původní práce

IPHOSPHAMIDE AND ETOPOSIDE BASED CHEMOTHERAPY AS SALVAGE AND MOBILIZING REGIMENS FOR POOR PROGNOSIS LYMPHOMA

ZÁCHRANNÉ A PERIFERNÍ BUŇKY MOBILIZUJÍCÍ CHEMOTERAPIE ZALOŽENÁ NA KOMBINACI IFOSFAMIDU A ETOPOSIDU U NEMOCNÝCH LYMFOMEM SE ŠPATNOU PROGNÓZOU

MAYER J., VÁŠOVÁ I., KOŘÍSTEK Z., NAVRÁTIL M., KLABUSAY M., DOUBEK M., VODVÁŘKA P.[1], PETRÁKOVÁ K.[2], VORLÍČEK J., ČERNILOVÁ I.

DEPARTMENT OF INTERNAL MEDICINE - HEMATOONCOLOGY, UNIVERSITY HOSPITAL, BRNO
[1]DEPARTMENT OF RADIOTHERAPY AND CLINICAL ONCOLOGY, FACULTY HOSPITAL, OSTRAVA
[2]MASARYK MEMORIAL CANCER INSTITUTE, BRNO

Abstract: ***Background:*** Treatment of early relapsing or resistant non-Hodgkin's lymphoma (NHL) and Hodgkin's disease (HD) is not satisfactory. High dose chemotherapy followed by autologous peripheral blood stem cell (PBSC) transplantation offers the possibility to improve prognosis. We adopted the strategy of using salvage chemotherapy as debulking as well as PBSC mobilizing treatment. We used the regimens based on ifosfamide and etoposide because these drugs are not frequently used in the front-line treatment. ***Patients and treatment:*** Patients with NHL (n=32) received MINE chemotherapy (n=43, mesna, ifosfamide 1330 mg/m^2 and etoposide 65 mg/m^2 by i.v. infusions on days 1-3, mitoxantrone 8 mg/m^2 i.v. on day 1). The same schedule, but higher doses were used for PBSC mobilization (n=32, ifosfamide 1700 mg/m^2, etoposide 175 mg/m^2, mitoxantrone 10 mg/m^2). Patients with HD (n=50) received VIM chemotherapy (n=116, mesna, ifosfamide 1200 mg/m^2 by i.v. infusion on days 1-5, etoposide 90 mg/m^2 by i.v.infusion on days 1, 3, and 5, methotrexate 30 mg/m^2 i.v. on days 1 and 5). After both VIM and MINE, mobilization chemotherapy was followed by G-CSF at the dose 5-16 μg/kg/day (mainly 10 μg/kg/day), depending on pretreatment of the patients. ***Results:*** The responses after VIM and MINE were CR 36% and 40%, PR 20% and 30%, and SD 24% and 4%, respectively. In both groups, patients with relapsing disease responded more better than those with primary progressive disease. Both regimens exhibited excellent mobilizing capacity. We performed 229 aphereses (median 3 leukaphereses per patient) starting on either day 13 (median; VIM), or on day 12 (median; MINE). In the vast majority of patients, the collection started in the time interval: median ± 1 day (n=70, 85%). The median yields were 10.7x10^6 CD34$^+$ cells/kg and 52.9x10^4 CFU-GM/kg for VIM, and 12.5x10^6 CD34$^+$ cells/kg and 51.5x10^4 CFU-GM/kg for MINE. We did not collect at least 2.5x10^6 CD34$^+$ cells/kg in only 7 patients (9%), and the harvested amount of CD34+ cells was lower than 1.0x10^6/kg in only 2 patients (2%). The toxicity of all 191 VIM and MINE chemoterapies was minimal. PBSC collections were not complicated by chemotherapy-induced trombocytopenia. Forty-seven HD patients (94%) and 26 NHL patients (81%) were transplanted (BEAM or BuCy2 regimens). The recovery of hematopoiesis was rapid in both groups of patients. Median time for reaching white blood cell count 1.0x10^9/l was 10 days for both HD and NHL patients, and for thrombocytes > 50x10^9/l 12 days for HD and 11 days for NHL patients, respectively. ***Conclusion:*** VIM and MINE are well-tolerated regimens providing significant anti-lymphoma effect and low toxicity. In combination with G-CSF, they also provide very good PBSC mobilizing capability in a predictable time interval. These regimens are also advantageous because of short-time leukopenia and mild thrombocytopenia, which is critical for ensuring uneventful apheresis. Compared with other regimens, VIM and MINE seem to offer multiple advantages

Key words: Non-Hodgkin's lymphoma, Hodgkin's disease, peripheral blood stem cells, bone marrow transplantation, etoposide, ifosfamide

Souhrn: ***Úvod:*** Léčba časně relabujících a nebo rezistentních lymfomů (ne-Hodgkinovy lymfomy, NHL a Hodgkinova choroba, HD) je velmi neuspokojivá. Vysokodávková léčba s autologní transplantací periferních kmenových buněk (PBSC) nabízí možnost, jak prognózu zlepšit. Na našem pracovišti jsme použili strategii podávání záchranné a zároveň PBSC mobilizační léčby s využitím ifosfamidu a etoposidu. Tyto léky nejsou běžně používány v úvodní léčbě nemocných s lymfomy. ***Soubor nemocných a metodika:*** Pacienti s NHL (n=32) byli léčeni MINE chemoterapií (n=43, mesna, ifosfamid 1330 mg/m^2 a etoposid 65 mg/m^2 i.v. infuze dny 1-3, mitoxantron 8 mg/m^2 i.v. den 1). Stejné schéma, ale s vyššími dávkami, bylo použito pro mobilizaci: (n=32, ifosfamid 1700 mg/m^2, etoposid 175 mg/m^2, mitoxantron 10 mg/m^2). Nemocní s HD (n=50) byli léčeni kombinací VIM (n=116, mesna, ifosfamid 1200 mg/m^2 i.v. infuze dny 1-5, etoposid 90 mg/m^2 i.v.infuze dny 1, 3, a 5, methotrexat 30 mg/m^2 i.v. dny 1 a 5). Po aplikaci mobilizační chemoterapie VIM nebo MINE následovalo podávání růstového faktoru G-CSF v dávce 5-16 μg/kg/den (většinou 10 μg/kg/den) v závislosti na předléčenosti nemocných. ***Výsledky:*** Léčebné odpovědi po VIM a MINE byly: CR 36% a 40%, PR 20% a 30%, SD 24% a 4%. Pro obě skupiny lymfomů platilo, že nemocní s relabující chorobou měli daleko lepší léčebnou odpověď než nemocní s chorobou rezistentní. Provedli jsme celkem 229 leukaferéz (medián 3 leukaferézy u jednoho nemocného) se zahájením den 13 (medián; VIM), nebo den 12 (medián; MINE). U naprosté většiny nemocných byl sběr PBSC zahájen v časovém intervalu: medián ± 1 den (n=70, 85%). Výtěžnosti (medián) byly: 10.7x10^6 CD34$^+$ buněk/kg a 52.9x10^4 CFU-GM/kg pro VIM a 12.5x10^6 CD34$^+$ buněk/kg a 51.5x10^4 CFU-GM/kg pro MINE. Alespoň 2.5x10^6 CD34$^+$ buněk/kg se nepodařilo nasbírat pouze u 7 nemocných (9%) a získané množství CD34+ buněk bylo nižší než 1.0x10^6/kg pouze u 2 nemocných (2%). Toxicita všech 191 VIM a MINE chemoterapií byla minimální. Sběr PBSC nebyl komplikován trombocytopenií indukovanou chemoterapií. Čtyřicet sedm nemocných s HD (94%) a 26 nemocných s NHL (81%) bylo transplantováno (režimy BEAM nebo BuCy2). Obnova krvetvorby byla rychlá u obou skupin nemocných. Medián dosažení leukocytů > 1.0x10^9/l byl 10 dnů pro nemocné s NHL i HD a medián dosažení trombocytů > 50x10^9/l byl 12 dnů pro nemocné s HD a 11 dnů pro nemocné s NHL. ***Závěr:*** VIM a MINE jsou velmi dobře tolerované režimy se signifikantní protilymfomovou aktivitou při nízké toxicitě. V kombinaci s G-CSF také vykazují velmi dobrou mobilizační kapacitu ve velmi dobře odhadnutelném časovém intervalu. Jejich výhoda je také v jen krátkodobé leukopenii a mírné trombocytopenii, což je důležité pro bezproblémové leukaferézy. Ve srovnání s jinými režimy jeví VIM a MINE chemoterapie mnohé výhody.

Klíčová slova: Ne-Hodgkinovy lymfomy, Hodgkinova choroba, periferní kmenové buňky, transplantace kostní dřeně, etoposid, ifosfamid

Introduction

Advanced-stage Hodgkin's disease (HD) and some non-Hodgkin's lymphomas (NHL) are neoplasias potentially curable with standard chemotherapy, but many patients do not achieve remission or their disease relapses (Cannelos et al., 1992, Fisher et al., 1993). One option how to improve these results is to intensify front-line chemotherapy, either by dose-escalation of conventional therapy (Diehl et al., 1998), or by adding high-dose chemotherapy with hematopoietic progenitor cell support (Carella et al., 1995, Haioun et al, 1999). Treatment of relapsing disease with conventional chemo- and/or radiotherapy is unsatisfactory, especially in early relapsing patients (Longo et al., 1992, Salles et al., 1994). However, there is also the possibility to improve prognosis of these patients by implementing the high dose chemotherapy into the treatment plan (Philip et al., 1995, Schmitz et al., 1999). Furthermore, high-dose chemotherapy is the effective salvage strategy for HD patients who do not enter remission after induction treatment (Sweetenham et al., 1999). For progressive disease of NHL patients, however, this kind of therapy usually fails (Mills et al., 1995). For above-mentioned reasons, the high dose therapy has become a standard part of management of many patients with lymphomas.

It is not entirely clear when to proceed to the high dose therapy (Phillips et al., 1997). Several reports showed better prognosis of those patients who were transplanted at the time of minimal tumor burden (Prince et al., 1996, Horning et al., 1997, Moskowitz et al., 1999). However, response to conventional salvage chemotherapy might only represent a good prognostic marker and it is not known whether deliberate maximization of pretransplant chemotherapy can improve the prognosis of patients. Some data, however, support the effort to achieve the maximum response before transplantation (Bosly et al., 1997).

During the last years, we have witnessed a dramatic replacement of bone marrow by peripheral blood stem cells (PBSC) for autotransplantation (Gratwohl et al., 1999). Usage of PBSC brings faster hematopoietic recovery and can be even cost saving (Smith et al., 1997). Growth factors (G-CSF or GM-CSF, granulocyte or granulocyte-macrophage colony stimulating factors) and/or a higher dose of cyclophosphamide are standards for adequate mobilization of PBSC, although other combinations of polychemotherapy and growth factors can be used. A combination of chemotherapy and growth factors is more effective than growth factors or chemotherapy alone and the intensity of chemotherapy correlates with the degree of PBSC mobilization (Demirer et al., 1996). In lymphoma patients, PBSC can also be mobilized with growth factors alone or by combination of high-dose cyclophosphamide and growth factors, but these regimens have little or no activity against malignant disease. Therefore, the best option seems to be the usage of the regimen that embodies proven mobilization ability and adequate anti-neoplastic activity, combining PBSC mobilization with tumor mass cytoreduction prior to administration of the conditioning regimen.

Few salvage regimens have been examined in combination with growth factors-stimulated PBSC mobilization (Fermé et al., 1994, Olivieri et al., 1995, Kröger et al., 1998, Donato et al., 1999, McQuaker et al., 1999, Moskowitz et al., 1999, Petit et al., 1999). In our study, we used the VIM regimen (VP-16, ifosfamide, methotrexate) (Nowrousian et al., 1987) for HD disease patients and MINE (mesna, ifosfamide, mitoxantrone - Novatrone, etoposide) (Rodriguez et al., 1995) for NHL patients. VIM is similar to the IMVP-16 regimen (Cabanillas et al., 1982). In this report, we have extended our observations published previously (Mayer et al., 1999) for a larger series of patients and for an analysis of engraftment kinetic.

Patients and methods

Patients

From September 1995 to September 2000, we treated 32 NHL and 50 HD patients, who were primarily refractory (n=21, 26%), partial responders to first-line therapy (usually ABVD and CHOP, n=17, 20%) relapsed (n=39, 48%) , or very high risk after achieving complete remission (CR, n=5, 6%). Patients' characteristics are shown in table 1.

Many patients were referred to our department from other departments for salvage therapy only. Therefore, we did not

Table 1. Patients' characteristics at the time of start of VIM or MINE salvage chemotherapy.

Patients' characteristics	HD (n=50)	NHL (n=32)
Age (years)	19-56, median: 29	18-63, median:43
Male: female	27:23	13:19
Number of previous chemotherapy courses	2-32, median: 6	2-15, median: 7
Previous radiotherapy	n=31; 62%	n=8; 25 %
Progressive disease	n=16; 32%	n=5; 16%
Partial remission after induction treatment	n=4; 8%	n=12; 38%
Stable disease after induction treatment	n=1; 2%	n=0
1st relapse	n=17; 34%	n=11; 34%
2nd or subsequent relapse	n=9; 18%	n=2; 6%
Complete remission in very high risk patients	n=3; 6%	n=2; 6%

Table 2. Chemotherapy VIM (salvage and/or mobilization).

Etoposide (Vepesid - Bristol - Myers Squibb)	90 mg/m^2 i.v. infusion	once daily on days 1,3,5
Ifosfamide (Holoxan - Asta Medica)	1200 mg/m^2 i.v. infusion	once daily on days 1 - 5
Methotrexate (Methotrexat Lachema – Lachema CZ)	30 mg/m^2 i.v.	once daily on days 1 and 5

Filgrastim (Neupogen - Amgen Roche) 5-16 μg/kg s.c. in two divided doses (for the doses >5 μg/kg/day) daily to mobilize PBSC from day 7.

Table 3. Chemotherapy MINE used for mobilization.

Ifosfamide (Holoxan - Asta Medica)	1700 mg/m^2 i.v. infusion	once daily on days 1 - 3
Mitoxantrone (Refador - Spofa CZ)	10 mg/m^2 i.v.	on day 1
Etoposide (Vepesid - Bristol - Myers Squibb)	175 mg/m^2 i.v. infusion	once daily on days 1 - 3

Filgrastim (Neupogen - Amgen Roche) 5-16 μg/kg s.c. in two divided doses (for the doses >5 μg/kg/day) daily from day 5 until aphereses are finished.

Table 4. Chemotherapy MINE used as salvage only.

Ifosfamide (Holoxan - Asta Medica)	1330 mg/m^2 i.v. infusion	once daily on days 1 - 3
Mitoxantrone (Refador - Spofa CZ)	8 mg/m^2 i.v.	on day 1
Etoposide (Vepesid - Bristol - Myers Squibb)	65 mg/m^2 i.v. infusion	once daily on days 1 - 3

have precise REAL classification for all NHL patients. Among the 32 NHL patients, there were 11 patients with diffuse large B-cell lymphoma, 5 patients with peripheral T-cell lymphoma, 5 patients with diffuse centroblastic-centrocytic lymphoma, 3 patients with centroblastic lymphoma, 2 patients with mantle cell lymphoma, 1 patient with immunoblastic lymphoma, 1 patient with centrocytic lymphoma, 2 patients with follicular lymphoma, and 2 patients with unclassified NHL.
In patients who received at least two courses of salvage chemotherapy the response was estimated according to published criteria (Cheson et al., 1999). For high dose therapy, we used standard BEAM or BuCy2 regimens in the majority of cases.

Chemotherapy VIM and MINE
Details are shown in tables 2, 3, and 4. Continuous hydration (250 ml per hour) and mesna (in 100% of ifosfamide dose) were started 3 hours prior to starting ifosfamide infusion. The higher dose of filgrastim (16 μg/kg/day) for mobilization was given only in highly pretreated patients (previous extensive radiotherapy and/or intensive polychemotherapy). According to the published data, MINE chemotherapy is not very myelotoxic (Rodriguez et al., 1995). Therefore, in an effort to improve the mobilizing capacity of MINE, we increased the doses of cytostatics in MINE chemotherapy used for mobilization.

Leukaphereses and cryoconservation
Aphereses began during recovery from myelosuppression when leukocytes exceeded at least 1 x 10^9 cells/l and the peripheral blood $CD34^+$ cell count approached 20 cells per μl. Collections were performed with a COBE Spectra cell separator (software version 3.6, later 5.1) with collection pump speed 0.9 ml/min. We chose as the threshold for satisfactory harvest 5 x 10^6 $CD34^+$ cells/kg (when achieved in one procedure, we collected at least 2.5 x 10^6 $CD34^+$ cells/kg in the second apheresis as a back-up). Usually, during one apheresis 2.5-3 total blood volumes of the patient were processed.
Cells were cryopreserved with a Sy-Lab Glacier device (SY-LAB, Austria) in a mixture of autologous plasma, Hank's balanced salt solution (without Ca and Mg; Sigma) and DMSO (Sigma; final concentration of 10%), and were stored in liquid nitrogen.

$CD34^+$ cell estimation and CFU-GM assay
These analyses were performed as previously described (Mayer et al., 1999). However, from May 1999 untill now, the number of CFU-GM has been evaluated in a routine colony-assay systems using complete methylcellulose-based medium MethoCult HCC-4434 (StemCell Technologies Inc., Vancouver, Canada). A sample of the leukapheresis product was diluted using heparinized (50 UI/ml) 4% human serum albumin in Hank's balanced salt solution (Sigma, St.Louis, USA) and analyzed for blood cell count. Volume containing $0.5 10^6$ of white blood cells was then mixed with Iscoves's modified Dulbecco's medium (StemCell Technologies Inc., Vancouver, Canada) to get 0.5 ml of cell suspension. In the end, 3 ml of MethoCult medium HCC-4434 was well mixed with 0.3 ml of the cell suspension and plated in duplicate according to the manufacturer's instructions. CFU-GM colonies were scored after 14-16 days of incubation at 37° C in 5% CO_2 humidified atmosphere. Results were expressed as the number of CFU-GM in one ml of analyzed sample.

Results

Toxicity of VIM and MINE therapy
A total of 191 courses of salvage ifosfamide and etoposide-based regimens were administered, 116 courses of VIM (median 2 per patient; range 1 - 4) and 75 courses of MINE (median 3 per patient; range 1 - 4).
The tolerance of chemotherapy was excellent, side effects

Table 5a. Toxicity of VIM regimen (WHO scale) administered as a salvage only (n=66).

Toxicity	Hematologic – leukocytes	Hematologic – thrombocytes	Hematologic – hemorrhage	Gastrointestinal – liver enzymes	Gastrointestinal – oral	Gastrointestinal – nausea/vomiting	Gastrointestinal – diarrhea	Renal – hematuria	Others
Grade 0	47	57	66	54	64	43	65	58	One patient developed severe mucositis, one patients developed febrile neutropenia.
Grade 1	6	2	0	10	1	18	1	8	
Grade 2	8	3	0	2	0	5	0	0	
Grade 3	2	3	0	0	0	0	0	0	
Grade 4	3	1	0	0	1	0	0	0	
Median	0	0	0	0	0	0	0	0	
Minimum	0	0	0	0	0	0	0	0	
Maximum	4	4	0	2	4	2	1	1	
Average	1	0	0	0	0	0	0	0	

Table 5b. Toxicity of VIM regimen (WHO scale) administered as a salvage and PBSC mobilization (n=50).

Toxicity	Hematologic – leukocytes	Hematologic – thrombocytes	Hematologic – hemorrhage	Gastrointestinal – liver enzymes	Gastrointestinal – oral	Gastrointestinal – nausea/vomiting	Gastrointestinal – diarrhea	Renal – hematuria	Others
Grade 0	30	18	48	30	46	28	50	39	Five patients (10%) needed platelet transfusions (1-5 units)
Grade 1	6	10	2	16	3	16	0	11	
Grade 2	3	9	0	3	0	4	0	0	
Grade 3	2	8	0	1	1	2	0	0	
Grade 4	9	5	0	0	0	0	0	0	
Median	0 (4x10^9/l)	1 (79.5x10^9/l)	0	0	0	0	0	0	
Minimum	0 (14.4x10^9/l)	0 (295x10^9/l)	0	0	0	0	0	0	
Maximum	4 (0.04x10^9/l)	4 (9x10^9/l)	1	3	3	3	0	1	
Average	0 (4.39x10^9/l)	1 (91.2x 10^9/l)	0	1	0	1	0	0	

Table 5c. Toxicity of MINE regimen (WHO scale) administered as a salvage only (n=43).

Toxicity	Hematologic – leukocytes	Hematologic – thrombocytes	Hematologic – hemorrhage	Gastrointestinal – liver enzymes	Gastrointestinal – oral	Gastrointestinal – nausea/vomiting	Gastrointestinal – diarrhea	Renal – hematuria	Others
Grade 0	22	33	43	43	41	31	43	34	
Grade 1	5	6	0	0	1	8	0	9	
Grade 2	5	3	0	0	0	2	0	0	
Grade 3	6	1	0	0	1		0	0	One patient developed febrile neutropenia.
Grade 4	5	0	0	0	0	0	0	0	
Median	1	0	0	0	0	0	0	0	
Minimum	0	0	0	0	0	0	0	0	
Maximum	4	3	0	0	3	3	0	1	
Average	2	0	0	0	0	0	0	0	

Table 5d. Toxicity of MINE regimen (WHO scale) administered as a salvage and PBSC mobilization (n=32).

Toxicity	Hematologic – leukocytes	Hematologic – thrombocytes	Hematologic – hemorrhage	Gastrointestinal – liver enzymes	Gastrointestinal – oral	Gastrointestinal – nausea/vomiting	Gastrointestinal – diarrhea	Renal – hematuria	Others
Grade 0	0	10	32	27	30	20	32	21	
Grade 1	4	3	0	5	1	6	0	11	
Grade 2	3	9	0	0	1	4	0	0	
Grade 3	9	9	0	0	0	2	0	0	Five patients (16%) needed platelet transfusions (2-5 units)
Grade 4	16	1	0	0	0	0	0	0	
Median	4 (1.0x10^9/l)	2 (69x10^9/ l)	0	0	0	0	0	0	
Minimum	1 (3.45x10^9/l)	0 (148x10^9/l)	0	0	0	0	0	0	
Maximum	4 (0.09x10^9/l)	4 (23.5x10^9/l)	0	1	2	3	0	0	
Average	3 (1.31x10^9/l)	1 (75.6x10^9/l)	0	0	0	0	0	0	

were not serious. For better outcome of salvage therapy or when VIM and MINE failed, we administered a total of 51 courses of other regimens (mini-dexa-BEAM, DHAP). Detailed information concerning toxicity of MINE and VIM regimens are given in the tables 5a – 5d.

Outcome of VIM and MINE salvage therapy

There were 41 HD and 27 NHL patients who received at least two courses of MINE or VIM salvage chemotherapy, and in whom all necessary data were available for response evaluation. Administration of salvage VIM chemotherapy resulted in 56% of overall response rate in HD patients and the responses were as follows: CR 36%, partial remission (PR) 20%, stable disease 24%, and progressive disease 20%. For NHL patients, the administration of MINE chemotherapy resulted in 70% of overall response rate and the responses were as follows: CR 40%, PR 30%, stable disease 4%, and progressive disease 26%. We observed striking differences between subgroups of patients and the data are displayed in the table 6. The detailed analysis of efficacy and toxicity of further salvage regimens (mini-dexa-BEAM, DHAP) is beyond the scope of this article. Briefly, these regimens, administered as second salvage chemotherapy, have substantial toxicity and limited efficacy (Vášová et al., 1999).

Leukaphereses and yields

We performed 229 leukaphereses (median 3 per patient; range 1-7) in total. We did not collect at least 2.5x10^6 $CD34^+$ cells/kg in only 7 patients (9%), and the harvested amount of $CD34^+$ cells was lower than 1.0x10^6/kg in only 2 patients (2%).

In 50 HD patients primed with VIM + G-CSF, we started to collect PBSC between the days +11 and +19 (median: +13). In 44 from 50 patients (88%), the harvest started between the days +12 - +14, so the timing of aphereses using VIM was advantageously predictable. The mean collection yield was 10.6x10^6 $CD34^+$ cells/kg (range 0.3 - 38.8 x10^6) and 53.1 x 10^4 CFU-GM/kg (range 0.6 - 216.0 x10^4). The dose of G-CSF used for mobilization was 5 μg/kg/day in 9 patients, 10 mg/kg/day in 29 patients, and 16 μg/kg/day in 12 patients.

We started to collect PBSC between days +10 and +15 (median: +12) in 32 NHL patients primed with MINE + G-CSF. In 26 from 32 patients (81%), the harvest started between the days +11 - +13. Again, the timing of aphereses using MINE was easily predictable. The mean collection yield was 12.5x10^6 $CD34^+$ cells/kg (range 0.8 - 48.1 x10^6) and 51.5x10^4 CFU-GM/kg (range 4.2 – 180.8 x10^4). The dose of G-CSF used for mobilization was 5 μg/kg/day in 5 patients, 10 μg/kg/day in 25 patients, and 16 μg/kg/day in 2 patients.

Engraftment after high dose therapy

Forty-seven of 50 HD patients (94%) and 26 of 32 NHL patients (81%) were transplanted (BEAM – 54 times, BuCy2 – 15 times). The recovery of hematopoiesis was rapid in both groups of patients. Median time for reaching white blood cells > 1.0x10^9/l was 10 days for both HD and NHL patients, and for thrombocytes > 50x10^9/l 12 days for HD and 11 days for NHL patients, respectively.

Table 6. Response rates in subgroups of HD and NHL patients after VIM and MINE salvage chemotherapy.

Disease status before salvage therapy	Disease status after salvage therapy							
	VIM (Hodgkin's disease)				MINE (non-Hodgkin's lymphomas)			
	SD	PR	CR	Progression	SD	PR	CR	Progression
Progression	5/14	2/14	2/13	5/14	0/5	1/5	0/5	4/5
	36%	14%	14%	36%	0%	20%	0%	80%
Relapse	4/25	6/25	12/25	3/25	0/11	4/11	6/11	1/11
	16%	24%	48%	12%	0%	36%	55%	9%
D, PR	1/2	–	1/2	–	1/11	3/11	5/11	2/11
	50%		50%		9%	27%	46%	18%

Discussion

Our data shows that VIM and MINE are effective regimens for therapy of relapsed or refractory lymphoma patients, have excellent PBSC mobilization capacity in a predictable time interval and are of low toxicity. Rodriguez et al. (1995) reported a response rate of 48% (CR 21%) in NHL patients treated with MINE. The main toxicity was myelosuppression, nephrotoxicity and neurotoxicity were also reported. Nowrousian et al. (1987) observed 34% CR and 43% PR in lymphoma patients treated with VIM±bleomycin. In 6% of patients, the authors observed stomatitis, and septic complications in 5%. The response rate of MINE in the present study was 70% (CR 40%, PR 30%). We also observed myelosuppression as the main form of toxicity. The response rate to VIM was 56%; 36% CR, and 20% PR. As with MINE, myelosuppression was the dominant form of toxicity. We also observed urotoxicity, nausea, vomiting and occasionally a transient increase in serum SGOT/SGPT levels. Keeping in mind heterogeneity of patient populations in different reports it seems that our results concerning efficacy and toxicity of VIM and MINE regimens are comparable with those published previously.

Several previous publications have demonstrated the PBSC

Table 7. Usage of lymphoma salvage chemotherapies as mobilizing regimens.

Study	Patients	Regimen	Collection	Toxicity
Olivieri et al., 1995	14 NHL and HD patients	DHAP + 5 μg/kg/day G-CSF	Start on days +11 - +18; 10 (1.1-162.3) $x10^4$/kg CFU-GM, 2.6 (0.2-18.3) $x10^6$/kg $CD34^+$	Thrombocytopenia <$20x10^9$/l in 4 patients (28%).
Petit et al., 1999	22 NHL and HD patients	ESHAP + 5 μg/kg/day G-CSF	Start on days +12 - +19; 7.4 (0.78-50.9) $x10^6$/kg $CD34^+$	Thrombocytopenia <$25x10^9$/l lasted 1 (0-8) days
Kröger et al., 1998	17 NHL and HD patients	Mini-dexa-BEAM + 5 μg/kg/day G-CSF	Start on days +13 - +21; 6 (0.8-90) $x10^4$/kg CFU-GM, 5.1 (0.3-24.8) $x10^6$/kg $CD34^+$	Hospitalization 21 (18-24) days, i.v. antibiotics 6 (0-7) days, number of platelet transfusions 1 (1-2)
Fermé et al., 1994	30 relapsed or refractory HD patients	Mitoguazone, ifosfamide, vinorelbine, etoposide + 5 μg/kg/day GM-CSF	Start on days +11 - +22; sufficient graft in 20/23 patients (87%)	Grade 4 neutropenia 85%, grade 3-4 thrombocytopenia 53%, febrile neutropenia 32%
McQuaker et al., 1999	42 NHL and HD patients	IVE (ifosfamide, etoposide, epirubicin) + G-CSF 300 μg/day	6.78 (0.19-36) $x10^6$/kg $CD34^+$	Not analyzed
Moskowitz et al., 1999	163 NHL patients	ICE (ifosfamide, carboplatin, etoposide) + G-CSF 10 μg/kg/day; collection started after the third ICE cycle. After previous two cycles, G-CSF 5 μg/kg/day was given.	In 91 of the 100 patients, who actually underwent PBSC harvest, the collection started on days +11 or +12. Collection: 8.4 (0.1-40) $x10^6$/kg $CD34^+$	After the stimulation ICE, 10% and 36% of patients needed platelet and red blood cell transfusion, respectively
Donato et al., 1999	36 HNL patients	Ifosfamide total dose 10 g/m^2, etoposide total dose 900 mg/m^2 + G-CSF 10 μg/kg/day.	Start on days +16 - +23; 13.1 (4.1-148) $x10^6$/kg $CD34^+$	Thrombocytes on the day of the first collection: 38 (11-315) $x10^9$/l, 17% of patients developed febrile neutropenia
Present study	82 NHL and HD patients	VIM for HD and MINE for NHL patients + G-CSF 5-16 μg/kg/day (mainly 10 μg/kg/day).	In 85% of patients, collection started on median (13-VIM, 12-MINE) ±1 day. In only 9% of patients, at least $2.5x10^6$/kg $CD34^+$ cells were not collected, and in only 2% of patients at least $1x10^6$/kg $CD34^+$ cells were not collected.	Very low, see "Results"

Although there were not many studies published so far studying anti-tumor efficacy, toxicity, and stimulating capacity of ifosfamide-based salvage regimens, data summarized in table 6 show very encouraging results. Based on our experience we conclude that the VIM and MINE are well-tolerated regimens providing significant anti-lymphoma effect and low toxicity. VIM and MINE in combination with 5-16 μg/kg/day of filgrastim also provide good PBSC mobilizing capability in a predictable time interval. These regimens are also advantageous because of short-time leukopenia and mild thrombocytopenia, which is critical to ensure an uneventful apheresis. Compared with other regimens, VIM and MINE seems to offer multiple advantages.

mobilization capacity of salvage regimens; some regimens were rather toxic, however, while others failed to show sufficient mobilizing capacity. The poor predictability of the harvest window after mobilization chemotherapy observed in other studies presents logistic problems. Data from some reports are summarized in the table 7.

Acknowledgements
We thank colleagues from our department for the excellent care for patients, V. Hoffová and J. Čerňanská for cooperation with stem cell harvest, and J. Adler and D. Havranová for cooperation with progenitor cell counting and cryopreservation.

Literature

1. Bosly A., Sonet A., Salles G., Brice P., Haioun C. et al.: Late intensification is superior to early intensification in relapsing/refractory aggressive non-Hodgkin's lymphoma. A randomized study from the GELA: LNH RP 93. Exp. Hematol. 25, 1997, 732. Abstract No 16.
2. Cabanillas F., Hagemeister F.B., Bodey G.P., Freireich E.J.: IMVP-16: an effective regimen for patients with lymphoma who have relapsed after initial combination chemotherapy. Blood 60, 1982, 693-697.
3. Canellos G.P., Anderson J.R., Propert K.J., Nissen N., Cooper M.R. et al.: Chemotherapy of advanced Hodgkin's disease with MOPP, ABVD, or MOPP alternating with ABVD. N. Engl. J. Med. 327, 1992, 1478-1484.
4. Carella A.M., Pollicardo N., Pungolino E., Frassoni F., Giordano D. et al.: Autologous stem cell transplantation as adjuvant treatment vs no further therapy for poor-risk Hodgkin's disease in first complete remission after MOPP/ABVD. Leukemia Lymphoma 15, 1995, Suppl. 1, 59-61.
5. Cheson B.D., Horning S.J., Coiffier B., Shipp M.A., Fisher R.I. et al.: Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. J. Clin. Oncol. 17, 1999, 1244-1253.
6. Demirer T., Buckner C.D., Bensinger W.I.: Optimization of peripheral blood stem cell mobilization. Stem Cells 14, 1996, 106-116.
7. Diehl V., Franklin J., Hasenclever D., Tesch H., Pfreundschuh M. et al.: BEACOPP, a new dose-escalated and accelerated regimen, is at least as effective as COPP/ABVD in patients with advanced-stage Hodgkin's lymphoma: interim report from a trial of the German Hodgkin's Lymphoma Study Group. J. Clin. Oncol. 16, 1998, 3810-3821.
8. Donato M.L., Champlin R.E., van Besien K.W., Korbling M., Cabanillas F. et al.: Intensive dose ifosfamide and etoposide with G-CSF for stem cell mobilization in patients with non-Hodgkin's lymphoma. Leuk. Lymphoma 35, 1999, 317-324.
9. Fermé C., Brice P., Gabarre J., Diviné M., Detourmignies L. et al.: MINE chemotherapy plus rhGM-CSF as salvage and peripheral blood progenitor cell mobilizing regimen for relapsed or refractory Hodgkin's disease. Blood 84, 1994, Suppl. 1, Abstract No 635.
10. Fisher R.I., Gaynor E.R., Dahlberg S., Oken M.M., Grogan T.M. et al.: Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N. Engl. J. Med. 328, 1993, 1002-6.
11. Gratwohl A., Passweg J., Baldomero H., Hermans J.: Blood and marrow transplantation activity in Europe 1997. Bone Marrow Transpl. 24, 1999, 231-245.
12. Haioun C., Lepage E., Gisselbrecht C., Salles G., Coiffier B. et al.: Survival benefit of high dose therapy over sequential chemotherapy in poor risk aggressive non-Hodgkin's lymphoma. Final analysis of the prospective LNH 87-2 protocol. A GELA study. Blood 94, 1999, Suppl. 1, Abstract No 2711.
13. Horning S.J., Chao N.J., Negrin R.S., Hoppe R.T., Long G.D. et al.: High-dose therapy and autologous hematopoietic progenitor cell transplantation for recurrent or refractory Hodgkin's disease: analysis of the Stanford University results and prognostic indices. Blood 89, 1997, 801-813.
14. Kröger N., Zeller W., Fehse N., Hassan H.T., Krüger W. et al.: Mobilizing peripheral blood stem cells with high-dose G-CSF alone is as effective as with Dexa-BEAM plus G-CSF in lymphoma patients. Brit. J. Haematol. 102, 1998, 1101-1106.
15. Longo D.L., Duffey P.L., Young R.C., Hubbard S.M., Ihde D.C. et al.: Conventional-dose salvage combination chemotherapy in patients relapsing with Hodgkin's disease after combination chemotherapy: the low probability for cure. J. Clin. Oncol. 10, 1992, 210-218.
16. Mayer J., Kořístek Z., Vášová I., Vorlíček J., Vodvářka P.: Ifosfamide and etoposide-based chemotherapy as salvage and mobilizing regimens for poor prognosis lymphoma. Bone Marrow Transplant. 23, 1999, 413-419.
17. McQuaker I.G., Haynes A.P., Stainer C., Byrne J.L., Russel N.H.: Mobilisation of peripheral stem cells with IVE and G-CSF improves CD34+ yields and engraftment in patients with non-Hodgkin's lymphomas and Hodgkin's disease. Bone Marrow Transpl. 24, 1999, 715-722.
18. Mills W., Chopra R., McMillan A., Pearce R., Linch D.C., Goldstone A.H.: BEAM chemotherapy and autologous bone marrow transplantation for patients with relapsed or refractory non-Hodgkin's lymphoma. J. Clin. Oncol. 13, 1995, 588-595.
19. Moskowitz C.G., Bertino J.R., Glassman J.R., Hedrick E.E., Hunte S. et al.: Ifosfamide, carboplatin, and etoposide: a highly effective cytoreduction and peripheral-blood progenitor-cell mobilization regimen for transplant-eligible patients with non-Hodgkin's lymphoma. J. Clin. Oncol. 17, 1999, 3776-3785.
20. Nowrousian M. R., Schoetensack B., Pfeiffer R., Niederle N., Anders C. et al.: Combination chemotherapy with etoposide (VP-16), ifosfamide, methotrexate, and bleomycin (VIM(B)) for refractory or recurrent lymphomas. Contr. Oncol. 26, 1987, 414-422.
21. Olivieri A., Offidani M., Ciniero L., Cantori I., Ombrosi L. et al.: DHAP regimen plus G-CSF as salvage therapy and priming for blood progenitor cell collection in patients with poor prognosis lymphoma. Bone Marrow Transplant 16, 1995, 85-93.
22. Petit J., Boqué C., Cancelas J.-A., Sarrá J., Muñoz J. et al.: Feasibility of ESHAP + G-CSF as peripheral blood hematopoietic progenitor cell mobilisation regimen in resistant and relapsed lymphoma: a simgle-center study of 22 patients. Leuk. Lymphoma 34, 1999, 119-127.
23. Philip T., Guglielmi C., Hagenbeek A., Somers R., van der Lelie H. et al.: Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N. Engl. J. Med. 333, 1995, 1540-1545.
24. Phillips G.L., Reece D.E., Wolf S.N., Goldie J.H.: The use of conventional salvage chemotherapy before dose-intensive cytotoxic therapy and autologous transplantation for aggressive-histology lymphoma: a case for re-evaluation. Leukemia Lymphoma 26, 1997, 507-513.
25. Prince H.M., Imrie K., Crump M., Stewart A.K., Girouard C. et al.: The role of intensive therapy and autologous blood and marrow transplantation for chemotherapy - sensitive relapsed and primary refractory non-Hodgkin's lymphoma: identification of major prognostic groups. Brit. J. Haematol. 92, 1996, 880-889.
26. Rodriguez M.A., Cabanillas F.C., Hagemeister F.B., McLaughlin P., Romaguera J.E. et al.: A phase II trial of mesna/ifosfamide, mitoxantrone and etoposide for refractory lymphomas. Ann. Oncol. 6, 1995, 609-611.
27. Salles G., Shipp M.A., Coiffier B.: Chemotherapy of non-Hodgkin's aggressive lymphomas. Semin. Hematol. 31, 1994, 46-69.
28. Schmitz N., Sextro M., Pfistner B., Hasenclever D., Tesch H. et al.: High-dose therapy followed by hematopoietic stem cell transplantation for relapsed chemosensitive Hodgkin's disease: final results of a randomized GHSG and EBMT trial (HD-R1). Proc. ASCO 18, 1999, Abstract No 5.
29. Smith TJ., Hillner B.E., Schmitz N., Linch D.C., Dreger P. et al.: Economic analysis of a randomized clinical trial to compare filgrastim-mobilized peripheral-blood progenitor-cell transplantation and autologous bone marrow transplantation in patients with Hodgkin's and non-Hodgkin's lymphoma. J. Clin. Oncol. 15, 1997, 5-10.
30. Sweetenham J.W., Carella A.M., Taghipour G., Cunningham D., Marcus R. et al.: High-dose therapy and autologous stem-cell transplantation for adult patients with Hodgkin's disease who do not enter remission after induction chemotherapy: results in 175 patients reported to the European Group for Blood and Marrow Transplantation. J. Clin. Oncol. 17, 1999, 3101-3109.
31. Vášová I., Navrátil M., Mayer J. et al.: Mini(dexa)BEAM jako konvenčně dávkovaná záchranná chemoterapie druhé řady v léčbě relabujících a rezistentních lymfomů. XII. český a slovenský hematologický a transfuziologický sjezd s mezinárodní účastí, Olomouc, 1.-4. 9. 1999, abstrakt č. P19. posílám náš příspěvek do Klinické onkologie po akceptování všech drobných připomínek recenzenta, spolu s disketou.