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A prospective study of α-interferon and autologous bone marrow transplantation in chronic myeloid leukemia

THE ITALIAN CO-OPERATIVE STUDY GROUP ON CHRONIC MYELOID LEUKEMIA
WRITING COMMITTEE: GIOVANNA MELONI,* DOMENICO RUSSO,° MICHELE BACCARANI,° NICOLETTA TESTONI,# GIOVANNI MARTINELLI,# RENATO FANIN,° ELIANA ZUFFA,@ GIANANTONIO ROSTI,# GIULIANA ALIMENA,* GIUSEPPE SAGLIO,^ FRANCO MANDELLI,* SANTE TURA#
**Department of Cellular Biotechnologies and Hematology, University "La Sapienza", Rome; °Division of Hematology and Department of Bone Marrow Transplantation, Udine University; #Department of Hematology and Medical Oncology "L. and A. Seràgnoli", University of Bologna; @Division of Hematology, S. Maria delle Croci Hospital, Ravenna; ^Department of Internal Medicine, University of Turin, Italy*

ABSTRACT

Background and Objective. **α-interferon (αIFN) can induce cytogenetic remissions in chronic myeloid leukemia (CML). Hemopoietic progenitors can be collected from the marrow in remission and utilized for autologous repopulation after high dose chemotherapy. This study was designed with the purpose of evaluating the feasibility of a combined treatment policy of αIFN followed by autologous bone marrow transplantation (autoBMT).**

Design and Methods. **A prospective study of αIFN and autoBMT was begun in 1989. Two hundred and seventy-two consecutive previously untreated non-blastic Ph positive chronic myeloid leukemia (CML) patients, who were less than 56 years old, were enrolled over a 3-year period (1989-1991) and were assigned to receive human recombinant αIFN 2a (Roferon-A) at a dose of 9 MIU daily for at least one year. If they achieved a cytogenetic response consisting in a percentage of Ph neg metaphases of more than 25%, they were eligible for marrow harvesting and subsequent autografting after high dose busulfan (16 mg/kg) and melphalan (60 mg/m²).**

Results. **Seventy-six patients (28%) were eligible for a marrow harvest but the marrow was harvested in only 37 cases (14%), and only twenty-three patients (8%) were actually autografted. One patient died of infection nine days after autoBMT. The other patients recovered and did not suffer any late adverse events. Five patients progressed to blastic phase, six are alive in complete hematologic remission and eleven are alive in complete hematologic and cytogenetic remission. αIFN treatment was reinstituted after autoBMT in 18 of 22 cases, but four patients who are in continuous complete cytogenetic remission were not given αIFN anymore. The progression-free survival of the autografted patients is 65% 8 years after registration.**

Interpretation and Conclusions. **This study shows that bone marrow hemopoietic progenitors (Ph neg and Ph pos) can be collected from patients who respond to αIFN and can be used to rescue hemopoietic activity after high dose chemotherapy. Though some complete and durable cytogenetic remissions were obtained, the treatment could be applied only to a small group of good risk patients, highlighting that selection is very important and results cannot be extrapolated to the average patient.**

Key words: chronic myeloid leukemia, interferon, bone marrow transplantation

Correspondence: Michele Baccarani, M.D., Division of Hematology, Udine University Hospital, 33100 Udine, Italy.
Phone: international +39-0432-559662-4 – Fax: international +39-0432-559661 – E-mail: ematologia@drmm.uniud.it

The term autologous bone marrow transplantation (autoBMT) covers a number of procedures based on intense cytotoxic treatment followed by the reinfusion of autologous hemopoietic progenitors to *rescue* the patient from marrow aplasia. In chronic myeloid leukemia (CML) autoBMT was first proposed and applied with the aim of reverting leukemia from the blastic to the chronic phase.[1,2] The intervention was successful in some cases and was subsequently also applied during the chronic phase, because it was observed that the harvest, both from the marrow and from the peripheral blood, contained more Ph neg cells than expected and that Ph neg cells could prevail over Ph pos. cells, leading in some cases to a partial or even complete cytogenetic response, albeit transient.[3-7]

In the mid eighties it was reported that α-interferon (αIFN) could induce hematologic and cytogenetic responses[8] and the Italian Group promoted a prospective study for the comparison of αIFN with conventional chemotherapy.[9,10] When the group members realized that a substantial number of patients achieved a cytogenetic response with αIFN, it was decided to promote another study with the purpose of evaluating whether the cytogenetic response to αIFN could be exploited and improved

by collecting the hematopoietic cells at the time of the response and by reinfusing them after high dose chemotherapy. At the time the study was planned it was not clear whether αIFN itself was a good treatment nor was it known whether an efficient marrow harvest could be obtained after αIFN nor whether the procedure of autoBMT would be safe or would create unexpected difficulties. The planned study was, therefore, prospective, but not randomized, and was designed with the aim of evaluating the feasibility and identifying the complications of a policy of autoBMT in αIFN responsive patients on a nation-wide basis. This is the final analysis of the study, which has previously been reported only at specialized meetings.[11] The analysis shows that the procedure is feasible and that some long term results are good, but highlights that there is a very important selection, based not only on disease features but also on patients' and doctors' compliance and logistic factors.

Design and Methods

Treatment protocol

The treatment protocol prescribed a short treatment with hydroxyurea (HU) 1,500 mg/sqm/day until the white blood cell count (WBC) fell below 10×10^9/L, followed by one-year treatment with human recombinant interferon α2a (Roferon-A) given s.c. or i.m. at a dose of 3 MIU/day for the first 2 weeks, 6 MIU/day for the third and fourth week, and 9 MIU/day thereafter. After one year the cytogenetic response was assessed by evaluating at least 20 marrow metaphases. If the percentage of Ph neg metaphases was more than 25% the protocol provided for marrow cell collection and autoBMT. Any subsequent treatment was at the investigators' discretion, both for the patients who were autografted and for those who were not. The dose of αIFN was adapted to tolerance, as previously reported.[9,10] HU could be administered at the investigators' discretion at any time, if and when it was felt to be necessary or useful for disease control. An option for allogeneic bone marrow transplantation (alloBMT) was open at any time and in any phase of the disease.

Marrow cells were harvested by multiple iliac bone punctures under general or spinal anesthesia, separated and cryopreserved according to local institutional protocols. The minimum number of nucleated cells that was required to proceed to the transplant was 1.0×10^8/kg body weight. Treatment for autoBMT was busulfan 16 mg/kg p.o. in 4 days and melphalan 60 mg/sqm i.v., single dose. For autoBMT the patients were cared for and nursed according to local institutional protocols, in reverse-isolation wards, and received supportive treatment as required.

Eligibility criteria

Eligibility criteria for this protocol were that the patients had Ph pos chronic phase CML, were less than 56 years old, were previously untreated or only minimally pretreated with conventional chemotherapy for less than 6 months and that they gave their informed consent to treatment, in a written or oral form, according to local institutional rules. Exclusion criteria included presentation in accelerated or blastic phase, pregnancy and any detectable disease or condition that at the investigators' discretion was a contraindication to the scheduled treatment.

Definitions and techniques

For the purpose of this study, the accelerated and blastic phases of leukemia were considered together and were defined by at least two of the following criteria: a peripheral blood sample containing more than 10% blast cells or more than 30% blast cells and promyelocytes; a bone marrow aspirate containing more than 15% blast cells or more than 50% blast cells and promyelocytes; a spleen that could be palpated more than 10 cm below the costal margin with a WBC of less than 25×10^9/L; involvement of the central nervous system, bone, lymph nodes or other extrahaematologic sites; and cytogenetic abnormalities including double Ph, trisomy 8 or isochromosome 17. The patients were stratified by risk using the formula proposed by Sokal *et al.*[12] that is based on age, spleen size, platelet count and percentage of blast cells in peripheral blood. The cytogenetic response was defined according to the proportion of Ph neg metaphases, as complete (Ph neg 100%), major (Ph neg 66-99%), minor (Ph neg 33-65%), minimal (Ph neg 1-32%) or none (Ph neg 0). A cytogenetic study was done at registration and after the first year of αIFN treatment. Thereafter, a study was repeated at least every 6 months in the patients who were autografted and at least every 12 months in the patients who were not autografted and were in complete hematologic response.

Qualitative reverse-transcriptase polymerase chain reaction (RT-PCR) was performed as described elsewhere.[13,14] RNA was extracted from mononuclear cells that were separated from the marrow on Ficoll gradient, kept in a guanidinium isothiocyanate solution, stored and shipped at –20°C. The quality and the quantity of RNA was checked by gel electrophoresis analysis and spectrophotometric measurement for integrity and quantity, cDNA was prepared from 1 µg RNA templates. The amplification cycles and the sensitivity of this assay (about 1×10^{-6} cells) have already been reported.[13,14] In some patients, a competitive assay described by Cross *et al.*[15] was used, with some modifications,[16] with a sensitivity close to 1×10^{-8} cells.

Statistics

Survival was calculated by the method of Kaplan and Meier[17] from the date of autoBMT, or from the date of registration, to death or to last contact. All the cases were updated as of December 1997; no patient was lost to follow-up and the observation period of the 120 living patients ranges between 70

and 120 months (median 92 months). The rates of other events, such as progression to accelerated or blastic phase, loss of response and so on, were calculated by the same method. Since the study was designed with the purpose of evaluating the feasibility of a policy of autoBMT in the patients who were responsive to αIFN and not of establishing whether a particular treatment was better or worse, any comparisons between treatment results in this report are descriptive and not statistically evaluable.

Results

Case selection

Six hundred and seventy-six consecutive patients were registered during the study period. Two hundred and seventy-seven (41%) were not eligible because of their age and 68 (10%) because of other causes, such as presentation in accelerated or blastic phase (21 cases), other diseases (13 cases), pregnancy (3 cases), refusal (20 cases) and other reasons (11 cases). Therefore 331 patients were eligible, but 39 were not enrolled because they were immediately referred for alloBMT and 20 for several other reasons. The remaining 272 patients were enrolled, corresponding to 40% of total registered patients and to 82% of total eligible patients. Ninety of the 272 enrolled patients (33%) did not complete the scheduled year of αIFN treatment. Of the remaining 182 patients, 174 were evaluated cytogenetically and 68 were eligible for marrow harvest (i.e. had more than 25% Ph neg metaphases). However, only 37 patients were harvested and only 23 completed the whole treatment protocol and proceeded to autoBMT. The main reasons that were given for justifying the exit from the treatment protocol are listed in Table 1. Opting for alloBMT accounted for the loss of 76 patients (23%). In 45 cases continuation of the treatment plan was refused, at the beginning because it was thought that chemotherapy would be better than αIFN and later because it was thought that the continuation of αIFN could be more appropriate than autoBMT. In 33 cases the protocol was not applied for technical reasons, and in 23 cases because of the side-effects and the complications of the treatment. True treatment failure overall accounted for the loss of 39% of cases, either due to early progression to blastic phase (21 cases) or lack of cytogenetic response (106 cases). The progressive selection of the cases autografted is shown in Table 2; an autoBMT was performed in 7% of the cases who were eligible, in 8% of the cases who were enrolled, in 13% of the cases who were evaluable for marrow harvest and in 62% of the patients who were harvested. The selection was not by chance, but was related to the cytogenetic response and the risk. Table 3 shows that none of the 8 patients with a minimal cytogenetic response (Ph neg 25-32%) was actually autografted, vs. 14% of those with a minor response (Ph neg 33-65%) and 57% of those with a major response (Ph neg 66-99%). Table 4 shows that low risk patients had twice as high a probability of autografting, while autoBMT was actually performed in only very few intermediate and high risk patients.

αIFN treatment

The details of the cytogenetic response after one year of αIFN treatment are reported in Table 5. Over-

Table 1. Main reasons for abandoning the treatment protocol at each subsequent protocol step. Only 23 of the 331 eligible patients were actually autografted.

	Before enrolment	*Before or during αIFN treatment*	*Before marrow harvest*	*After marrow harvest*	*Total*
No. of cases	331	272	76	37	331
Refusal or choice of another treatment					
alloBMT	39 12%	31 11%	5 6%	1 3%	76 23%
other treatments	0	20 7%	16 21%	9 24%	45 13%
Technical problems					
karyotype non evaluable	0	//	8 10%	//	8 2%
marrow fibrosis	0	//	4 5%	0	4 1%
logistic and other	20 6%	0	0	1 3%	21 6%
Complications and side-effects					
αIFN side effects	//	18 7%	//	//	18 5%
other complications	//	0	5 6%	0	5 1%
Treatment failure					
progression to blastic phase	//	21 8%	0	0	21 6%
no cytogenetic response (Ph neg < 25%)	//	106 39%	//	//	106 32%
rapid loss of cytogenetic response	//	0	1 1%	3 8%	4 1%
TOTAL	59 18%	196 72%	39 51%	14 38%	308 93%

Table 2. The table shows the progressive selection of the patients who were actually submitted to autoBMT. It should be remembered that during the 3-year study period, another 345 patients were registered but were not eligible because of age or other reasons.

	A	B	C	D	E	F	G
(No. of cases)	(331)	(272)	(182)	(174)	(68)	(37)	(23)
Percent eligible	100	82	55	52	20	11	7
Percent enrolled	//	100	67	64	25	14	8
Percent completed 1 year of αIFN	//	//	100	96	37	20	13
Percent evaluable for harvest	//	//	//	100	39	21	13
Percent eligible for harvest	//	//	//	//	100	54	34
Percent of harvested	//	//	//	//	//	100	62

A = Eligible; B = Enrolled; C = Completed 1 year of αIFN; D = Evaluable; E = Eligible for harvest; F = Marrow harvested; G = AutoBMT.

Table 3. The distribution by cytogenetic response of the patients who were harvested and autografted shows a trend for a negative relationship, the smaller the response the higher the probability of skipping the procedure. However, 6 of the 10 patients with a complete cytogenetic response were either not harvested (n = 4) or not autografted (n = 2), mainly because their physicians advised the patients to continue treatment with αIFN.

Grade of cytogenetic response	Minimal	Minor	Major	Complete	Total
(Ph neg metaphases)	(25-32%)	(33-65%)	(66-99%)	(100%)	(25-100%)
Total eligible	8 100%	21 100%	28 100%	10 100%	67 100%
Total harvested	3 38%	8 38%	20 71%	6 60%	37 54%
Total autoBMT	0 //	3 14%	16 57%	4 40%	23 34%

all, the median total dose of αIFN was 1,570 MIU/sqm b.s. vs. 1,800 scheduled, or 4.2 MIU/sqm b.s./day vs. 4.9 scheduled. The administered dose was ≥ 75% of the scheduled dose in 65% of cases, 50 to 75% in 20% of cases and less than 50% of scheduled in 15% cases. αIFN was discontinued for ever in 18 cases because of side-effects (Table 1) and was discontinued temporarily one to three times in another 40 cases. Hydroxyurea or other drugs were administered temporarily in 78 and 4 cases respectively.

Marrow harvest and autoBMT

Sixty-eight patients were eligible for marrow harvest based on cytogenetic response (Table 3) but the marrow was harvested from only 37 of 68 cases (54%) and the autoBMT was performed in only 23 of these 37 cases (62%). The reasons that were given to justify not performing either the harvest or the autoBMT are listed in Table 1. The median time from αIFN discontinuation to marrow harvest was 26 days (range 7 to 90 days) and the median number of cells that were cryopreserved was 1.7×10^8/kg b.w. (range 0.8 to 3.2) for total nucleated cells and 4.2×10^4/kg b.w. (range 0.2 to 15.0) for granulocyte-monocyte colony-forming cells. The median time from marrow harvest to autoBMT was 1 month, but two patients were transplanted much later, one after 13 and one after 26 months. One patient died of Gram negative septicemia 9 days after autoBMT. Another patient was still completely aplastic one month after autoBMT, was reinfused with autologous peripheral blood cells that were collected at diagnosis, and recovered with a Ph pos hemopoiesis. The hematologic recovery of the remaining 21 patients is shown in Table 6. The patients were discharged 18 to 50 days after autoBMT (median 28 days). No major complications were observed subsequently, and the performance status (Karnofsky's index) remained ≥ 90% in all patients.

Table 4. The distribution of the patients by risk (Sokal *et al.*, 1984) was identical at enrolment and after one year of αIFN treatment. Subsequently, low risk patients became prevalent and accounted for 74% of the autografts.

	relative risk						
	low		intermediate		high		total
At enrolment	128	47%	83	30%	61	22%	272 100%
At the end of one year of aIFN	91	50%	55	30%	36	20%	253 100%
Eligible for harvest	41	61%	20	30%	6	9%	67 100%
Harvested	29	76%	5	13%	3	8%	37 100%
Autografted	17	74%	3	13%	3	13%	23 100%

Table 5. Cytogenetic response rate after the completion of one year of αIFN treatment.

Grade of cytogenetic response	*No. of cases*	*% of enrolled (n =272)*	*% of evaluable (n = 174)*
None (Ph neg 0)	69	25%	40%
Minimal (Ph neg 1-32%)	46	17%	26%
Minor (Ph neg 33-65%)	21	8%	12%
Major (Ph neg 66-99%)	28	10%	16%
Complete (Ph neg 100%)	10	4%	6%
TOTAL	174	64%	100%

Table 6. Hematologic recovery after autoBMT. No growth factors were used.

Time to	*Mean±SD (days)*	*Median (days)*	*Interval (days)*
Hb > 100 g/L	75±59	70	0–238
neutrophils > 0.5×10^9/L	25±10	22	14–46
neutrophils > 1.0×10^9/L	32±11	29	8–51
neutrophils > 2.0×10^9/L	73±107	34	24–491
platelets > 20×10^9/L	24±15	27	0–64
platelets > 50×10^9/L	64±67	34	14–276
platelets > 100×10^9/L	99±80	73	25–303

Course and treatment of the patients who were submitted to autoBMT

Figure 1 shows overall survival and progression-free survival after autoBMT; after 7 years 71% of patients were alive and 65% were progression-free. Figure 2 shows the time from autoBMT to loss of complete hematologic response and to loss of complete or major cytogenetic response; after 7 years 58% of the patients were in continuous complete hematologic remission and 55% in continuous complete or major cytogenetic remission.

Table 7 reports the cytogenetic course and status of the patients. Leaving apart the patient who died of autoBMT, the complete cytogenetic response rate increased from 3/22 (14%) before autoBMT to 16/22 (73%) after the autoBMT. αIFN was given again in 18/22 cases, 3 to 48 months (median 19 months) after autoBMT for several reasons, including the failure to achieve or to maintain the cytogenetic response or decrease of the cytogenetic response from complete to major. The dose of αIFN was 3 MIU and was given either daily or every other day. After αIFN six patients failed to improve, two patients remained stable and the cytogenetic response recovered or improved in eight. Overall, a complete cytogenetic response after autoBMT was achieved in 17/22 cases and is currently maintained in 11/22 cases. It should be noted (Table 7) that four patients were in continuous complete cytogenetic remission 51 to 91 months after autoBMT, without having received any subsequent treatment. During complete cytogenetic remission nested PCR allowed detection of the bcr/abl transcript in all seven patients who were tested with a more sensitive technique, while five of the ten patients who were tested with the less sensitive technique were occasionally or continuously negative.

Course and treatment of the patients who did not proceed to autoBMT

The number of the patients who did not proceed to autoBMT was 249. Twenty-one of them had progressed to accelerated or blastic phase during the first year. Sixty-eight patients were treated with alloBMT in chronic phase. Twelve patients were submitted to a non-scheduled procedure of autoBMT, 8 to 65 months after registration, the cells being collected from the marrow (5 cases) or from the peripheral blood without or with prior chemotherapy (3 and 4 cases respectively). Cytogenetic response improved, from minor to major, in only one of these 12 patients. The remaining 148 patients have been treated either with chemotherapy (HU in 41 cases, HU and BUS in 10 cases), with HU and αIFN (64 cases) or with αIFN alone (33 cases). In these patients the number of complete cytogenetic responses increased from 6 at one year to 18 after more than one year.

Allo BMT was performed in chronic phase in 68 of 272 patients (25%), 4 to 75 months after registration (median 14 months). The median age at alloBMT was 35 years. Fifty-two patients received the marrow from an HLA-identical sibling, with T-depletion in 14 of 52 cases. Four patients received T-depleted marrow from a partially matched family

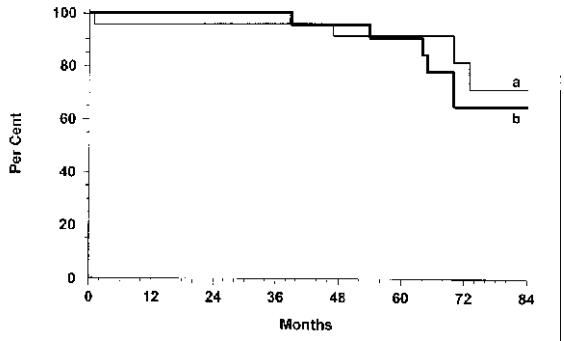

Figure 1. Survival (a) and progression-free survival (b) of the 23 autografted patients. Kaplan-Meier's plot from the date of autoBMT. The median observation time of living patients is 73 months, with 8 cases still alive and in chronic phase after more than 84 months.

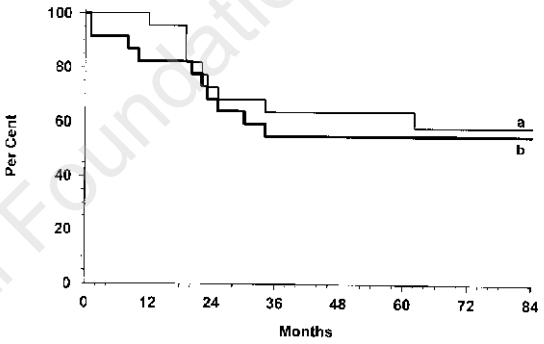

Figure 2. Time to the loss of complete hematologic response (a) and to the loss of complete or major cytogenetic response (b) in the 23 autografted patients. Kaplan-Meier's plot from the date of autoBMT. The median observation time of non-relapsed patients is 72 months, with 6 cases still in remission after 72 months.

Table 7. The twenty-three patients who were autografted are ordered according to the degree of the last cytogenetic response. One patient died of autoBMT, 3 patients died in blastic phase, 2 patients are alive in accelerated phase, 6 patients are alive in chronic phase and the remaining 11 patients are alive in complete hematologic and cytogenetic remission. Eighteen patients were treated again with αIFN, 3 to 48 months after autoBMT (median 19 months).

Case No.	*Cytog. response before autoBMT^*	*Best cytog. resp. after autoBMT, before αIFN^*	*αIFN after autoBMT°*	*Cytog. resp. at the reinstitution of αIFN^*	*Best cytog. resp. after αIFN^*	*Best cytog. resp. overall^*	*PCR#*	*Last cytogenetic response^ and status@*
1/101	100%	/ /	/ /	/ /	/ /	100%	ND	/ /, died of autoBMT
2/82	35%	63%	19 m.	0	20%	63%	+	0, died in ABP
3/103	44%	48%	8 m.	5%	0	48%	+	0, died in ABP
4/85	78%	90%	8 m.	33%	25%	90%	+	0, died in ABP
5/3	75%	88%	10 m.	28%	20%	88%	+	0, alive in ABP
6/37	100%	100%	7 m.	70%	11%	100%	+*	0, alive in ABP
7/272	80%	100%	24 m.	85%	85%	100%	+*	0, alive in CHR
8/60	80%	100%	24 m.	12%	20%	100%	+	20%, alive in CHR
9/190	70%	90%	25 m.	25%	85%	90%	+	85%, alive in CHR
10/104	94%	100%	26 m.	55%	100%	100%	+	90%, alive in CHR
11/33	66%	100%	19 m.	92%	96%	100%	+*	92%, alive in CHR
12/22	78%	98%	9 m.	81%	100%	100%	+*	97%, alive in CHR
13/253	83%	100%	29 m.	0	100%	100%	+	100%, alive in CHR and CCR
14/52	100%	100%	3 m.	81%	100%	100%	+*	100%, alive in CHR and CCR
15/152	80%	100%	5 m.	75%	100%	100%	±	100%, alive in CHR and CCR
16/130	100%	100%	10 m.	94%	100%	100%	+*	100%, alive in CHR and CCR
17/89	82%	100%	25 m.	95%	100%	100%	+*	100%, alive in CHR and CCR
18/218	95%	100%	39 m.	95%	100%	100%	±	100%, alive in CHR and CCR
19/182	90%	100%	48 m.	70%	100%	100%	+	100%, alive in CHR and CCR
20/78	81%	100%	/ /	/ /	/ /	100%	+	100%, alive in CHR and CCR
21/84	57%	100%	/ /	/ /	/ /	100%	-	100%, alive in CHR and CCR
22/86	69%	100%	/ /	/ /	/ /	100%	-	100%, alive in CHR and CCR
23/250	90%	100%	/ /	/ /	/ /	100%	-	100%, alive in CHR and CCR

^Cytogenetic response is given as percent of Ph neg metaphases; °time (months) from autoBMT to the subsequent administration of αIFN.; #PCR = nested polymerase chain reaction, with a sensitivity of about 1x10^6 and of about 1x10^8 in the cases that are marked with an asterisk; @ABP = accelerated or blastic phase, CHR = complete hematologic remission, CCR = complete cytogenetic remission.

donor and the remaining 12 patients received unmanipulated marrow from a matched unrelated person. The overall survival at 7 years after alloBMT ranges from 65% for the patients who received unmanipulated marrow from an HLA-identical sibling to 28% and 37% for the patients who received T-depleted marrow and for those who received marrow from an unrelated person, respectively.

Discussion

This study confirms the therapeutic effects of αIFN and provides specific information concerning the feasibility of a combined program of αIFN and autoBMT in Ph pos CML, but also raises and brings into focus the important, general problem of the selection that can take place during the execution of an advanced and a complex treatment program and of the influence of that selection on treatment results.

The questions that were asked when the protocol was planned in 1988 have been answered, because it has been shown that the early chronic phase CML patients who are responsive to αIFN can be submitted to a policy of treatment intensification with autologous hemopoietic progenitor rescue. The progenitors could be collected from the marrow after one year of αIFN treatment at a median dose of 4.2 MIU/sqm b.w./day, without resorting to chemotherapy or growth factors. Transplant-related mortality was 4% (1/23). Hemopoietic reconstitution was stable and the subsequent course was not complicated by either infections or any other adverse events.

Sixty-five per cent of the 23 patients who were actually transplanted were alive and progression-free 7 years after autografting and 8 years after registration. These patients were expected to have a good prognosis because many of them (17/23) were low risk by Sokal's score and all of them were responsive to αIFN.[18] As a matter of fact, their survival and progression-free survival was by chance identical to that of the patients who had achieved a comparable cytogenetic response but were not autografted (Figure 3). Although a statistical comparison cannot be made, the data point out that when autoBMT is applied to a selected cohort of good risk patients a very large number of cases and a long observation are required to evaluate whether survival is affected, either positively or negatively. The long-term effect of autoBMT itself is even more difficult to evaluate because many

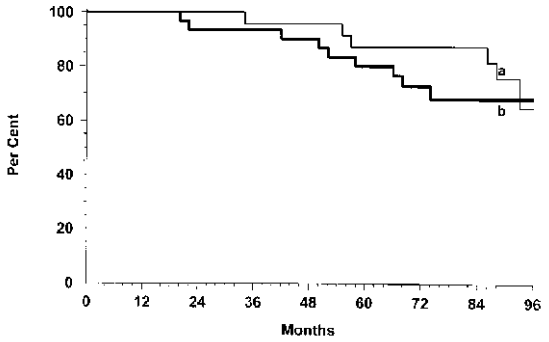

Figure 3. Progression-free survival of the patients who achieved a cytogenetic remission after the completion of one year of αIFN treatment. Curve *a* concerns the 23 patients who were autografted and curve *b* the 36 patients who were not autografted. The median observation time of living patients is 92 months. Note that the calculation is made from the date of the registration, and not from the date of autoBMT as in Figures 1 and 2.

of the patients who proceeded to autoBMT were given αIFN again after autoBMT, as happened in many prior studies.[19-23] It is important to note that all the patients who were tested with a sensitive PCR technique were positive confirming that in this therapeutic setting a complete molecular remission is rarely, if ever, achieved.[24-25]

This first application of autoBMT in CML was limited to the treatment of blastic phase with peripheral blood progenitors that had been collected either at diagnosis or during the chronic phase.[1-4,7,19,26] This approach was largely abandoned and the same or a similar procedure was applied already during the chronic phase, with the purpose of reducing the leukemic cell mass.[6-7,20,22,26-28] Some methods were developed to select normal cells and to clean or purge the harvest.[5,20,23,29,30] More recently, a procedure of collection of normal cells with chemotherapy and growth factors was shown to be effective when it was performed prior to other treatments and has been tested in some pilot trials.[4,22,27,28,31,32] Attention is now focused on two points. The first point concerns the cleaning of the cells. It is likely that the cleaner the harvest the better the outcome[4,22,29,33,34] and there are data showing a relationship between the number of Ph pos cells in the graft and cytogenetic repopulation *in vivo*.[34,35] However, many studies report major and complete cytogenetic responses also without any *in vitro* or *in vivo* purging, and our study shows that a complete cytogenetic response could be achieved and maintained for a long time, with or without αIFN, also when the cells were collected from patients who did not have a complete cytogenetic response. The second point concerns the observation that any prior treatment, including αIFN, or even the disease course or duration by itself, can adversely affect the collection of normal cells.[4,22,27,32,36,37] This is an important point, because it is crucial to the timing of harvesting, but it is still controversial. A recent report by Archimbaud *et al.*[35] showed that Ph neg hemopoietic progenitors could be mobilized with granulocyte-colony-stimulating factor and collected from the peripheral blood of patients who had been treated with αIFN for 11 to more than 120 months (median 28 months). The results of our study confirm that treatment with αIFN allows collection of normal hemopoietic progenitors.

The data that have been discussed so far cannot settle the question of the value of autoBMT in CML, a question that has been put for several years[21,38-42] and was fed by many reports suggesting that autoBMT could be advantageous.[3,6,7,22,27,41] The results of the present study can be viewed encouraging, but they are presented in the perspective of a prospective study, in which the patients were enrolled with the specific purpose of autografting and where it is possible to evaluate and to measure selection. Selection is likely to be so important that the results cannot be extrapolated to *average* patients and only prospective randomized studies will provide an answer to the question of whether autoBMT can prolong survival.

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GM, DR and MB planned and supervised the study over a 10-year period. NN and GA were responsible for the cytogenetics. GM and GS were responsible for the molecular biology. RF, EZ and GR were responsible for data collection and analysis. FM and ST chaired the study. The manuscript was prepared by MB, DR and GM and was reviewed by all the other authors.

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Appendix

The members who participated actively in the study are:

E. Montefusco, G. Alimena, G. Meloni, Catt. Ematologia, Univ. La Sapienza, Roma; N. Testoni, A. Martinelli, G. Rosti, Ist. Ematologia "L. e A. Seràgnoli", Univ. Bologna; D. Damiani, M. Michieli, Catt. Ematologia, Univ. Udine; G. Specchia, V. Liso, Serv. Ematologia, Univ. Bari; A. D'Emilio, F. Rodeghiero, Div. Ematologia, Osp. Vicenza; A. Di Tucci, G. Broccia, Osp. Oncologico Businco, Cagliari; C. A. Bod-

enizza, M. Carotenuto, Div. Ematologia, Casa Sollievo della Sofferenza, S. Giovanni Rotondo; A. Ambrosetti, G. Perona, Catt. Ematologia, Univ. Verona; S. Tringali, I. Majolino, Div. Ematologia, Osp. Cervello, Palermo; L. Luciano, B. Rotoli, Div. Ematologia, Univ. Napoli; S. Merante, M. Lazzarino, Catt. Ematologia, Univ. Pavia; P. Viero, A. Rambaldi, Div. Ematologia, Osp. Bergamo; F. Ronco, F. Nobile, Div. Ematologia, Osp. Reggio Calabria; S. Rupoli, P. Leoni, Clinica di Ematologia, Univ. Ancona; L. Gargantini, A. Nosari, Div. Ematologia, Osp. Niguarda, Milano; A. Montuoro, A. De Laurenzi, Div. Ematologia, Osp. S. Camillo, Roma; I. Gentilini, R. Quaini, Div. Ematologia, Osp. Bolzano; D. Dini, G. Torelli, Ematologia, Univ. Modena; F. Papineschi, M. Petrini, Clinica Medica I, Univ. Pisa; A. Liberati, E. Donti (Ist. Clinica Medica) e A. Tabilio, M. Martelli (Ist. Ematologia), Univ. Perugia; S. Pardini, M. Longinotti, Ist. Ematologia, Univ. Sassari; L. Mangoni, C. Carlo-Stella, Catt. Ematologia, Univ. Parma; P. Galieni, F. Lauria, Catt. Ematologia, Univ. Siena; A. Abbadessa, L. Bruzzese, serv. Ematologia Oncologica, Univ. Napoli, F. Leoni, S. Ciolli, Catt. Ematologia, Univ. Firenze; M. Cantonetti, S. Amadori, Catt. Emat., Univ. Tor Vergata Roma; S. Sica, G. Leone, Ematologia, Univ. Cattolica Roma; D. Ferrero, A. Pileri, Catt. Ematologia, Univ. Torino; V. Zagonel, A. Pinto, Centro Rif. Oncologico, Aviano; F. Palmieri, E. Volpe, Div. Ematologia, Avellino; A. Di Francesco, D. Quaglino, Ist. Sem. Medica, Univ. L'Aquila; P. Avanzini, L. Gugliotta, Div. Ematologia, Osp. Reggio Emilia; P. Pregno, M. Bertini, Div. Ematologia, Osp. Molinette Torino; A. Novarino, W. Piacibello, Ist. Medicina Interna, Univ. Torino; G. Rege Cambrin, P. Sivera, Ist. Pat. Medica, Univ. Torino; G. Pinotti, G. Venco, Div. Medicina, Osp. Varese; A. Capucci, T. Izzi, Div. Ematologia, Osp. Brescia; S. Morandi, C. Bergonzi, Div. Medicina, Osp. Cremona; G. L. Scapoli, G. L. Castoldi, Catt. Ematologia, Univ. Ferrara; E. Capussela, Div. Ematologia, Osp. Foggia; M. Pizzuti, F. Ricciuti, Div. Ematologia, Osp. Potenza; A. Capaldi, M. Aglietta, Div. Oncologia e Ematologia, Osp. Umberto I, Torino.

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