Allogeneic non-myeloablative hematopoietic stem cell transplantation for treatment of metastatic renal cell carcinoma – single center experience

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We evaluated the efficacy of allogeneic non-myeloablative stem cell transplantation (NST) in patients with metastatic renal cell carcinoma (RCC). A total of 5 patients received blood stem cells from HLA identical siblings. Conditioning consisted of: cyclophosphamide 60 mg/kg/d, days -7 to -6 and fludarabine 25 mg/m^2/d for consecutive days [days -5, -4, -3, -2, -1].

The median CD34+ cell dose was 3.34 million/kg. Immunosuppression consisted of cyclosporine A and methotrexate. Among all, four patients achieved full donor chimerism with a median of 89 days. One patient rejected the graft and received the second transplantation. Grade II-III acute GVHD occured in 3 patients. None of patients achieved complete or partial response and there were only two mixed responses. All patients died due to cancer progression. There were no transplant-related deaths. Summarising, NST regimen allows allogeneic engraftment with low treatment related mortality in this high-risk population of patients. Acute and chronic GVHD are the major morbidities. Progression is common after NST in unselected patients with advanced RCC. However, regression of some metastases suggests that the graft versus tumor effect may occur after this type of treatment. At present such a procedure should be considered as an experimental approach.

Key words: hematopoietic stem cell transplantation, non-myeloablative conditioning regimen, metastatic renal cell carcinoma

Despite continuous progress in the treatment of patients suffering from solid tumors, a large number of them die from cancer progression. Allogeneic stem cell transplantation has shown its potential in treatment of malignant blood diseases. It is now known that the major antitumor effect is due to the immune activity of the allogeneic graft on leukemic cells. This means that allogeneic transplant represents a unique form of cellular immunotherapy. Nonablative hematopoietic cell transplantation is becoming a preferred treatment for those recipients in whom the potential toxicity of standard ablative allogeneic therapy may be unacceptable. Recently, investigators have begun to explore the potential of allogeneic stem cell transplantation to generate analogous graft versus tumor (GVT) effects in patients with metastatic tumors refractory to other treatments. Regression of disease compatible with a GVT effect has recently been described in patients with metastatic breast, prostate, ovarian, colon and renal cell carcinoma (RCC) following low intensity allogeneic stem cell transplantation [1, 7, 9–11, 25].

Metastatic RCC has an ominous prognosis. Conventional chemotherapy is ineffective in this disease. Immunotherapy with recombinant human cytokines may induce response in up to 20% of patients, but most of them will succumb with a few months. Therefore this disease may be an ideal model for exploring new kind of cellular immunotherapy-nonablative hematopoietic cell transplantation from HLA identical donor especially sibling.

Several authors reported recently a promising response rate of 60% with this novel therapy [10–12, 19].

This study was designed to evaluate the feasibility and safety of nonmyeloablative allogeneic stem cell transplantation in patients with metastatic renal cell cancer and to evaluate efficacy of this procedure with respect to engraftment and tumor regression.

Patients and methods

Since the beginning of 2002 a protocol for treatment with

nonmyeloablative allogeneic peripheral blood stem cell transplantation has been available for metastatic RCC in our hospital. Patients had to sign an informed consent as approved by the institutional review board. Inclusion criteria were as follows: age between 18–65 years; metastatic renal cell carcinoma refractory or progressive after two courses of immunotherapy with interferon α and interleukin 2; lesions not amenable to complete surgical resection; attainable radiological evaluation of the disease; ECOG 0–1; no brain metastases; no hypercalcemia; normal renal, liver and cardiac function and a suitable HLA-identical sibling donor. All patients had multiple metastases in lungs, liver and lymph nodes, one patient had also bone metastases. Pretransplant work-up included CT scan of chest, abdomen, left ventricular ejection fraction, pulmonary function testing, renal and liver function tests as well as infectious disease serologies. Between January 2002 and August 2003 six transplantations were performed (one patient received two transplantations because of graft rejection).

Preparative regimen. HLA-identical transplant recipients received cyclophosphamide 60 mg/kg/day days -7 to -6 and fludarabine 25 mg/m^2/day for five consecutive days (days -5, -4, -3, -2, -1). All donors were full matched siblings.

Donor stem cell mobilization and stem cell processing. A total of five donors for HLA-identical siblings underwent stem cell mobilization using G-CSF 5 μg/kg every 12 h subcutaneously until the time of last leukapheresis procedure. Cobe Spectra equipment was used for stem cells separation. Collection was started on day 5 and continued daily until the targed CD34+ cell dose of 3x 10^6/kg was collected. Number of collections was 2 to 3/per donor. These stem cells were infused without further manipulations.

Post transplant gvhd prophylaxis. All patients received graft versus host disease prophylaxis consisting of cyclosporine A and short course of methotrexate. Methotrexate schedule was 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, +11. Cyclosporine A 3 mg/kg i.v. as 4–6 h infusion was given from day -4 and after the day +14 was administered at a bioequivalent amount of the oral formulation in two divided doses. The dose was adjusted to achieve levels of 200–350 ng/ml. Decisions regarding post-transplantation cyclosporine withdrawal were based on the speed and degree of engraftment of donor cells. Patients with 100% donor T-cell chimerism in peripheral blood samples on day 30 after transplantation received full dose of cyclosporine A by the day +60 and then the dose was decreased slowly and discontinued by day +100 (if symptoms of graft versus host disease did not occured). In patients with mixed donor chimerism on day 30, the dose of cyclosporine was rapidly tapered over a two week period.

Supportive care and growth factors. A total of six patients received G-CSF 10 μg/kg b.i.d. subcutaneously starting on day +7 stopped by the third day when a neutrophil count of >1.5x10^9/l was reached.

Trimethoprim/sulfamethoxazole 2x960/daily three times a week were given from day +30 till the end of immunosuppressive therapy. Acyclovir was administered to all patients 800 mg p.o. t.i.d. for 6 months, except for those receiving ganciclovir. All patients were screened weekly for CMV reactivation and pre-emptive therapy with ganciclovir was started when number of CMVpp65 positive cells was at least 20/1x10^5 leukocytes.

After transplantation all patients were located in a room with high-efficiency, particulate-free air (HEPA) filters with strict reverse isolation. Irradiated and leukocyte depleted blood products were administered to maintain a hemoglobin level of greater than 80 g/l and platelet count >20x10^9/l.

Assessment of graft versus host disease and chimerism. The severity of graft versus host disease was graded according to the criteria of the International Bone Marrow Transplant Registry.

The degree of donor-recipient chimerism in both myeloid and T-cell lineages was assessed by polymerase chain reaction assay. The first blood sample for T-cell chimerism was estimated on day +30 and then monthly until complete T-cell chimerism.

Donor lymphocyte infusions. Indications for donor lymphocyte infusion were as follows: 1. lack of complete donor T-cell chimerism after the withdrawal of cyclosporine (until achieving complete T-cell chimerism); 2. patients with stable or progressive disease after the withdrawal of cyclosporine.

When no evidence of graft versus disease >2nd degree was observed patients received donor T lymphocytes in escalating doses: from 5x10^6 to 5x10^7 CD3+ T cells per kilogram of the recipient's weight.

Response to treatment. All patients underwent computed tomographic (CT) scanning within 30 days before transplantation and then on days +30, +60, +100 after transplantation; thereafter monthly for the first year and every 3 months later.

A response was defined as complete if all measurable tumors disappeared and as partial if the sum of the products of the longest perpendicular diameters of metastatic lesions that could be evaluated decreased by at least 50% for a period of at least 30 days.

Results

Between January 2002 and August 2003 six transplantations from HLA identical siblings were performed (one patient received two transplantations because of graft rejection). The patients ranged in age from 24 to 54 years. All patients had nephrectomy and two had metastatectomy as a part of their previous therapy. They had to have radiographically documented progressive disease after treatment with cytokines (interleukin-2 + interferon alpha). The characteristics of 5 patients and outcomes of transplantation are listed in Tables 1 and 2. Median of 3.34x10^6 CD34+ cells per kilogram (range 3.0–4.5) was administered. The neutrophil count fell to less than 100 per μl in all patients and rose to more than 0.5 G/l a median of 13 days (range 6–18) after transplanta-

Table 1. Characteristics of the patients

No.	sex	age	No. of metastatic sites	No. of prior therapy	Sex of donor	No. of CD34+ cells separations	No. of CD34+ cells transfused/kg	No. of days with ANC<0.5G/l	No. of days with platelet <20G/l	No. of days with hgb<85g/l	No. of packed erythrocytes s transfused	No. of packed platelets s transfused	Acute GVHD
1	M	54	4	2	M	2	3.00	18	19	27	10	22	II-skin,gut
2a	M	44	4	3	F	3	3.17	14	13	12	2	2	I-skin
2b	M	44	4	4	F	3	3.84	12	12	14	14	5	–
3	M	31	3	2	M	6	4.50	13	13	6	2	3	II-liver
4	F	24	5	2	M	2	3.95	7	0	12	2	0	–
5	M	30	3	2	M	3	3.5	6	0	1	0	0	III-skin, liver, gut

Patient No. 2 received two transplantations because of graft rejection; the first 04.04.2002; the second 31.10.2002.
Metastases: 1. muscles of the back + lungs + lymph nodes of the mediastinum + retroperitoneal lymph nodes + local relapse (site after nephrectomy). 2. lungs + lymph nodes of the mediastinum + local relapse (site after nephrectomy) + neoplastic obstruction of inferior caval vein. 3. lungs + lymph nodes of the mediastinum + retroperitoneal lymph nodes. 4. lungs + lymph nodes of the mediastinum + retroperitoneal lymph nodes + skin + deep and superficial cervical lymph nodes. 5. lungs + bones + retroperitoneal lymph nodes.

Table 2. Characteristics of patients and outcome of transplantation

nr	Chronic graft versus host disease	stage	Involved organs	treatment	Chimerism Day +100	Number of DLI	Day of reaching 100% donor chimerism	Death- day after transplantation	The best disease status after transplantation
1	+	localized	Skin; liver, oral mucosa	Prednisone	95	3	109	315	Progressive disease
2a	–	–	–	–	5	3	68 days after second transplantation	494 after the day of first transplantation	Stable disease
2b	–	–	–	–	100	0	68	287 after the day of second transplantation	Mixed response
3	+	extensive	Skin; liver; oral mucosa	prednisone	95	4	152	531	Stable disease
4	+	extensive	Skin;liver; gut; eyes; oral mucosa	Prednisone; mycophenolate mofetil; thalidomide	100	–	69	415	Mixed response
5	not concerning	not concerning	Not concerning	–	65	–	Not reached	103	Progressive disease

tion. Two patients had not got platelet count $20x10^9$/l. In other three it took a median of 12.5 days (range 0–15) after transplantation for the platelet count to exceed $20x10^9$/l. None of the patients reached total donor chimerism on +30 day after transplantation. Four had a full donor T-cell chimerism after a median of 89 days (range 68–152) after cyclosporine therapy has been discontinued and infusions of donor lymphocytes (DLI) had been made. DLI were performed in three patients. Each patient received 3 escalating doses of donor lymphocytes at 30-day intervals (or 4 in one case) because of mixed chimerism and lack of improvement of their disease. DLI did not enhance GVHD in any patient.

Table 3 lists transplantation-associated adverse events. Graft versus host disease was the most serious complication. None of the patients died of transplantation related complications.

Out of the 5 patients none had regression of all metastases. We observed only mixed responses in two patients. Patient no. 2 had regression of size and number of metastases in the lungs but progression of metastases in the liver at the same time. Regression of lung metastases occurred after the second infusion of donor lymphocytes. He died on day +494 after transplantation because of massive haemorrhage from pathological lesions in the liver. Patient no. 4 had generalized

Table 3. Transplantation-related adverse events

Adverse event	No. of patients	Comment
Neutropenic fever	2	Patient nr 2- septic shock during neutropenic period of second transplantation (Escherichia coli)
Pneumonitis	1	
Cytomegalovirus reactivation	5	
Cytomegalovirus infection	0	
Acute GVHD	3	
II°	1	
III°	2	
Chronic GVHD	3	
Limited	1	
extensive	2	
Diarhhoea (WHO)	3	
I°	2	
II°	1	
Liver toxicity (WHO)	4	
I°	3	
II°	0	
III°	1	
Nephrotoxicity (WHO)	2	
I°	1	
II°	1	
Transplantation related mortality	0	

chronic graft versus host disease involving the skin, intestine and the liver, regression in size and number of metastases in her lungs and unchanged status of the majority of abdominal metastases. However she developed metastases in the brain and in the bones at the same time and died on day +415 because of intracranial hypertension refractory to treatment. All responses occurred 5 to 7 months after transplantation.

As of 02 April, 2004 all patients died due to progressive disease. They were alive 103–531 days after transplantation (median 365).

Discussion

Recently allogeneic haemopoietic cell trasplantation with nonablative conditioning regimen has been investigated as a new treatment option for patients with advanced solid tumors, especially with metastatic renal cell carcinoma (RCC) [2, 4, 6, 8, 15]. According to the American Cancer Society the estimated number of new cases of RCC in 2002 is 31800, with 11600 expected deaths. Early stage renal cell carcinoma can be cured by nephrectomy, but most cases present as advanced disease. Metastatic renal cell cancer remains a treatment resistant disease that can slightly respond to different forms of immunotherapy. Interleukin-2 and interferon alpha when given alone or with chemotherapy can induce measurable responses in up to 20% of cases [9, 19]. These responses have been linked to cellular immune reactions induced by T cells and natural killer cells. In addition, since metastases can regress even spontaneously, tumor growth should be controlled immunologically in patients with renal cell carcinoma. Among those patients who show a complete response to immunotherapy, a significant fraction could remain disease free. Nevertheless, the 5-year survival rate is less than 10%, with little hope of cure for patients in whom standard immunotherapy failed [7, 9, 14, 19].

Renal cell cancer may be susceptible to a GVT effect as T lymphocytes have been shown to be an important component of the antitumor immunological response. In addition to the known effect of cytokines in this disease, it has been reported that clonally expanded cytotoxic T lymphocytes are present in primary and metastatic renal cell cancer specimens and demonstrate HLA-restricted cytotoxicity against RCC cell lines. To optimize the possibility of generating a graft versus tumor effect *in vivo*, rapid and complete engraftment of the donor immune system is required [7, 9, 14, 19]. This goal was achieved in pioneer studies by CHILDS and coworkers with the use of fludarabine- cyclophosphamide nonablative conditioning regimen along with rapid withdrawal of the post-transplant immunosuppression and infusions of donor lymphocytes in the aim to convert mixed to full donor T-cell chimerism. Peripheral blood was used in this study as the source of stem cell because of high T-cell doses [10–12].

After receiving this regimen 19 of 42 patients had a measurable response and four patients enjoyed complete, long lasting responses. Importantly, disease regression typically occurred only after cyclosporine had been withdrawn and chimerism had transitioned from mixed to predominantly donor T-cell. Interestingly, only clear cell carcinoma and not other histologic types of renal cancer appeared to be a target of a graft versus tumor effect. Moreover, patients who had failed to respond to interferon alfa before transplantation had disease response when they were retreated with interferon alfa after transplantation.

These encouraging results have aroused interest for this approach in patients with metastatic clear cell renal carcinoma refractory to other therapeutic approaches [10–12]. Since a few data are available, we reported here our five cases. They constituted the group of unselected patients with metastatic clear cell carcinoma of the kidney, refractory to at least two courses of chemoimmunotherapy. All of them received stem cells from HLA identical sibling. Conditioning regimen, indications for early discontinuation of immunosuppressive therapy and for donor lymphocyte infusions were the same as described by CHILDS et al. Cyclosporine and short course of methotrexate were used to prevent graft versus host disease. Donor T-cell chimerism was reached in four patients. There was no treatment related mortality. Acute and chronic GVHD was the most common complication after transplantation. Moderate to severe acute GVHD occured in 3 (no grade IV was observed) and extensive chronic graft versus host disease in 2 patients. Donor lymphocyte infusion did not enhance GVHD in any patient.

No complete or partial tumor response was observed is our cases. Only two mixed response were reached. In the latter two cases regression of metastases in the lungs and mediastinal lymph nodes was occompanied by the progression in other organs. No tumor response occured after donor lymphocyte infusions.

To sum up, this reduced intensity regimen allow relatively safe allogeneic engraftment in this high-risk population of patients. Acute and chronic GVHD were common but no fatalities occured.

The efficacy of allogeneic HSCT in unselected patients with advanced solid tumors is recently widely documented [4, 5, 9–12, 20–25]. It is generally accepted that debulking of the primary tumor and metastases before stem cell transplantation decrease the risk of rapid tumor progression and allow donor T-cells to induce the anti-cancer response. Because of the higher response rate seen in the lungs than in the liver or bones, at first patients with isolated metastases to lungs should be considered as candidates to nonablative stem cell therapy. Overall, the reported experiences in metastatic renal cell carcinoma suggest that younger, otherwise healthy patients with low-volume, slow growing disease are the best candidates for allogeneic transplantation. However, this procedure should still be considered as an experimental approach for selected cases [4, 9, 15, 23].

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