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ORIGINAL ARTICLE

A phase II single institution single arm prospective study with paclitaxel, ifosfamide and cisplatin (TIP) as first-line chemotherapy in high-risk germ cell tumor patients with more than ten years follow-up and retrospective correlation with ERCC1, Topoisomerase 1, 2A, p53 and HER-2 expression

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Summary

Purpose: *One half of high-risk germ cell tumor (HRGCT) patients relapse after standard chemotherapy. This phase II study evaluated prospectively the toxicity and efficacy in first-line of the paclitaxel-ifosfamide-cisplatin combination (TIP) in HRGCT patients and tried to identify biomarkers that may allow patient-tailored treatments.*

Methods: *Between October 1997- September 2000, 28 chemo-naive HRGCT patients were enrolled. Patients received 4 cycles of TIP (paclitaxel 175 mg/m^2 day 1; ifosfamide 1.2 g/m^2/day, days 1-5; Mesna 1.2 g/m^2/day, days 1-5; and cisplatin 20 mg/m^2/day, days 1-5 every 3 weeks). A non-randomized comparison was made between HRGCT patients treated in the same period with first-line TIP and bleomycin-etoposide-cisplatin (BEP) (28 patients vs 20).*

In 17 HRGCT patients treated between 1998-2006, ERCC1, Topoisomerase 1 and 2A, p53 and HER-2 expression was retrospectively analysed by immunohistochemistry (IHC) (7 patients with TIP, 10 with BEP), and correlations were made with response to chemotherapy and survival.

Results: *With a median follow-up of 72 months [range 48+...89+], 5-year disease free survival (DFS) was 55%, with 95% CI 36-72, and the overall survival (OS) was 63%, with 95% CI 44-78. In June 2015, with a median follow-up of 196.47 months (range 177.30-209.27) (>15 years), 12 [%?] patients were alive and disease-free, and 16 [%?] had died (12 specific causes). There was no significant correlation between the expression of ERCC1, Topoisomerase 1 and 2A, HER-2 and p53 and response to treatment.*

Conclusion: *Long-term follow-up showed no difference in OS between TIP vs BEP as first-line therapy. Both regimens had mild toxicity.*

Key words: *BEP, ERCC1, high risk germ cell tumor, phase II study, TIP, topoisomerase*

Introduction

Germ-cell tumors (GCTs) are the most common cancers in young men and, even metastatic, are highly curable with cisplatin-based chemotherapy and resection of residual masses, if necessary [1,2].

Patient prognosis is assessed according to International Germ Cell Consensus Classification Group system (IGCCCG) [3] with three prognostic

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groups (low-, intermediate-, and high-risk) defined on the basis of primary tumor site, the presence of extrapulmonary metastases, and serum tumor marker levels. Allocation to the appropriate prognostic category is important to define the intensity of chemotherapy [4-6].

The proportion of high-risk patients who achieve a long-term complete response (CR) to standard chemotherapy is approximately 40% with objective responses of 75% and 41% 5-year progression-free survival (PFS) and 48% 5-year OS, but the high-rate of approximately 50% of relapses constitutes a challenge [3]. Since about one half of poor-prognosis patients die of their cancer, alternative treatments were evaluated in order to improve this dismal outcome.

TIP combination chemotherapy has shown a CR rate of 70%, with a 2-year PFS rate of 65% in patients with favorable prognostic factors as second-line chemotherapy [7].

Attempts were made to improve the results in this high-risk population, with either TIP in first-line in poor- and intermediate-prognosis patients [8], or classic BEP in the intermediate-risk group [9].

A randomized trial comparing first-line standard BEP and first-line with high-dose chemotherapy (HDCT) in poor-prognosis patients with GCTs, showed no benefit for HDCT in improving survival over 4 cycles of standard BEP [10].

Paclitaxel was substituted for vinblastine and added to ifosfamide and cisplatin, on the basis of in vitro studies that showed synergy for the three drugs against resistant GCTs [11] and single-agent activity in phase II trials [12,13].

As salvage therapy, multiple treatments were studied: combinations of gemcitabine, ifosfamide and cisplatin [14,15], gemcitabine, oxaliplatin and cisplatin [16].

High levels of ERCC1 (excision repair cross-check protein 1) were associated with non-sensitivity to cisplatin in GCTs [17-20]. DNA topoisomerase 1 is the target for several drugs and a potential candidate for chemo-refractory GCTs, and DNA topoisomerase 2A is the target for epipodophyllotoxines and for DNA intercalators such as anthracyclines. The sensitivity of cells to the topoisomerase-targeted drugs is related to the nucleus levels of topoisomerase [21-23]. The topoisomerase 2A gene is linked to that of the c-erB-2 oncogene (HER-2) on chromosome 17 and overexpression and co-amplification of both proteins have been observed in invasive breast cancer and ovarian cancer [22]. Because of the high expression of topoisomerase 2A in testicular seminoma, we stained blocks for HER-2 to detect co-expression of both genes. We also studied the IHC expression of genes involved in apoptosis (p53). The pattern of expression is consistent with the high sensitivity of GCTs to apoptotic stimuli, such as chemotherapy, and the expression of p53 was found to vary between tumors [24].

This article reports the long-term results of TIP combination as first-line chemotherapy in patients with poor-prognosis GCTs, treated prospectively in a phase II, single-institution study. A non-randomized comparison was made with HRGCT patients treated in the same period with standard BEP.

In 17 HRGCT treated with either TIP or BEP, we retrospectively analyzed the expression of ERCC1, Topoisomerase 1 and 2A, HER-2 and p53.

Methods

Twenty-eight male patients were enrolled in this prospective phase II single-arm single-institution study between October 1997 and September 2000, on the basis of high-risk classifications according to the IGCCCG criteria [3].

Eligible patients had histologically and serologically confirmed high-risk GCTs, according to IGCCCG score, or stage IIIC and were chemo-naive. The high-risk criteria according to IGCCCG score are: S3 serum marker (Mk) levels (i.e. Alpha-fetoprotein: AFP>10,000; beta human chorionic gonadotrophin: bHCG>50,000; and lactate dehydrogenase: LDH>10x N), M2 (nonpulmonary metastases, i.e. bone, liver or brain), and primary mediastinal tumor [3].

Written informed consent was obtained from all patients, and the trial was approved by the institutional review board, and the Ethics Committee of the University and of our Institute. Retrospective immunohistochemical analysis was also approved by the University and Institute`s Ethics Committee.

Patients were also required to be ≥18 years old, to have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 3 and adequate hematologic function (absolute granulocyte count ≥ 1,500/uL and platelet count ≥100,000/uL), liver function (AST and ALT <2.5 x normal and bilirubin <2.0 mg/dL) and serum creatinine level <1.2 mg/dL at baseline.

The pretreatment evaluation included physical examination, measurement of serum tumor markers AFP, bHCG and LDH, complete blood count, serum biochemistry for liver and renal function and a computed tomography of the chest, abdomen, and pelvis. A brain scan was performed if the patient had central nervous system symptoms.

If the primary tumor was testicular, orchidectomy was performed before chemotherapy.

The treatment consisted of paclitaxel 175 mg/m^2 intravenously (IV) in 3-hr infusion with antialergic premedication (dexamethasone 8 mg IV 1 hr before, diphenhydramine 50 mg IV and cimetidine 300 mg IV or ranitidine 50 mg IV) on day 1; ifosfamide 1.2 g/m^2/day IV in 3-hr infusion, days 1 through 5 with hydration and alkaline protection; Mesna 1.2 g/m^2/day IV in 3 doses days 1 through 5; and cisplatin 20 mg/m2/day IV in 1-hr infusion days 1 through 5 with hydration and antiemetic prophylaxis (ondansetron 8 mg IV + dexamethasone 8 mg IV). Therapy was repeated every 21 days for 4 cycles, with a maximum of 6 cycles, if continuing response was seen. No granulocyte colony stimulating factor (G-CSF) was given as primary prophylaxis for neutropenia.

Day 1 of a new TIP cycle began only if the absolute neutrophil count was ≥1,500/uL, white blood cell count was ≥3,000/uL and platelet count was ≥100,000/uL. Therapy was delayed until recovery to grade 1 toxicity.

Assessment of response was performed after 4 cycles, including physical examination, a comprehensive serum biochemistry panel, measurements for AFP, bHCG and LDH, and computed tomography scans for initially involved sites.

Response was classified as complete (CR) in case of disappearance of all clinical, radiographic and biochemical evidence of disease (normalization of serologic tumor markers), or when all resected masses contained necrotic debris, fibrosis or mature teratoma. A partial response (PR) with negative markers (Mk-) was defined as radiographic tumor decrease by at least 50% of the sum of all measured lesions in the absence of progression of any lesion or the appearance of any new lesion and normalization of tumor markers. In those patients, surgery of residual lesions was performed.

In partial responders with positive markers, second-line chemotherapy with BEP was administered. The standard BEP regimen was delivered, every 21 days (i.e. bleomycin 30 mg/days 1, 8, 15, IV; etoposide 100 mg/m^2/day IV, days 1-5; and cisplatin 20 mg/m^2/day IV, days 1-5 with premedication and hydration).

We made a non-randomized comparison of the results for the HRGCT patients treated in the same period at our Institute with first-line TIP regimen (28 patients) and standard BEP chemotherapy (20 patients).

We retrospectively analyzed the expression of ERCC1, Topoisomerase 1 and 2A, p53 and HER-2 by IHC if tumor blocks from primary tumor or resected metastases were available. The findings were correlated with response to chemotherapy and survival in 17 patients (7 with first-line TIP, 10 with BEP). The levels were based on the intensity of staining when compared with internal controls (0 indicates no reactivity of the stained cells in tumor cells, 1+ :reactivity is less than in control cells, 2+ : reactivity is similar, and 3+ : reactivity is greater in tumor cells). The result was considered as `positive` if > 10% of tumor cells were 3+, and 2+ and` negative` if < 10% were 3+, or the score was 0, 1+. HER-2 immunohistochemical expression was assessed according to ASCO-CAP HER-2 Test Guideline Recommendations 2013.

Statistics

The level of significance was set at 0.05 for all types of tests used and for the interval estimates. For the differences concerning the distribution of prognostic factors between the chemotherapy regimens x^2 test was used with Yates correction for small number of cases whenever necessary [25]. OS and PFS were evaluated by Kaplan-Meier method and differences between curves were compared by log-rank test [25].

Results

From October 1997 to September 2000, 28 chemo-naive HRGCT male patients were enrolled. Patient baseline characteristics are shown in Table 1.

The treatment protocol consisted of orchidectomy, followed by 4 cycles of TIP combination chemotherapy. Secondary surgery in partial responders with negative markers was performed. For partial responders with positive markers or for patients with progressive disease, treatment was switched to standard BEP. For brain metastases, external beam radiation with 50Gy was delivered.

A hundred and eleven cycles of chemotherapy were administered. The median number of cycles per patient was 4 (range 2-6).

There were no toxic deaths. The toxicity was mild, except grade 3-4 anemia, neutropenia and neurotoxicity. The main toxicities were: nausea and vomiting (53%), anemia (44%), leucopenia (27%), thrombocytopenia 8%. Figure 1a shows the acute toxicities for TIP.

Objective responses were seen in 75% (95%CI 58-92; 21/28 patients), with 7 patients experiencing CR (25%; 95%CI 8-42), and 14 (50%) patients PR. Eight patients with PR and negative markers underwent surgery for residual tumors (5 retroperitoneal, 2 pulmonary and one retroperitoneal + pulmonary).

Histopathologic findings of resected lesions were: necrosis/fibrosis/teratoma in 7 (87.5%) patients, and one (12.5%) patient had active tumor.

After chemotherapy and surgery, 15/28 (53.57%) patients were considered as complete responders (95% CI 29-67).

Survival

With a median follow-up of 72 months [range 48+...89+], 5-year DFS was 55% (95%CI 36-72), and 5-year OS was 63% (95%CI 44-78). As of Oc-

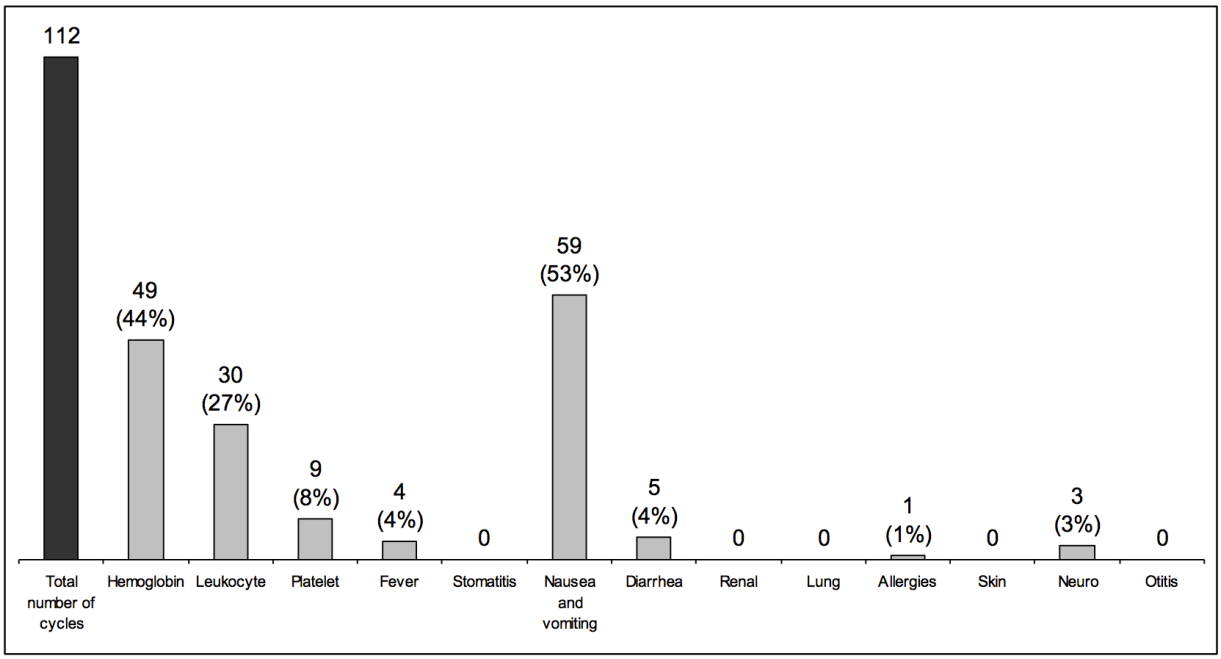

Figure 1. Toxicities of TIP chemotherapy.

Table 1. Patient characteristics treated with TIP (N=28)

Characteristics (N=28)
Sex
male = 28(100%)
Age (years)
median 29 (range 19-68); age<30=17 vs >30=11
S3 markers
9
M2(extrapulmonary)
7 (liver:5, bone:1,brain:1)
S3+M2: 9
Primary mediastinal:3
ECOG PS
1 (11) vs 2 (11) vs 3 (6)
Histology
Containing mainly choriocarcinoma (4) vs yolk sac (10) vs embryonal (12) vs seminoma (2)
Weight loss
0% (12) vs 0-5% (8) vs 5-10% (1) vs >10% (7)

M: metastasis, ECOG PS: Eastern Cooperative Oncology Group, S: serum positive tumor markers (LDH, AFP, HCG)

tober 2005, 15 patients were alive, 14 in CR, 9 in second CR after second-line chemotherapy, one patient in PR after third-line chemotherapy, and 13 have died (12 due to progressive disease, one from unrelated disease). The 12 patients who died due to GCTs had the following sites of disease: 5 metastatic, one T+N+M+ (second gonadal primary) and 6 had nodal metastatic disease.

A 10-year follow-up analysis, (median: 142 months; range 123-155) revealed: 12 patients are alive and free of disease, 16 have died (in addition to those 13 registered in October 2005, 3 of unrelated causes, but one due to coronary ischemia, and one due to secondary cancer: small cell lung carcinoma, probably due to smoking). The 10-year OS was 56% (95% CI: 37-73).

Furthermore, as of June 2015, with a median follow-up of 196.47 months (range 177.30-209.27) (>15 years), the results were similar: 12 patients alive and disease-free, and 16 dead (12 disease specific and 4 of other causes). The specific disease survival was 56% and the DFS 48% (unchanged vs analysis at 10 years) (Figure 1b). One patient fathered two healthy children after treatment completion.

Univariate analysis of DFS and OS was performed according to pretreatment variables (histology, age, weight loss, performance status), and response to chemotherapy (Table 2).

Statistically significant improvement was noted in DFS and OS among patients who didn`t experienced weight loss, age under 30 and positive response to chemotherapy (Table 2).

The 10-year OS according to the response to TIP regimen was: 80% for CR, 59% for PR and 36%

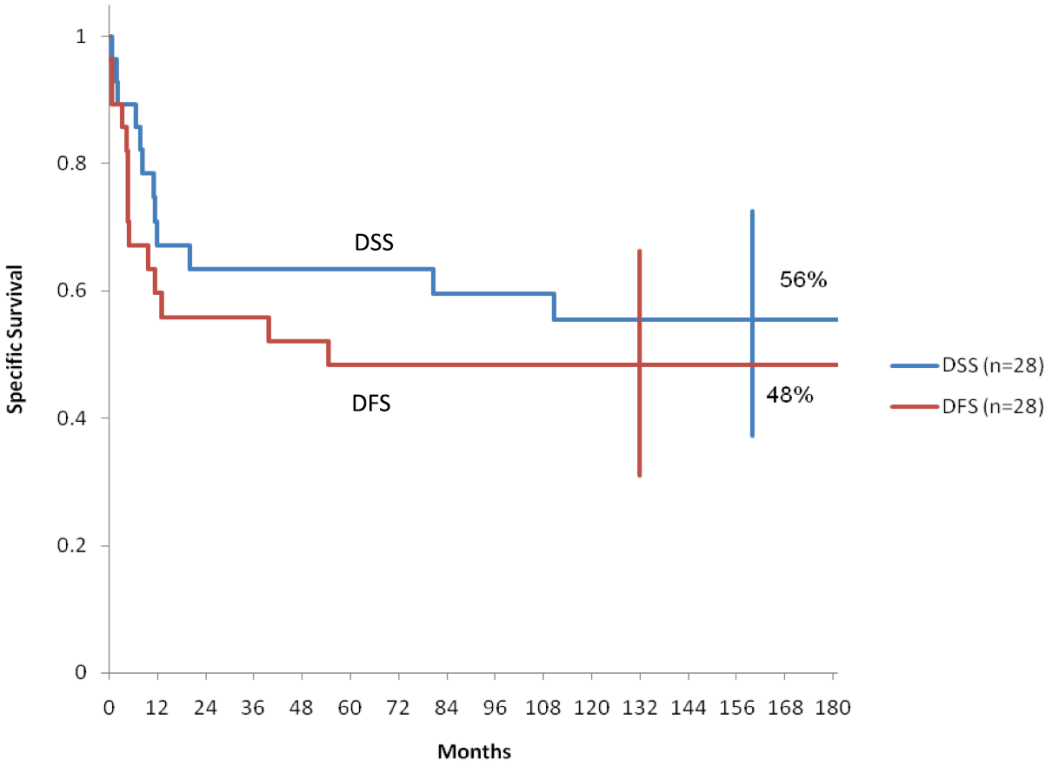

Figure 2. Disease specific survival (DSS) (N=28), and disease free survival (DFS) (N=28) at 15 years in TIP patients.

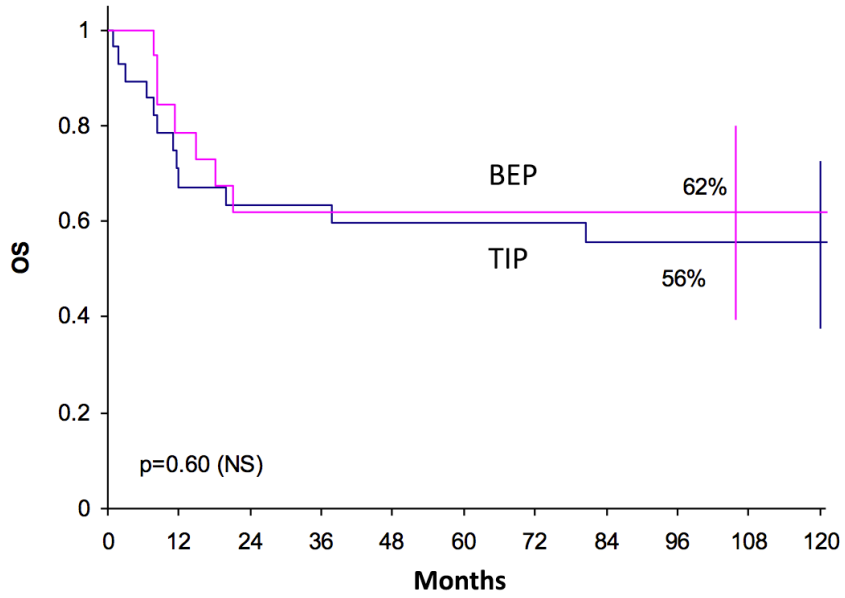

Figure 3. Overall survival for TIP (N=28) vs BEP (N=28) chemotherapy (p=0.06).

Table 2. Overall survival and disease-free survival at 10 years for patients treated with TIP

	N	*10-year OS %*	*p value (Yates)*	*10-year DFS %*	*p value (Yates)*
Histology			0.84		0.78
Embryonal	12	56		48	
Yolk sac	10	60		50	
ECOG PS			0.99		0.69
0-1	11	51		42	
2-4	17	59		53	
Weight loss			0.13		0.05
Positive	12	73		74	
Negative	16	43		31	
Age, years			0.05		0.02
≤30	17	69		63	
>30	11	36		27	
Chemotherapy response			0.05		0.01
ST+PD	11	36		27	
PR	26	59		42	
CR	11	50		82	

OS: overall survival, DFS: disease-free survival, PD: progressive disease, CR: complete response, PR: partial response

Table 3. Prognostic factors in relation to the regimens administered

	BEP N (%)	TIP N (%)	Total N	p value (Yates)
Histology				0.31
Embryonic	12 (50)	12 (50)	24	
Yolk sac	5 (33.33)	10 (66.67)	15	
Yolk sac tumor				0.43
Positive	5 (33.33)	10 (66.67)	15	
Negative	15 (45.45)	18 (54.55)	33	
ECOG PS				0.51
0-1	6 (35.29)	11 (64.71)	17	
2-4	14 (45.16)	17 (54.84)	31	
Weigh loss				0.58
Yes	7 (36.84)	12 (63.16)	19	
No	13 (44.83)	16 (55.17)	29	
Age, years				0.51
≤30	14 (45.16)	17 (54.84)	31	
>30	6 (35.29)	11 (64.71)	17	
Chemotherapy response				0.97
SD+PD	4 (36.36)	7 (63.64)	11	
PR	12 (46.15)	14 (53.85)	26	
CR	4 (36.36)	7 (63.64)	11	

For abbreviations see footnote of Table 2

Table 4. Patient characteristics regarding Excision Repair Cross-Complementation Group 1 gene expression, Topoisomerase 1 enzyme, Topoisomerase 2A enzyme, receptor tyrosine-protein kinase erbB-2 status, tumor protein p53 score, chemotherapy regimen, response to treatment and patient status

Patients, N=17	*ERCC1 score*	*TOPO1 score*	*TOPO2A score*	*HER-2 score*	*p53 score %*	*First-line chemo*	*Response to first-line chemo*	*Patient status*
1	0	0	0	0	0	BEP	CR	alive
2	0	0	0	0	20	BEP	non CR	dead
3	2+	0	3+	0	10	BEP	non CR	alive
4	2+	1+	3+	0	60	BEP	non CR	alive
5	2+	0	2+	0	60	BEP	CR	alive
6	2+	0	3+	0	80	BEP	CR	alive
7	2+	0	3+	0	90	BEP	CR	alive
8	1+	0	2+	0	100	BEP	CR	alive
9	2+	0	3+	0	ND	BEP	non CR	dead
10	3+	3+	3+	0	ND	BEP	non CR	alive
11	1+	0	2+	0	70	TIP	non CR	alive
12	1+	0	3+	0	80	TIP	CR	alive
13	3+	0	3+	0	80	TIP	non CR	alive
14	2+	1+	2+	0	100	TIP	non CR	dead
15	2+	2+	2+	0	100	TIP	non CR	alive
16	3+	0	2+	0	ND	TIP	non CR	dead
17	2+	0	2+	0	ND	TIP	non CR	alive

ERCC1: Excision Repair Cross-Complementation Group 1 gene expression, TOPO1: Topoisomerase 1 enzyme, TOPO2A: Topoisomerase 2A enzyme, HER-2 Receptor tyrosine-protein kinase erbB-2, p53: tumor protein p53, CR: complete response, non-CR: non complete response according to RECIST 1.1; BEP: bleomycin plus etoposide plus cisplatin, TIP: paclitaxel plus ifosfamide plus cisplatin, ND: not done

for SD+PD, p=0.05 (significant).

The PFS according to the response to chemotherapy was of 82% for complete responders, 40% for partial responders and 27% for patients experiencing stable disease and progressive disease (p=0.01).

Comparing best responses, the 10-year OS was 80% for CR and 52% for PR+SD+PD alltogether.

We made a nonrandomized comparison between the two regimens (TIP and BEP) administered in poor-risk GCTs patients: 28 with TIP and 20 with BEP, for DFS and OS (Figure 1c) and found no difference between these 2 regimens. Despite the fact that the study was not randomized, the prognostic factors were well balanced (Table 3).

In order to find new prognostic factors, we retrospectively performed ERCC1, Topoisomerase 1 and 2, p53 expression and HER-2 status on available tumor blocks for patients treated with TIP and in the same period with BEP and found 17 tumor samples (7 patients treated with TIP and 10 with BEP) (Table 4).

All 17 patients were HER-2 negative. From the 7 patients treated with first-line TIP, 2 had 1+ score for ERCC1 and are alive (one with CR), 3 had 2+ score and 2 were with 3+ score. The remaining 4 needed second-line BEP, and/or surgery and were free of disease. From 10 patients treated with first-line BEP 6 were ERCC1 positive with 2+ score and 5 are alive, and one had a 3+ score and is alive. Nine were Topoisomerase 1-negative or 1+ with 7 alive/2 dead, one with 3+ score is alive. Six patients were positive (3+) for Topoisomerase 2A (1 progressed and died from GCT, 5 are alive). For patients where IHC expression of ERCC1, topoisomerase 1 and 2A, p53 and HER-2 was assessed, with a median follow-up of 123 months (range 140-241), 13/17 are alive and CR, 5/7 TIP arm, 1/5 TIP arm has died after CR of other causes, and 2/17 in the BEP arm have progressed and died of GCT.

Retrospective analysis of p53 expression was also performed in 13 patients and the best prognostic cut-off was 60%. The distribution of p53 values according to these cut-off values is illustrated in Table 5.

The p53 cut-off seemed to distinguish the treated population into two different groups regarding CR. The group of patients with cut-off >60% had 50% CR vs those with cut-off <60% who had only 20% CR after initial chemotherapy, but with no statistical significance.

In multivariate analysis, which included combined expression of ERCC1, Topoisomerase 1 and 2A, HER-2 and p53 and the chemotherapeutic regimen, IHC expression failed to show independent prognostic significance for CR (Table 6).

Regarding the patient status (alive vs dead), multivariate IHC analysis of ERCC1, topoisomerase 1 and 2A, HER-2 and p53 expression failed to

Table 5. Tumor protein p53 mutation and response to chemotherapy (N=13 patients)

Overall response	*CR N (%)*	*Non- CR N (%)*	*Total N*	p value (Yates)
p53 cutoff N				
≤60	1 (20)	4 (80)	5	p=0.62
>60	4 (50)	4 (50)	8	
p53 cutoff				
≤25	1 (33.3)	2 (66.7)	3	p=0.64
>25	4 (40)	6 (60)	10	
Total	5	8	13	

CR: complete response, non-CR: non-complete response according to RECIST 1.1, p53: tumor protein p53

Table 6. Excision Repair Cross-Complementation Group 1 (ERRC1) gene expression, Topoisomerase 1, Topoisomerase 2A, receptor tyrosine-protein kinase erbB-2 status, tumor protein p53 cutoff and response to first-line chemotherapy (TIP and BEP regimens)

Response to first-line chemotherapy	*CR N (%)*	*Non-CR N (%)*	*Total N*	*p value (Yates)*
ERRC1 score				p=0.75
0	1 (50)	1 (50)	2	
>0	5 (33.3)	10 (66.7)	15	
TOPO1 score				p=0.75
0	6 (42.6)	7 (53.8)	13	
>0	0	4 (100)	4	
p53 cutoff (%)				p=0.83
≤60	2 (40)	3 (60)	5	
>60	4 (50)	4 (50)	8	
Regimens				p=0.32
BEP	5 (50)	5 (50)	10	
TIP	1 (14.3)	6 (85.7)	7	
BEP & ERRC1 >0	4 (50)	4 (50)	8	
BEP & ERRC1=0	1 (50)	1 (50)	2	
TIP & ERRC1 >0	1 (14.3)	6 (85.7)	7	
BEP & TOPO1=0	1 (50)	1 (50)	2	
BEP & TOPO1 >0	4 (50)	4 (50)	8	
TIP & TOPO1 >0	1 (14.3)	6 (85.7)	7	
BEP & TOPO2A=0	1 (50)	1 (50)	2	
BEP & TOPO2A >0	4 (50)	4 (50)	8	
TIP & TOPO2A >0	1 (14.3)	6 (85.7)	7	

Table 7. Excision Repair Cross-Complementation Group 1 gene expression, Topoisomerase 1, Topoisomerase 2A, tumor protein p53 cutoff, patient status and chemotherapy regimen

Patient status	*Alive N (%)*	*Dead N (%)*	*Total N*	*p value (Yates)*
ERRC1 score				0.96
0	1 (50)	1 (50)	2	
>0	12 (80)	3 (20)	15	
TOPO1 score				0.55
0	10 (76.9)	3 (23.1)	13	
>0	3 (75)	1 (25)	4	
TOPO2A score				0.96
0	1 (50)	1 (50)	2	
>0	12 (80)	3 (20)	15	
p53 cutoff (%)				0.67
≤60	4 (80)	1 (20)	5	
>60	7 (87.5)	1 (12.5)	8	
Regimens				0.86
BEP	8 (80)	2 (20)	10	
TIP	5 (71.4)	2 (28.6)	7	
BEP & ERRC1 >0	7 (87.5)	1 (12.5)	8	
BEP & ERRC1=0	1 (50)	1 (50)	2	
TIP & ERRC1 >0	5 (71.4)	2 (28.6)	7	
BEP & TOPO1=0	1 (50)	1 (50)	2	
BEP & TOPO1 >0	7 (87.5)	1 (12.5)	8	
TIP & TOPO1 >0	5 (71.4)	2 (28.6)	7	
BEP & TOPO2A=0	1 (50)	1 (50)	2	
BEP & TOPO2A >0	7 (87.5)	1 (12.5)	8	
TIP & TOPO2A >0	5 (71.4)	2 (28.6)	7	
	13 (76.5)	2 (23.5)	17	

For abbreviations see footnote of Table 4

show independent prognostic significance (Table 7).

Discussion

Despite the significant advances made in the management of poor-prognosis GCTs over the last three decades, this group of patients continues to be a major therapeutic challenge.

In this nonrandomized, prospective phase II single-arm study of first-line TIP chemotherapy (instead of standard BEP) in poor-prognosis GCTs patients, we found that 75% had overall response, with 25% CR post-chemotherapy and a total of 53.57% were free of disease after chemotherapy and surgery. Because GCTs are infrequent tumors and the subgroup of poor-risk represents only 16-26% of non-seminomas, the accrual was slow and the number of patients small. This was a study testing the TIP regimen as first-line, already tested in second-line or salvage treatment, but also, in first-line in intermediate and poor-risk patients (again a small number: total 44, 79% RFS) [8].

The rationale for testing paclitaxel (found active as single agent in about 25% of cases) combined with ifosfamide and cisplatin in front-line, was to validate a first-line standard-dose regimen, without the toxicity of high-dose chemotherapy [7,11-13].

In a trial with the combination of paclitaxel, ifosfamide, and cisplatin as second-line therapy for 30 favorable prognostic group relapsed patients, 77% achieved CR and 11 had PR, Mk- which underwent resection of residual tissue, with necrosis in 10 patients and teratoma in one [26].

In our series, 14 patients underwent operation for residual tumors after RP with negative Mk, with necrosis/fibrosis/teratoma in 7 patients (87.5%) while one patient had active tumor and the results are at least non-inferior compared with literature reports.

Attempts were made to overcome the issue of drug resistance inherent in poor-prognosis GCTs.

In a salvage setting, sequential dose-intensive paclitaxel, ifosfamide, carboplatin, and etoposide therapy with peripheral blood-derived stem-cells (PBSCs) in 37 unfavorable prognostic patients was conducted to evaluate efficacy and toxicity. Twenty-one (57%) patients achieved a CR and an additional 2 (5%) PR with normal Mk; therefore, 23 (62%) achieved a favorable response, with myelosuppression being the main toxicity [27].

In a phase III trial comparing paclitaxel-BEP (T-BEP) with standard BEP on 337 intermediate-risk patients, the results showed no statistical significance (p=0.15) and the accrual was very slow [9].

TI-CE regimen (paclitaxel plus ifosfamide followed by high-dose carboplatin, etoposide with PBSCs support) was found to be effective in relapsed patients predicted to have a poor-prognosis with conventional-dose salvage chemotherapy. Beyer and Einhorn models can assist in predicting outcome [28]. Characteristics at initial diagnosis that portended poor DFS included mediastinal primary (p=0.003) and IGCCCG risk status before first-line chemotherapy (p=0.03) [28]. The Beyer model [29] uses 5 prognostic features to predict outcome: progressive disease before HDCT (1 point), mediastinal primary site (1 point), refractory disease (1 point), absolute refractory disease (2 points),

and HCG >1,000 U/mL (2 points). Patients with 0 points were predicted to achieve a good outcome (good-risk); those with 1 or 2 points, an intermediate outcome (intermediate-risk); and those with 3 or more points, a poor outcome (poor-risk) with respect to both OS and DFS [28,29]. The Einhorn and the Lorch models use prognostic factors like: third-line chemotherapy, platinum refractoriness or advanced IGCCCG tumors or Lorch (histology, primary tumor site, response to first-line chemotherapy, progression-free interval, levels of AFP and HCG and non-pulmonary metastases) [30,31].

In our analysis, we identified the following prognostic factors in poor-risk GCTs: chemotherapy response, PS, weight loss and age, with better outcome in respect to DFS and OS for PS 1 vs 2-3, weight loss 0% vs >0%, age <30 and CR vs PR vs others.

The main toxicities with TIP chemotherapy were gastrointestinal (nausea/vomiting 53%), and hematological (anemia 44%, leucopenia 27%, and thrombocytopenia 8%). Neurotoxicity was only 3%.

Neurotoxicity associated with paclitaxel and cisplatin combinations was anticipated, however, neurotoxicity can occur with vinblastine, and the other toxicities associated with high-doses of vinblastine in the VeIP regimen, including gastrointestinal toxicities, were avoided [32,33].

Toxicity was more severe in patients treated with BEP+HDCT with 6 of 219 patients dying of toxicity during treatment vs 4 deceased treated in the standard BEP [10].

The PFS at 2 years in patients treated with standard BEP (45%; 95% CI 35-65%) was similar to that predicted by the IGCCCG criteria (51%).

In the long-term follow-up of 15 years, as of June 2015, the results are superimposed with those of December 2010, with a specific survival of 56% and a DFS of 48%.

ERCC1 staining and response to cisplatin-based chemotherapy and cancer-specific death: of the 17 tested patients, 8 were ERCC1-negative and 9 ERCC1-positive. In a study made on 76 patients, 59 (77.6%) were ERCC1-negative and 17 (22.4%) were ERCC1-positive (p=0.05) [20]. In this study, bivariate analysis showed that ERCC1-positive patients had a 2.37-fold greater objective responses of non-sensitivity to cisplatin-based chemotherapy compared with ERCC1-negative patients [20]. In our study, the IHC expression of ERCC1, topoisomerase 1 and 2A and p53 failed to show independent prognostic significance for CR and OS probably due to the small number of patients. In the mentioned study, the OS was influenced by the response to cisplatin-based chemotherapy and this was strongly associated with the presence of ERCC1 by IHC. The median survival was the lower in the non-cisplatin sensitive patients who were either ERCC1-positive (1.27 years) or ERCC1-negative (1.30 years) compared with the cisplatin-respondent patients, who had a median survival of 6.31 years independent of their ERCC1 status [20]. The 5-year OS of the ERCC1-negative and cisplatin-sensitive patients was greater than in patients with ERCC1-positive and non-cisplatin responders (p<0.001) [17-20].

Topoisomerase 1 DNA is the target for several drugs and a potential candidate for chemorefractory GCTs, and topoisomerase 2A DNA is the target for epipodophyllotoxines and DNA intercalators such as anthracyclines [20-22], so we evaluated their expression retrospectively on patients` paraffin blocks. Topoisomerase 1 and 2A IHC expression failed to show independent prognostic significance for CR, irrespective of the chemotherapy regimen. This may be due to the low number of patients.

The gene for topoisomerase 2A is linked to that of the c-erB-2 oncogene (HER-2) on chromosome 17 and overexpression and co-amplification of both proteins were seen in invasive breast cancer and ovarian cancer [22]. Because of the high expression of topoisomerase 2A in seminomas, each case was also stained for HER-2, to detect co-expression of both genes, but all 17 patients were HER-2 negative.

p53 expression can demonstrate two different patterns correlating with TP53 mutation. The usual pattern, and the one most common, is strong diffuse nuclear staining in approximately 60% or greater of cells. The pattern correlates with a missense mutation. The other pattern is the complete absence of staining which correlates with a nonsense mutation resulting in a truncated protein that is not detected by the p53 antibody. TP53 alterations correlated in 88 patients with cisplatin resistance and predicted PFS independent of risk [34,35]. In our analysis, the group of patients with p53 cut-off value >60% had 50% CR vs only 20% CR for those with a value <60%, but with no statistical significance.

The increasing understanding of the pathogenesis and molecular changes in GCTs as well as of chemotherapy resistance will translate into improvements in the treatment and prognosis of high-risk patients.

TIP combination chemotherapy had promis-

ing activity as first-line in poor-risk GCTs in terms of response rate, DFS, and OS, and the toxicity was mild. There is need for a randomized trial to compare this regimen with the standard BEP schedule in this setting.

ERCC1, topoisomerase 1, 2A and p53 expression may become prognostic markers and might help identifying patients likely to respond to platinum-based therapy, or different classes of drugs in case of resistance.

In conclusion our analysis identified the following prognostic factors in poor-risk GCTs: PS, weight loss, age and chemotherapy response, with better outcome in respect to DFS and OS for PS 1 vs 2-3, weight loss 0 vs >0%, age <30 years and CR vs PR vs others. The chemotherapy regimen (TIP vs BEP) did not prove to be an independent factor for OS in our analysis. And looking at the literature we may say that there might not be any difference in OS in first-line either with BEP or TIP. The toxicities of TIP were mild and manageable.

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Conflict of interests

The authors declare no confict of interests.

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