Eur J Cancer Clin Oncol, Vol. 24, No. 5, pp. 903–908, 1988.
Printed in Great Britain

0277-5379/88$3.00+0.00

Ifosfamide in Previously Untreated Disseminated Neuroblastoma

Results of Study 3A of the European Neuroblastoma Study Group

STEWART J. KELLIE,* JAN DE KRAKER,‡ JOHN S. LILLEYMAN,§ ANGELA BOWMAN† and JON PRITCHARD*

*Departments of Haematology/Oncology and †Pharmacy, Hospitals for Sick Children, Great Ormond Street, London WC1N 3JH, U.K., ‡Werkgroep Kindertumoren, Emma Kinderziekenhuis, Spinozastraat 51, 1018 HJ, Amsterdam, Netherlands, §Department of Haematology, Sheffield Children's Hospital, Western Bank, Sheffield S10 2TH, U.K.

Abstract—*A prospective study of the effectiveness of ifosfamide as a single agent in the management of previously untreated patients with Evans stage IV neuroblastoma was undertaken. Eighteen children aged more than 1 year were treated with ifosfamide (IFX) 3 g/m² daily for 2 days immediately after diagnosis and 3 weeks later. Treatment was continued with combination chemotherapy using vincristine, cyclophosphamide, cisplatinum and etoposide (OPEC) or a variant. Mesna (2-mercaptoethane sulphonate) was given to all patients during IFX treatment to prevent urotoxicity.*

Eight of the 18 patients (44%) responded to IFX. Nine had >66% reduction in baseline tumor volume. Of 15 evaluable patients with raised pre-treatment urinary catecholamine excretion, six (40%) achieved >50% reduction in pretreatment levels. Two of 10 patients evaluable for bone marrow response had complete clearance. Toxicity was mild in all patients. Upon completing 'first line' therapy, only four patients (22%) achieved a good partial remission (GPR) or complete response (CR). Median survival was 11 months. There was a lower rate of attaining GPR and shortened median survival in patients receiving phase II IFX before OPEC or variant, compared to patients with similar pre-treatment characteristics treated with OPEC from diagnosis in an earlier study.

INTRODUCTION

IN CHILDREN over a year old, disseminated neuroblastoma (DNBL) is one of the most lethal cancers. Effective new agents are urgently required, but phase II and III studies can take a long time to complete for two reasons. Firstly, neuroblastoma is relatively uncommon, comprising less than 7% of all childhood neoplasms [1]; and secondly, experimental therapy is often difficult to justify in infants and children with recurrent tumors. A further relevant point is that the response rates to new agents in heavily pretreated patients are often low and may not reflect response rates in previously untreated patients, and the toxicity of potentially valuable new agents may be exaggerated following intensive treatment. For these reasons in 1984 the European Neuroblastoma Study Group (ENSG) decided to measure response rates to single agent ifosfamide (IFX) (Boehringer Ingelheim) at diagnosis, before proceeding to standard combination chemotherapy. ENSG Study 3A was thus designed to measure the activity of IFX in previously untreated children with stage IV disease.

PATIENTS

Between March 1985 and January 1986, 18 consecutively diagnosed patients from three participating institutions were registered on ENSG Study 3A. Each patient satisfied the ENSG diagnostic criteria (Table 1). To be eligible for the study, patients had to satisfy four criteria: (1) age >1 year at diagnosis; (2) Evans [2] stage IV disease; (3) no prior chemotherapy or radiotherapy and (4) no evidence of spontaneous tumor maturation as indicated by the natural history or histology of the tumor prior to chemotherapy [3]. Patient characteristics, details of primary tumors, sites of metastases and

Accepted 22 December 1987.
SJK receives support from the Leukaemia Research Fund, and JP from the Imperial Cancer Research Fund.
Address correspondence and reprint requests to: Dr. Stewart J. Kellie, FRACP, Department of Hematology/Oncology, St. Jude Children's Research Hospital, P.O. Box 318, Memphis, TN 38101 U.S.A.

Table 1. ENSG scoring system for diagnosis of neuroblastoma

	Points
Histopathological confirmation	2
VMA and/or HVA >2× normal level for age	1
Characteristic radiology	1
Tumor cells in bone marrow	1
Two points required for diagnosis	

Tumor response was assessed in all patients immediately before combination chemotherapy using a scoring system devised by the ENSG (Table 3). Response at the primary site was defined as >66% reduction in the product of three perpendicular diameters of the mass measured by computerized tomography or ultrasound. Bone marrow examination from at least two sites was required to assess response. Response duration and survival times were calculated from the first day of IFX to the documentation of disease progression or death, respectively.

Table 2. Patient characteristics at diagnosis and response to ifosfamide

Patient	Age (months)	Sex	Site of primary	Site of metastases			Raised catechols	Response score				Overall response
				BM	Bone	Other		Primary	Catechols	BM*	Other	
1	64	M	T	+	+	DLN	+	1	1	0	1	R
2	27	F	A	+	+	DLN	+	0	0	1	−†	NR
3	21	F	A	+	+	−	+	0	0	0	−	NR
4	33	F	A	+	+	Li	+	0	0	N/A	0	NR
5	40	F	A	+	+	DLN	+	1	N/A	0	1	R
6	29	F	A	−	+	DLN	+	1	1	−	1	R
7	53	F	A	+	+	E	−	0	−	1	−†	R
8	27	F	A	+	+	Li	+	1	0	N/A	0	NR
9	51	M	A	+	+	−	+	1	1	0	−	R
10	60	M	A	−	+	−	+	0	0	−	−	NR
11	54	M	A	+	+	DLN Li	+	1	N/A	0	1	R
12	17	F	A	+	+	DLN	+	0	0	0	1	NR
13	40	M	A	−	+	Li Lu	+	0	1	−	0	NR
14	52	M	T–A	+	+	−	+	0	0	0	−	NR
15	32	M	A	+	+	DLN	+	0	0	N/A	0	NR
16	19	M	A	+	+	−	+	1	0	N/A	0	NR
17	50	F	A	+	+	DLN	+	1	1	N/A	1	R
18	16	M	A	+	+	−	+	1	1	0	−	R

T, thoracic; A, abdominal; DLN, distant lymph nodes; Li, liver; E, extradural; Lu, lung; N/A, not assessed.
*Response not assessed unless ≥2 sites examined.
†Excisional biopsy of metastatic disease.
NR = no response.
R = response.

response to IFX are shown in Table 2. No patients had skin or intracranial involvement.

METHODS

Before receiving IFX, patients were prehydrated with 10% dextrose/0.225% saline infused at 125 ml/m^2/h for 4 h, followed by a bolus i.v. injection of mesna 600 mg/m^2 (sodium 2-mercaptoethane sulphonate, Urimetexan Asta-Werke AG, Bielefeld F.R.G.). IFX 3 g/m^2 plus mesna 100 mg/m^2 diluted to 125 ml/m^2 with glucose 4%/sodium chloride 0.18% were infused over 1 h, and followed by an infusion of 100 mg/m^2/h of mesna in 125 ml/m^2 of 10% dextrose/0.225% saline every hour for 23 h. The sequence of administration was repeated without interruption for a further 24 h. All patients received antiemetics which varied from patient to patient and center to center.

RESULTS

Seventeen patients each received two courses of IFX/mesna with a median interval of 17 days (range 14–26 days) between courses. A second course was omitted in patient 4 because of progressive disease. She is included in subsequent analyses. The median interval between starting IFX and the commencement of combination chemotherapy was 38 days (range 21–50 days).

The overall response rate to IFX was 44% (8/18) (Table 2); no complete responses (CR) were achieved after two courses. Two patients (Nos. 2 and 4) experienced disease progression during IFX. Patient 2 developed a supraclavicular lymph node metastasis; course 2 followed excisional biopsy. Patient 4 received OPEC after only one course of IFX because of accumulating malignant ascites. Nine patients (50%) demonstrated >66% reduction

*Table 3. ENSG: assessment of response**

Disease parameter	Score 0	Score 1
Primary tumor Reduction in baseline tumor volume	<66%	≥66%
Catecholamines Reduction in urinary VMA excretion	<50%	≥50%
Bone marrow Minimum two sites assessed	Positive ≥1 sites	Negative all sites
Other disease Bone scan reduction in number of sites	<50%	≥50%
Other metastatic disease Reduction in baseline tumor area	<50%	≥50%

*Defined as occurring when 50% or more of total possible score is obtained

Table 4. Toxicity of ifosfamide

Toxic effect	No. of patients (%)	Median duration (days) (range)
Hematologic		
WBC		
$<2.0 \times 10^9$/l	10 (56)	10 (2–17)
$<1.0 \times 10^9$/l	5 (28)	4 (3–8)
Platelets		
$<50 \times 10^9$/l	1 (6)	12
Infection		
Fever with neutropenia		
temp >38.5°C	8 (44)	4 (1–11)
i.v. antibiotics	6 (33)	8 (7–16)
Gastrointestinal		
Vomiting (grade 3+)	6 (33)	
Weight loss 1–10%	7 (39)	
Weight gain 1–10%	9 (50)	
Hematuria		
Microscopic	0 (0)	
Renal		
Raised BUN	0 (0)	
Raised serum creatinine	0 (0)	
CNS	0 (0)	

in the baseline tumor volume. No correlation was found between response and patient age, sex, size of primary or the presumed structure of origin.

Toxicity

Side-effects of IFX administration were acceptable in all patients (Table 4). There were no treatment-related deaths or adjustments in dosage because of drug toxicity. Mild myelosuppression was the most commonly observed toxicity. Six episodes of febrile neutropenia requiring i.v. antibiotics complicated 35 treatment courses. Vomiting was satisfactorily controlled with antiemetics, except in patient 13 who initially presented with persisting vomiting and failure to thrive. No episodes of microscopic hematuria, renal dysfunction or neurotoxicity were recorded.

Subsequent treatment and outcome (Table 5)

Following completion of the phase II study with IFX, all patients received combination chemotherapy in accordance with each institution's cur-

Table 5. Treatment after ifosfamide—response and survival

Patient No.	IFX–OPEC* interval (days)	Chemotherapy Drugs	Courses	Surgery	Final response	Relapse free survival (weeks)
1	35	OPEC	7	No surgery	PD	31
2	43	OPEC	6	No surgery	PD	33
3	35	OPEC	7	>95% excision	PD	34
4	21	OPEC	9	No surgery	PD	36
5	42	OPEC	9	Incomplete excision	GPR	100
6	42	CVI	6	Extradural decompression	PD	50
		OPEI	1			
7	35	OPEC	8	>90% excision	GPR	27
8	38	OPEC	4	No surgery	PD	25
9	39	OPEC	7	>90% excision	SD	40
10	35	OPEC	6	>90% excision	†	30
11	29	OPEC	6	>90% excision	PD	49
12	41	OPEC	2	No surgery	PD	11
13	30	OPEC	6	No surgery	PD	38
14	35	CVI	7	>90% excision	CR	66
15	33	OPEI	3	No surgery	PD	40
16	42	CVI	3	Incomplete excision	PD	34
		OPEI	3			
17	40	CVI	6	>90% excision	CR	64
18	50	OPEC	5	No surgery	PD	29

OPEC: vincristine, *cis*-platinum, etoposide, cyclophosphamide; OPEI: vincristine, *cis*-platinum, etoposide, ifosfamide; CVI: *cis*-platinum, vincristine, ifosfamide; IVAD: ifosfamide, vincristine, adriamycin; PD: progressive disease; GPR: good partial response; CR: complete response; SD: stable disease.

*Or variant.

†Postoperative death.

rent DNBL protocol. Fourteen of 18 patients received OPEC [4] or OPEI, denoting IFX substitution for cyclophosphamide (CPA). The remaining patients received cisplatin, vincristine and IFX (CVI). The timing of surgical resection of the primary tumor was discussed individually with our surgical colleagues, but resolution of metastatic disease was a prerequisite for surgery. At the completion of planned 'first line' therapy (IFX–OPEC–surgery–OPEC), two patients were in CR and two patients had minimal residual disease (GPR) (CR + GPR = 22% of the study population). There was no correlation between age, response to IFX, number of bony metastases or selection of post-IFX chemotherapy with outcome. Patients in GPR or CR after induction chemotherapy and surgery underwent high-dose 'consolidation' comprising chemotherapy or chemoradiotherapy with autologous bone marrow transplantation. Median relapse-free survival and overall survival were 7 months and 11 months, respectively.

DISCUSSION

New agents are urgently required in the treatment of DNBL, particularly in patients aged 1 year or more at diagnosis. The outlook for a child aged over 1 year with DNBL remains poor, with survival rates of 3–20% following conventional chemotherapy [4–6]. Improved median survival following treatment with high dose chemotherapy, total body irradiation and allo- or autologous bone marrow transplantation has been reported recently [7, 8], although considerable toxicity was encountered. Further follow-up is required to determine whether long-term DFS will be significantly improved by 'high-dose' therapy. Combination chemotherapy protocols including cisplatin have also been associated with ototoxicity and nephrotoxicity in survivors [9, 10], and second malignancies, possibly therapy related, involving translocations of chromosome 11 have been reported [11, 12]. New therapeutic approaches which improve both median and long-term survival, with acceptable toxicity, are needed.

IFX, an alkylating oxazophosphorine, is a structural analog of CPA in which one chloroethyl group is present on both nitrogen atoms. The altered chemical structure is reflected by the different preclinical and clinical pharmacokinetics of the two agents. In laboratory animals IFX, by comparison with CPA, has a higher therapeutic index with respect to acute toxicity, a broader spectrum of antitumor activity, and incomplete cross resistance of IFX and CPA has also been demonstrated [13]. In phase II trials IFX has been shown to have activity against many pediatric tumors including

neuroblastoma, soft tissue sarcoma, Wilms' tumor, osteosarcoma, Ewing's sarcoma, retinoblastoma, dysgerminoma, B cell lymphoma and primitive neuroectodermal tumors [14–22]. In an earlier study by our Group (ENSG Study 2), up to three courses of IFX 3 g/m^2 daily for 2 days were administered to 25 children with recurrent DNBL or evidence of progressive disease during treatment [15]. No responses were observed in children with progressive disease at the time of treatment with IFX. Partial responses were seen in two patients who were off treatment when their disease recurred. Each of these children had had complete responses lasting over 6 months to 'first line' therapy. The overall response rate was low (two of 25 patients), but justified further study of IFX. In ENSG Study 3A, the antitumor activity and toxicity of two courses of IFX were assessed in previously untreated children to eliminate the influence of heavy prior chemotherapy and reduce the impact of low performance status, due to progressive disease, on response and toxicity with IFX therapy. The potential influence of these factors on the assessment of response in Phase II trials involving six common adult cancers has been described by Wittes *et al.* [23].

This study demonstrated a response to IFX in 44% of previously untreated patients, with only mild toxicity. The median interval from diagnosis to start of combination chemotherapy was 38 days, and was usually determined by recovery from myelosuppression. The sole exception was a patient whose second IFX course was delayed by the excision of new disease appearing after course 1. Hematologic toxicity was minimal. Mesna was given by continuous infusion for 23 h after completion of IFX because of the prolonged urinary excretion of IFX compared to the short half-life of mesna [14], and was effective in preventing both hemorrhagic cystitis and IFX associated nephropathy, characterized by glomerular and tubular toxicity [24–26]. The urothelial toxicity of IFX, most probably related to the total dose, is mediated by acrolein, 4-hydroxyifosfamide, other alkylating decomposition products, and possibly metabolites formed from side-chain metabolites [27]. Cisplatin-related renal insufficiency was associated with potentiation of IFX-related neurotoxicity, hematotoxicity and nephrotoxicity in children receiving IFX in a recently reported Phase II trial [26].

We are concerned that initial single-agent IFX administration may have contributed to the disappointing overall rate of response and survival in these patients. Though a prospective, randomized study (IFX followed by OPEC vs. OPEC from diagnosis) is required to settle this issue, we have made a retrospective comparison of the results of this study with 16 comparable patients treated from diagnosis with combination chemotherapy (OPEC) [4]. Diagnostic criteria and staging procedures in that study were identical to the current study. The patients were slightly older, median age 3 years 9 months (range 1 year 2 months to 6 years 5 months), but the pattern and frequency of metastatic disease was similar; bone marrow was involved in 13 out of 16 patients (81%), bone in 14 patients (88%), liver in four patients (25%), distant lymph nodes in six patients (38%). Fourteen patients (88%) achieved GPR following OPEC and surgery whereas only two out of 14 patients (14%) in ENSG 3A who were treated with 4-drug chemotherapy following IFX achieved GPR at the completion of 'first line' treatment. Five out of six patients who responded to IFX and then received OPEC failed to achieve GPR. Of the remaining five patients (two IFX responders, three IFX nonresponders), who subsequently received an IFX containing combination (OPEI), only one patient, an initial IFX responder, achieved GPR/CR. Similar observations have been made in a group of small cell lung cancer patients treated, at diagnosis, with single agent idarubicin, then, 'standard' combination chemotherapy [28]. Although we cannot exclude other mechanisms, it is possible that single agent IFX may have induced either pleiotropic drug resistance or resistance to alkylating agents. Despite the unfavorable final outcome in this study, the demonstration of the activity of single agent IFX in DNBL warrants its further evaluation in combination with other active agents.

Acknowledgements—We wish to thank Professor Ann Barrett for helpful discussion and Adrianne Prior and Jessyca Mosby for typing the manuscript.

REFERENCES

1. Evans AE. Natural history of neuroblastoma. In: Evans AE, ed. *Advances in Neuroblastoma Research.* New York, Raven Press, 1980, 3–12.
2. Evans AE, D'Angio GJ, Randolph J. A proposed staging for children with neuroblastoma. *Cancer* 1971, **27**, 374–378.
3. Sitarz AL, Santulli TV, Wigger HJ. Complete maturation of neuroblastoma with bone metastases in documented stages. *J Pediatr Surg* 1975, **10**, 533–536.
4. Shafford EA, Rogers DW, Pritchard J. Advanced neuroblastoma: improved response rate using a multiagent regimen (OPEC) including sequential cisplatin and VM-26. *J Clin Oncol* 1984, **2**, 742–747.
5. Coldman AJ, Fryer CJH, Elwood JM, Sonley MJ. Neuroblastoma: influence of age at

diagnosis, stage, tumor site, and sex on prognosis. *Cancer* 1980, **46**, 1896–1901.
6. Kinnier-Wilson LM, Draper GJ. Neuroblastoma, its natural history and prognosis: a study of 487 cases. *Br Med J* 1974, **3**, 301–307.
7. Philip T, Bernard JL, Zucker JM *et al.* High dose chemoradiotherapy with bone marrow transplantation as consolidation treatment in neuroblastoma: an unselected group of stage IV patients over 1 year of age. *J Clin Oncol* 1987, **5**, 266–271.
8. Pritchard J, Germond S, Jones D, de Kraker J, Love S. Is high dose melphalan (HDM) of value in treatment of advanced neuroblastoma (AN)? Preliminary results of a randomized trial by the European Neuroblastoma Study Group (ENSG). *Proc Am Soc Clin Oncol* 1986, **5**, 205 (Abstract).
9. Reddel RR, Kefford RF, Grant JM, Coates AS, Fox RM, Tattersall MHN. Ototoxicity in patients receiving cisplatin: importance of dose and method of drug administration. *Cancer Treat Rep* 1982, **66**, 19–23.
10. Womer R, Pritchard J, Barrett TM. Renal toxicity of cisplatin in children. *J Pediatr* 1985, **106**, 659–663.
11. Secker-Walker LM, Stewart EL, Todd A. Acute lymphoblastic leukemia with *t*(4;11) follows neuroblastoma: a late effect of treatment? *Med Pediatr Oncol* 1985, **13**, 48–50.
12. Weh HJ, Kabisch H, Landbeck G, Hossfeld DK. Translocation (9;11) (*p*21;*q*23) in a child with acute monoblastic leukemia following 2-1/2 years after successful chemotherapy for neuroblastoma. *J Clin Oncol* 1986, **4**, 1518–1520.
13. Brock N. Contributions to the pharmacology of ifosfamide. Proc Int Holoxan Symposium, Dusseldorf, Asta-Werke, Bielefeld, 1977, 15–19.
14. Brade WP, Herdrich K, Varini M. Ifosfamide—pharmcology, safety and therapeutic potential. *Cancer Treat Rev* 1985, **12**, 1–47.
15. de Kraker J, Pritchard J, Hartmann O, Ninane J. Single-agent ifosfamide in patients with recurrent neuroblastoma (ENSG Study 2). *Paediatr Hematol Oncol* 1987, **4**, 101–104.
16. de Kraker J, Voute PA. Effect of iphosphamide (IPP) on soft tissue sarcomas in childhood. *Proc Am Soc Clin Oncol* 1983, **2**, 82 (Abstract).
17. Otten J, Flamant F, Rodary C *et al.* Effectivness of combination of ifosfamide, vincristine, and actinomyin D in inducing remission in rhabdomyosarcoma in children. *Proc Am Soc Clin Oncol* 1985, **4**, 236 (Abstract).
18. de Kraker J, Voute PA. Ifosfamide, mesna and vincristine and pediatric oncology. *Cancer Treat Rev* 1983, **10**, 165–166 (Suppl. A.).
19. Magrath IT, Sandlund JT, Rayner A, Rosenberg SA, Arasi V, Miser JS. Treatment of recurrent sarcomas with ifosfamide (IF). *Proc Am Soc Clin Oncol* 1985, **4**, 136 (Abstract).
20. Pinkerton CR, Rogers H, James C *et al.* A phase II study of ifosfamide in children with recurrent solid tumors. *Cancer Chemother Pharmacol* 1985, **15**, 258–262.
21. Marti C, Kroner T, Remagen W, Berchtold W, Cserhati M, Varini M. High-dose ifosfamide in advanced osteosarcoma. *Cancer Treat Rep* 1985, **69**, 115–117.
22. Pratt CB, Green AA, Horowitz ME *et al.* Central nervous system toxicity following the treatment of pediatric patients with ifosfamide/mesna. *J Clin Oncol* 1986, **4**, 1253–1261.
23. Wittes RE, Marsoni S, Simon R, Leyland-Jones B. The phase II trial (Editorial). *Cancer Treat Rep* 1985, **69**, 1235–1239.
24. Bryant BM, Jarman M, Ford HT, Smith IE. Prevention of isophosphamide-induced urothelial toxicity with 2-mercaptoethane sulphonate sodium (mesnum) in patients with advanced carcinoma. *Lancet* 1980, **ii**, 657–659.
25. Falkson G, Van Dyk JJ, Stapelberg R, Falkson HC. Mesnum as a protector against kidney and bladder toxicity with high-dose ifosfamide treatment. *Cancer Chemother Pharmacol* 1982, **9**, 81–84.
26. Goren MP, Wright RK, Pratt CB *et al.* Potentiation of ifosfamide neurotoxicity, hematotoxicity and tubular nephrotoxicity by prior *cis*-diamminedichloroplatinum (II) therapy. *Cancer Res* 1987, **47**, 1457–1460.
27. Wagner T, Heydrich D, Jork T *et al.* Comparative study on human pharmacokinetics of activated ifosfamide and cyclophosphamide by a modified fluorometric test. *J Cancer Res Clin Oncol* 1981, **100**, 95–104.
28. Cullen MH, Smith SR, Benfield GFA, Woodroffe CM. Testing new drugs may prejudice treatment: a phase II study of oral idarubicin in extensive disease small cell lung cancer. *Cancer Treat Rep* (in press).