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FLUDARABINE IN UNTREATED AND PREVIOUSLY TREATED B-CLL PATIENTS: A REPORT ON EFFICACY AND TOXICITY

Mauro Spriano, Marino Clavio*, Paola Carrara*, Letizia Canepa*, Maurizio Miglino*, Ivana Pierri*, Lidia Celesti*, Edoardo Rossi, Renato Vimercati, Roberta Bruni, Gino Santini, Anna Maria Gatti*, Paola Stura*, Riccardo Ghio*, Franco Patrone*, Eugenio Damasio, Marco Gobbi*

*I Division of Hematology, S. Martino Hospital, Genoa; *Department of Internal Medicine, University of Genoa, Italy*

ABSTRACT

Background. **It has been shown that fludarabine (FLU) is superior to conventional treatment in B-CLL for rate and quality of response, leading to CR even at the molecular level. In this paper we report our preliminary results with this drug in B-CLL patients.**

Methods and Patients. **Twenty-seven B-CLL patients (16 refractory to previous therapy, 7 responsive and treated for subsequent disease reexpansion, 4 untreated with active disease) were administered FLU at a dose of 25 mg/sqm for 5 days every 4 weeks.**

Results. **Twenty-five patients were evaluable and 14 of them (56%) were responsive. All four untreated patients responded: 1 CR (PCR analysis showed the persistence of clonal VDJ rearrangement) and 3 PR, while 67% of the previously responsive group again showed a reaction: 2 PR (33%) and 2 nodular PR (33%). Among the refractory patients we recorded 6 responses (39%): 1 CR (6%) and 5 PR (33%). Besides 2 cases of lethal myelotoxicity, we observed 2 cases of encephalopathy and 2 cases of heart failure. Four deaths may have been related to FLU therapy (15%).**

Conclusions. **We confirm the effectiveness of FLU and the improved outcome, in terms of toxicity and response rate, it provides in untreated B-CLL patients. Further studies are needed to explore the possible negative effects of FLU on neuronal and heart function, and the impact of this drug on survival in selected groups of patients.**

Key words: fludarabine, CLL, toxicity, chemotherapy

B-cell chronic lymphocytic leukemia (B-CLL) has an extremely heterogeneous prognosis, ranging from cases with a benign clinical course whose survival is similar to that of an age-matched healthy control population (the so-called *smouldering CLL* according to Montserrat),[1,2] to those in advanced stages that show an aggressive course and a median survival of less than two years. These latter cases may achieve a transient response and enjoy a short period of progression free survival (PFS), either after standard chemotherapy with chlorambucil (CHL) plus prednisone (PDN)[3,4] or after more aggressive regimens such as cyclophosphamide-vincristine-prednisone (COP)[5,6] or CHOP.[7] Relapsing and resistant patients usually show a very poor prognosis, and their survival is scarcely affected by the chemotherapy regimens.[8,9]

Fludarabine monophosphate, 9-β-D-arabinofuranosyl 2-fluoroadenine-5' phosphate (FLU), a fluorinated analogue of vidarabine (ara-A) relatively resistant to deamination by adenosine deaminase,[10,11] has recently been used

Correspondence: Prof. Marco Gobbi, Cattedra di Ematologia DIMI, viale Benedetto XV 6, 16132 Genoa, Italy.
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Table 1. Clinical characteristics of patients.

Pt		Group	Sex	Age	time dx-tx	Rai S
1	FG	UN	M	45	26	1
2	OA	UN	M	69	24	2
3	VL	UN	F	52	1	2
4	CA	UN	M	60	12	4
5	BA	SR	F	68	48	1
6	BI	SR	F	72	24	4
7	GG	SR	F	61	56	4
8	SG	SR	M	48	14	4
9	LG	SR	M	53	44	4
10	FG	SR	M	48	62	2
11	BE	SR	M	45	58	4
12	BE	RD	M	63	3	3
13	BM	RD	F	70	4	2
14	VE	RD	F	75	132	4
15	SR	RD	M	56	8	4
16	VF	RD	M	57	13	2
17	NF	RD	M	58	30	2
18	AS	RD	M	58	132	4
19	TA	RD	F	74	65	3
20	LG	RD	M	59	103	4
21	RA	RD	F	59	114	3
22	DG	RD	M	51	34	4
23	BA	RD	F	72	162	4
24	TC	RD	F	44	66	4
25	MO	RD	M	45	79	1
26	GL	RD	M	46	50	0
27	VE	RD	M	54	42	2

Legend: Group = according to previous therapy; UN = non pretreated; SR = pretreated and relapsedm potentially sensitive disease; RD = pretreated resistant disease; Time dx-tx = time (months) from diagnosis to FLU treatment

extensively in the treatment of low grade lymphoproliferative diseases.[12-15] FLU therapy in CLL results in a response rate of 50 to 55% in previously treated patients, whereas the activity of this agent appears to be very impressive in untreated B-CLL patients, achieving a remission rate of about 70%.[16] Patients with refractory CLL seem to experience more toxicity and show a lower response rate.[17,18]

In December, 1990 we started a phase II trial using FLU in 23 previously treated and 4 untreated B-CLL patients; herein we report the results in term of response rate and toxicity, comparing pretreated patients, with responsive or resistant disease, and untreated ones.

Table 2. Treated patients (summary).

		Group UN	Group SR	Group RD
N. pts		4	7	16
Age (58)		45-60 (56)	45-72 (56)	44-75
Sex M/F		3/1	4/3	10/6
Time dx-flu treat.		1-26	4-62	3-132
RAI stage	0	0	0	1
	I	1	1	1
	II	2	1	4
	III	0	0	3
	IV	1	5	7

Materials and methods

From December, 1990 to June, 1993, twenty-seven patients with CLL were entered in a phase II clinical trial with fludarabine (FLU) as single agent led by the 1st Division of Hematology, Ospedale S. Martino and the Department of Internal Medicine, University of Genoa.

Diagnostic criteria

All patients fulfilled the NCIWG diagnostic criteria for CLL.[19] Monotypic expansion of lymphoid cells was confirmed by an immunophenotype analysis performed with a panel of monoclonal antibodies including CD3, CD4, CD8, CD5, CD10, CD19, CD20, CD56, HLA-DR, anti-k and anti-λ. Only patients with CD19, CD20, CD5-positive CLL were considered. Pretreatment evaluation included a medical history, physical examination, complete blood cell count, differential analysis, chemical survey, Ig quantitation, bone marrow aspirate and biopsy examination.

Inclusion criteria

Patients who fulfilled at least one of the following criteria were enrolled into our study:

1. presence of active disease, defined according to NCI criteria;[19]
2. refractoriness to an alkylating agent-containing regimen;
3. age less than 50 years.

Patient characteristics

Tables 1 and 2 summarize patient characteristics. Median age was 58 years with a range from 44 to 75. There were 17 males and 10 females. Distribution according to Rai stage (defined

Table 3. Therapeutic results.

PT	GR	N	R	TOX	RL	A/D	Cause of death	Surv.
1	UN	6	CR	–	3+	A	–	8+
2	UN	6	PR	–	16+	A	–	21+
3	UN	6	PR	–	4+	A	–	9+
4	UN	5	PR	–	1+	A	–	5+
5	SR	6	PR	–	6	D	progr.	21
6	SR	3	PR	–	10	D	progr.	18
7	SR	3	NR	Myel.	–	D	myel.	1
8	SR	1	NE	Myel.	–	D	myel.	1
9	SR	4	NR	–	–	D	progr.	20
10	SR	12	nPR	PN	1+	A	–	25+
11	SR	10	nPR	–	9+	A	–	22+
12	RD	4	PR	myel.	3	A	–	22+
13	RD	3	NR	heart	–	D	progr.	3
14	RD	1	NR	–	–	D	progr.	3
15	RD	3	NR	heart	–	A	–	13+
16	RD	6	PR	–	1+	A	–	7+
17	RD	3	NR	–	–	A	–	3+
18	RD	3	PR	–	1+	A	–	3+
19	RD	8	CR	–	22	A	–	32+
20	RD	6	NR	–	–	A	–	7+
21	RD	4	PR	–	10	D	progr.	19
22	RD	1	NE	CNS	–	D	CNS	1
23	RD	4	PR	CNS	2	D	CNS	4
24	RD	5	NR	–	–	D	progr.	7
25	RD	3	NR	–	–	D	progr.	21
26	RD	2	NR	–	–	D	progr	8
27	RD	2	NR	–	–	D	progr.	3

Legend: N = number of FLU cycles; GR = group; R = response Myel = myelotoxicity; NR = no response; R.L.= response length; nPR = nodular; PR A/D = alive/dead; PR = partial response SURV = survival (months); CR = complete response; PN = peripheral neuropathy; NE = not evaluable; CNS = central nervous system toxicity

before the start of FLU therapy) was: 0=1; 1=3; 2=7; 3=3; 4=13. Time from initial diagnosis of B-CLL to the beginning of FLU treatment ranged from 1 to 132 months.

At the start of FLU treatment three groups of patients could be distinguished according to previous therapy:

– four patients enrolled at diagnosis (untreated; UN);
– seven patients who had already received conventional therapy, and achieved a transient response lasting at least three months, followed by disease reexpansion (sensitive relapse; SR);
– sixteen patients with refractory disease, defined as lack of response or progression of disease after an alkylating agent-containing regimen (resistant disease; RD). Patients included in this group generally had a long history of treatment and advanced disease (stages III and IV), with some exceptions. Patient #26, although in stage 0, began receiving FLU because, following an extended period in which the disease was well controlled with alkylating agents, lymphocyte count soon increased and the patient became symptomatic and refractory to conventional therapy. Patients #12 and 13 were rapidly considered refractory (after 3 and 4 months) because of disease progression under conventional therapy (chlorambucil plus steroid, cyclophosphamide).

Treatment

FLU was administered as a single agent at a dose of 25 mg/sqm per day by 30-minute infusion for 5 consecutive days; treatment was repeated every 4 weeks for 6 courses. Complete evaluation was carried out after the third and sixth course and at the end of therapy, using the same tests employed before starting FLU. Patients with nodular partial response after the sixth course received further therapy for a maximum of 6 cycles, in order to reach a complete response.

Response criteria

Patients who completed at least three courses of therapy were considered evaluable for response. Those in whom FLU was stopped after 1 or 2 courses because of disease progression were considered non responders. Response criteria were previously defined by the NCIWG.[19]

In particular, complete response (CR) was defined by the disappearance of any palpable

Table 4. Responses, deaths, survival.

	Group UN	Group SR	Group RD
N. evaluable pts	4	6	15
CR (%)	1 (25)	0	1 (6)
PR (%)	3 (75)	2 (33)	5 (33)
nPR (%)	0	2 (33)	0
NR (%)	0	2 (33)	9 (60)
Deaths	0	5	9
Response length	1-16 (6)	1-10 (6)	1-22 (7)
Survival	4-21 (10)	1-25 (15)	1-32 (9,6)

masses, normalization of blood parameters with a neutrophil count greater than 1.5×10^9/L, platelets greater than 100×10^9/L, hemoglobin higher than 11 g/dL and less than 30% bone marrow lymphocytosis; immunophenotype analysis of bone marrow and peripheral blood mononuclear cells had to be negative for monotypic lymphoid cells. Nodular PR (nPR) differed from CR for the persistence of lymphoid nodules in the bone marrow only.[20]

Partial response (PR) required a decrease in the palpaple disease and lymphocyte count of more than 50% and a greater than 50% improvement in at least one of the abnormal blood parameters. Patients with stable (SD) or progressive disease (PD) were considered non responders (NR). Patients had PD if they showed an increase of more than 50% in organomegaly and/or lymphocyte count.

Toxicity was evaluated using the standard toxicity criteria developed by the ECOG.[21]

Molecular biology studies

High molecular weight DNA was extracted from bone marrow according to standard methods.[22] DNA amplification was performed according to Nizet et al.[23] Briefly, 40 cycles of PCR were performed using two oligonucleotides recognizing the V and J consensus sequences. The amplification products were analyzed on ethidium bromide-stained 2.5% agarose gel with a molecular weight marker. Normal bone marrow DNA and a mixture without DNA were analyzed in parallel with test DNA as control for contamination of reagents with PCR products.

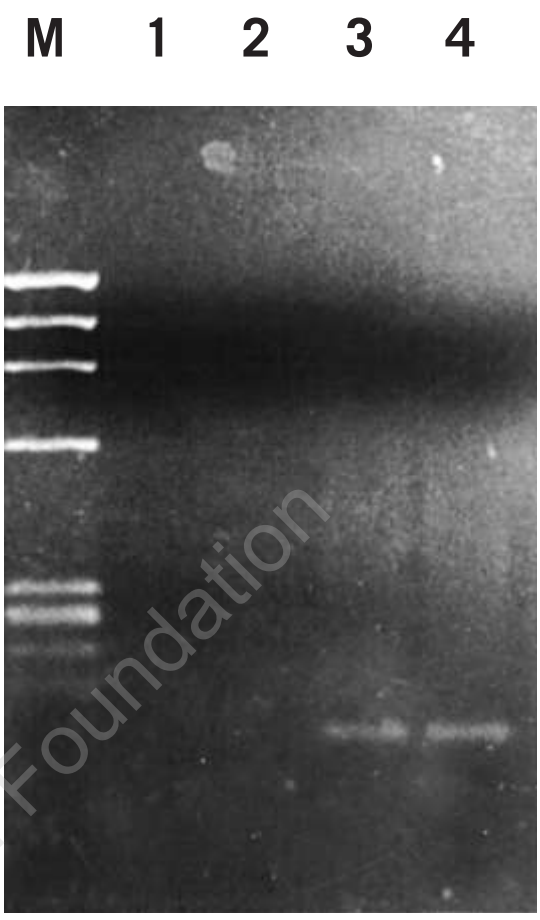

Figure 1. Detection of clonal VDJ rearrangement in patient #1. Lanes: M=marker; 1=amplification mix without DNA; 2 = normal; 3 = patient #1 after 3 cycles of therapy; 4 = patient #1 at the end of therapy.

Results

The date of analysis was September 30, 1993. Two of the 27 enrolled patients were not evaluable for response: 1 for severe myelotoxicity after the first cycle, 1 for central nervous system toxicity. Of the 25 evaluable patients, 4 were untreated, 6 relapsed and 15 had resistant disease.

Four patients did not complete the therapeutic program. The causes were: unavailability of FLU (patient #6), myelotoxicity (#12), patient refusal (#17 and #21). Therapy is ongoing in two patients (#4 and #18).

Tables 3 and 4 report data on responses, deaths, response length and survival.

Overall, 14 patients (56%) were responsive to the treatment: 2 reached CR (8%) and 12 PR (48%). All previously untreated patients responded (1 CR and 3 PR). Among the 6 relapsed patients we observed 4 responders (67%): 2 PR and 2 nPR; the other 2 (33%) were resistant. Among the 15 refractory patients, we recorded 6 responses (39%) (1 CR and 5 PR). The other 9 in this group were non responders (60%).

Biomolecular analysis carried out at the end of FLU treatment in the patients of the first group who achieved complete remission, docu-

mented the persistence of clonal VDJ rearrangement (Figure 1).

Concerning the RAI stage, we observed 2/2 responders in low stage (I-II) and 2/4 responders in advanced stage (III-IV); in refractory patients we obtained 1/6 responders in stage I-II and 5/9 in stage III-IV.

All the untreated patients are still maintaining the response after 1, 3, 4, 16 months of follow-up. Among relapsed patients, 2 responders showed disease reexpansion, at 6 and 10 months, whereas 2 are still maintaining response at 1 and 9 months (both are nPR). Four refractory patients relapsed at 1, 1, 10 and 22 months; the other 2 responders have a follow-up of only one month.

Toxic effects

In untreated patients no toxic effects were recorded. Two cases of severe and lethal myelotoxicity (WHO 4) were observed in the relapsed group. In one patient (case 10, Table 3) a peripheral neuropathy was well documented with clinical and neurophysiological studies; it developed after 4 courses of FLU therapy, was mild and transient and caused only a delay of the therapeutic program.

In two refractory patients we recorded, after 1 and 4 cycles of therapy, the onset of a neurologic syndrome characterized by progressive mental deterioration, psychomotor restlessness, worsening mental confusion, coma and death, without focal signs at neurological examination. Instrumental data were unfortunately not available: in one case the patient was at home at the beginning of the syndrome and relatives refused hospitalization; in the other the clinical condition of the patient rapidly worsened so that EEG, CAT and NMR could not be performed.

Moreover, two patients in this group developed congestive heart failure after 1 and 3 courses of therapy; in one patient a severe reduction in the ejection fraction was documented.

Fourteen out of 27 treated patients died (5 and 9 in the relapsed and refractory groups, respectively). In the group of relapsed patients the causes of death were progression (3 pts, 2 of which after PR) and myelotoxicity (2 pts); in refractory patients the causes were progression (7 pts, one of which after a PR), and CNS toxicity (2 pts). Besides progression of disease other complications which contributed to patient deaths were infections (1 pneumonia, 2 sepsis), heart failure (2 cases), stroke (1 case), liver failure (1 case).

Discussion

The treatment of CLL has not changed significantly during the last two decades; combination chemotherapy regimens such as CHOP have not yielded better results than those obtained by chlorambucil, with or without steroid.[6-8] On the other hand, α-interferon, which is very effective in hairy cell leukemia (HCL),[24] is active in early phases of the disease when low-dose CHL or even no treatment at all are recommended.[25-28]

Flu is the only new agent whose activity emerged as clearly superior to conventional treatment in preliminary reports,[12-16, 29] both in terms of percentage of responding patients and quality of response. In fact, fludarabine produced a considerable number of CR, which were evaluated according to more stringent criteria than in the past. Moreover, in some cases the disappearance of the neoplastic clone was documented even at the molecular level after treatment with FLU.[30,31]

This paper reports our initial experience in CLL patients. Although the overall results are comparable with other similar reports some points deserve further discussion.

First, while all previously untreated patients responded, as expected,[17] previous treatment reduced the percentage of response. However, it should be pointed out that 1 CR of long duration (22 months) was recorded even in resistant group. In the present series a correlation between the number of previous treatments and response to FLU, as described by De Rossi et al.[9] was not evident, nor was the impact of clinical stage on response rate clear, in keeping with an observation by Zinzani et al. in a similar study.[17] Moreover, in our experience other prognostic parameters such as age and time

from diagnosis and FLU treatment did not seem to influence the outcome of therapy. The limited number of enrolled patients and the short follow-up period do not allow confirmation of the better trend toward progression-free survival in untreated and relapsed patients observed by others.[18, 32]

Second, toxicity was not negligible and 4 patients (15%) died as a direct effect of treatment. Similar figures for toxic related effects are reported in all previously published experiences.[13,14,33] Notably, 2 toxic deaths were due to myelotoxicity, which is the major cause of death in reported series.[9,17,18] Interestingly the cause of death in the remaining 2 patients was CNS toxicity. This complication was relatively frequent in early clinical studies in acute leukemia patients where the dosage of the drug was at least three times that used in all studies in CLL.[34,35] CNS toxicity has rarely been observed at similar dosages and usually transient.[36] The extremely severe toxic effects seen in these two patients may be linked to a particular sensitivity to the drug or to the coexistence of an unsuspected factor favoring the devastating effect of FLU on CNS. A similar neurologic complication was observed by our group in a patient affected by mycosis fungoides after the first course of the Fludarabine program.

A non lethal complication observed in two additional patients was the development of congestive heart failure. To the best of our knowledge this is the first report of this adverse effect. More data are necessary to establish whether the occurence of this phenomenon is related to FLU therapy or merely occasional.

Finally, as FLU is able to induce real CR, it appears mandatory to study these events with more sophisticated parameters than standard morphologic assessment of bone marrow aspirates and biopsies; even immunophenotipic evaluation of minimal residual disease may be inadequate. Molecular biology studies using PCR appear at present the most suitable methodology to evaluate these cases. In patient #2, who obtained CR after FLU as first treatment, the persistence of the clone was seen with PCR amplification of VDJ rearrangement; however the complete disappearance of the disease after FLU treatment was documented by Johnson et al.[31] It would be of interest to evaluate the real weight of this finding in the long term outcome of treated patients. For this purpose it could be helpful to distinguish *hematological CR* from *PCR CR*.

In conclusion, FLU is an effective drug, both in untreated cases and in previously sensitive to conventional therapy or refractory patients, even if the best results are reached in untreated patients. However, the toxic effects of the drug appear remarkable, especially on bone marrow, CNS and possibly heart. It seems reasonable to avoid FLU administration in patients with severely impaired bone marrow function (i.e. platelets $< 50\times10^9$/L). The reported suspected toxicity on CNS demands for further studies on the possible effects of this drug on cerebral metabolism and neuronal function. It should be underlined that another purine nucleoside analogue, 2-chloro-2'-deoxyadenosine (CdA) has been reported to be very usefull in chronic lymphoproliferative disorders. Randomized studies compairing FLU and CdA will indicate which of these two agents could be most appropriate for the treatment of B-CLL patients refractory to conventional therapy, also considering the minor toxic effects reported by some authors for CdA.[37,38]

Further clinical trials, with considerable follow up are needed to understand if FLU can improve survival in certain groups of patients (young patients and/or patients with aggressive disease). A biomolecular approach is needed to define with more precision the kind of clinical responses and the relationship with time to progression and survival.

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HEMATOLOGICAL RECOVERY AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR HIGH-GRADE NON HODGKIN'S LYMPHOMAS: A SINGLE CENTER EXPERIENCE

Maria Cristina Miggiano, Filippo Gherlinzoni, Giuseppe Visani, Annarita Belardinelli, Paolo Ricci, Gianantonio Rosti, Patrizio Mazza, Maria Rosa Motta, Simonetta Rizzi, Roberto Massimo Lemoli, Pier Luigi Zinzani, Sante Tura*

*Institute of Hematology "L. & A. Seràgnoli", St. Orsola Hospital, University of Bologna; *Department of Hematology, Ospedale Civile di Taranto, Italy*

ABSTRACT

Background. **Both rhGM-CSF and rhG-CSF can accelerate hematological recovery after high-dose therapy and autologous bone marrow transplantation in patients with high grade non Hodgkin's lymphoma and reduce transplant-related morbidity after ABMT.**

Methods. **The clinical course of 23 non randomized patients was analyzed and compared with a historical control group of 10 patients. Ten patients received GM-CSF at a dose of 10 μg/kg in a 6-h IV infusion, and 13 received G-CSF at a dose of 5 μg/kg subcutaneously. Control patients received no GFs.**

Results. **Mean granulocytic recovery to 0.5×10^9/L was obtained 13.1±3.2 days after marrow reinfusion in the G-CSF arm vs 16±2.7 in GM-CSF pts ($p = 0.03$) and vs 19.6±7.6 in controls ($p < 0.01$); this reduction led to a statistically significant shorter duration of fever and parenteral antibiotic therapy. Platelet recovery to 20×10^9/L was not significantly influenced by GFs.**

Conclusions. **These results indicate that only G-CSF accelerates hematological recovery after high-dose chemotherapy and autologous bone marrow transplantation and induces a significant decrease in terms of infection morbidity and duration of hospital stay.**

Key words: high-grade non Hodgkin's lymphoma, autologous bone marrow transplantation, growth factors

Intensive chemotherapy (CHT) followed by autologous bone marrow transplantation (ABMT) finds progressively widespread utilization in the treatment of several hematological malignancies and solid tumors.[1,2] Unfortunately, ABMT is still associated with a relatively high rate of morbidity and mortality because of severe myelosuppression.[3] In fact, the most frequent causes of transplant-related death are infections, followed by the hemorrhagic syndrome and major organ toxicity.[4,5] The incidence of severe infections is strictly related to the duration of neutropenia. Recombinant human growth factors (GFs) are a family of glycoproteic hormones that regulate blood cell production and differentiation.[6] In particular, granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) are now commonly used to accelerate neutrophil recovery after myeloablative chemotherapy[7] and ABMT.[8-12] Here we report our experience with 23 non-randomized patients (pts) with high grade non Hodgkin's lymphoma (NHL) submitted to ABMT and treated with GFs immediately after marrow reinfusion. Their hematological recovery was compared with a historical control group of 10 matched pts who did not

Correspondence: Dr. Maria Cristina Miggiano, Institute of Hematology "Lorenzo & Ariosto Seràgnoli", Policlinico S. Orsola, via G. Massarenti 9, 40138 Bologna, Italy. Tel. international +39.51.390413, Fax international +39.51.398973.
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