32	242	16, maximum	35	12 at 10 y*	1.2
	P32 + HU	219			21 at 10 y*	2.1
FSPG, 1997 (older than 65) ⁵	HU	150	16, maximum	13	5 at 10 y*	0.5
	Pipobroman	142			Similar in both groups	
Observational prospective studies						
PVSG-08, 1986 ^{6,7}	HU	51	8.6	5	9.8	1.1
Weinfeld et al, 1994 ⁸	HU	30	10	3	10	1
Tarstarsky and Sharon, 1997 ⁹	HU	71	10.9	4	5.6	0.5
Brusamolino et al, 1984 ¹⁰	Pipobroman	100	5	6	6	1.2
Passamonti et al, 2000 ¹¹	Pipobroman	163	10	11	7	0.7
Kiladjian et al, 2003 ¹²	Pipobroman	164	11.4	32	19.5	1.7
Passamonti et al, 2003 (younger than 50) ²⁴	Pipobroman, 73% HU, 9% Phlebotomy, 18%	70	14	5	7	0.5
ECLAP, 2004	Phlebotomy, 63% HU, 48% P32, 3% Interferon, 4% Alkylators, 10%	1638	2.8	22	1.3	0.5
Observational retrospective studies						
Halnan and Russell, 1965 ²⁸	No P32	127	19, maximum	0	0	0
	P32	107		0		
Najejan et al, 1988 ¹⁴	Phlebotomy, 22% P32, 61% Cytoreductive drugs, 17%	288	10, minimum	33	11.4	1.1
GISP, 1995 ²⁵	Phlebotomy, 31% Alkylators, 59% Other cytoreductive drugs, 10%	1213	5.3	28	2.3	0.43
Brodman et al, 2000 ²⁶	HU, 63% Alkylators, 19% P32, 5% Phlebotomy, 70%	59	3.7	1	1.7	0.5
Nielsen and Hasselbalch, 2003 ²⁷	No HU	13	8	1	7.7	0.9
	HU, alone or with busulphan	29		7	24	3.0

Estimated annual incidence rate is based on number of cases per 100 persons per year.

*Actuarial risk.

treated with HU⁶⁻⁹ and between 0.7% and 1.7% in patients treated with pipobroman.¹⁰⁻¹² Our results show an increased risk in patients receiving pipobroman compared with those treated with HU. However, the only randomized clinical trial performed in patients younger than 65 showed similar incidences of AML/MDS in patients treated with the 2 drugs.⁵

The PVSG 01 trial compared the benefit-risk ratio of phlebotomy with that of chlorambucil and of P32. AML/MDS developed in 1.5% of patients who underwent phlebotomy during a median follow-up of 8.6 years (incidence rate, 0.17% cases per year).^{7,19} This estimate, however, pertains only to the 10% of patients who were allocated to phlebotomy, had no thrombotic events, and continued the same treatment until the 10th year of study.³⁰ Therefore, such low occurrence of AML/MDS could not be the true incidence rate of AML/MDS in patients undergoing only phlebotomy because it reflects a select group of patients who likely had a less proliferative form of PV.³¹ The only other clinical trial comparing cytoreductive drugs with no intervention was conducted in essential thrombocythemia.³² After a median follow-up of 73 months, the incidence rates of AML/MDS in this study were 0.0%, 0.6%, and 5.4% per year for no pharmacologic treatment and cytoreduction with HU and HU plus busulphan, respectively.³³

Because our study design prevented us from assessing the total exposition time to cytoreductive drugs, we could not rule out the possibility that HU could be leukemogenic in the long run. However, our results are reassuring and suggest that if such a risk

exists, it is probably not much greater than the natural tendency of the disease to progress to AML/MDS and is definitely lower than that of pipobroman, busulphan, and P32. Our study had the power to capture the risk for AML/MDS associated with drugs used by less than 15% of the patients. Because HU was prescribed to approximately half the population and was found not to be associated with the development of AML/MDS, we could safely rule out the possibility of a large leukemogenic effect of this drug in PV patients.

We found that low levels of total blood cholesterol at recruitment were strongly associated with the development of AML/MDS. Some small studies have found low levels of cholesterol in patients affected by hematologic malignancies,³⁴⁻³⁷ and some of these reports include patients with PV.^{34,36-37} It has been observed that the lowest levels of blood cholesterol are observed in advanced stages of proliferative diseases, and some have suggested the use of this parameter as a marker of disease activity.³³⁻³⁵ Given that the average total blood cholesterol level in a 35- to 75-year-old Mediterranean population, such as Italian, is 206 mg/dL,³⁸ the average cholesterol level of the ECLAP population (187.4 mg/dL) is surprisingly low and is indicative of the proliferative nature of the disease. In addition to patients with progression to AML/MDS, others who acquired myelofibrosis also had more noticeable hypocholesterolemia (data not shown). These findings resemble the increased risk for death from cancer observed in patients with low cholesterol levels, and they suggest that low cholesterol could be a

precocious marker of neoplastic diseases in otherwise healthy persons undergoing no cholesterol-lowering treatment.³⁹

Although the relationship between high WBC counts at diagnosis and at recruitment with the later development of AML/MDS was not statistically significant in our study, this association has been observed in some studies,¹²⁻¹⁴ and such findings suggest that high WBC counts could mark a different pathway of proliferation in some patients, indicating a subset of subjects with a distinct natural risk for progression to AML/MDS.

Relationships between the progression to AML/MDS and markers of cellular proliferation, such as low blood cholesterol levels, and the administration of alkylating drugs, alone or in combination with other drugs, should not be viewed as independent phenomena because they may indicate that some PV patients may have a more aggressive disease with a higher proliferation rate, making them more likely to be resistant to cytoreductive treatments and, in the end, to progress to AML/MDS. If so, these patients are also more likely to be exposed to recurrent changes in therapy, often toward more aggressive options, for improved control of the disease. Because such work-up bias cannot be eliminated in observational studies and because the effect of unmeasured and unknown confounders cannot be adjusted for in multivariate analyses, new randomized trials are needed for correct evaluation of the efficacy and safety of cytoreductive treatments in PV to determine a final answer to the debated issue of the supposed leukemogenic effect of frequently used drugs such as HU.

Appendix

Principal ECLAP Investigators (recruiting 40 or more patients in the study): Austria, Vienna: H Gisslinger. Italy, Bergamo: T Barbui, G Finazzi, S Pusterla, A Falanga, M Galli. Bologna: S Tura, C Finelli, G Marino. Monza: E Pogliani, I R Miccolis, A La Rocca, E Elli. Padova: M L Randi. Reggio Emilia: L Gugliotta, F Ilariucci. Venezia: T Chisesi, A Polacco. Venezia-Mestre: T Chisesi, G Capnist. Sweden, Göteborg: J Kutti, H Wadenvik. Other ECLAP Investigators: Austria, Innsbruck: G Gastl, Ch Ludescher, M Fiegl. Linz: D Lutz, M Girschikofsky, G Michlmayr, E Rechberger. Wr Neustadt: H Niessner, E Ivanschik. France, Paris: J D Rain, C Chomienne-Thomas. Germany, Mannheim: R Hehlmann, G Engelich. Ulm: E Kohne, A Kramer. Greece, Thessaloniki: J I Christakis, M Papaioannou, G Gerotzi-afas, J Korantzis, P Marinaki, A Kioumi, I Katodritou. Ireland, Dublin: D

McCarthy, R O'Donnell. Israel, Afula: M Bennett. Ashkelon: G Lugassy. Kfar Saba: M Ellis. Tel-Aviv: A Eldor, † E Naparstek, R Marilus. Italy, Ancona: P Leoni, S Rupoli, A R Scortechini, V Agostini. Avellino: E Volpe, F Palmieri, A Volpe, G Storti, A Ciampa. Bari: F Dammacco, V M Lauta, G Ranieri, R Rizzi. Brescia: G Rossi, C Almici, A Capucci, F Zanetti. Castrovillari CS: F Laghi, G Micciulli. Catania: R Giustolisi, RR Cacciola, E Cacciola. Catanzaro: A Peta, D Magro. Como: G Frigerio, F Alberio, A Beretta. Cuneo: M Bonferroni, Raviolo. Firenze: P L Rossi Ferrini, A Grossi, A Fabbri. Genova: M Gobbi, I Pierri, M Cavaliere. Latina: S Nardelli, A Centra. Messina: C Musolino, G Bellomo, O Tringali, G Spatari. Milano: P Foa, G Gerli, M C Carraro, A Zanella, A Iurlo, F Barraco. Modena: G Torelli, M Marietta. Montebelluna TV: A Puglisi, G Sardeo. Napoli: B Rotoli, V Martinelli, R Ciancia, A Abbadesse, O Villani, R Cimino, A Fasanaro. Parma: V Rizzoli, C Caramatti, Gaeta. Pavia: M Lazzarino, F Passamonti, M Lazzola, L Malabarba. Pescara: D Natale, G. Davi, S Pulini. Roma: R Landolfi, E De Candia, S Amadori, F Buccisano, F Mandelli, E Montefusco, M C Petti, A Spadea, M Carotenuto, † A Morelli, M Nobile. Sassari: M Longinotti, S M Pardini. Siena: F Lauria, A Buccalossi, S Gentili. Taranto: P Mazza, M Cervellera, A Maggi. Teramo: A Di Francesco, E Pasqualoni. Vicenza: F Rodeghiero, M Ruggeri. Udine: M Baccarani, D Russo. Spain, Barakaldo: B Arizabalaga. Barcelona: A Remacha. Burgos: B Pérez De Mendiguren. La Laguna (Santa Cruz de Tenerife): L Hernández-Nieto, M T Hernández-García, G González-Brito, P Machado. León: G Garcia. Madrid: A Villegas, A Peña, A González Fernández. Valencia: F Carbonell, A Del Arco. Sweden, Borås: H Bäck. Danderyd: L Stenke. Eksjö: S Hansen. Huddinge: J Samuelsson. Hudiksvall: L Nezadal. Kristianstad: G Larsson. Kungälv: G Strömblad. Luleå: B Lauri. Motala: B O Ryden. Norrköping: T End. Örebro: O Linder. Örnsköldsvik: B G Lundholm. Säffle: O Lannemyr. Skövde: J Väart. Sundsvall: M Strandberg. Uddevalla: B Andréasson, D Stockelberg. Värnamo: L Engquist. Västerås: F Pasquariello. Switzerland, Basel: A Tichelli. Oldenburg: B Otremba, H F Hinrichs. Schweiz: W Weber-Stadelmann. The Netherlands, Heerlen: M M F Fickers. Rotterdam: J J Michiels. United Kingdom, Belfast: M F McMullin. Birmingham: D Bareford. Bournemouth: D G Oscier, N Bowey. Cambridge: T A R Green. London: M Messinezy. South Yorks: P C Taylor. Steering committee: R Landolfi, T Barbui, G de Gaetano, R Marchioli, Y Najean, C Patrono, TC Pearson. Scientific and organizing secretariat: R Marchioli, S Atashkar, V Caruso, A Di Blasio, G Finazzi, H Gisslinger, E Mari, D Tamayo, G Tognoni. Data management and analysis: G Borrelli, B Ferri, R M Marfisi, M Olivieri, A Polidoro, R Spoltore. Event adjudicating committee: R Landolfi, G Levantisi, R Di Mascio, G Finazzi, G Miceli, G Sperti. Writing committee: V Caruso, G Finazzi, R Landolfi, R Marchioli, J Kutti, H Gisslinger, C Patrono, R Marilus, A Villegas, G Tognoni, T Barbui.

References

- Berk PD, Goldberg JD, Silverstein MN, et al. Increased incidence of acute leukemia in polycythemia vera associated with chlorambucil therapy. *N Engl J Med*. 1981;304:441-447.
- Haanen C, Mathe G, Hayat M. Treatment of polycythemia vera by radiophosphorus or busulfan: a randomized clinical trial. *Br J Cancer*. 1981;44:75-80.
- Najean Y, Rain J-D. A very long-term evolution of polycythemia vera: an analysis of 318 patients initially treated by phlebotomy or 32P between 1969 and 1981. *Semin Hematol*. 1997;34:6-16.
- Najean Y, Rain J-D for the French Polycythemia Study Group. Treatment of polycythemia vera: use of P32 alone or in combination with maintenance therapy using hydroxyurea in 461 patients greater than 65 years of age. *Blood*. 1997;89:2319-2327.
- Najean Y, Rain JD for the French Polycythemia Study Group. Treatment of polycythemia vera: the use of hydroxyurea and pipobroman in 292 patients under the age of 65 years. *Blood*. 1997;90:3370-3377.
- Donovan PB, Kaplan ME, Goldberg JD, et al. Treatment of polycythemia vera with hydroxyurea. *Am J Haematol*. 1984;17:329-334.
- Fruchtman SM, Mack K, Kaplan ME, et al. From efficacy to safety: a Polycythemia Vera Study Group report on hydroxyurea in patients with polycythemia vera. *Semin Hematol*. 1997;34:17-23.
- Weinfeld A, Swolin B, Westin J. Acute leukaemia after hydroxyurea therapy in polycythemia vera and allied disorders: prospective study of efficacy and leukaemogenicity with therapeutic implications. *Eur J Haematol*. 1994;52:134-139.
- Tatarsky I, Sharon R. Management of polycythemia vera with hydroxyurea. *Semin Hematol*. 1997;34:24-28.
- Brusamolino E, Salvaneschi L, Canevari A, Bernasconi C. Efficacy trial of pipobroman in polycythemia vera and incidence of acute leukemia. *J Clin Oncol*. 1984;2:558-561.
- Passamonti F, Brusamolino E, Lazzarino M, et al. Efficacy of pipobroman in the treatment of polycythemia vera: long-term results in 163 patients. *Haematologica*. 2000;85:1011-1018.
- Kiladjian JJ, Gardin C, Renoux M, Bruno F, Bernard JF. Long-term outcomes of polycythemia vera patients treated with pipobroman as initial therapy. *Hematol J*. 2003;4:198-207.
- Nand S, Messmore H, Fisher SG, et al. Leukemic transformation in polycythemia vera: analysis of risk factors. *Am J Hematol*. 1990;34:32-36.
- Najean Y, Deschamps A, Dresch C, et al. Acute leukemia and myelodysplasia in polycythemia vera: a clinical study with long-term follow-up. *Cancer*. 1988;61:89-95.
- Diez-Martin JL, Graham DL, Pettitt RM, Dewald GW. Chromosome studies in 104 patients with polycythemia vera. *Mayo Clin Proc*. 1991;66:287-299.
- Landaw SA. Acute leukemia in polycythemia vera. In: Wasserman LR, Berk PD, Berlin NI, eds. *Polycythemia Vera and the Myeloproliferative Disorders*. Philadelphia, PA: WB Saunders; 1995:154-165.
- Swolin B, Weinfeld A, Westin J. A prospective long-term cytogenetic study in polycythemia vera in relation to treatment and clinical course. *Blood*. 1988;72:386-395.
- Landolfi R, Marchioli R, Kutti J, et al. Efficacy and

- safety of low-dose aspirin in polycythemia vera. *N Engl J Med*. 2004;350:114-124.
19. Berk PD, Goldberg JD, Donovan PB, Fruchtman SM, Berlin NI, Wasserman LR. Therapeutic recommendations in polycythemia vera based on Polycythemia Vera Study Group protocols. *Semin Hematol*. 1986;23:132-143.
 20. Pearson TC, Messinezy M. The diagnostic criteria of polycythemia rubra vera. *Leuk Lymphoma*. 1996;22(suppl 1):87-93.
 21. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the acute leukaemias: French-American-British (FAB) Cooperative Group. *Br J Haematol*. 1976;33:451-458.
 22. Bennett JM, Catovsky D, Daniel MT, et al. Proposal for the classification of myelodysplastic syndromes. *Br J Haematol*. 1982;51:189-199.
 23. Ghali WA, Quan H, van Melle G, et al. *JAMA*. 2001;286:1494-1497
 24. Passamonti F, Malabarba L, Orlandi E, et al. Polycythemia vera in young patients: a study on the long-term risk of thrombosis, myelofibrosis and leukemia. *Haematologica*. 2003;88:13-18.
 25. Gruppo Italiano Studio Policitemia. Polycythemia vera: the natural history of 1213 patients followed for 20 years. *Ann Intern Med*. 1995;123:656-664.
 26. Brodmann S, Passweg JR, Gratwohl A, Tichelli A, Skoda RC. Myeloproliferative disorders: complications, survival and causes of death. *Ann Hematol*. 2000;79:312-318.
 27. Nielsen I, Hasselbalch HC. Acute leukemia and myelodysplasia in patients with a Philadelphia chromosome negative chronic myeloproliferative disorder treated with hydroxyurea alone or with hydroxyurea after busulphan. *Am J Hematol*. 2003;74:26-31.
 28. Halnan KE, Russell MH. Polycythemia vera: comparison of survival and causes of death in patients managed with and without radiotherapy. *Lancet*. 1965; 2:760-763.
 29. Linet MS, Devesa SS. Epidemiology of leukemia: overview and patterns of occurrence. In: Henderson ES, Lister TA, Greaves M, eds. *Leukemia*. 7th ed. Philadelphia, PA: WB Saunders; 2002: 131-151.
 30. Najean Y, Dresch C, Rain JD. The very-long-term course of polycythaemia: a complement to the previously published data of the Polycythaemia Vera Study Group. *Br J Haematol*. 1994;86:233-235.
 31. Pearson TC, Green AR, Reilly JT, Harrison G. Leukemic transformation in polycythemia vera. *Blood*. 1998;92:1837-1842.
 32. Cortelazzo S, Finazzi G, Ruggeri M, et al. Hydroxyurea in the treatment of patients with essential thrombocythemia at high risk of thrombosis: a prospective randomized trial. *N Engl J Med*. 1995; 332:1132-1136.
 33. Finazzi G, Ruggeri M, Rodeghiero F, et al. Second malignancies in patients with essential thrombocythemia treated with busulphan and hydroxyurea: long-term follow-up of a randomized clinical trial. *Br J Haematol*. 2000;110:577-583.
 34. Marini A, Carulli G, Azzara A, Grassi B, Ambrogi F. Serum cholesterol and triglycerides in hematological malignancies. *Acta Haematol*. 1989;81:75-79.
 35. Pandolfino J, Hakimian D, Rademaker AW, Tallman MS. Hypocholesterolemia in hairy cell leukemia: a marker for proliferative activity. *Am J Hematol*. 1997;55:129-133.
 36. Gilbert HS, Ginsberg H, Fagerstrom R, Brown WV. Characterization of hypocholesterolemia in myeloproliferative disease: relation to disease manifestations and activity. *Am J Med*. 1981;71: 595-602.
 37. Aixala M, Sarandria CN, Speroni JG. Hypocholesterolemia in hematologic neoplasms. *Sangre (Barc)*. 1997;42:7-10.
 38. The Italian Cardiovascular Epidemiological Observatory. *Ital Heart J*. 2004;5(suppl 3):49S-92S.
 39. Sherwin RW, Wentworth DN, Cutler JA, Hulley SB, Kuller LH, Stamler J. Serum cholesterol levels and cancer mortality in 361,662 men screened for the Multiple Risk Factor Intervention Trial. *JAMA*. 1987; 257:943-948.