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Chemotherapy with Adriamycin and Vincristine Alternated with Cyclophosphamide and Actinomycin D in Testicular Germ Cell Tumors Refractory to Cisplatin, Vinblastine and Bleomycin*

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Abstract—*Eighteen patients with testicular cancer refractory to cisplatin, vinblastine and bleomycin (PVB) were treated with a non-cross-resistant regimen including adriamycin,* 60 mg/m^2 *i.v. on day* 1, *and vincristine,* 1.2 mg/m^2 i.v. on days 1 *and* 8, *alternated q* 3 weeks *with cyclophosphamide,* 600 mg/m^2 *i.v. on days* 1 *and* 8, *and actinomycin D,* 1 mg/m^2 *i.v. on days* 1 *and* 8. *The median number of administered cycles was* 8 *(range* 3–14). *The results were analyzed according to previous response to PVB. One of two patients relapsing after the first-line therapy obtained a transient second complete response (CR) (duration* 7 months). *None of seven patients who showed no response to PVB obtained a CR; in* 3/9 *patients with a partial response (PR) after PVB, the achievement of CR could not be definitely attributed to salvage therapy. Toxicity was mild; no cardiac failure or drug-related deaths were observed. In conclusion, these two alternating regimens were well tolerated, but this treatment was not found to be useful in patients not responsive to PVB, for whom new and alternative therapies are required. The favorable impact on prognosis, evident only in the subgroup of patients with PR, was probably attributable to PVB rather than to this salvage regimen.*

INTRODUCTION

GREAT improvements in the treatment of advanced testicular tumors have been made during the last decade. About 55–70% of patients have a long-lasting complete remission (CR) after cisplatin, vinblastine and bleomycin (PVB) combination chemotherapy, often followed by surgery [1–5]. However, a few patients (30–45%) require second-line treatment. The choice of salvage therapy still represents a difficult problem.

In 1977 our initial approach at the Istituto Nazionale Tumori of Milan in the first group of patients considered to be resistant to PVB was that of using a non-cross-resistant combination chemotherapy containing adriamycin and vincristine (AV), alternated with cyclophosphamide and actinomycin D (CA). This study was mainly based on clinical and biological criteria. Adriamycin [6], vincristine [7, 8], actinomycin D [9, 10] and cyclophosphamide [11–14] have been shown to be active in testicular carcinoma when administered as single agents and have also been included in several combination chemotherapy regimens [15–19]. Alternating combination chemotherapy with non-cross-resistant drugs was demonstrated to be effective in neoplasms such as lymphomas [20], whose kinetic characteristics are similar to those of testicular tumors [21]. This treatment allowed us to use several drugs at full dose in an attempt to eliminate single-drug resistance and to limit toxicity. When we began this study in 1977, etoposide (VP-16), a drug which produces overall responses in about 20–46% of pretreated patients (22, 23), was still in an experimental phase and was not yet available in Italy.

MATERIALS AND METHODS

From June 1977 to December 1981, 18 patients with disseminated testicular germ-cell tumors believed to be refractory to PVB combination chemotherapy were treated at the Istituto Nazionale Tumori of Milan with AV alternated with CA. At the time this clinical study was conducted, patients were classified as resistant to PVB in cases of relapse after CR, progressive (PD) or stable disease

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(SD), or in cases of partial remission (PR). PVB was administered initially to 12 patients with a slightly modified schedule of PVB [24] (cisplatin, 50 mg/m^2 on days 1 and 2) compared to that originally reported by Einhorn and Donohue [1]; the other six patients received cisplatin at the dosage of 20 mg/m^2/day × 5. Bleomycin was administered in all cases at the dosage of 15 mg/m^2 on days 2, 9 and 16, and vinblastine at 6 mg/m^2 was repeated on days 1 and 2. Previous treatment also included surgery in five patients, radiotherapy in three cases, and both modalities in one.

Salvage chemotherapy was administered with the following schedule: adriamycin, 60 mg/m^2 i.v. on day 1, and vincristine, 1.2 mg/m^2 i.v. on days 1 and 8 (one cycle). These drugs were alternated every 3 weeks with another cycle of cyclophosphamide, 600 mg/m^2 i.v. on days 1 and 8 and actinomycin D, 1 mg/m^2 i.v. on days 1 and 8.

All patients had primary gonadal tumors. The median age was 28 yr. Four patients had embryonal carcinoma, one had teratoma, one had seminoma, one had yolk sac tumor, one had choriocarcinoma and the ten remaining patients had mixed germ-cell tumors.

Table 1 shows the definition of extent of disease presently used at our institute. To allow a better description of the tumor burden, metastatic disease was subdivided into minimal, intermediate and far advanced. Since in this patient population, clinical characteristics, results of previous treatments and response to secondary chemotherapy are strictly correlated, all the data have been listed in Table 2.

Prior to each course of therapy a complete physical examination was performed, and the size of all measurable lesions was determined. A complete blood count with platelets, BUN, creatinine, SGOT, SGPT, alkaline phosphatase, bilirubin, urinalysis and LDH tests were performed before each treatment. BHCG and alpha-fetoprotein were determined before treatment and then periodically repeated if an initially abnormal elevation was present. Preteatment electrocardiograms were obtained and repeated if clinically indicated. Radiologic examinations (chest X-ray, lymphangiogram) were performed before the first cycle and rechecked after about 2 months. Extensive radiologic examination (CT scan, ultrasonography, urography, cavography) was performed when clinically indicated. In responding patients, restaging was accomplished by repeating all tests that had been positive at the start of therapy. Chemotherapy was discontinued if progression of the disease was noted.

CR was defined as complete disappearance of all evident disease for at least 2 months. A PR was defined as more than a 50% reduction in the sum of the product of the perpendicular diameters of all measurable lesions and of any previously abnormal tumor marker for at least 2 months. SD was defined as a minor regression (<25%) as compared with pretreatment values for at least 3 months. PD was defined as an increase in 25% or more of any measurable lesion. Survival was measured from the first day of this salvage treatment. Median follow-up for all patients was 25.5 months (range 5–70 months).

Table 1. Definition of extent of disease in metastatic testicular tumors [3]

Minimal metastatic disease:
Only persistently elevated serum tumor markers (occult disease)
No more than 5 pulmonary nodules in each lung field; none larger than 2 cm, with or without lymph node metastases; none larger than 5 cm (small volume disease)
Intermediate–advanced disease:
More than 5 pulmonary nodules per lung field, or at least one lung metastasis between 2 and 5 cm in diameter (bulky pulmonary disease)
Far-advanced disease:
invasion of more than 50% of pulmonary fields, or at least one lung metastasis larger than 5 cm in diameter. Lymph node metastases larger than 10 cm in diameter (very bulky disease) metastases outside of lymph nodes and lung (extra-pulmonary disease)

RESULTS

Treatment response

Table 2 reports the initial response and the subsequent evolution of the disease after salvage therapy in 18 patients judged to be resistant to PVB. With only two exceptions (Nos 3 and 13), all patients initially presented with far-advanced disease. Taking into account the relationship between extent of disease at salvage therapy and response, CR was observed in two (Nos 1 and 18) of six patients with far-advanced disease and in two (Nos 16 and 17) of four patients with minimal metastatic disease. All cases with intermediate advanced disease failed to obtain a CR after salvage chemotherapy.

A further analysis of the relationship between response to initial treatment with PVB and subsequent response to the second-line chemotherapy was carried out on the basis of the data presented in detail for each patient in Table 2. In two patients (Nos 1 and 2) who relapsed after PVB, one transient CR was obtained. The responding patient (No. 1) had relapsed within 90 days of completing PVB chemotherapy, in the lung and brain (cortical and subcortical site of the right parieto-occipital lobe). With salvage chemotherapy, pulmonary lesions disappeared and brain metastases were controlled by radiotherapy. After

Table 2. Characteristics and results of PVB and salvage chemotherapy

Case No.	Presalvage: Histology	Presalvage: Initial extent of disease*	Presalvage: PVB† No. of cycles	Presalvage: PVB† Response‡	Salvage: Site of disease	Salvage: Extent of disease	Salvage: AV/CA No. of cycles	Salvage: AV/CA Response	Survival (months): Alive disease free	Survival (months): Alive with disease	Survival (months): Dead
1	Yolk sac tumor	FAD	5	CR	CNS, lung	FAD	10	CR§			13
2	Choriocarcinoma + seminoma	FAD	6	CR§	lung	IAD	3	PD			5
3	Embryonal carcinoma	IAD	5	PD	lung	IAD	7	PD			34
4	Teratocarcinoma	FAD	6	PD	retroperitoneum, lung	FAD	10	PD			11
5	Embryonal carcinoma	FAD	4	PD	retroperitoneum	FAD	6	PD		70+	
6	Teratoma	FAD	5	SD	retroperitoneum	IAD	8	PR		48+	
7	Teratocarcinoma	FAD	4	SD§	retroperitoneum, lung	IAD	4	SD		36+	
8	Choriocarcinoma + seminoma	FAD	5	SD§	CNS, lung	FAD	8	SD			17
9	Teratocarcinoma	FAD	5	SD‖	retroperitoneum, peritoneum	FAD	8	SD			6
10	Teratocarcinoma	FAD	6	PR‖	lung	IAD	5	PD			6
11	Embryonal carcinoma + seminoma	FAD	5	PR‖	retroperitoneum	IAD	4	PD			5
12	Embryonal carcinoma + seminoma	FAD	5	PR‖	lung	MMD	5	PD			8
13	Choriocarcinoma	IAD	4	PR‖	retroperitoneum	IAD	10	SD	46+¶		
14	Teratocarcinoma	FAD	6	PR	retroperitoneum, lung	MMD	5	SD			14
15	Seminoma	FAD	5	PR	retroperitoneum	IAD	9	SD		48+	
16	Teratocarcinoma + seminoma	FAD	5	PR‖§	retroperitoneum	MMD	10	CR	40+		
17	Embryonal carcinoma	FAD	5	PR	retroperitoneum	MMD	8	CR	36+		
18	Embryonal carcinoma	FAD	5	PR	retroperitoneum	FAD	14	CR	67+		

*FAD, far-advanced disease; IAD, intermediate advanced disease; MMD, minimal metastatic disease.
†CDDP, 20 mg/m^2 × 5 days (case Nos 1, 2, 4, 7, 12, 13); CDDP, 50 mg/m^2 days 1 and 2 (case Nos 3, 5, 6, 8–11, 14–18).
‡CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.
§Plus radiotherapy.
‖Plus surgery.
¶CR was obained with third-line chemotherapy.

7 months there was a second lung relapse. Therefore, of the seven patients (Nos 3–9) who clearly did not respond to PVB (SD or PD), only one PR was achieved. This responding patient (No. 6), whose initial pathology was teratoma, is alive with disease after 48+ months, whereas another patient (No. 7) with SD after first- and second-line chemotherapy is alive with disease after 36+ months. In only 3/9 patients (Nos. 10–18) with PR after PVB was the therapy with AV/CA followed by a clinically (Nos. 16 and 17) or pathologically (No. 18) documented CR.

A detailed description of each of three cases presently free of disease is necessary to evaluate the effect of salvage chemotherapy. Patient No. 16 had residual retroperitoneal disease but negative markers after five cycles of PVB. Partial surgical debulkment showed residual teratoma; the patient was then treated with radiation therapy with further reduction but not radiological disappearance of the tumor residual mass. After four cycles of AV/CA, the lymphangiogram was considered as negative. Patient No. 17 had a radiologic (lymphangiogram) PR after five cycles of PVB. Although markers were negative, he could not be operated on because retroperitoneal gross vessels were infiltrated by the tumor. Lymphangiogram and cavography were judged to be negative after four cycles of salvage therapy. Patient No. 18 had a CR of pulmonary metastases after PVB for five cycles; markers were negative but retroperitoneal metastases were unchanged. Debulking surgery was ruled out because of tumoral extension to the duodenum and vena cava. He then received 14 cycles of AV/CA, which was followed by the disappearance of duodenal infiltration. The radiologic examination of the vena cava was ambiguous. He thus underwent debulking of a retroperitoneal necrotic mass (pathologic CR).

The remaining six patients with PR after PVB presented clear-cut progression or SD. Among them, one patient (No. 13) with a PR and negative markers after PVB and SD after salvage treatment was judged to be in CR (lymphangiogram) after

third-line treatment containing vincristine, 0.7 mg/m^2 i.v. days 1 and 2, 5-fluorouracil, 300 mg/m^2 i.v. on days 3–7, and procarbazine, 120 mg/m^2 p.o. on days 3–7. A similar therapy has been used by Brulé [25].

Toxicity

Patients received 3–14 cycles (median number, eight cycles) of AV/CA. Nausea, vomiting, alopecia and mild paresthesia occurred in all of them. Hemoglobin was less than 10 mg/100 ml in 32% of the patients; two patients required red blood cell transfusions. The white blood cell count was lower than 2500/mm^3 in 79%; no patient developed sepsis. Twenty per cent had thrombocytopenia lower than 100,000/mm^3; no bleeding occurred. No liver, renal or heart failure was observed. There were no drug-related deaths; all deaths were attributed to progression of disease.

DISCUSSION

This is one of the few reports on second-line chemotherapy after PVB that does not include VP-16, but drugs of known activity used more in the past for advanced testicular carcinomas. At the time the study was started, not many alternatives were available. VP-16 was not yet readily available in our country, and its role in the therapy of cases refractory to PVB was still to be established.

In this study a true but transient CR was only achieved in one of two relapsing patients. In the other three cases with PR after PVB, the value of the CR after salvage therapy may be certainly questioned, since fibrosis and tumor necrosis after first-line chemotherapy has been described several times in patients clinically judged as partial responders before debulking surgery [26–28].

A comparison between our results and those reported in the literature is difficult because of the small number of patients in our series as well in others, and for the differences in some variables (histology, first-line treatment and response, extent of disease, and drugs used for salvage treatment). Whereas iphosphamide and VP-16 alone or in combination [29, 30] do not appear to be more active (0–11% CR) than our salvage regimen, second-line regimens containing cisplatin and VP-16 ± adriamycin ± bleomycin in patients previously treated with cisplatin combination chemotherapy [31–33] may be considered more effective (37–44% CR) than our alternating combination chemotherapy. However, these last favorable results should be interpreted according to the initial response to PVB.

It has been recently demonstrated that a close relationship exists between response to first- and second-line treatment. Such an analysis was first reported by Williams *et al.* [31] on 25 patients judged to be refractory to PVB ± adriamycin and treated with salvage chemotherapy containing cisplatin, VP-16 ± bleomycin ± adriamycin. Seven of ten (70%) patients who relapsed obtained a second CR (three recurrences) and 4–15 (26%) patients with unresectable PR also achieved a CR after a second-line chemotherapy (two recurrences), whereas only 1/6 (16%) patients with progression obtained a CR. Almost superimposable results were achieved by Lederman *et al.* [33] for patients in relapse after PVB or with PR using VP-16 and cisplatin ± bleomycin ± adriamycin. These results show that a second CR in patients relapsing after PVB can be relatively easily achieved.

In our case series we could only treat two patients relapsing after PVB. Therefore no evaluation of the efficacy of our salvage regimen can be given in this group of patients in comparison with the combinations including VP-16, cisplatin ± bleomycin ± adriamycin. However, the only CR was observed in this group of patients.

Considering patients with no response to PVB, a negligible activity has been evidenced with our combination as well as with the aforementioned regimens. In patients with unresectable PR after PVB becoming a so-called CR after salvage therapy, the achievement of a CR in the aforementioned series as well as in ours should be considered as an index of the clinical efficacy of the salvage treatment only in cases with pathological proven residual carcinoma or in patients with clinical PR and abnormal levels of tumor markers. In consideration of the experience accumulated in the last 5 yr with the surgical documentation of response after very active combinations (PVB, VAB6), what in this and other series was clinically thought to be a CR after second-line combination treatment could have been unresectable necrotic tissue that dissipated spontaneously during salvage chemotherapy. Therefore, no convincing cure rate can be achieved in patients with PR with any second-line combination chemotherapy, so that even the term, 'salvage chemotherapy', could be questioned.

In conclusion, so far positive results in patients refractory to PVB have only been observed in patients relapsing after first-line treatment. Patients with clinically documented PR and negative markers should not be used to assess the value of a second-line combination chemotherapy, since a CR may only reflect the delayed effect of PVB and not that of the salvage chemotherapy. However, only cases with a clinical PR, judged unresectable and with positive markers, with pathologically proven residual unresectable carcinoma, or with residual benign teratoma and positive markers, are suitable for evaluation of salvage therapy. Since it

is apparent that in patients refractory to PVB, with the exclusion of those in relapse after CR, there is presently no substantial cure rate with any salvage therapy, even trials reporting negative results such as ours should be published to stress the need for innovative treatments.

REFERENCES

1. Einhorn LH, Donohue JP. *Cis*-diamminedichloroplatinum, vinblastine and bleomycin combination chemotherapy in disseminated testicular cancer. *Ann Intern Med* 1977, **87**, 293–298.
2. Einhorn LH, Williams SD. Chemotherapy of disseminated testicular cancer. A random prospective study. *Cancer* 1980, **46**, 1339–1344.
3. Pizzocaro G, Salvioni R, Pasi M *et al.* Early resection of residual tumor during cisplatin, vinblastine, bleomycin combination chemotherapy in stage III and bulky stage II nonseminomatous testicular cancer. *Cancer* 1985, **24**, 31–37.
4. Stoter G, Vendrik CPJ, Struyvenberg A *et al.* Results of 5 years follow-up of patients with disseminated testicular non seminomas, treated with chemotherapy including cisplatin (P), vinblastine (V) and bleomycin (B). UICC Conference on Clinical Oncology, Lausanne, 28–31 October, 1981. Abstract No. 15–0481.
5. Samson MK, Fisher R, Stephens RL *et al.* Vinblastine, bleomycin and *cis*-diamminedichloroplatinum in disseminated testicular cancer: response to treatment and prognostic correlations. A South-west Oncology Group study. *Eur J Cancer* 1980, **16**, 1359–1366.
6. Monfardini S, Bajetta E, Musumeci R. Clinical use of adriamycin in advanced testicular cancer. *J Urol* 1972, **108**, 293–296.
7. Costa G, Hreshchyshyn MM, Holland JF. Initial clinical studies with vincristine. *Cancer Chemother Rep* 1962, **24**, 39–44.
8. Shaw RK, Bruner JA. Clinical evaluation of vincristine (NSC-67574). *Cancer Chemother Rep* 1964, **42**, 45–48.
9. Watne AL, Badillo J, Koite A *et al.* Clinical studies of actinomycin D. *Ann NY Acad Sci* 1960, **89**, 445–453.
10. Merrin NCE, Murphy GP. Metastatic testicular carcinoma. Single agent chemotherapy (actinomycin D) in treatment. *NY State J Med* 1974, **74**, 654–657.
11. Snyder W, Rodensky P, Lieberman B. Regression, relapse, and regression of metastatic seminoma by cyclophosphamide (NSC-26271). *Cancer Chemother Rep* 1964, **41**, 37–40.
12. Buckner CD, Clift RA, Fefer A *et al.* High-dose cyclophosphamide (NSC 26271) for the treatment of metastatic testicular neoplasms. *Cancer Chemother Rep* 1974, **58**, 709–714.
13. Mullins GM, Colvin M. Intensive cyclophosphamide (NSC 26271) therapy for solid tumors. *Cancer Chemother Rep* 1975, **59**, 411–419.
14. Bergsagel DE, Levin WC. A prelusive clinical trial of cyclophosphamide. *Cancer Chemother Rep* 1960, **8**, 120–134.
15. Blom J, Brodovsky HS. Comparison of the treatment of metastatic testicular tumors with actinomycin D, bleomycin and vincristine. *Proc AACR* 1975, **16**, 247 (Abstract No. 1107).
16. Monfardini S, Fossati V, Pizzocaro G, Bonadonna C. Studio pilota con due associazioni chemioterapiche nei carcinomi testicolari. *Tumori* 1976, **62**, 435–444.
17. Solomon J, Steinfeld JL, Bateman JR. Chemotherapy of germinal tumors. *Cancer* 1967, **20**, 747–750.
18. Williams C. Current dilemmas in the management of nonseminomatous germ-cell tumors of the testis. *Cancer Treat Rev* 1977, **4**, 275–297.
19. Jacobs EM. Combination chemotherapy of metastatic testicular germinal cell tumors and soft-part sarcomas. *Cancer* 1970, **25**, 324–332.
20. Santoro A, Bonadonna G, Bonfante V, Valagussa P. Alternating drug combinations in the treatment of advanced Hodgkin's disease. *N Engl J Med* 1982, **13**, 770–775.
21. Tubiana M, Malaise E. Comparison of cell proliferation kinetics in human and experimental tumors: response to irradiation. *Cancer Treat Rep* 1976, **60**, 1887–1895.
22. Cavalli F, Klepp O, Renard J, Röhrt M, Alberto P for the EORTC Early Clinical Group. A phase II study of oral VP-16-213 in non-seminomatous testicular cancer. *Eur J Cancer* 1978, **17**, 245–249.
23. Fitzharris BM, Kaye SB, Saverymuttu S *et al.* VP-16-213 as a single agent in advanced testicular tumors. *Eur J Cancer* 1980, **16**, 1193–1197.
24. Pizzocaro G, Zanoni F, Piva L *et al.* Bulk of disease and management of metastatic nonseminomatous germ cell tumors of the testis. In: Denis L, Murphy GP, Prout GR, Schröder F, eds. *Controlled Clinical Trials in Urologic Oncology*. New York, Raven Press, 1984, 79.
25. Brulé G. Combination treatment chemotherapy of cancer. In: *Symp Lugano 28 April–1 May 1964*. Amsterdam, Elsevier, 1964, 245–249.
26. Donohue JP, Einhorn LH, Williams SD. Cytoreductive surgery for metastatic testis cancer: considerations of timing and extent. *J Urol* 1980, **123**, 876–879.

27. Johnson DE, Bracken RB, Ayala AG, Samuels ML. Retroperitoneal lymphadenectomy as adjunctive therapy in selected cases of advanced testicular carcinoma. *J Urol* 1976, **116**, 66–68.
28. Vugrin D, Whitmore WP, Golbey RB. VAB-6 combination chemotherapy without maintenance in treatment of disseminated cancer of the testis. *Cancer* 1983, **51**, 211–215.
29. Wheeler B, Einhorn LH, Loehrer P, Williams S. Ifosfamide (IFOS), an active agent in refractory germ-cell tumors (RGCT). *Proc ASCO* 1984, **3**, 154 (Abstract No. C 602).
30. Scheulen NE, Bremer K, Niederle N *et al.* Ifosfamide and etoposide in refractory testicular tumors. UICC Conference on Clinical Oncology, Lausanne, 28–31 October, 1981, 129, Abstract No. 15–0373.
31. Williams SD, Turner S, Loehrer PJ, Einhorn LH. Testicular cancer: results of re-induction therapy. *Proc ASCO* 1983, **2**, 137 (Abstract No. C 536).
32. Mortimer J, Bukowski RM, Montie J, Hewlett JS, Livingston RB. VP16–213, *cis*platinum, and adriamycin salvage therapy of refractory and/or recurrent nonseminomatous germ cell neoplasms. *Cancer Chemother Pharmacol* 1982, **7**, 215–218.
33. Lederman GS, Garnick MB, Canellos GP, Richie JP. Chemotherapy of refractory germ-cell cancer with etoposide. *J Clin Oncol* 1983, **11**, 706–709.