Regression of Metastatic Osteosarcoma Following Non-Myeloablative Stem Cell Transplantation. A Case Report

We report the evidence of regression of multiple metastases following non-myeloablative stem cell transplantation (NST) from an HLA-identical sibling in a case of relapsed fibroblastic osteosarcoma. The course of NST was well tolerated. Full donor chimerism was achieved on day +150 both for CD15+ and CD3+ cells. Complete remission was achieved on day +116. On day +210 the patient relapsed with a scapular metastasis that was unresponsive to four doses of donor lymphocyte infusion (DLIs).To our knowledge, this is the first reported case showing the achievement of complete remission following NST in an osteosarcoma patient.

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Introduction.

Relapsed osteosarcoma with multiple bone metastases is generally considered incurable using conventional treatment.[1] High dose chemotherapy and autologous stem cell transplantation (ASCT) have improved the remission rate but convincing evidence of disease free survival (DFS)[2] has never been shown. Progress in the understanding of the immune response to cancer together with an increased understanding of the basic mechanism of cellular immunology have combined to open new opportunities for the development of effective immunotherapies for cancer.Patients with leukaemia undergoing allogeneic stem cell transplantation (SCT) and developing acute and, especially chronic GvHD run a lower risk of relapse than those without GvHD.[3] Furthermore, T cell depletion of the graft increases the risk of relapse. Recent reports suggest that donor lymphocytes, transferred with the graft, may also produce a clinically meaningful graft versus tumour (GvT) effect in patients with refractory solid tumours, such as renal cell carcinoma,[4] breast cancer,[5] colonic cancer,[6] non-small cell lung cancer,[7] ovarian cancer[8] and Ewing sarcoma.[9] Based on these data, attempts have been made to diminish transplant related mortality and, possibly, morbidity associated with conventional myeloablative allogeneic stem cell transplantation. Recently, attention has been addressed to less cytotoxic conditioning regimens to obtain a mixed chimerism over which allogeneic cells might display an anti-tumour effect.In this report, we describe the evidence of the regression of multiple metastases following non-myeloablative transplantation (NST) in a case of relapsed fibroblastic osteosarcoma.

Patient and Donor.

In February 1997 a 12 year-old boy was diagnosed a grade IV fibroblastic osteosarcoma of the right distal femur. No metastasis was found at diagnosis. He received neo-adjuvant chemotherapy according to the ISG-SSG1 protocol (an Italian-Scandinavian chemotherapy and surgery protocol) including high dose Methotrexate, high dose Ifosfamide, Adriamycin and Cis-platinum. In June 1997 he underwent resection of the distal femur and insertion of a Kotz prothesis. The histological examination showed 100% of tumour necrosis. The patient finished post-surgery chemotherapy in October 1997. The Adriamycin, Methotrexate, Cisplatinum and Ifosfamide total doses administrated were 330 mg/m^2, 48 g/m^2, 480 mg/m^2 and 60 g/m^2, respectively. In October 2000 the patient underwent left lung metastasectomy (+ 44 months from diagnosis). In June 2001 (+ 52 months from diagnosis) the total body scan with 99Tc showed multiple osseous metastases. A CT scan showed a retro-orbital mass, whereas the lungs were negative (Tab 1).[10] Neoplastic cells were also found in the bone marrow biopsy.The patient underwent a modified ISG-AIEOP (Italian Sarcoma Group-Italian Association of Paediatric Haematology Oncology) for very high risk osteosarcoma patients, that was approved by the Hospital Ethical Committee. The patient was treated with three cycles of high dose Methotrexate cycles (12 g/m^2), a Cisplatinum-Adriamycin cycle (120 mg/m^2 and 75 mg/m^2, respectively) and a Cis-platinum cycle (120 mg/m^2). In November 2001 the total body scan with ^{99}Tc-Osteosol showed the partial uptake reduction, while the retro-orbital cavity CT scan was unchanged. As he had several healthy siblings, his 22-year-old HLA-identical brother was chosen as a stem cell donor.

Allogeneic stem cell transplantation. The patient received Fludarabine 30 mg/m^2/day over four days and Cyclophosphamyde 30 mg/Kg/day over two days. The graft versus host disease (GvHD) prophylaxis was: Cyclosporin-A (Cy-A) 3 mg/Kg i.v. from day -1 to +84 to maintain blood values between 100-250 ng/ml and Mycofenolate Mofetil (MMF) 15 mg/Kg b.d. orally from day 0 to +28. Cy-A tapering was started from day +84 until day +180 (7% weekly). Acyclovir was given for viral prophylaxis 500 mg/m2 three times a day from day &endash;5 and liposomal anphotericin 1 mg/Kg was given for antifungal prophylaxis from day -1. For Pneumocystis carinii prophylaxis aerosolized pentamidine 300 mg and, thereafter, trimethoprim-sulfametoxazole 5 mg/Kg over two consecutive days each week was given.The graft was: nucleated cells 10x10^8/Kg, CD34$^+$ 13.^{8}x106/Kg, CFU-GM 60x10^4/Kg, CD3$^+$ 2.22x10^8/Kg, CD3$^+$CD4$^+$ 1.04x10^8/Kg, CD3$^+$CD8$^+$ 1.02x10^8/Kg, CD19+ 2.8x10^7/Kg, CD56+ 3.2x10^7/Kg.

Immune reconstitution following. NST. Monthly, peripheral blood samples were analysed for CD3$^+$, CD4$^+$, CD8$^+$, CD19$^+$ and CD56$^+$ lymphocyte reconstitution. Cells were analysed on a Becton Dickinson Facscan cytometer.

Molecular studies to monitor chimerism. DNA from patient

Table 1.

	Days following NST										
	Immune reconstitution following NST										
	18	37	66	91	112	150	180	210	240	270	300
CD4$^+$	ND	368	330	384	329	364	402	549	492	661	319
CD8$^+$	ND	148	451	362	495	436	458	405	429	552	251
CD56$^+$	ND	309	255	270	304	382	307	213	320	359	124
CD19$^+$	ND	3	112	103	95	142	143	174	210	177	70
CD3$^+$CD45RA$^+$	ND	177	377	313	318	460	438	ND	619	661	370
CD3$^+$CD45RO$^+$	ND	292	386	446	464	552	543	ND	619	624	378
	Chimerism analysis following NST										
Unseparated BM cells	<50%	ND	>80%	ND	ND	ND	ND	ND	ND	ND	ND
Unseparated PB cells	ND	ND	>80%	>70%	ND	>97%	ND	<90%	>97%	>97%	ND
CD3$^+$ PB	ND	ND	>80%	>70%	ND	>97%	ND	<90%	>97%	>97%	ND
CD15$^+$ PB	ND	ND	>80%	>70%	ND	>97%	ND	<90%	>97%	>97%	ND

and donor pre-transplant samples was obtained using a standard protocol. (Qiagen, Hilden, Germany). The separation of $CD3^+$ and $CD15^+$ cells from peripheral blood was performed with immunomagnetic beads (Miltenyi Biotec srl, Italy). Before freezing, 30 mM Tris buffer was added to each cell pellet. After thawing, 60 μL of lysis buffer was added to each bead-selected cell sample. These samples were then digested at 37°C overnight on a shaker, and subsequently diluted, as previously described elsewhere. The cell lysate samples were heated to 70°C, then spun and cooled on ice. Polymerase chain reaction (PCR) amplification of two different variable number tandem repeats (VNTRs) were chosen for mixed chimerism analysis.

Evaluation of tumour regression. Table 1 shows the results of the re-staging performed periodically after transplantation.

Results.

Transplant related toxicity. The patient did not develop acute and chronic GvHD. Transplant related toxicity was 0 according to the Bearman score.

Haemopoietic engraftment. The patient achieved neutrophil engraftment (>500/μL) on day +14. The lower platelet count was 119.000/μL. One packed red cell transfusion was performed on day +1 (before transfusion Hgb level 8.1 g/dL).

Immune reconstitution following NST. The CD4+ cell reconstitution was monitored from day +27, however since day+16 the total lymphocytes were >500/μL. As reported in Table 2, also the $CD19^+$ population showed a quick recovery following NST.

Chimerism analysis. The PCR study for the VNTRs showed from day+18 >50% of recipient cells in the bone marrow. On day +37 the peripheral blood analysis for mixed chimerism showed $CD15^+$ and $CD3^+$ cells with >60% of donor origin and on day +84 the unseparated bone marrow cells, the peripheral blood $CD15^+$ and CD3+ cells showed >70% of donor origin. On day +150 the patient achieved full donor chimerism. No evidence of rejection emerged, even at the time of relapse.

Tumour response. On day +116 the head CT scan showed complete remission of the retro-orbital mass, and the total body scan with 99Tc-Osteosol showed no active metastases. The complete remission status was maintained until day +180. On day +210 following NST, the patient relapsed with a single right scapular metastasis. He received four, monthly, escalating doses of donor lymphocyte infusion (DLIs): $1x10^7$/Kg, $5x10^7$/Kg, $7x10^7$/Kg, $1x10^8$/Kg $CD3^+$ lymphocytes. No tumour response or GvHD developed. The patient is now alive with progressive disease on day +420 following transplantation.

Discussion.

Patients with osteosarcoma and multiple bone metastases have been shown to have a DFS of 0% at 4 years follow-up 1. In a recently reported series, for patients with early relapse, high dose chemotherapy with stem cell rescue and pre or post-surgery, had a high remission rate, but DFS remained low (12%) at 3 year follow-up 2. Here, we report the evidence of metastases regression in a patient with advanced osteosarcoma, in which the immunological anti-osteosarcoma effect was independent of the GvHD reaction. Evidence that regression of metastatic osteosarcoma cells was mediated by the graft versus tumour (GvT) effect is compelling. First, the Fludarabine and Cyclophosphamide preparative regimen was administrated at immunosoppressive dosages, to allow the donor's immune cell engraftment, while avoiding the substantial side-effects of myeloablative therapy. Indeed, the conditioning regimen did not affect the tumour growth as shown by the re-staging done on day +30. Secondly, the regression of metastatic sites was delayed as complete remission was not achieved before day +116 following NST. Thirdly, the anti-tumour response developed only after a mixed chimerism status was obtained. A greater immunological difference between donor lymphocytes and recipient tumour cells may be the reason why allogeneic cells have an unequivocal anti-osteosarcoma effect in the NST setting in the absence of any signs of GvHD. This phenomenon might be explained considering that initially a mixed chimera is formed, and the recipient and host T and B lymphocyte and dendritic cells locate the thymus and delete host and donor reactive T cells. This may result in a tolerant T cell repertoire for both host and donor cells. Thus, the formed mixed-chimera serves as a platform for adoptive immunotherapy in which the risk for GvHD is reduced as the severity of its clinical manifestation is also related to the conditioning regimen and subsequent cytokine release. The GvT effect is maintained by the host antigen

Table 2.

	At relapse	Before transplant	+30	+48	+116	+150	+180	+210	+240	+270	+300
Retro-orbital mass by skull CT scan	2.5x1.5 cm	2.5x1.5 cm	2.5x1.5 cm	0.5x0.5 cm	CR	CR	CR	CR	CR	CR	CR
Bone metastases by ^{99}Tc scan:											
skull,	−+−	−+	Not	+	-	-	-	-	-	-	-
scapula,	++	+	Done	-	-	-	-	-	-	-	-
pelvis,	++	+		-	-	-	-	-	-	-	-
femur, proximal	+	+									
right tibia	+	+									
right scapula								+	+	+	+
Scapular metastasis by CT scan	Negative	Negative	Negative	Negative	Negative	Negative	Negative	1.5x1 cm	5x4 cm	6x4 cm	12x10 cm
Immunosuppressive treatment	No	No	Yes	Yes	Yes	No	Yes	No	No	No	No
Chimerism	NA	NA	Mixed	Mixed	Mixed	Full donor	Full donor	Full donor	Full donor	Full donor	Full donor

presenting cell, which elicits the overall GvT effect by recognising neoplastic antigens to donor lymphocytes. We conclude that this first evidence of regression following NST suggests a role for immunological control of osteosarcoma by allogeneic cells.

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