Repeated infusions of donor-derived cytokine-induced killer cells in patients relapsing after allogeneic stem cell transplantation: a phase I study

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ABSTRACT

Background and Objectives

Cytokine-induced killer (CIK) cells have shown anti-leukemic activity and little graft-versus-host disease (GVHD) in several animal models. The safety of these cells in autologous settings has been shown. We performed a phase I study of allogeneic (donor's) CIK cells in patients relapsing after allogeneic haematopoietic stem cell transplantation (HSCT).

Design and Methods

Eleven patients with acute myelogenous leukemia (n=4), Hodgkin's disease (n=3), chronic myelomonocytic leukemia, (n=1), pre-B acute lymphoblastic leukemia (n=1) and myelodysplasia (n=2), all of whom had relapsed after sibling (n=6) or matched unrelated donor (n=5) HSCT, entered this study.

Results

Before CIK administration, six patients had received other salvage treatments including chemotherapy (n=5), radiotherapy (n=1) and unmanipulated donor lymphocytes (n=6) without any significant tumor response. The median number of CIK infusions was two (range 1-7) and the median number of total CIK cells was 12.4 x10^6/kg (range 7.2-87.4). The infusions were well tolerated and no acute or late infusion-related reactions were recorded. Acute GVHD (grade I and II) was observed in four patients, 30 days after the last CIK infusion, and progressed into extensive chronic GVHD in two cases. Disease progression and death occurred in six patients. One patient had stable disease, one had hematologic improvement and three achieved complete responses.

Interpretation and Conclusions

This study shows that the production of allogeneic CIK cells is feasible under clinical-grade conditions, well tolerated and may contribute to clinical responses.

Key words: CIK, DLI, GVHD, allo HSCT.

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Hematopoietic stem cell transplantation (HSCT) is a curative therapy for a variety of hematologic malignancies, although disease recurrence remains a formidable obstacle. Allogeneic HSCT can induce disease eradication through both the conditioning regimen and the immunologic effect of the graft (graft-versus-tumor effect, GVT), and represents the first robust demonstration that immunotherapy can, in fact, control human tumors.[1-3] A further improvement of the allogeneic immune effect was provided by the administration of donor leukocyte infusions (DLI), which proved to be particularly successful for some leukemic subtypes such as chronic myeloid leukemia (CML) at relapse.[4,5] Unfortunately, DLI are complicated by unwanted immune-reaction of the donor's cells against the recipient's normal tissues, leading to potentially fatal graft-versus-host disease (GVHD).

One way to overcome these problems is linked to the possibility of selecting leukocyte subpopulations with an effective graft-versus-leukemia (GVL) effect and reduced or absent GVH reactivity.[6,7] Natural killer (NK) cells have potent antitumor cytotoxicity which is under the control of both activating and inhibitory molecules, and their effectiveness in allogeneic combinations is yet to be fully understood.[8] This uncertainty, in addition to the difficulty of expanding NK cells *in vitro* and the necessity of co-administering high doses of interleukin-2 (IL-2) with its associated toxicity, has severely restricted their use in the clinic so far.[9-11]

Other cell types have, however, more recently been characterized which may provide useful therapeutic tools to treat leukemia patients who relapse after allogeneic HSCT. Indeed, it has been demonstrated that cells expressing both the T-cell marker CD3 and the NK-cell marker CD56 show potent MHC non-restricted cytotoxicity against several tumor targets. Cells with this phenotype, originally described by Griffin *et al.*, represent a rare subset of circulating lymphocytes (from 1% to 5% of peripheral bood lymphocytes).[12-14] More recently, a simple method has been established to rapidly and reproducibly expand *in vitro* cells showing a $CD3^+CD56^+$ phenotype, called cytokine-induced killer cells (CIK), starting from the peripheral blood of normal donors or leukemia/lymphoma patients[15-18]. *In vitro* expanded murine or human CIK cells are mostly $CD3^+CD56^+$ and show potent cytotoxic activity against a number of tumor cell lines or freshly isolated tumor samples, but not against normal $CD34^+$ stem cells.[17-22] Furthermore, CIK cells have shown potent anti-tumor activity *in vivo* in several animal models, and protected SCID mice more effectively than lymphokine-activated killer (LAK) cells.[15-17,19,23-25] Moreover, the same cells did not interfere with bone marrow engraftment *in vivo* and had little GVHD activity in several allogeneic models.[19,23]

Two phase I studies have been performed so far, using single or repeated injections of autologous CIK cells in a total of 22 patients, including lymphoma and hepatocellular carcinoma patients.[26-28] The total number of cells inoculated ranged from 3×10^9 to 22×10^9. No toxicity was observed except for low-grade fever lasting a few hours. Two partial responses were observed in two Hodgkin's lymphoma patients, as well as a significant decrease in hepatitis B virus load in hepatocarcinoma patients.

Here we present the results of a phase I study of the use of allogeneic CIK cells in patients with hematologic neoplasms, who had relapsed after allogeneic HSCT. The study allowed for the first time an exploration of the feasibility of expanding such allogeneic CIK cells from donors' peripheral blood or apheresis material, under Good Manufacturing Practice conditions and within hospital-based facilities, and an evaluation of their toxicity, with particular attention to the induction of acute and chronic GVHD and activity of these cells after infusion into patients at relapse.

Design and Methods

Eligibility criteria

Patients with hematologic malignancies (excluding CML) who showed hematologic, cytogenetic or molecular relapse after allogeneic HSCT (including loss or insufficient lymphoid chimerism) were eligible for this study. Immunosuppressive therapy had to be withdrawn or reduced to a minimum prior to infusion of the CIK cells. Patients with acute GVHD greater than grade II were excluded from this study.

Study design

The study was designed as a phase I study to test the safety and feasibility of repeated infusions of *in vitro* expanded allogeneic CIK cells given at 3 to 4 week intervals to patients who had relapsed after allogeneic HSCT. For patients with hematologic recurrence, tapering of cyclosporine A (CsA) and the most appropriate chemotherapy treatment could be performed to achieve the best hematologic response and to allow the laboratory preparation of CIK cells. For patients with molecular recurrence or loss of full donor chimerism, tapering of CsA was performed and CIK cells infused thereafter. In some cases, a conventional program of DLI (from a minimum of 1×10^6/kg up to 10×10^6/kg) was performed before the CIK infusions. The minimum amount of CIK cells given during each infusion was set at 3×10^6/kg up to a maximum of 15×10^6/kg. All patients who received at least one CIK infusion were considered evaluable. In the case of infusion-related toxicity or secondary acute GVHD (> grade 2), no further CIK infusions could be given. In all the remaining patients, after a minimum of 21 days, further CIK infusions could be performed at the same time interval and up to the best clinical response, emerging toxicity or occurrence of GVHD.

Expansion of CIK cells

For one patient, 30 mL of peripheral blood were separated over Ficoll Hypaque (Eurobio) and mononuclear cells were used. In all other cases the starting material was represented by unmanipulated leukapheresis samples (10-100 mL). Cells were resuspended in X-VIVO serum-free medium containing glutamine and gentamicin (Cambrex Bioscience), at a concentration of 5-10×10^6 cells/mL and placed in sterile flasks (Greiner BioOne). On day 0, 1000 U/mL recombinant human interferon-γ (rhIFNγ) (Imukin) were added and cells incubated at 37°C in a 5% CO_2 atmosphere. On day 1, OKT3 antibody (Orthoclone) was added at a final concentrations of 50 ng/mL and rhIL-2 (Proleukin) at 500 U/mL. Every 2-4 days, cells were counted and diluted to 1×10^6/mL by adding fresh X-VIVO medium in the presence of rhIL-2 at a concentration of 500U/mL. After 20-26 days of culture (median 22 days), expansion was stopped and cells were pooled into 250 mL conical tubes (Falcon), washed in clinical grade saline solution (SALF) supplemented with 2% human serum albumin (Kedrion) and resuspended in fully screened human homologous plasma. Cells were divided into clinical-grade cryopreservation bags (Fresenius), in order to allow the administration of 3-14×10^6 $CD3^+CD56^+$ CIK cells/kg in one to four infusions, and finally cryopreserved in an automated machine (Ice Cube 15M) after addition of clinical grade DMSO (Wak-Chemie Gmbh) at a final concentration of 10%.

Results

Patients' characteristics

Eleven patients (five males, six females) were enrolled into this study; their main clinical findings are summarized in Table 1. The median age of patients was 53 years (range 24-62) and their diagnoses were acute myelogenous leukemia (AML, n=4), Hodgkin's disease (HD, n=3), pre-B acute lymphoblastic leukemia (pre-B ALL, n=1), chronic myelomonocytic leukemia (CMML, n=1) and myelodysplastic syndrome (MDS, n=2, in one case secondary to a previous non-Hodgkin's lymphoma). With the exception of two patients, all patients had been previously heavily pretreated before allogeneic transplantation and six had also undergone autologous stem cell transplantation (Table 1). The conditioning regimens before allogeneic transplantation were based on reduced intensity protocols in all but two patients. The allogeneic donors were HLA identical siblings in six cases and unrelated donors in the other five (Table 1). The best clinical responses after allogeneic transplantation were complete responses (CR) in nine patients, partial response (PR) in one case and progressive disease (PD) in one patient. The reasons for additional therapy were progression of disease or clinical relapse in nine and cytogenetic relapse with reduced donor chimerism in two patients. Disease progression or relapse required treatment with chemotherapy alone in two patients, local radiotherapy in one and infusion of unmanipulated DLI, alone or in addition to chemotherapy, in six patients (Table 2). Before CIK cell infusions, all patients had clinical or laboratory evidence of active disease in spite of all the previous therapies (Table 2).

Characterization of expanded CIK cells

Eigtheen different batches of CIK cells were prepared under Good Manufacturing Practice conditions for this phase I study and were released to treat 11 patients after successful execution of all quality controls. The mean number of nucleated cells (TNC) used to start CIK cell preparations was 678×10^6 (range 58-1500×10^6). The final cell number at the end of expansion was a mean of 3192×10^6 (range 660-5300×10^6) corresponding to a median 4.8-fold expansion (*data not shown*). The mean percentage of $CD3^+/CD56^+$ cells in the starting population was 5.9% (range 0.7-9.9%) (Figure 1A), corresponding to an absolute mean number of 47.6×10^6 $CD3^+CD56^+$ CIK cells (range 1.2-136×10^6) (Figure 1B). After a median 22 days of *in vitro* culture, a mean percentage of 49% $CD3^+CD56^+$ cells (range 40-71%) was obtained (Figure 1A) corresponding to an absolute mean value of 1460x10^6 (range 422-2782×10^6) total CIK cells (Figure 1B). Thus, the median fold expansion of $CD3^+CD56^+$ cells was 43.2 fold (range 9.3-553) (Figure 1C). With respect to total $CD3^+$ T lymphocytes, the mean percentage of total $CD3^+$ cells in the starting population was 52.8% (range 33-75%) (Figure 1A), corresponding to an absolute number of 385×10^6 $CD3^+$ (range 21-871×10^6) (Figure 1B). These cells expanded by a median of 8-fold (range 3.1 to 32.8, Figure 1C), thus giving a mean absolute number of 3083×10^6 (range 660-5247×10^6) and mean 96.7% $CD3^+$ cells (range 92-100%) (Figures 1A and B). The cytotoxic capacity of expanded CIK cells was verified by performing standard cytotoxicity assays against the NK cell line target K562. Measurable cytotoxicity was observed with all batches, with a mean target cell killing of 56.8% at an effector to target ratio of 30:1 (*data not shown*). In two cases (patients 5 and 10) leukemic samples were also available to perform cytotoxic assays. Cytotoxic activity against these leukemic cells was equivalent to that observed against the K562 cell line (23% and 44% for patients 5 and 10, respectively, at a 30:1 ratio).

These data are in agreement with those from a previous series of experimental CIK preparations (n=9) from donors, which consistently showed comparable cytotoxicity against K562 and blasts of host origin (as shown in Figure 2, panel A) at all tested effector:target cell ratios. Moreover, within an even larger series of experimental CIK cells from healthy donors (n=16) we observed that, in spite of strong cytotoxic activity against K562, there was little if any cytotoxic activity against *normal* autologous or allogeneic T PHA blasts (Figure 2, panel B).

Administration of allogeneic CIK cells to patients and clinical responses

The median time from allogeneic HSCT to relapse or progression was 250 days (range, 90 to 1182) while a median of 315 days (range 132-1224) elapsed from transplantion to the first CIK infusion. Table 2 shows the total amount of CIK cells infused and the number of infusions for each patient, with patients listed according to the total amount of cells infused from the lowest to the highest. On the basis of cell recovery at the end of *in vitro* expansion, the first administration of CIK cells in each patient ranged from 3.6 up to 12.4 x 10^6/kg (*data not shown*). Three patients received only one CIK cell preparation (patients 2, 4 and 5). Three patients received two CIK infusions at an interval of

Table 1. Patients' characteristics.

Pt No. and sex	Age at allogeneic HSCT	Diagnosis	Lines of previous chemotherapy[a]	Previous autologous HSCT	Intensity of conditioning regimen	Donor[b]	HLA mismatches (locus)	Months from diagnosis to allogeneic HSCT	Best response after allogeneic HSCT (days)[c]
1/ F	24	HD	3 + RT	yes	Reduced	MUD	1 allelic class I (B)	23	PR (+41)
2/F	38	MDS	0	no	Myeloablative	SIB	0	5	CR (+29)
3/M	58	AML	1	yes	Reduced	MUD	0	9	CR (+33)
4/F	54	AML	1	no	Reduced	SIB	0	5	CR (+80)
5/M	54	sMDS	2	yes	Reduced	SIB	0	1	PD
6/F	57	AML	2	no	Reduced	SIB	0	4	CR (+80)
7/F	62	AML	2	no	Reduced	SIB	0	5	CR (+42)
8/F	40	Pre B ALL	1	yes	Myeloablative	MUD	0	48	CR (+36)
9/M	37	HD	4 + RT	yes	Reduced	MUD	1 antigenic class I (C)	128	CR (+77)
10/M	53	CMML	0	no	Reduced	SIB	0	6	CR (+27)
11/M	36	HD	3 + RT	yes	Reduced	MUD	1 allelic class I (A)	29	CR (+30)

[a]RT: radiotherapy; [b]MUD: unrelated donor; SIB: sibling donor. [c]CR: complete response; PR: partial response; PD: progressive disease. Numbers in parentheses refer to days from the date of the allogeneic HSCT.

Table 2. Patients' hematologic status, treatment and responses.

Pt No.	Days from HSCT to progression relapse	Additional therapy before CIK infusion[b]	Status pre CIK infusion	First CIK infusion	CIK cells infused $\times 10^9$/kg (n. of inf.)	Acute GVHD	Chronic GVHD	Best Clinical response [c]	Further therapy	Last follow-up
1	(+167)	None	Active	(+169)	7.2 (2)	No	No	NR	DLI 10×10^6/kg	Death (+314)
2	(+405)	DLI 85×10^6/kg (+411, +433, +466, +496, +559)	Cytogenetic relapse and mixed chimerism	(+603)	7.6 (1)	II (+624)	Extensive (+666)	CR	No	Alive (+880)
3	(+103)	Chemo (+157)	Active	(+165)	9.6 (2)	No	No	NR	No	Death (+188)
4	(+245)	Chemo (+275) DLI 5×10^6/kg (+280)	Active	(+315)	10 (1)	No	No	NR	No	Death (+330)
5	NAa	None	Active	(+147)	12.4 (1)	I (+157)	No	HI	2nd allo HSCT (+ 190)	Death (+310)
6	(+180)	Chemo (+205)	Active	(+270)	14.5 (2)	No	No	NR	No	Death (+345)
7	(+1182)	DLI 10×10^6/kg (+1194)	Active	(+1224)	24 (3)	No	No	NR	No	Death (+1327)
8	(+637)	Chemo DLI 10×10^6/kg (+663, +692)	Active	(+741)	26.4 (4)	No	No	NR	No	Death (+829)
9	(+257)	RT (38 Gy) (+315)	Active	(+374)	51 (7)	No	No	SD	Chemo (+640)	Alive (+1000)
10	(+90)	DLI 10×10^6/kg (+102)	Cytogenetic relapse and mixed chimerism	(+132)	53 (6)	II (+195)	Extensive (+235)	CR	No	Alive (+782)
11	(+455)	Chemo (+462) DLI 1×10^6/kg (+516)	Active	(+537)	87.4 (6)	II (+525)	No	CR	Chemo (+572)	Alive (+979)

[a]NA: not applicable (never achieved partial or complete response); [b]RT: radiotherapy; DLI: donor lymphocyte infusion; [c]CR: complete response; SD: stable disease; NR: no response; HI: hematologic improvement.[35] Numbers in parentheses refer to days from the date of the allogeneic HSC

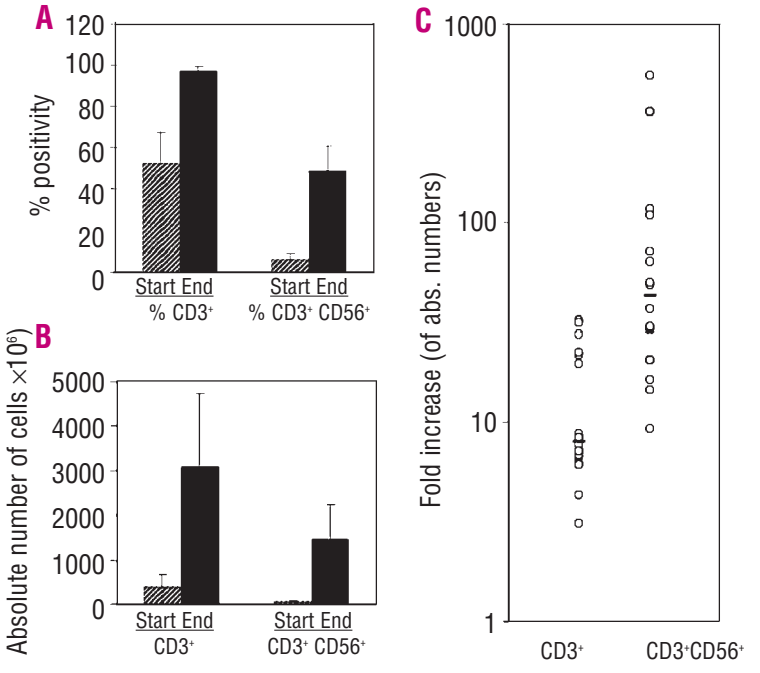

Figure 1. *In vitro* CIK expansion. **A.** The percentages of $CD3^+$ and $CD3^+/CD56^+$ cells at the start (striped bars) and at the end (black bars) of culture are indicated. The results are the mean and standard deviations of 18 preparations. **B.** Absolute numbers of $CD3^+$ and $CD3^+/CD56^+$ cells at the start (striped bars) and at the end (black bars) of cultures are indicated. The results are the mean and standard deviations of 18 preparations. **C.** Fold increase of absolute numbers of $CD3^+$ and $CD3^+/CD56^+$ cells are shown as circles for each single expansion. The horizontal bars indicate the median fold increases for the two populations.

19-21 days according to the study protocol (patients 1, 3, and 6). Patients 7 and 8 received three and four CIK infusions, respectively. Two patients (10 and 11) received six CIK infusions and finally one patient (n. 9) received seven CIK infusions. Each infusion was well tolerated and no immediate adverse effects were observed.

Six patients (1, 3, 4, 6, 7 and 8) showed no evidence of clinical response, had rapidly progressive disease and died. One patient (n. 5) with secondary MDS (following previous non-Hodgkin's lymphoma treated with high dose chemotherapy and autologous transplantation), was elected to CIK treatment because of early relapse after a reduced intensity allogeneic transplant. At the time of infusing CIK cells, a trilinear dysplasia was documented in the bone marrow showing a normal cellularity of full patient origin. CIK cells induced a hematologic improvement,[30] as documented by the induction of marrow aplasia, with cytogenetic analysis showing 50% donor chimerism. A subsequent infusion of donor allogeneic PBSC was performed to rescue the hematologic function but disease progression occurred and the patient died on day + 310 (Table 2). One patient with HD (n.9) received a total of 51×10^6/kg CIK cells and the disease did not require further treatment for almost 1 year. On day +640 disease progression occurred (supraclavicular and axillary lymph nodes) so that chemotherapy was given (11 cycles of gemcitabine). This patient remains alive at day + 1000 with stable disease. In another HD patient (n. 11), disease relapse after transplantation was documented on day + 455, so six cycles of gemcitabine followed by one unmanipulated donor lymphocyte infusion (1×10^6/kg) were given. Given the lack of clinical response and massive disease involvement of bone marrow, lung and mediastinal

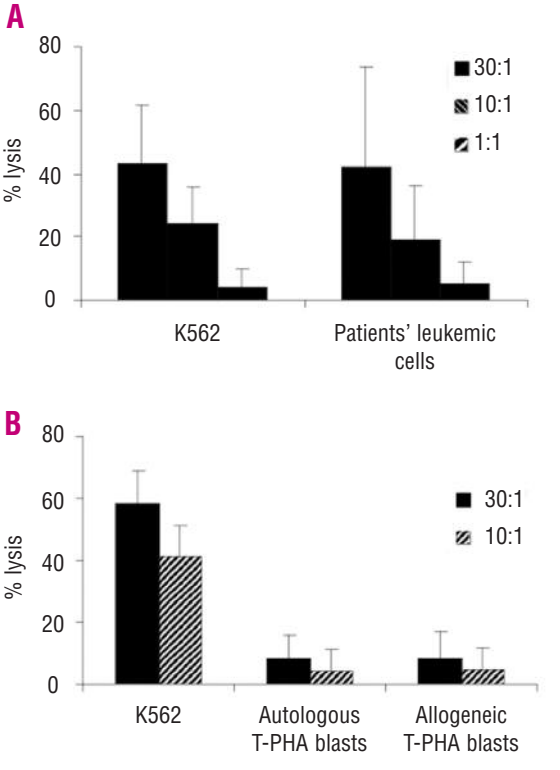

Figure 2. *In vitro* cytotoxic activity by CIK cells. **A.** CIK cells from nine bone marrow donors were prepared in experimental conditions and tested against K562 or leukemic samples from the HLA-matched allogeneic recipients at the indicated effector:target (E:T) ratios. **B.** CIK cells from 16 healthy donors were prepared in experimental conditions and tested against *normal* autologous or HLA-mismatched allogeneic T-PHA blasts at the indicated E:T ratios.

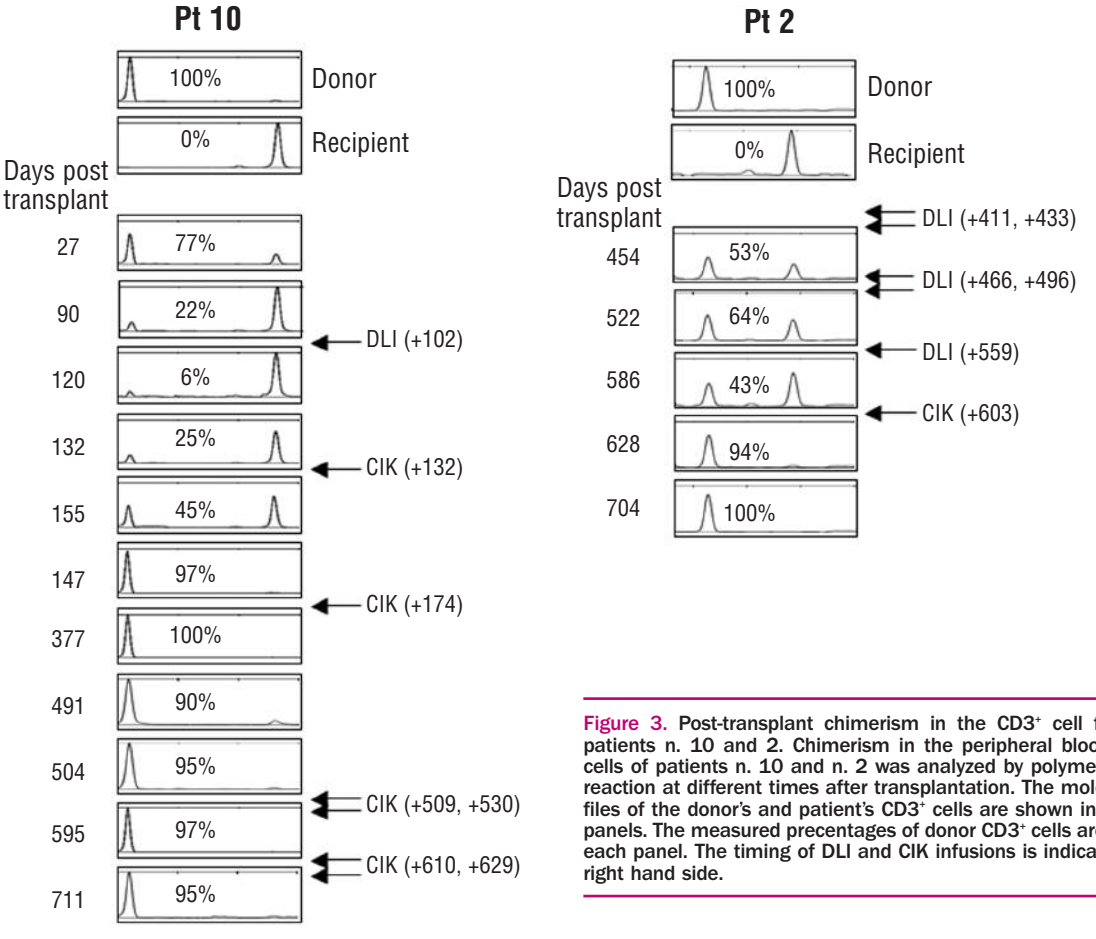

Figure 3. Post-transplant chimerism in the CD3$^+$ cell fraction of patients n. 10 and 2. Chimerism in the peripheral blood of CD3$^+$ cells of patients n. 10 and n. 2 was analyzed by polymerase chain reaction at different times after transplantation. The molecular profiles of the donor's and patient's CD3$^+$ cells are shown in the upper panels. The measured precentages of donor CD3$^+$ cells are shown in each panel. The timing of DLI and CIK infusions is indicated on the right hand side.

lymph nodes, four cycles of standard BEACOPP chemotherapy were administered from day +572. On top of this, six additional CIK infusions were given and a complete remission was documented by bone marrow biopsy, positron emission tomography and computed tomography scan on day +700 although modest cutaneous acute GVHD was documented. However, disease recurrence occurred at day +874. Further chemotherapy has been given and the patient remains alive at day + 969.

The patient with CMML (n. 10) showed evidence of bone marrow and peripheral blood mixed chimerism (22% donor CD3$^+$ lymphocytes at day +90 after allogeneic transplantation) so that one unmanipulated DLI was infused on day +102. Due to the lack of measurable response he was given additional therapy with CIK cells (9.5×10^6 CIK cells on days +132 and +174). Although full donor chimerism was documented 42 days after the first CIK infusion (day +174), extensive chronic cutaneous and oral mucosa GVHD developed so that recommencement of immune suppression with steroids, CsA and extracorporeal photopheresis were necessary. This treatment strategy was followed by a good response with regards to GVHD but at day +503 hematologic relapse occurred. Four additional CIK infusions did not promote a further hematologic response even though they were able to maintain an almost complete full donor chimeric status of peripheral blood CD3$^+$ lymphocytes (Figure 3), still documented on day + 595 and the patient remains alive at day + 782. The last patient (n. 2), upon cytogenetic and hematologic evidence of disease recurrence on day +405 after transplantation, entered a rescue program with dose escalating infusions of unmanipulated donor lymphocytes (five infusions). Because of the lack of any cytogenetic response and the mixed chimeric status documented on day +586 in the bone marrow (48% donor) and peripheral blood (43% donor CD3$^+$ lymphocytes), CIK cells were infused on day +603. A full donor chimeric status was documented on days +628 and +704 in both the bone marrow and the peripheral blood (CD3$^+$ cells) along with a complete cytogenetic response (Figure 3). The patient remains alive and a full responder at day +880 although extensive cutaneous and ocular chronic GVHD is present.

Discussion

Patients' characteristics

The aim of our study was to develop, under strict adherence to Good Manufacturing Practice guidelines, large numbers of MHC-unrestricted donor-derived allogeneic cytotoxic effector T cells. CIK cells are T cells with an NK phenotype which have shown *in vitro* and *in vivo* broad

antitumor activity.[31] More recently, two phase I studies have shown the feasibility and clinical safety of infusions of huge amounts of autologous CIK cells, with some preliminary evidence of antitumor activity.[26-28] Several different animal models have shown that allogeneic CIK cells of both human and animal origin do not mediate a significant GVHD effect but can retain a remarkable antitumor activity.[15-17,19,23-25] In this study we have shown that large numbers of allogeneic CIK cells can be produced in a 3-week time period and safely given to patients who have relapsed after allogeneic transplantation. This is the first report, to our knowledge, on the use of CIK cells in an allogeneic context for hematologic malignancies.

We have demonstrated that CIK cells could be obtained for 18 out of 18 consecutive batches to treat the 11 patients enrolled into this study, which was open for enrollment for 8 months. The feasibility of the laboratory procedure is also documented by the fact that the total amount of starting nucleated cells was relatively modest (a mean of less than 1×10^9 TNC obtained by donor leukapheresis) and in one case a small 30 mL peripheral blood sample was sufficient. It is important to note that the final yields and relative purity of CIK cells are comparable with those reported previously by others.[26-28]

The concomitant administration of other antineoplastic treatments such as chemotherapy or radiotherapy and the infusion of unmanipulated donor lymphocytes substantially prevents the possibility of evaluating the therapeutic activity of the allogeneic CIK cells that were infused, a problem also encountered in similar studies.[32] Within the limits of the study design, we can report the achievement of three complete responses (1 MDS, 1 CMML and 1 HD) and stable disease in one patient with HD. In one additional patient with secondary MDS, however, the infusion of CIK cells was the sole clinical intervention so that in this case the hematologic improvement,[30] documented by marrow aplasia with 50% donor chimerism, could be directly ascribed to the infused CIK cells.

Patients enrolled into this phase I study received variable amounts of CIK cells and despite the fact that some patients received more than 10×10^6/kg CIK cells as a first single infusion, acute toxicity was never observed. All patients received CIK cells after complete withdrawal of CsA and corticosteroids; acute GVHD developed in four patients but never exceeded grade II. However, progression to extensive chronic GVHD occurred in the two patients who showed the most significant clinical responses. Therefore, although the interpretation of chronic GVHD may be hindered by the preceeding infusions of unmanipulated DLI, the administration of allogeneic CIK cells may be associated with the development of GVHD. In this regard it is interesting to note that in the two documented cases, CIK administration was rapidly followed by the disappearance of host T lymphocytes. Since the patients who showed a clinical response as well as acute and chronic GVHD, were those who were also treated with unmanipulated DLI, the role of these latter in gaining a maximal therapeutic effect from CIK cells remains to be determined. Therefore, we cannot provide certain evidence of the antineoplastic activity of CIK cells *in vivo*, nor we can show that CIK cells are more effective than conventional DLI in rescuing patients experiencing disease recurrence after allografting. However, the degree of the toxicity of the observed GVHD may allow, in the near future, the planning of clinical studies with higher doses and repeated infusions of CIK cells in less clinically compromised HSCT patients. On the other hand, the use of allogeneic MHC-unrestricted cytotoxic effector cells has been recently suggested for haploidentical NK cells[33] and could also be explored with CIK cells, which share key molecules, such as NKG2D, crucial for effective target recognition and killing.[34]

In this report we confirm and extend previous observations on the *in vitro* antitumor activity of CIK cells against leukemic samples of host origin. Furthermore, our data support the idea that these cells have limited toxicity against normal cells, as evidenced by the resistance of T-PHA blasts of autologous or allogeneic origin.

All in all, the following comments can be made about the clinical results we observed in this small cohort of patients. First, the most interesting clinical results were recorded in patients with indolent diseases (MDS, HD, CMML) rather than in those with acute leukemias. Second, it seems unlikely that CIK cells alone can induce a major clinical response when a massive leukemia or lymphoma relapse is occurring. Finally, our results are in keeping with previous observations by Slavin and co-workers[35] who showed that, in patients resistant to DLI, remissions can be accomplished by adding donor peripheral blood lymphocytes activated *in vivo* or *in vitro* with IL-2. Nonetheless, a direct comparison in terms of efficacy and safety between the two studies is difficult to perform due to the different *in vitro* treatment protocols. Rather, cytoreductive chemotherapy or radiotherapy should usually preceed the administration of CIK cells given finally as a form of adoptive immunotherapy with the intention to treat minimal residual disease. Along the same lines, the preemptive use of CIK cells to treat a cytogenetic or molecular relapse also seems rational. Determining the effectiveness of this strategy will obviously be a major aim of a future clinical study with CIK cells.

Authors' Contributions

MI: responsible of the project, writer of the manuscript; GB: production of cells in GMP in Bergamo; EC: production of cells in GMP in Bergamo; MF: experimental data CIK cells; AMB: clinical administration of CIK cells; RB: experimental data CIK cells; ED, GG: production of CIK cells in GMP in Monza; GD'A: experimental data CIK cells; EB, MP, EMP, EMP, MP, DB: clinical administration CIK cells; OS: molecular analysis of chimerism; AG: clinical administration of CIK cells and patients registry; JG: quality control GMP facility; TB: responsible Department; AB: responsible Clinical Unit; AR: responsible Clinical Unit and CIK clinical design and writer of the manuscript.

Conflict of Interest

The authors reported no potential conflicts of interest.

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