Myeloablative conditioning in myelofibrosis using i.v. treosulfan and autologous peripheral blood progenitor cell transplantation with high doses of CD34+ cells results in hematologic responses – follow-up of three patients

Autologous transplantation after myeloablation for myelofibrosis with myeloid metaplasia provides a palliative therapy with a long term relief of symptoms. We have transplanted three patients with more than 5×10^6 CD34+ cells/kg body weight after myeloablation with treosulfan (total dose 42 g/m²) with a 18 months follow-up. Two of the patients had symptomatic splenomegaly and severe anemia. One patient had symptomatic splenomegaly and thrombocytopenia (<100×10^9/L). Granulocyte colony-stimulating factor-supported peripheral blood progenitor cell mobilization and collection was not associated with increased toxicity. Following transplantation we observed a prolonged reconstitution period of 28-38 days without fever or severe mucositis. All patients became free of erythrocyte transfusions or recovered to normal thrombocyte counts. There was a significant reduction of max. spleen size in one patient. We conclude that myeloablation with treosulfan and autologous PBPCT in these three patients with myelofibrosis was safe and useful.

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Agnogenic myeloid metaplasia (AMM), syn.: idiopathic myelofibrosis (IMF), one of the myeloproliferative disorders is characterized by marrow fibrosis, splenomegaly, extramedullary hematopoiesis (i.e. myeloid metaplasia), and a leukoerythroblastic peripheral blood smear. AMM is regarded as a stem cell disorder and is accompanied by bone marrow fibrosis as a secondary event. The other members of the group of chronic myeloproliferative diseases, which include essential thrombocytosis, and polycythaemia vera have the potential to progress to a clinical picture that is similar to AMM. The term myelofibrosis with myeloid metaplasia (MMM) encompasses AMM and the progressive, fibrotic phase of polycythaemia vera,[1] whereas essential thrombocytosis only exceptionally evolves into myelofibrosis. MMM is also frequently designated osteomyelofibrosis (OMF). The bewildering terminology with multiple different terms used in the past has recently been brought to standardized definitions.[2] Currently it is not possible to outline a single model of the cellular and molecular pathogenesis of AMM that justifies the proliferative advantage of the hematopoietic cells, the disruption of normal bone marrow extracellular texture with fibrosis, and the native extramedullary hematopoiesis.[3] The incidence of MMM is estimated at 0.73 per 100 000 person years in males and 0.4 per 100 000 person years in females. The median age at diagnosis ranges from 54 to 62 years in different reports. Median survival time from diagnosis ranges from 3.5 to 5.5 years. As only few patients are relatively young, allogeneic transplantation with maximal toxicity is not an option in the majority of patients. There have been a variety of criteria-sets for diagnosis of MMM, the latest being the Cologne Criteria[4] and the Italian Criteria.[3] A variety of clinical and biological prognostic parameters have been studied. Dupriez *et al.* presented a scoring system based on two adverse prognostic factors, namely Hb <10 g/dL and WBC <4 or >30×10^9/L, based on the survival data of 195 patients.[5] It was able to separate patients in three groups with low (0 factors), intermediate (1 factor) and high (2 factors) risk, associated with a median survival of 93, 26 and 13 months, respectively. The other recently proposed Scoring System by Cervantes *et al.*[6] identifies a high- and a low-risk prognostic group, based on the three factors constitutional symptoms, Hb < 10 g/dL, and circulating blasts. In these patients with none or one bad prognostic factor, MMM had an indolent course with a median survival of 98.8 months and a high-risk group with two or three factors, with a more aggressive disease and a median survival of 20.6 months. The only curative therapy to date is myeloablation followed by allogeneic transplantation. This approach, however, is associated with the risk of morbidity and mortality. After intensity-reduced conditioning, in the EBMT multicenter study, the 1-year treatment-related mortality was 37% and the relapse rate was 36% at 1 year, which included patients in acute transformation. The one year treatment related mortality was 18% with an overall survival of 82% amongst patients who were transplanted in chronic phase disease transplanted with HLA identical siblings.[7] The data presented by Guardiola et al. show that the prognosis after allogeneic transplantation is much better for younger patients. The five-year overall survival was 14 percent for patients older than 45 years vs. 62 percent for those <45 years old, $p<0.01$.[8,9] Deeg et al. reported on 56 patients with myelofibrosis who underwent allogeneic transplantation. The three year overall survival was 58%. Intriguingly, all 12 patients with a Dupriez score of 1 and only mild myelofibrosis are still alive.[10] These findings should be taken into account when a transplantation is considered. Especially for patients at low to intermediate who are less than 40 years old planning a transplantation after the age 45 years may not be the optimal strategy. Also, an allogeneic transplantation in early stage disease carries a relatively low risk. Taking these facts into account, autologous transplantation may therefore be a treatment alternative for older patients and patients with advanced stage disease or patients without a stem cell donor. *This approach is designed to reduce complications associated with the disease without causing undue morbidity or mortality. The mechanism for response among patients to this kind of therapy may include: reduction in fibrosis resulting in restored intramedullary hematopoiesis, reduction in spleen size resulting in reduced sequestation, preferential stimulation of nonclonal stem cells, and overall debulking of the disease burden resulting in decreased ineffective hematopoiesis. In the first study on this approach reported by Anderson et al. 2001[11] 21 patients underwent autologous transplantation after myeloablation with busulfan (Bu). 15 of these 21 patients had a clinical benefit from the procedure with improvements of anemia, thrombocytopenia or splenomegaly.*

To date this is the first study with autologous transplation for myelofibrosis. Busulfan was used as the single conditioning agent. Other agents used for conditioning in allogeneic trials with myeloablative conditioning include TBI (total body irradiation) alone or in combination with Bu, cyclophosphamide (Cy), thiotepa or etoposide or the combination of Bu plus Cy.[8,10] Myeloablative conditioning with these agents can lead to considerable non-hematologic toxicities like severe mucositis with TBI, hemorrhagic cystitis with Cy and veno-occlusive disease with Bu. Recently, also nonmyeloablative allogeneic transplantation was applied with combinations of TBI, fludarabin, Bu, BCNU, melphalan, thiotepa, Cy and antithymocyte/antilymphocyte globulin.[7]

We hypothesized that a conditioning wih treosulfan, a bifunctional alkylating drug, could result in reduced non-hematologic side effects with maintained therapeutic effects. Moreover,

treosulfan can be administered safely i.v with reliable pharmacokinetics compared to the oral application of busulfan.[12]

Case Report

To date 3 patients underwent autologous transplantation, all female (Table 1 and Table 2). Allogeneic transplantation was discussed for these patients. The overall performance status of patient #1 did not allow this treatment, there was no related donor for patient #2 and there is a related donor for patient #3, but she did not want to take the risk of allogeneic transplantation at that time. In this series no other patients were considered for transplantation. Mobilization failures did not occur. There were no other patients mobilized that did not go on to autologous transplantation.

Two of the patients (#1, #3) had symptomatic splenomegaly and severe anemia. Patient #2 had symptomatic splenomegaly and thrombocytopenia (< 100x109/L). Patients were stimulated with granulocyte colony-stimulating factor (G CSF, Neupogen™, 16 µg/kg) daily for 4 days and subsequent leukapheresis of a minimum of 5×10^6 CD34+ cells/kg was performed. Patient #1 required two leukapheresis sessions, patient #2 four and patient #3 six. The cumulated yields were 5.6 to 6.2 CD34+ cells$\times10^6$ /kg BW. *Patient #1 and patient #3 required transfusion of 2 units of packed erythrocytes each. The leukapheresis was clinically well tolerated in each case.* The following myeloablation consisted of treosulfan infusions of 14 g/m^2 for three consecutive days (total dose 42 g/m^2) and subsequent autologous peripheral blood progenitor cell transplantation (PBPCT). The data for PBPCT-associated toxicity are presented in Table 3. The time to reconstitution of leucocytes > 1/nl post transplantation was 28 days (#1, #2) and 38 days (#3) and the time for reconstitution of thrombocytes > 50/nl was 36 (#1), 22 (#2) and 33 (#3) days. The prolonged reconstitution period may have been due to the myelofibrosis and has also been observed after busulfan conditioning and PBPCT by others.[11] There were no fever or other severe toxicity and no parenteral nutrition was needed. The patients have been observed for 18 months post transplantation now. Marrow fibrosis remained unchanged as investigated by bone marrow biopsies pre-transplantation and after six and twelve months post-transplantation. The first patient (#1), who required erythrocyte transfusions twice weekly pretransplant received her last erythrocyte transfusion on day 56; her Hb-value is 11,2 g/dL at last follow-up. The second patient (#2) recovered to platelet counts higher than pre transplantation (58×10^9/L) at 3 months.($143/\times10^9$/L) and had $103/\times10^9$/L at last follow-up. Pat. #3 showed a marked reduction of max. spleen size and a rise in Hb from 9 g/dL to 12 g/dL after 12 months, the Hb value at 18 months is 8.7 g/dL and allogeneic transplantation is considered now.

Discussion

Our data show that treosulfan conditioning followed by autologous PBPCT is a safe and efficient treatment for patients with myelofibrosis. The basis for this treatment design was a study on autologous transplantation after myeloablation with high-dose oral busulfan. The authors reported that autologous transplantation provided a palliative approach which can lead to a long term relief of symptoms of the disease and is associated with acceptable morbidity and mortality.[11] However, busulfan pharmacokinetics after oral administration can vary between patients and increased toxicity is encountered. In this study mucositis, diarrhea, fever, vomiting, nausea or rash was observed in all patients that underwent transplantation. After transplantion, 6 patients died: 3 of nonrelapse causes (1 within 100 days of PBSC infusion died of graft failure) and 3 of disease progression. Erythroid response (hemoglobin > or = 10 g/dL without transfusion for > or = 8 weeks) occurred in 10 of 17 anemic patients. Four of 8 patients with a platelet count less than 100×10^9/L responded with a durable platelet count more than 100×10^9/L. Symptomatic splenomegaly improved in 7 of 10 patients. As this included patients with several improvements, altogether 15 out of 21 patients who underwent autologous transplantation showed a clinical benefit. Other myeloablative regimens included TBI alone or in combination with Bu, Cy, thiotepa or etoposide or the combination of Bu plus Cy.[8,10] High dose therapy wih these agents can lead to considerable non-hematologic toxicities like severe mucositis with TBI, hemorrhagic cystitis with Cy and veno-occlusive disease with Bu. In contrast to busulfan, treosulfan, a bi-functional alkylating drug, can be administered safely i.v with reli-

Table 1. Patient characteristics – clinical and prognostic data.

	Patient #1 (f)	*Patient #2 (f)*	*Patient #3 (f)*
Hepatomegaly at diagnosis (y/n)	no	yes	no
Dupriez Score [D] / Cervantes [C] score at diagnosis	D: low risk C: low risk	D: low risk C: low risk	D: intermediate risk C: low risk
Treatments from diagnosis to mobilization	3a	1b	0
Interval between diagnosis and G-CSF mobilization	81 month	18 month	28 month
Hepatomegaly at mobilization (y/n)	yes	yes	yes
Dupriez Score [D] / Cervantes [C] score at mobilization	D: intermediate risk C: high risk	D: low risk C: low risk	D: intermediate risk C: high risk
Date of PBPCT	21.03.2002	14.05.2002	10.06.02
Hepatomegaly at PBPCT (y/n)	yes	yes	yes
Dupriez Score / Cervantes score at PBPCT	D: intermediate risk C: high risk	D: low risk C: low risk	D: intermediate risk C: high risk
treatments after PBPCT	0	0	allogeneic transplantation from sister 08/2004

Prior treatments: a: 1996-2001 intermittent therapy with hydroxyurea, 1996-1999 interferon alfa, 12/2001 spleen irradiation ; b: 11/2000 hydroxyurea.

Table 2. Patient characteristics - hematological response.

		Time						
		at diagnosis	*before mobilization*	*before transplantation*	*3 months follow-up*	*6 months follow-up*	*12 months follow-up*	*18 months follow-up*
Patient #1 (f)	age	52	62	62			63	
	Hb (g/dl)	10.8	8.8	8.8	8.6	9.5	11.4	11.2
	leukocyte count (x10^9/L)	15.7	9.23	11.6	3.85	9.01	12.6	28.6
	thrombocyte count (x10^9/L)	413	88	154	142	210	209	181
	PB blasts (%)	0	0	1	2	0	2	0
	spleen size (length in cm)	20	24,5	29	27	26	27	n.a.
	erythrocyte transfusions	0	16/month	16/month	4/month	0	0	0
	constitutional symptoms (y/n)	no	yes	yes	yes	yes	yes	yes
Patient #2 (f)	age	48	50	50			51	
	Hb (g/dl)	11.3	14.4	12.8	14.2	14.1	12.1	12.8
	leukocyte count (x10^9/L)	13.8	7.61	8.57	4.5	5.1	5.06	7.8
	thrombocyte count (x10^9/L)	314	58	58	140	108	98	103
	PB blasts (%)	0	0	0	0	0	0	0
	spleen size (length in cm)	12	24,2	24,3	18	25	31	32
	erythrocyte transfusions	0	0	0	0	0	0	0
	thrombocyte transfusions	0	0	0	0	0	0	0
	constitutional symptoms (y/n)	no	yes	yes	no	no	yes	yes
Patient #3 (f)	age	45	48	48			49	
	Hb (g/dl)	9.2	10.1	9	9,8	11.8	12	8.7
	leukocyte count (x109/L)	8.1	7.53	6.1	1.89	2.69	2.86	4.04
	thrombocyte count (x109/L)	223	193	222	119	231	207	176
	PB blasts (%)	0	0	0	0	0	0	0
	spleen size (length in cm)	25	23,7	23	n.a.	18.5	17.5	18.1
	erythrocyte transfusions	0	2/month	2/month	0	0	0	0
	constitutional symptoms (y/n)	no	yes	yes	yes	no	no	yes

n.a. - not assessed.

Table 3. Patient characteristics – transplantation associated toxicity.

	Leukocyte nadir after PBPCT	*Time to reconstitution to > 1×10^9/L leukocytes (days) after PBPCT*	*Time to reconstitution to > 0.5×10^9/L granulocytes (days) after PBPCT*	*Time to reconstitution to > 1×10^9/L granulocytes (days) after PBPCT*	*Time to reconstitution to > 50×10^9/L thrombocytes [days] after PBPCT*	*days with use of opiates*	*Number of transfusions after PBPCT erythrocyte*	*Number of transfusions after PBPCT thrombo-cyte*	*Additional EPO or G-CSF*	*Hospitalization time [days]*
Patient #1 (f)	0.06/nl	33	n.a.	33	36	0	14	3	no	37
Patient #2 (f)	0.28/nl	28	n.a.	32	22	0	0	0	no	35
Patient #3 (f)	0.09/nl	38	38	n.a.	33	0	34	4	no	45

n.a. - not assessed

able pharmacokinetics. The maximal tolerated dose (MTD) without stem cell support is 10 g/m^2 in man.[12]The myeloablative dose range has been explored recently at escalating doses from 20 g/m^2 up to 56 g/m^2 with subsequent PBPCT.[13] The maximum tolerated dose of treosulfan in this trial was 47 g/m^2. A split dose regimen was explored with doses of 3×10 g/m^2 to 3×14 g/m^2.[14]This seemed attractive for conditioning of myelofibrosis patients. Moreover, treosulfan doses of 3×14 g/m^2 combined with other high-dose cytotoxic drugs were safely administered to patients with breast- and ovarian cancer as well as NHL or MM prior to autologous stem cell transplantation.[13,15,16] In our study in myelofibrosis patients treosulfan was well tolerated. Patients did not experience severe non-hematological toxicity such as mucositis and did not require total parenteral nutritition. None of the patients developed an infection Response was maximal at 12 months in patients #2 and #3 with a subsequent drop of values. It is not arguable that complete remissions in myelofibrosis can only be expected with allogeneic blood stem cell transplantation. The study of Anderson *et al.*[11] showed that autologous PBPCT in myelofibrosis is feasible and provides palliation. An improvement in prognosis was not formally demonstrated. This remains difficult, as it would require randomization. After a longer follow-up a matched-pair comparison with controls should be performed. *Alternative agents that have been tested clinically include pegylated interferon, the tyrosine-kinase inhibitor imatinib mesylate, the farnesyl transferase inhibitor R115777 (Zarnestra), the TNF-α receptor etanercept and the tyrosine kinase inhibitor of the VEGF receptor SU5416 as reviewed by Hennessy et al. [17] A large body of data has been acquired for thalidomide, with response rates of up to 56% as reported by Mesa et al.[18] at a relatively low daily dose of 50 mg in combination with prednisone. Other trials that used higher doses of thalidomide without prednisone did not report higher response rates, but a higher rate of side effects and significantly more withdrawals occurred.[17] Thus the question currently remains whether thalidomide/prednisone should be used as a front-line treatment for MMM. One possible concept including thalidomide and autologous transplantation could be to first evaluate allogeneic tranplantation. If this is not feasible one could pursue autologous transplantation followed by thalidomide treatment or one of these regimes. It has to be kept in mind, that thalidomide is currently not licensed in the European Union and therefore the treating physician is liable for prescrip-*

tion outside of a clinical trial. We conclude that myeloablation with treosulfan and autologous PBPCT in these three patients with myelofibrosis was safe and useful. Survival of these patients is promising up to date. *The single experienced toxicity was an aplasia time of approximately four weeks, which is in our eyes justifiable for the probable palliation of symptoms for at least one year.* The results presented warrant further exploration of this approach in a clinical study which has been initiated by our group.

(cf. http://leukaemie.krebsinfo.de/kn_home/Studien/studie_101.html).

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