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Thoracic Stop-flow Perfusion for Refractory Lymphoma: A Phase I-II Evaluation Trial

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Abstract. *Background: Management of patients with heavily pretreated malignant lymphoma failing front-line treatment and salvage high-dose chemotherapy and autologous peripheral stem cell rescue is problematic. A phase I-II evaluation trial was conducted to evaluate thoracic stop-flow perfusion. Patients and Methods: nine refractory patients were enrolled in the study. The schedule of thoracic stop-flow perfusion was based on cisplatin (100 mg/m^2) and melphalan (25 mg/m^2). Melphalan pharmacokinetic analyses were performed. All patients received hemofiltration during each procedure. Results: Overall 18 cycles of perfusional chemotherapy were administerd. During the procedures there were no technical, hemodynamic, or vascular complications, and no deaths occurred during surgery. Hematological and non-hematological toxicity was mild in relation to the use of hemofiltration during the procedures. All 9 patients responded favourably to stop-flow therapy. We observed 5 CR and 4 PR. Four out of five patients in CR relapsed. Three out of these four died of progressive disease after a response duration of 9, 7 and 7 months, respectively. One patient had a duration of CR of 10 months before relapse. He attained a new PR (+ 3 months). The remaining complete responder is still in remission aftre 37+ months. The 4 patients in PR progressed and died after a response duration of 4, 6, 2 and 1 month, respectively. To date, 8 out of 9 patients have died and one is still alive. Conclusion: Thoracic stop-flow perfusion seems very active in this kind of patient.*

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Chemotherapy is the mainstay treatment modality in malignant lymphoma. A total of 80% of Hodgkin's disease and about 40% of intermediate and highgrade non-Hodgkin's lymphomas are cured by combination chemotherapy (1, 2). However, patients who fail to achieve a complete remission or develop recurrent disease have little chance for cure with further conventional therapies. The response rates to conventional salvage regimens range from 20% to 60% but durable remissions are rarely observed (3). Trying to improve on survival after relapse, the addiction of new drugs, increasing dose-intensity, different chemotherapy combination, radioimmunotherapy, and first of all, highdose chemotherapy with hemopoietic stem cell support are also being used with results that are largely dependent upon patient selection (4-8). These therapeutical systems have had an impact, but the effect on the number of long-term survivors has been limited. The search for new active therapeutical systems is therefore needed for the therapy of malignant lymphomas. Particularly problematic is the management of patients with heavily pretreated malignant lymphomas failing front-line therapy and salvage treatments. Despite a possible residual chemosensitivity, often no further intensive therapy can be delivered. In this setting, we proposed a phase I-II study, with melphalan pharmacokinetics, to evaluate the activity and toxicity of a new therapeutic system consisting of perfusion of the target district by means of the stop-flow technique. In the stop-flow technique drugs are delivered in the thorax for a period of approximately 20 minutes after endovascular balloon occlusion of both aorta and inferior cava vein. During this first phase of the procedure, drugs reach the target district at very high concentrations, significantly greater than those obtainable after intravenous administration. In the subsequent phase of the procedure, the same active substances are distributed in the greater circulation as in conventional intravenous administration.

Table I. *Patients characteristics*.

No. of patients	9
Male to female ratio	3/6
Age (years)	
Mean	34
Range	18-62
ECOG performance status	
0	1
1	7
2	1
Primary diagnosis	
NHL primary B cell mediastinal	1
NHL diffuse large B cell	1
ALCL	1
HD	6
Previous treatments	
Conventional standard chemotherapy	9
Radiotherapy	9
Conventional salvage chemotherapy	9
High-dose plus ABMT salvage therapy	3

NHL, Non Hodgkin's lymphoma; ALCL, anaplastic large cell lymphoma; HD, Hodgkin disease; ABMT, autologous bone marrow transplantation.

Patients and Methods

Patient selection. Patients aged 18-62 years were required to have a histologically confirmed recurrent/refractory malignant lymphoma with thoracic localisation of the disease, no probability of cure and wich was not amenable to conventional treatment. Eligible performance status (Eastern Cooperative Oncology Group, ECOG) was between 0 and 3. Patients with renal and/or liver failure, deep venous thrombosis, severe atherosclerosis, or coagulopathy were ineligible. Patients with lymphomatous involvement of bone marrow and/or brain and/or testis were also excluded. Written informed consent was obtained from all patients.

Patient characteristics. Demographics of the 9 assessable patients entered onto the study are listed in Table I and Table II. Seven out of nine patients had primary induction failure. Only two patients achieved a complete remission after first-line therapy but experienced an early relapse. All the patients had received salvage therapy. Three out of nine had also received high dose therapy with progenitor replacement. All the patients had received radiation therapy; 8 out of 9 receiving it as a part of front-line treatments. Before stop-flow therapy, four of nine had a persistence of bulkiness whereas five out of nine had extranodal disease. All the patients had a pretreatment PS (ECOG) between 0 to 2.

Study design. The study was a phase I-II evaluation trial. Primary end-points were response, tolerability, toxicity, and melphalan pharmacokinetic data. Secondary end-point was survival. The stop-flow schedule was based on cisplatin and melphalan at the dose of 100 mg/m^2 and 25 mg/m^2, respectively. Epirubicin, at the dose of 70 mg/m^2, was added for patients 1, 2, and 3 based on previously demonstrated efficacy. For the same reson, for patients 4, 5, 6, and 8, carmustine (BCNU), at the dose of 100 mg/m^2, was added. Cytarabine (Ara-C) at the dose of 2,000 mg/m^2, was further added during the second course in two cases (patient 5 and 6) who had a minimal response to the first cycle of stop-flow therapy. In patient 8, etoposide, at the dose of 100 mg/m^2, was added to BCNU, based on chemosensitivity tests and demonstrated resistance to cisplatin and melphalan. For similar reasons, taxol (140 mg/m^2) was added to cisplatin for patient 9.

Clinical assessment. All patient underwent uniform staging that included a physical examination, complete blood count with differential, serum chemistry panel, lactate dehydrogenase, serum B2 microglobulin level, chest radiography, computed tomography (CT) thorax, abdomen and pelvis, total body gallium scintigrphy or FDG-positron-emission tomography and bilateral bone marrow aspiration and biopsy. In addition, patients with gastrointestinal symptoms underwent endoscopy screening for lymphomatous involvement. Residual disease identified with imaging was studied by means of total body gallium scintigraphy and FDG-positron-emission tomography. When feasible, biopsy of any residual mass was reccomended. Effusions of pleura and pericardium, when present, were cytologically studied.

Response criteria. Complete response (CR) was defined as the disappearance of all clinical evidence of lymphoma for a minimum of 4 weeks with no symptoms related to the disease and normalization of those biochemical abnormalities definitively assignable to malignant lymphoma. Residual masses were required to be gallium scan and/or FDG-PET negative, and unchanged for 4 months or longer off treatment to be considered CR. Partial response (PR) was defined as a greater than 50% decrease in the sum of the products of the diameters of all measured lesions that persisted for at least 4 weeks and non-measurable lesions had to decrease by at least 50% . No lesions could increase in size and no new lesions could appear. Progressive disease (PD) was defined as any increase of greater than 25% in the sum of the products of the diameters of any measurable lesions or the appearance of a new lesion (9).

Stop-flow technique. The therapy concept consisted of multiple cycles of locoregional chemotherapy employing thoracic perfusion with a treatment interval of approximately 40 days. A thoracic perfusion means the limitation of the greater circulation to the target region such as the thorax, including the head, for a period of 20 minutes (Figure 1). Using an inguinal approach, the femoral vessels were surgically exposed and then, after systemic heparinisation with 150 IU heparin/K, a three lumen 12 French ballon-catheter was entered into the thoracic aorta as well as the inferior caval vein. Both catheters were positioned under X-ray control in such way that when leveling the diaghragm, a complete occlusion of both vessels on inflation of the balloons was possible. To achieve a further reduction of the circulation volume, two pneumatic cuffs were inflated at both roots of the arms (250 mm Hg). In order to avoid greater volume displacements both balloons were inflated simultaneously. During the stop-flow thoracic perfusion, continous monitoring of the thoracic aorta blood pressure was registered using an extensible wire linked to the guidewire lumen of the aortic catheter and connected by an arterial line transducter to a computer monitor. Cytotoxic drugs were delivered as bolus injection within the first 3 minutes of the perfusion using the guidewire line of the venous catheter. After deflating the balloons, catheters were used to activate an

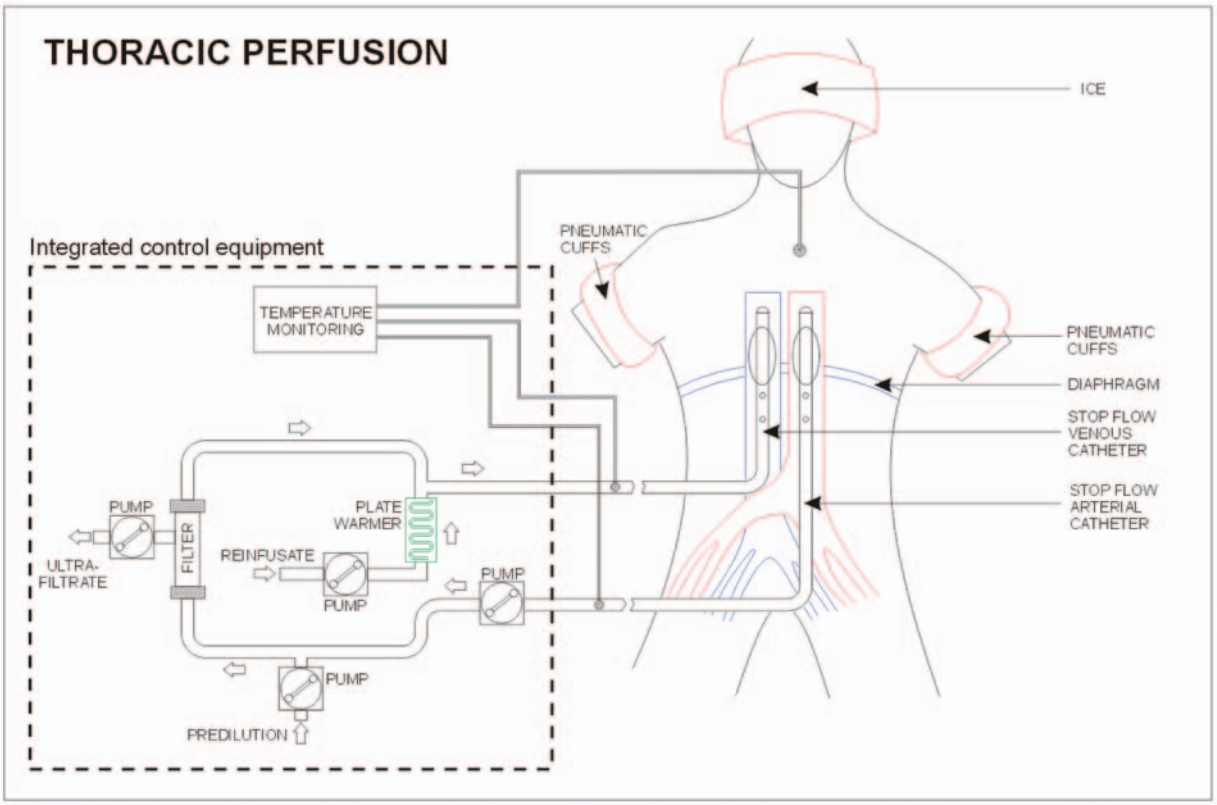

Figure 1. *Scheme of thoracic stopflow perfusion and extracorporeal circuit incorporating a hemofiltration system and a heater-cooler unit.*

extracorporeal circuitation according to criteria previously published (10), with the purpose of performing a hemofiltration to reduce systemic toxic effects. The duration of hemofiltration was approximately of 50 minutes. At the end of the procedure, the catheters were withdrawn and the vessels repaired. After a second thoracic stop-flow perfusion, further procedures were performed *via* iliac vessels, exposed *via* an abdominal extraperitoneal approach.

Anesthesia and hemodynamics. Thoracic stop-flow perfusion not require a routine pulmonary artery catheterization, except in high cardiac risk patients, However, central venous catheterization should be regarded as the minimum level of monitoring for such procedures. During thoracic stop-flow perfusion a temporary increase of approximately 25% of the mean arterial pressure has been previously reported (11), with a mean value of 120 mmHg in the thoracic district.

Melphalan pharmacokinetics. The pharmacokinetic analyses were performed in 6 patients submitted to thoracic stop-flow perfusion with melphalan at the dose of 25 mg/m^2, infused in the first 3 minutes after the aorto-caval occlusion. All blood samples were collected in 4-ml prelabeled lithium-heparin tubes. Peripheral blood samples were collected at the following time points: 0, 5, 7, 14, 17, 20, 25, 30, 45, 60 and 120 minutes while thoracic compartment blood samples were collected at 0, 5, 7, 14, 17 and 20 minutes. The 0-minute time point is defined as being before the drug was injected into thoracic compartment. After all samples were collected, they were centrifuged at 2,000 rpm at 4˚C for 10 minutes. The plasma was removed and aliquoted in 1 ml amounts into freezer tubes. Each tube was labelled with name, date and perfusion time point and stored at –80˚C until testing. Melphalan was quantified in plasma using high-performance liquid chromatography (12). For the pharmacokinetic analysis, a two-compartmental model for plasma melphalan disposition was chosen and applied to thoracic stop-flow perfusion chemotherapy. In this model, the drug distribution was divided into a first compartment (thoracic) and a second compartment (systemic). For each patient, plasma melphalan concentrations versus time were fitted to a biexponential equation using a nonlinear least-squares method. The main pharmacokinetic parameters evaluated were: C_{max} (maximal melphalan concentration); AUC (area under raw data of concentration time curve of melphalan); $T_{1/2}$ el (elimination half-life of melphalan); Vd (distribution volume of melphalan); MRT (mean residence time).

Results

Tolerability and toxicity. The nine patients received a total of 18 thoracic stop-flow perfusions. During the procedures, there were no technical, hemodynamic, or vascular complications, and no morbidity occurred during surgery. Damage of vital

Table II. *Patient and treatment characteristics*.

	First presentation features								Previous therapy			Stopf-low data	
Patient	Age (years)	Gender	Stage	Histology	Bulky disease	Extranodal disease	PS ECOG	LDH	Front-line	Standard salvage	High-dose	Sites of disease before stop-flow	Stop-flow schedule
1	18	M	IVB	NHL B cell primary mediastinal	Mediastinum	Lung, Pleura	1	High	CHOP-like + RT 44 Gy (mediastinum)	Ifosfamide, Epirubicin, Etoposide	BEAM+ APSC	Mediastinum, lung	Cisplatin Melphalan Epirubicin
2	26	F	IIB	HD SN1	Mediastinum	Absent	1	High	MOPP-ABVD hybrid + RT 36 Gy (mediastinum)	Vinorelbine Etoposide Lomustine	BEAM+ APSC	Mediastinum Neck nodes	Cisplatin Melphalan Epirubicin
3	34	M	IVB	HD SN2	Absent	Bone, Lung	1	High	MOPP-ABVD hybrid + RT 40 Gy (bone)	Ifosfamide, Epirubicin, Etoposide	BEAM+ APSC	Mediastinum Lung Neck nodes	Cisplatin Melphalan Epirubicin
4	44	F	IIA	ALCL Hodgkin-like	Absent	Absent	0	Normal	ABVD+ mantle RT 30 Gy	Ifosfamide, Epirubicin, Etoposide		Mediastinum, Lung, Neck nodes	Cisplatin Melphalan BCNU
5	25	F	IVB	HD SN1	Mediastinum	Lung	1	High	MOPP-ABVD hybrid + RT 36 Gy (mediastinum)	CHOP-like		Mediastinum, Lung, Pleura, Pericadium	Cisplatin Melphalan BCNU Ara-c
6	37	F	IVB	HD PL	Mediastinum	Lung, Pleura	1	High	MOPP-ABVD hybrid + RT 36 Gy (mediastinum)	Vinorelbine, Etoposide, Lomustine		Mediastinum Lung Pleura	Cisplatin Melphalan BCNU Ara-c
7	62	M	IIA	NHL diffuse Large B cell	Neck	Absent	1	High	CHOP-like+ RT 40 Gy (bulkiness)	MINE		Neck	Cisplatin Melphalan
8	30	F	IIB	HD SN1	Mediastinum	Pleura, Lung, Bone	2	High	Stanford v + RT 20 Gy, RT 36 Gy, palliative thoracic surgery, ABVD, Navelbine, RT 30 Gy	IGEV + Thiotepa, Alkeran, PBSC, BEACOPP, Di Bella therapy, Talidomide, Endoxan, Velcade, Tarciva, Gendicine		Mediastinum Lung Pleura, Neck	BCNU, Etoposide
9	29	F	IVB	HD SN1	Mediastinum	Lung	1	High	ESC BEACOPP	IGEV, mediastinal RT 30 Gy	Bad Mobilizer	Mediastinum	Cisplatin Taxol

Pt, Patient; RT, radiotherapy; Gy, Grays; APSC, Autologous peripheral stem cell; BEAM, BCNU 300 mg/m^2 *i.v.* on day 6, etoposide 200 mg/m^2 *i.v.* on day 5, Ara-c 400 mg/m^2 *i.v.* on days 5 to 2, melphalan 140 mg/m^2 *i.v.* on day 1; BCNU, carmustine; CEVOP-B, cyclophosphamide 750 mg/m^2 *i.v.* on day 1, vincristine 1.4 mg/m^2 *i.v.* on day 1, prednisone 100 mg *p.o.* day 1 to 7, bleomicin 10 mg/m^2 *i.v.* on day 5, epirubicin 75 mg/m^2 /96 h i.c., etoposide 140 mg/m^2 /96 h i.c.; MOPP, nitrogen mustard 6 mg/m^2 *i.v.* on day 1, vincristine 1.4 mg/m^2 *i.v.* on day 1, procarbazine 100 mg/m^2 *p.o.* days 1 to 7, prednisone 40 mg/m^2 *p.o.* days 1 to 14; EBVD, epirubicin 30 mg/m^2 *i.v.* on day 8, bleomicin 10 mg/m^2 *i.v.* on day 8, vinblastine 6 mg/m^2 *i.v.* on day 8; ABVD, adriblastine 25 mg/m^2 instead of epirubicin; IEV, ifosfamide 1800 mg/m^2 *i.v.* on day 1, epirubicin, etoposide; VEC, vinorelbine 25 mg/m^2 *i.v.* on days 1, and 8, Etoposide 120 mg/m^2 *i.v.* on day 1 to 3, CCNU 80 mg/m^2 *p.o.* day 1; LDH, lactate dehydrogenase; CHOP, cyclophosphamide-hydroxydorubicin-oncovin-prednisone; CHOP-like, ciclophosphamide- hydroxydorubicin-vincristine-prednisone; MINE, mitoguazone 500 mg/m^2 *i.v.* on days 1 to 3 ifosfamide 1.5 mg/m^2 *i.v.* on day, Vinorelbine 15 mg/m^2 *i.v.* for 30 minutes on day, etoposide150 mg/m^2 *i.v.* for 3 hours on day; HD, Hodgkin disease; NHL; non Hodgkin's lymphoma; ALCL, anaplastic large cell lymphoma; HD-NS1, Hodgkin lymphoma nodular sclerosis 1; HD-NS2, Hodgkin lymphoma nodular sclerosis 2; PS ECOG, performance status Eastern Cooperative Oncology Group; MOPP/ABDV hybrid, hybrid regimen (*versus* MOPP alternating ABDV); Stanford V, mechlorethamine, doxorubicin, vinblastine, vincristine, bleomycin, etoposide, prednisone; IGEV, ifosfamide 2000 mg/m^2 *i.v.* days 1-4, gemcitabine 800 mg/m^2 *i.v.* days 1-4, vinorelbine 20 mg/m^2 IV day 1, prednisolone 100 mg/m^2 days 1-4; BEACOPP, cyclophosphamide 650 mg/m^2 *i.v.* day 1, adriamycin 25 mg/m^2 *i.v.* day 1, etoposide 100 mg/m^2 *i.v.* day 1-3, oncovin 1.4 mg/m^2 (max 2 mg) *i.v.* day 8, procarbazine 100 mg/m^2 *p.o.* day 1-7, prednisone 40 mg/m^2 *p.o.* day 1-14; Di Bella therapy, Combination of somatostatin plus bromovriptine, vitamins and melatonin, and sometimes low dose of cyclophosphamide; ESC BEACOPP, escalated BEACOPP cyclophosphamide 1250 mg/m^2 on day *i.v.* day 1, adriamycin 35 mg/m^2 *i.v.* day 1, etoposide 200 mg/m^2 *i.v.* day 1-3, vincristine 1.4 mg/m^2 (max 2 mg) *i.v.* day 8, bleomycin 10 mg/m^2 *i.v.* day 8, procarbazine 100 mg/m^2 *p.o.* day 1-7, prednisone 40 mg/m^2 *p.o.* day 1-14; PBSC, peripheral blood stem cell.

organs such as heart, lung, liver, kidney or bowel, was not observed in any of the cases. Table III lists the adverse events observed in the 9 patients during a total of 18 cycles of stop-flow therapy. Overall, patients tolerated the therapy well. Treatment delays were infrequent. No dose reduction was necessary in the 18 courses of stop-flow therapy.

Out of a total of 196 observed adverse events (WHO), 142 (73%) were grade 1-2, while 47 (24%) and 7 (4%) were grade 3 and grade 4, respectively. No important renal or hepatic disfunction, or pulmunary toxicity were observed. A total 13 out of 54 (24%) adverse events of grade 3-4 were non hematological. Specifically, the most significant nonhematological toxicity included two cases of grade 3-4 stomatitis, two cases of grade 3 fever, one case of severe arrhythmia, one case of grade 3 asthenia, four cases of grade 3 gastrointestinal adverse events, and one case of grade 3 peripheral neuropathy; two patients developed documented infections of grade 3. All the mentioned adverse events were adequately treated and reversible. Myelosuppression was the major toxicity associated with stop-flow therapy. A total of 58 hematological adverse event were recorded: 41 out of 58 were of grade 3-4, representing 76% of any event of 3-4 grade; grade 4 myelosuppression was observed in 33% of cycles. One patient had 9 days of grade 4 neutropenia and 8 days of grade 4 thrombocytopenia during the first cycle. A second patient had 10 days of grade 4 neutropenia and 9 days of grade 4 thrombocytopenia during the second stop-flow cycle. This last patient received two transfusions of apheretic platelets because of an episode of severe epistaxis. Both patients received protracted broad-spectrum antimicromial therapy for neutropenic fever. Two patients experienced grade 4 anemia during the second stop-flow cycle and received packed RBCs. The mean nadir absolute neutrophil count was 410/l (range 120-1,400/l). The mean WBC/platelet count nadir were 74,000/l (range 4,000-202,000/l).

All patients showed recovery of blood counts after completation of therapy.

Table III. *Adverse events during stopflow therapy in the 9 patients (n. of cycles=18).*

Event	WHO Grade 1-2	WHO Grade 3	WHO Grade 4	% Of cycles with events
Any	142 (72%)	47 (24%)	7 (4%)	100 %
General				
Fever	8 (42%)	2 (11%)		53 %
Chills	3 (16%)			16 %
Headache	5 (26%)			26 %
Stomatitis	9 (48%)	1 (5%)	1 (5%)	58 %
Asthenia	16 (84%)	1 (5%)		89 %
Pain	3 (16%)			16%
Pruritus	3 (16%)			16%
Rash	2 (11%)			11%
Urticaria	1 (5%)			5%
Dizziness	1 (5%)			5%
Digestive				
Nausea	15 (79%)	2 (11%)		90%
Vomiting	3 (16%)			16%
Constipation	6 (32%)	1 (5%)		37%
Diarrhea	4 (21%)	1 (5%)		26%
Respiratory				
Bronchospasm	2 (11%)			11%
Dyspnea	4 (21%)			21%
Rhinitis	1 (5%)			5%
Cough increase	2 (11%)			11%
Cardiovascular				
Hypotension	3 (16%)			16%
Arrhythmia	4 (21%)	1 (5%)		21%
Other				
Acute infections	7 (37%)	2 (11%)		47%
Ototoxicity	2 (11%)			11%
Nephrotoxicity	3 (16%)			16%
Neuropathy	9 (47%)	1 (5%)		53%
Hematological				
Neutropenia	3 (16%)	14 (73%)	2 (11%)	100%
Thrombocytopenia	9 (47%)	8 (42 %)	2 (11%)	100%
Hemorrage		1 (5%)		5%
Hemoglobin	5 (26%)	12 (63%)	2 (11%)	100%

Disease status post-therapy. Overall response (OR) represented the end-point used to establish if this therapeutical approach could be considered an active program when compared with the current conventional salvage systems.

Table IV lists responses to the different treatments in the 9 patients. Seven out of nine patients may be categorized as having experienced primary induction failure since before stop-flow therapy they had never achieved a complete response (CR). Specifically, 3 out of 9 had progressed during initial front line-therapy, while the remaining 4 patients obtained only a brief partial response and rapidly progressed after stopping treatment. Two of the patients who achieved a CR had an early relapse (less than 6 months). All 9 patients responded favourably to stop-flow therapy. We observed 5 CR s and 4 PRs.

Survival. Patient 1. A 18-yr-old boy with a diagnosis of primary B cell stage IV B, with a bulky mediastinum of 14×12 cm and extranodal disease to lung and pleura failed front-line, conventional and high dose salvage therapy obtaining only brief partial responses after each treatment followed by rapid progression of lymphoma in the thorax. Figure 2 shows his mediastinal and lung lymphomatous lesions studied by means of computed tomography before stop-flow therapy (a) and 3 weeks after the first cycle of thoracic stop-flow perfusion (b). The patient received 3 courses of cisplatin, melphalan and epirubicin. He reached the uCR (Cotswold criteria) after the first course. CT examinations done at the beginning of third courses and 1

Table IV. *Responses to treatments*.

Response							
Patient	Disease	First line therapy	Standard salvage therapy	HD salvage therapy	Stop flow therapy (duration, months)	Overall survival from stop-flow	Status post-stop flow therapy and outcome
1	NHL primary mediastinal B cell	PR (3 mo)		Brief PR PD	CR (9)	15	Died from renal progression of lymphoma still being in thoracic CR from 9 months
2	HD SN1	PD	Brief PR PD	Brief PR PD	CR(7)	20	Thoracic recurrence→ new stop-flow therapy with NVB DHAD, Taxol,→2nd CR(4-months) Died of PD 9 months later
3	HD SN2	PR (3 mo)	Brief PR PD	Brief P R PD	PR (4)	19	Lung progression plus abdominal recurrence 4 months from PR. Died of PD 15 months later.
4	ALCL Hodgkin like	uCR (4 mo)	PD		CR (10)	14	Died for acute respiratory failure from lung progression of lymphoma
5	HD SN1	PD	Brief PR PD		PR (6)	20	Persistence of mediastinal bulkiness (67Ga+). Good general conditions and stable disease untill PD→death
6	HD PL	PD	PD		PR (2)	4	Rapidly fatal course characterised by acute progression of thoracic disease and ARDS
7	NHL diffuse large B cell	CR	(5 mo)	PR	CR (7)	18	Died of systemic progression of disease at 18 months from stop-flow therapy
8	HD SN1	CR (5 mo)	PD	PD	PR (1)	4	Died of brain progression of disease at 4 months from stop-flow therapy
9	HD SN1	PR (2 mo)	PD		CR (37+)	37+	Alive. In CR 37 months from stop-flow therapy

uCR=Undetermined Complete Response; PR=Partial Response; CR=Complete Response, PD=Progressive Disease; NHL=Non Hodgkin Disease; HD=Hodgkin Disease; HDSN1=Hodgkin Disease Nodular Sclerosis 1; HDSN2=Hodgkin Disease Nodular Sclerosis 2; ALCL=Anaplastic Large Cell Lymphoma; Mo=Months; OS=Overall Survival; PS=Performance Status; ECOG=Eastern Cooperative Oncology Group; ARDS=Acute Respiratory Distress Syndrome; NVB=Navelbine; DHAD=Mitoxantrone Hydrochloride.

month later were unchanged. The negativity of gallium scan and FDG-positron emission tomography studies confirmed the CR. Nine months later the patient had a bilateral renal relapse of lymphoma still being in thoracic CR as confirmed by new imaging studies (CT, gallium scan, and FDG-PET) which resulted unmodified. The patient died of progressive renal failure 15 months from the first stop flow therapy.

Patient 2. A 26-old-woman with a diagnosis of Hodgkin Lymphoma SN1 (BCNLI) stage IIB with bulky mediastinum progressed during initial front-line MOPP-ABVD hybrid regimen. Salvage therapy was attempted with conventional combination chemotherapy followed by BEAM plus APSC rescue and mantle RT (30 Gy) done two months after high dose program. The patient experienced a new supradiaphragmatic progression of lymphoma after a brief partial response.

She received 2 cycles of thoracic stop-flow perfusion of cisplatin, melphalan and epirubicin reaching CR one month later. The duration of CR was 7 months. The patient had a new thoracic recurrence of disease with severe life-threathining compression of tracheo-bronchial tract. She was treated by a new thoracic stop-flow perfusion of mitoxantrone (12 mg/m^2), vinorelbine (25 mg/m^2) and taxol (135 mg/m^2). The respiratory compression syndrome reversed 24 hours from the procedure and a 2nd CR was achieved. The duration of the 2nd CR was 4 months.The patient died of progressive disease 9 months later.

Patient 3. A 34-yr-old man affected by Hodgkin lymphoma SN2 (BCNLI) stage IV B because of extranodal localization of the disease to bone (iliac) and lung. He received 8 cycles of MOPP-ABVD hybrid regimen and 40 Gy of radiation

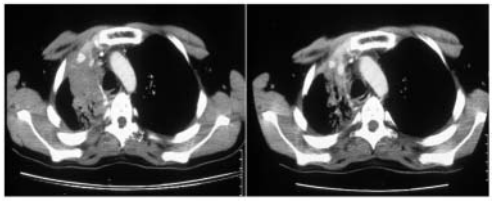

Figure 2. *Computed tomography scan of the thorax of a patient before (left) and after (right) thoracic stopflow perfusion.*

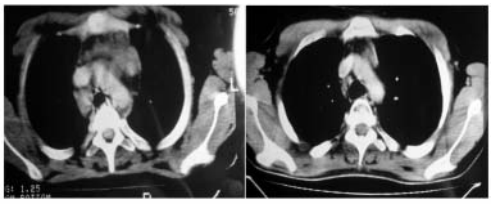

Figure 3. *Computed tomography scan of the thorax of a patient before (left) and after (right) thoracic stopflow perfusion.*

therapy to the bone lesion. He had a partial response of 3–month duration, then he experienced a progressive lymphomatous lung disease and lymphoadenopathy of neck and mediastinum. Conventional salvage therapy and BEAM high dose program were unable to obtain a durable response since the patient experienced a progressive lymphomatous lung disease and a new increase of neck and mediastinal lymphoadenopathy. He received 2 cycles of thoracic stop-flow perfusion of cisplatin, melphalan and epirubicin achieving a PR of 4-month duration. He died 15 months from stop-flow therapy because of progressive thoracic and abdominal disease.

Patient 4. A 44-yr-old woman with a diagnosis of anaplastic large cell lymphoma Hodgkin-like was treated, at first presentation, with ABVD and mantle radiation therapy (30 Gy). She obtained an undetermined complete response (Cotswold criteria) because of a residual mediastinal node of 1.8 cm (maximum longitudinal diameter). This residual node became Gallium positive 4 months after the completation of RT. One month later the patient was symptomatic and a CT study showed an enlargement of mediastinal residual mass. She received salvage combination chemotherapy but he progressed rapidly, during the second course of IEV (ifosfamide, epirubicin, etoposide), showing a marked lymphoadenomegaly of the neck and mediastinum and a new appearance of lung lesion. The patient was treated with 2 courses of thoracic stop-flow perfusion of cisplatin, melphalan and BCNU. She achieved a complete response but 10 months later she progressed at lung sites (Figure 3). Four months from the progression she died of acute respiratory failure.

Patient 5. A 25-yr-old woman with a diagnosis of Hodgkin lymphoma SN1 (BCNLI) stage IV B with a bulky disease to the mediastinum and extranodal localization to the the lung, failed the initial induction therapy because of progression during the 4th course of hybrid MOPP-ABVD regimen. She also failed salvage therapy obtaining only a brief partial response and progressing rapidly and symptomatically with a marked enlargement of mediastinal mass and conspicuous pleural effusion. The patient received a first course of thoracic stop-flow perfusion of cisplatin, melphalan and BCNU. A minor response was obtained and aracytin (2,000 mg/m^2) was added at the 2nd cycle of stop-flow therapy. The restaging showed a 50% reduction of mediastinal mass and the regression of pleural effusion; the mass was positive to gallium scan. This partial response had a duration of 6 months until thoracic progression of disease. The patient died 20 months from stop-flow therapy.

Patient 6. A 37-yr-old woman affected by Hodgkin lymphoma LP stage IVB with mediastinal bulky disease and extranodal localization to pleura and lung progressed during intial front-line MOPP-EBVD hybrid regimen before to intiate radiation therapy. Salvage therapy was attempted using a first salvage treatment with vinorelbine, etoposide, lomustine (VEC regimen) and a second with a CHOP-like schedule. Both regimens failed as the patient continued to have a thoracic progression of the disease characterized by an enormous mediastinal mass, and massive effusions of pleura and pericardium. She received a first course of thoracic stop-flow perfusion of cisplatin, melphalan and BCNU. A minimal response was seen and Ara-c (2,000 mg/m2) was added to the 2nd cycle. The patient responded most favourably as the mass had a further reduction and a major regression of effusions was seen. However this partial response was brief since she experienced a rapidly fatal progression characterized by massive enlargement of the mass and massive effusions of pleura and pericardium. Death occured few months later because of acute respiratory distress syndrome.

Patient 7. A 62-yr-old man with a diagnosis of diffuse large B-cell lymphoma stage II A, presenting a nodal bulky disease to the neck, received 5 cycles of CHOP-like regimen and radiation therapy (40 Gy) on bulky site of the disease. He had a partial response of 4-month duration then he experienced a disease progression at bulky site. Conventional salvage therapy was unsuccessfull since the patient had no response to MINE regimen. Stopflow therapy was planned and the patient received two courses of cisplatin and melphalan. The patient obtained a good partial response of 7-mo duration, with reduction of the lymphomatous masses over 75% . He had a systemic progression of disease a died at 18-mo from the stop-flow therapy.

Patient 8. A 30-year-old woman with a diagnosis of Hodgkin Lymphoma SN1 (BCNLI) stage II B, initially had a complete response after STANFORD V regimen and RT (20 Gy). 5 months later relapsed with bulky mediastinic disease and extranodal localization to the lung. In the six next years, she slowly progressed after different regimens, reirradiation, thoracic surgery, gene therapy (Table II and IV). Thoracic stop-flow perfusion was planned 7 years after the first diagnosis of lymphoma and the patient received a first course with BCNU and etoposide based on indication of chemosensitivity tests. She obtained a partial response as shown by imaging studies, which, unfortunately, evidentiated also a new localisation of lymphoma to the brain. The patient had a rapid fatal progression and death occured two months later.

Patient 9. A 29-yr-old woman with a diagnosis of Hodgkin Lymphoma SN1 (BCNLI) stage IV B, with bulky mediastinic disease and extranodal localization to the lung, failed the initial induction therapy based on 6 cicles of escalated BEACOPP because of persistence of PET/TC positive lesions. At the end of salvage therapy based on 4 cicles of combination chemotherapy (ifosfamide, gemcitabine, vinorelbine, and etoposide), the patient obtained a partial response with residual disease at mediastinum. The patient failed CD34+ mobilisation. 30-Gy consolidation radiotherapy was given to mediastinal residual mass (maximum longitudinal diameter 6 cm). Six months later the patient had a progression in this site without any other evidence of lymphomatous localisation, as shown from imaging studies (PET and TC). Thoracic stop-flow perfusion was planned and the patient received a first course of cisplatin and taxol. Forty-eight hours after perfusion the patient showed a severe and persistent polyuria. This clinical condition necessitated a continuous support. Seven days later the patient was well but refused any other treatment of lymphoma. At two months from stop-flow therapy imaging studies showed a persistence of a residual PET-negative mass (Figure 4), defined as undetermined complete response (UCR). The patient was monitored every six months with clinical and imaging studies. Two year later the residual mass was smaller and the patient considered in complete response, according to Cotswolds criteria. When last seen in April 2008, she was well in 37-mo+ continous complete remission.

Outcome. Major tumor responses (CR+PR) occurred in all 9 patients who received stop-flow perfusions. Five patients attained CRs, while four attained PRs.

Four out of five patients in CR relapsed (patients n.1, 2, 4 and 7 of Table IV). Three of these four relapsed and died of progressive disease after a response duration of 9, 7 and 7 months, respectively. The third patient had a duration of complete response of 10 months before relapse and attaining a new PR (+3 months) by combination chemotherapy. The remaining complete responder (Patient 9 of Table IV) in remission for 37+ months. The patients in PR progressed and died after a response duration of 4, 6, 2 and 1 month, respectively. To date, 8 out of 9 patients have died and 1 is still alive. This last patient is in complete remission at 37 months from stop-flow therapy.

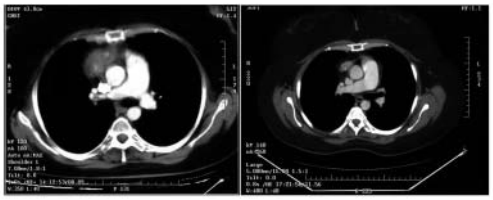

Figure 4. *Computed tomography scan of the thorax of a patient before (left) and after (right) thoracic stopflow perfusion.*

Melphalan pharmacokinetic data. In Table V are reported all pharmacokinetic data. In particular, during the stop-flow perfusion, the mean values of the ratios between the melphalan AUCs of the thoracic compartment in comparison to the systemic one (thoracic AUC_{0-20} / systemic AUC_{0-20}) demonstrated that the aorto-caval occlusion exposed the tumor to a 10-fold higher concentration than that measured in the systemic compartment. The values of melphalan C_{max} in the thoracic compartment averaged 4,010±2,005 ng/ml; the mean AUC_{0-20} was 66,933 ng/ml min±42,897 ng/ml min; the melphalan distribution volume (Vd) ranged from 3.7 to 14.4 l with a mean of 7.4 l. The values of melphalan C_{max} in the systemic compartment averaged 619 ng/ml±305 ng/ml; the mean AUC_{0-20} was 6,258 ng/ml min±2828 ng/ml min; the mean $T_{1/2}$ el was 30 min; the mean melphalan plasma clearance was 1,009 ml/min. For 120 minutes, the mean melphalan AUC_{0-120} was 26,145 ng/ml min.

Discussion

The common chemotherapy salvage regimens for treatment of relapsed/recurrent aggressive lymphomas rarely produce long-term survivors. In addition, despite the encouraging results obtained by the use of monoclonal antibodies, stem cell transplantation, included transplant-lite, and new drugs, the response rate and survival in heavily pretreated and recurrent/refractory patients still remain unsatisfactory. Different efforts may be undertaken to improve survival. In this setting, we are evaluating the impact of thoracic stop-flow perfusion with cisplatin/melphalan-based regimens. Clinical studies, concerning thoracic perfusion by means of the stop-flow technique, have shown promising results in advanced noncurable malignancies such as lung cancer

Table V. *Melphalan pharmacokinetic analyses in 6 patients.*

Thoracic compartment								
Patient	1	2	3	4	5	6	Mean	s.d.
Cmax (ng/ml)	5860	2420	6010	1510	5520	2740	4010	2005
AUC_{0-20} (ng/ml^{x}min)	136000	51200	78100	17900	86900	31500	66933	42897
Vd(l)	4	9.6	3.7	14.4	4.8	7.9	7.4	4.2
Clearance (ml/min)	183	488	320	1390	288	793	577	452
Systemic compartment								
Cmax (ng/ml)	625	552	912	291	1030	303	619	306
AUC_{0-20} (ng/ml$^{x/min}$)	6516	6510	9633	2575	8887	3430	6259	2828
AUC_{0-120} (ng/ml$^{x/min}$)	27908	21360	39011	13042	36059	19488	26145	10058
Vd(L)	36	35	27	76	28	54	43	18.9
Clearance (ml/min)	790	1140	570	1750	613	1190	1009	446.4
t½ el (min)	32	22	33	30	32	32	30	4.2
MRT (min)	40	33	41	42	41	44	40	3.7
Ratios (thoracic/systemic)								
AUC_{0-20}/AUC_{0-20}	20.9	7.9	8	6.9	9.8	9.2	10.5	5.2
C_{max}/C_{max}	9.4	4.4	6.6	5.2	5.4	9	6.7	2.1

S.d.=standard deviation; C_{max}=maximal concentration; AUC_{0-20}=area under melphalan concentration-time curve, during 20 minutes thoracic perfusion; min=minutes; Vd=volume distribution; AUC_{0-120}=area under melphalan concentration-time curve, during 120 minutes; t½ el=half life of melphalan; MRT=mean residence time.

(13). In this technique, it is possible to distinguish two phases. The first phase consists of 20 minutes perfusion of a target district (*i.e.* thorax and head) obtained by endovascular balloon occlusion of both aorta and inferior vena cava. The isolation of the compartment is not complete, with bidirectional fluid passage. Our procedures gave good results, in fact the pharmacokinetic data of thoracic melphalan distribution volume (Vd) and clearance (Table V) confirm that the leakage to the systemic compartment was limited. Moreover, the mean value of the thoracic versus systemic AUC_{0-20} ratios, confirmed a 10-folds higher melphalan exposure in the thoracic compartment. During this first phase, in the thoracic compartment drugs reach very high concentrations with C_{max} greater than those obtainable by comparable intravenous administration. In particular, the mean C_{max} detected (approximately 4000 ng/ml), can be reached with intravenous systemic injection of 80 mg/m^2 (14). Moreover, the levels of both oxygenation and mean arterial pressure are higher in comparison to the physiological state (11). During conventional intravenous administration, heart, liver, kidney, brain, and other highly perfused organs receive most of the drug during the first few minutes after injection. In stop-flow perfusion, drugs are given as a fast bolus and have a smaller central volume. In addition, during stop-flow perfusion, the drugs are not modified by exposure to organs not included in the target district (*i.e.* the liver is excluded during thoracic perfusion). As a consequence, during the 20-minute duration of the stop-flow perfusion, there is a significant variation of absorption rate, biotransformation, volume of distribution and half-life. As consequence of both high mean arterial pressure and interstitial pressure, drugs easily penetrate into the tumor mass and, based on high oxygenation level, are absorbed very rapidly into cells. At the end of the first phase, after deflating the catheter ballons, the drugs are distributed to a final (larger) volume. During this second phase, the pharmacokinetic features are not comparable to a conventional intravenous administration because of the use of hemofiltration, a techinique able to decrease the drug systemic biodisposition and toxicity. In particular, our pharmacokinetic data (Table V) for the systemic compartment gave a melphalan $t_{1/2\ m}$ el mean value of 30 minutes, significantly lower than that reported by Pinguet *et al*. (15) (approximately 50 minutes) after intravenous administration. Moreover, our clearance mean value of 1,009 ml/min was significantly higher than their 380 ml/min (15). In the high-dose regimens for lymphoma, the properties of certain drugs such as doxorubicin, epirubicin and cisplatin make them attractive for use in high-dose therapy but the ability to escalate doses is limited by their cardiac or renal toxicity. Other drugs such as melphalan, aracytin, BCNU and or mitoxantrone have better pharmacological features for escalation of doses. In the high-dose setting, with or without progenitor cell replacement, cisplatin is only escalated from 100 (conventional dose) up to 200 mg/m^2. On the contrary, intravenous melphalan may be escalated from 30-40 mg/m^2 to 160-200 mg/m^2 with a significant pharmacokinetic impact. The standard half-times of unbound cisplatin and

melphalan are brief. The plasma $t_{1/2}$ el of melphalan is reportedly between 40 minutes and 2 hours. Clearance of cisplatin is triphasic in nature, with elimination half-life ($t_{1/2}$beta) of 43 min, and terminal half-life ($t_{1/2}$gamma) of 5.4 days for total platinum. As a consequence, during exposure to conventional and high-dose cisplatin there are only moderate differences of AUCs, while high-dose therapy with intravenous melphalan determines a significantly higher exposure of tumor lesions to the cytotoxic action of the drug than conventional therapy (16-18). During the first phase of the stop-flow procedure, drugs achieve very high concentrations in the targed district, as shown by pharmacokinetic data. This means that for 20 minutes, the tumor lesions are exposed to a concentration gradients of cytotoxic drugs resulting in significantly greater concentrations than that expected from the intravenous administration at the same dosages (14). This is particularly interesting when perfused drugs are used which cannot be sufficiently escalated such as epirubicin, or cisplatin due to the risk of fatal nonhematological toxicity. Reasonably, as a consequence of these pharmacokinetic profiles, higher intracellular concentrations of active cytotoxic substances may be achieved with a possible circumvention of resistance mechanisms. As evident from the study of adverse events during the 18 administered courses, stop flow therapy was well tolerated. Despite the high concentration reached in vital organs such as lung, or heart, we recorded only a minority of nonhematologic adverse events of grade 3-4 toxicity. All events were reversible when promptly treated. Myelosuppression was the major toxicity associated with stop-flow therapy. Pancytopenia was common, but relatively brief in duration, and always reversible. This limited toxicity is likely related to the use of hemofiltration during the thoracic stop-flow perfusion. All nine patients responded to the treatment despite almost all having failed primary induction therapy and salvage treatments, also including, in three patients, the use of high-dose therapy with replacement of progenitor cells. Importantly, we recorded five CRs. Three of these five patients had never achieved a CR before this new treatment. Interestingly, one patient who achieved CR and relapsed 6 months later obtained a second CR by means of new stop-flow perfusion with taxol, mitoxantrone, and vinorelbine. The majority of patients had a relatively brief duration of response. However, for every patient this was the "least worst" result. More importantly, to date, we have one CR of 37 months duration, as never seen before in any of the studied patients. The clinical and pharmacokinetic results of the study seem to indicate that this therapeutical approach is feasible, well-tolerated and active in the salvage therapy of recurrent malignant lymphoma. It permits higher concentration of drugs in the target district such as the site of bulkiness to be achieved and, since recurrence of lymphoma is more common in these previous sites of disease, stop-flow therapy may play an important role in this therapeutic setting alone, or in combination with other therapeutical tools. In addition, the good tolerance to this therapy may give room for possible improvement of results by means of certain modification of the procedure such as escalation of doses, new combinations of drugs, the use of modulators (*e.g.* hyperthermia, oxygen, chemicals) and/or the formulation of schedules which include the stop-flow perfusion as a part of a multiple-day program of combination chemotherapy or in the high-dose setting. Further clinical and pharmacological studies on a larger cohort of patients are needed to confirm the validity of this experience and to establish if this system is able to improve survival of patients with recurrent malignant lymphoma.

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