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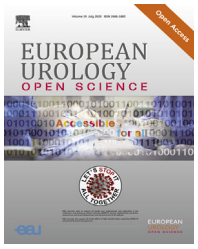

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# Paclitaxel, Ifosfamide, and Cisplatin in Patients with Poor-prognosis Disseminated Nonseminomatous Germ Cell Tumors with Unfavorable Serum Tumor Marker Decline After First Cycle of Chemotherapy. The GCT-SK-003 Phase II Trial

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**Abstract**

***Background:*** Germ cell tumors represent highly curable disease even in metastatic stage. However, poor-risk patients with an unfavorable serum tumor marker (STM) decline after the first cycle of chemotherapy represent a subgroup with dismal prognosis, with approximately 50% cure rate using bleomycin, etoposide, and cisplatin (BEP).

***Objective:*** The aim of this study was to determine the efficacy and safety of paclitaxel, ifosfamide, and cisplatin (TIP) in this patient population.

***Design, setting, and participants:*** This was an open-labeled, nonrandomized, single-center phase II trial to study the efficacy and toxicity of TIP in the first-line treatment of germ cell tumor patients with an unfavorable decline of STMs. Nineteen patients with a poor prognosis according to the International Germ Cell Cancer Collaboration Group classification and an unfavorable STM decline after the first cycle of chemotherapy were included in this phase II study (NCT02414685). The treatment regimen consisted of paclitaxel 250 mg/m$^2$ on day 1, ifosfamide 1200 mg/m$^2$ on days 1–5, and cisplatin 20 mg/m$^2$ on days 1–5, totally for four cycles.

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***Outcome measurements and statistical analysis:*** The primary endpoint was complete response (CR) rate. An optimal Simon two-stage design was used with a type I error of 5% and study power of 80%. If fewer than six CRs to study therapy have been observed among the first 19 patients, the study was to be terminated.
***Results and limitations:*** A CR was achieved in four (21.1%) patients; therefore, the study was terminated in the first stage. A favorable response rate (CR or partial remission with negative tumor markers) was observed in 14 (78.9%) patients. At a median follow-up period of 35.2 mo (range, 5.6–62.1 mo), ten (52.6%) patients experienced disease progression and eight patients (42.1%) died. The 2-yr progression-free and overall survival was 41.2% (95% confidence interval [CI] 16.8–65.7) and 72.7% (95% CI 48.9–96.4), respectively. TIP was well tolerated, and no unexpected toxicity was observed. No informative biomarkers, including miR-371a-3p was identified.
***Conclusions:*** Treatment modification from the BEP to the TIP regimen in patients with an unfavorable STM decline after the first cycle of chemotherapy was not associated with improved outcome, and four cycles of BEP remain the standard treatment option in this patient population.
***Patient summary:*** Poor-risk patients with an unfavorable serum tumor marker decline after the first cycle of chemotherapy represent a subgroup with dismal prognosis, with an approximately 50% cure rate using bleomycin, etoposide, and cisplatin (BEP). Treatment modification from the BEP regimen to the paclitaxel, ifosfamide, and cisplatin regimen in patients with an unfavorable serum tumor marker decline after the first cycle of chemotherapy was not associated with improved outcome, and four cycles of BEP remain the standard treatment option in this patient population.


## 1. Introduction

Germ cell tumors (GCTs) are the most common tumors in young males aged between 20 and 40 yr with a rising incidence [1]. These represent highly curable disease even in metastatic stage [2]. Tumor primary, pretreatment level of serum tumor markers (STMs), and histology categorize metastatic patients into good, intermediate, and poor prognostic groups with long-term survival of 96%, 89%, and 67%, respectively [3,4].

Three to four cycles of bleomycin, etoposide, and cisplatin (BEP) is the standard treatment for GCTs for >30 yr [5]. Numerous attempts were made to improve treatment results, especially in the poor prognostic group, including: (1) substitution of bleomycin and etoposide with other drugs, (2) utilizing a dose-dense regimen, and/or (3) utilizing a high-dose regimen. Unfortunately, all these efforts failed to improve overall survival (OS) compared with standard BEP [6–14].

Paclitaxel, ifosfamide, and cisplatin (TIP) is considered one of standard salvage conventional dose regimens in GCTs with long-term survival from 36% to 63%. A higher dose of paclitaxel was associated with higher efficacy compared with the standard dose [15–17]. In a phase II study with intermediate and poor prognostic groups in the first-line setting, TIP demonstrates promising results with estimated 3-yr progression-free survival (PFS) and OS rates of 72% (poor risk, 63%; intermediate risk, 90%) and 91% (poor risk, 87%; intermediate risk, 100%), respectively [18].

However, poor-risk patients with an unfavorable STM decline after the first cycle of chemotherapy represent a subgroup with dismal prognosis, with approximately 50% cure rate with BEP [19]. Dose-dense chemotherapy regimen utilizing BEP with new drugs, including paclitaxel, oxaliplatin, and ifosfamide, improved PFS in this setting, at the cost of increased toxicity, without an impact on OS [3,14].

As TIP is an active salvage regimen in relapsed GCTs as well as associated with promising first-line data, we hypothesized that it might have increased efficacy in patients with an unfavorable STM decline after the first cycle of BEP. The aim of this study was therefore to determine the efficacy and safety of the TIP regimen in the first-line treatment of patients with poor-prognosis nonseminomas.

## 2. Patients and methods

### 2.1. Patients

Eligible patients were older than 16 yr, with evidence of nonseminomatous germ cell tumor (NSGCT) based on an histological examination or clinical evidence and elevated serum beta–human chorionic gonadotropin (βHCG) or alpha-fetoprotein (AFP) levels.

Patients should have an unfavorable decrease of STMs after the first cycle of BEP, as described previously [14]. Patients with major lung involvement by GCTs (including those with the "choriocarcinoma syndrome") [20] might not receive bleomycin and/or full dose of BEP during the first cycle of chemotherapy to avoid acute respiratory distress syndrome (ARDS). In case of clinical emergency, therapy can be started before a pathological sample is obtained if tumor markers are highly elevated. Patients with primary testicular, retroperitoneal, or mediastinal primary GCTs were eligible. Disease should be classified to have poor prognosis according to International Germ Cell Cancer Collaboration Group (IGCCCG) criteria [3]: primary mediastinal NSGCT or nonpulmonary visceral metastases or HCG >50 000 UI/l, or AFP >10 000 ng/ml, or lactate dehydrogenase (LDH) more than ten times the upper normal value. Other inclusion criteria included adequate renal and liver function as well as hematological parameters (for more details, see clinicaltrials.gov). Exclusion criteria included patients infected by the human immunodeficiency virus (HIV) and female patients.

This study was approved by the Ethical Committee of the National Cancer Institute in Bratislava, Slovakia. This study has been registered in the Database of Clinical Trials, and the ClinicalTrials.gov identifier is NCT02414685. All patients provided signed informed consent before enrollment.

### 2.2. Pretreatment evaluation

Pretreatment evaluation included medical history, physical examination, electrocardiogram, complete blood count (CBC), 12-h urine collection for the determination of creatinine clearance rate, measurement of STMs (LDH, AFP, and βHCG), serum screening biochemistry panel, and computed tomograms of the chest, abdomen, and/or pelvis. An unfavorable decrease of STMs was calculated using the formula described previously [14].

### 2.3. Treatment program

Treatment consisted of four cycles of TIP given 21 d apart. Paclitaxel 250 mg/m$^2$ was administered on an inpatient basis by 3-h infusion on day 1 after standard premedication that consisted of dexamethasone, bisulepin-HCl, and ranitidine. Ifosfamide 1200 mg/m$^2$ and cisplatin 20 mg/m$^2$ were administered by infusion on an inpatient basis on days 1 through 5. Ifosfamide was administered after paclitaxel on day 1 and before cisplatin on days 1 through 5.

Mesna was administered in three infusions, with the first infusion administered together with ifosfamide, and second and third infusions administered 4 and 8 h thereafter, respectively, as described previously [16]. All patients received pegfilgrastim on day 6, 24 h after the last dose of chemotherapy. Antiemetics including 5HT3 receptor and NK1 receptor antagonists were used before chemotherapy. Patients with fever were evaluated with CBC. Patients with neutropenic fever had blood cultures, urianalysis, urinary culture, and chest x-ray, and were treated with broad-spectrum antibiotics according to standard recommendation for the treatment of neutropenic fever. Platelet transfusion and red cell transfusion were used according to the NCI Slovakia policy on transfusions.

Each cycle was initiated if the clinical status and hematological parameters (absolute granulocyte count >1000/mm$^3$, platelets 100 000 mm$^3$) granted it. No general criteria based on grade of toxicity for dose reduction were established as GCTs are highly curable, and dose reduction may compromise cure rate. Dose adjustments were discussed with the primary investigator on a case-by-case basis. STMs were examined before each cycle and after completion of chemotherapy; CT scans of the thorax, abdomen, and pelvis were done after the completion of chemotherapy.

### 2.4. Management after completion of chemotherapy

Patients with complete remission after four cycles of TIP were followed without additional therapy. Patients with radiographically detectable residual mass and normal STMs (partial remission with negative STMs) were planned for exploratory surgery to take out any residual masses. Patients with viable GCTs in completely resected masses were managed by either immediate postoperative chemotherapy or surveillance alone with chemotherapy at relapse [21,22].

Patients with brain metastases and residual masses after chemotherapy were treated with surveillance, neurosurgery, or radiotherapy. The choice of treatment was based upon the extent of metastases, histological subtype of the tumor, and patients' underlying medical presentation. Patients with evidence of a growing teratoma syndrome were managed by complete resection of residual masses.

### 2.5. Evaluation of response and toxicity

Response was assessed after completion of therapy (chemotherapy with or without surgical resection), as described previously [14]. Toxicity was assessed before each cycle of chemotherapy according to the NCI Common Toxicity Criteria (CTC) version 4.1.

### 2.6. Plasma miR-371a-3p

Peripheral blood samples were collected from all translational study participants into BD Vacutainer EDTA tubes at baseline in the morning of day 1 of the first cycle of TIP ($n$ = 16). Patients' blood samples (5 ml) were centrifuged at 2300$g$ for 10 min to separate the plasma and blood cells. Collected plasma samples were afterward filtered through a 0.2 μm filter to remove larger particles. Plasma aliquots were stored at −80 °C until further analysis. The mir-371a-3p level was determined as described earlier [23].

### 2.7. CBC and inflammation-based scores

CBC and CBC-derived inflammation-based scores were calculated as described previously [24,25]. For CBC-derived inflammation-based scores, identical cutoff values were calculated as published previously [24,25].

### 2.8. Statistical considerations

#### 2.8.1. Statistical and analytical plan

This was a phase II study to investigate the efficacy of TIP in the first-line treatment of GCTs in patients with an unfavorable decline of STMs. A two-stage phase II design was used for patient accrual. The protocol planned to accrue up to 37 eligible patients. The primary endpoint of this study was complete response (CR) rate. An intention-to-treat analysis was used.

#### 2.8.2. Study design, significance level, and power

This was an open-labeled, nonrandomized, single-center phase II trial to study the efficacy and toxicity of TIP in the first-line treatment of GCTs patients with an unfavorable decline of STMs. A patient was eligible for evaluation after the administration of at least one treatment dose. Any patient who was not eligible for survival evaluation was replaced for this primary evaluation in order to maintain adequate sample size and power. Sample size and power calculations were based on response rate according to the RECIST criteria. An optimal Simon two-stage design was used to determine the number of patients required. Assuming a response

rate of clinical interest of ≥50%, a minimal response rate of 30%, a probability of 5% for rejecting an active drug combination (type II error), and a probability of 20% to further evaluate an ineffective drug combination (type I error), 19 patients have been enrolled into the first cohort. If fewer than six CRs to study therapy have been observed among the first 19 patients, the study was to be terminated. If the CR has occurred in at least seven patients, the study has to be continued with a second cohort of 20 patients. If <16 responses to study therapy have been observed among 39 patients, then no further investigation of the TIP is warranted.

#### 2.8.3. Statistical analysis

The study population was summarized using descriptive statistics. Pearson's or Spearman's correlation tests were used according to the normality of data. The patients' characteristics were summarized using the median (range) for continuous variables and frequency (percentage) for categorical variables. The nonparametric Mann-Whitney U test or Kruskal-Wallis H test was used for non-normally distributed data. The median follow-up period was calculated as the median observation time among all the patients and among those still alive at the time of their last follow-up, using the reverse Kaplan-Meier method [26]. PFS was calculated from the date of starting the treatment with TIP to the date of progression or death or to the date of the last follow-up. OS was calculated from the date of starting the treatment with TIP to the date of death or last follow-up. PFS and OS were estimated using the Kaplan-Meier product-limit method. Statistical analyses were performed using NCSS 10 (2015) software (2015; Hintze J, Kaysville, UT, USA).

## 3. Results

### 3.1. Patients' characteristics

Patients' characteristics are summarized in Table 1. From November 2015 to January 2020, 19 patients with a median age of 30 yr (range 24–44 yr) were enrolled. Eleven (57.9%) patients had nonpulmonary visceral metastases and five (26.3%) had βHCG above 100 000 IU/ml. The median number of metastatic sites was 3 (range 1–7). Seven patients (36.8%) did not receive full course of full-dose BEP during the first cycle of chemotherapy due to very advanced lung involvement to avoid ARDS; one patient received only carboplatin as a cytoreduction due to very advanced disease and poor renal function. Patients who did not receive full dose of BEP during the first cycle had significantly higher pretreatment βHCG (median: 141 305.5 vs 210.9 IU/ml, $p = 0.02$) and lower AFP (median: 997.95 vs 14 064.5 mIU/ml, $p = 0.03$), while there were no differences in lactate dehydrogenase (median: 15.3 vs 15.6 μkat/l, $p = 1.00$). Three (16.8%) patients started treatment without histology, based on high STMs, due to symptomatic disease as oncological emergency.

**Table 1 – Patients' characteristics**

| Variable | *N* | % |
|---|---|---|
| All patients | 19 | 100.0 |
| Histology | | |
| Seminoma | 1 | 5.3 |
| Nonseminoma | 15 | 78.9 |
| No histology (nonseminoma based on serum tumor markers) | 3 | 15.8 |
| Tumor primary | | |
| Testis | 18 | 94.7 |
| Retroperitoneum | 1 | 5.3 |
| First cycle of therapy | | |
| Carboplatin (cytoreduction) | 1 | 5.3 |
| EP (2–3 d) | 6 | 31.6 |
| BEP | 12 | 63.2 |
| Metastases | | |
| Retroperitoneal lymphadenopathy | 16 | 84.2 |
| N stage | | |
| N0 | 3 | 15.8 |
| N1 | 0 | 0.0 |
| N2 | 1 | 5.3 |
| N3 | 15 | 78.9 |
| Mediastinal lymphadenopathy | 5 | 26.3 |
| Other lymphadenopathy | 7 | 36.8 |
| Lung | 13 | 68.4 |
| Liver | 10 | 52.6 |
| Brain | 3 | 15.8 |
| NPVM | 11 | 57.9 |
| Baseline serum tumor markers before 1st cycle of chemotherapy | | |
| AFP, mIU/ml (range) | 3890.6 (1.1–54 216.0) | |
| βHCG, IU/ml (range) | 72 102.6 (0.2–1 027 097.0) | |
| LDH, μkat/l (range) | 15.5 (2.8–33.2) | |
| Serum tumor markers before TIP | | |
| AFP, mIU/ml (range) | 781.3 (0.0–7430.0) | |
| βHCG, IU/ml (range) | 1884.9 (0.0–337 104.0) | |
| LDH, μkat/l (range) | 5.9 (1.8–18.2) | |

AFP = alpha-fetoprotein; βHCG = beta–human chorionic gonadotropin; BEP = bleomycin, etoposide, and cisplatin; EP = etoposide and cisplatin; LDH = lactate dehydrogenase; N stage = nodal stage; NPVM = nonpulmonary visceral metastases; TIP = paclitaxel, ifosfamide, and cisplatin.

### 3.2. Treatment outcome

A CR to chemotherapy was achieved in three (15.8%) patients, while one patient (5.3%) achieved a CR after chemotherapy and subsequent surgery with teratoma in resected retroperitoneal metastases; therefore, a CR was achieved in four patients in total (21.1%). As a result, the study was terminated in the first stage according to the statistical plan. A favorable response rate (CR or partial remission with negative tumor markers) was observed in 14 (73.7%) patients (Table 2).

Postchemotherapy surgery was performed in 12 (63.2%) patients, including delayed orchiectomy in five (26.3%) patients, retroperitoneal lymphadenectomy in nine (47.4%) patients (including four patients who had orchiectomy and retroperitoneal lymphadenectomy together), and resection of residual lung metastases in one (5.3%) patient. In resection specimens, viable cancer was present for three (15.8%) patients, teratoma was present for four (21.1%) patients, while necrosis/fibrosis was present for five (26.3%) patients. In two (10.5%) of the patients, postchemotherapy surgery also included resection of GCT residues in the liver, and in one (5.3%) patient, left-sided nephrectomy was needed due to a bulky mass in the retroperitoneum.

**Table 2 – Response to treatment**

| Response | | | Relapsed | |
|---|---|---|---|---|
| | *N* | % | *N* | % |
| Favorable | 14 | 73.7 | 5 | 35.7 |
| Unfavorable | 5 | 26.3 | 5 | 100.0 |
| CR | 4 | 21.1 | 1 | 25.0 |
| PRnm– | 10 | 52.6 | 4 | 40.0 |
| PRnm+ | 5 | 26.3 | 5 | 100.0 |

CR = complete remission; PRnm– = partial remission with negative serum tumor markers; PRnm+ = partial remission with positive serum tumor markers.

The median follow-up period of all patients was 35.2 mo (range 5.6–62.1 mo). During follow-up, ten (52.6%) patients experienced disease progression and eight patients (42.1%) died (Table 2). The 2-yr PFS and OS were 46.8% (95% confidence interval [CI] 24.1–69.5%) and 69.8% (95% CI 44.9–88.7%), respectively (Fig. 1A and B). The median PFS was 18.4 mo (95% CI 5.5–18.4 mo), while median OS was not reached. Patients who received the full course of full-dose BEP during the first cycle had nonsignificantly better outcome than patients who did not receive the full course of full-dose BEP (hazard ratio [HR] = 0.65, 95% CI 0.18–2.31, $p = 0.49$ for PFS, and HR = 0.42, 95% CI 0.09–1.92, $p = 0.20$ for OS; Fig. 2A and B, respectively). A further subgroup analysis revealed that patients with mediastinal lymphadenopathy ($p = 0.04$) had inferior PFS, while those with liver metastases had inferior OS ($p = 0.003$) compared with patients without these metastases. All patients with an unfavorable response to therapy experience disease recurrence, in contrast to 35.7% of patients with a favorable response. One patient with recurrent disease was treated with salvage chemotherapy with autologous stem cell transplantation, while the rest were treated with the standard-dose salvage chemotherapy regimen.

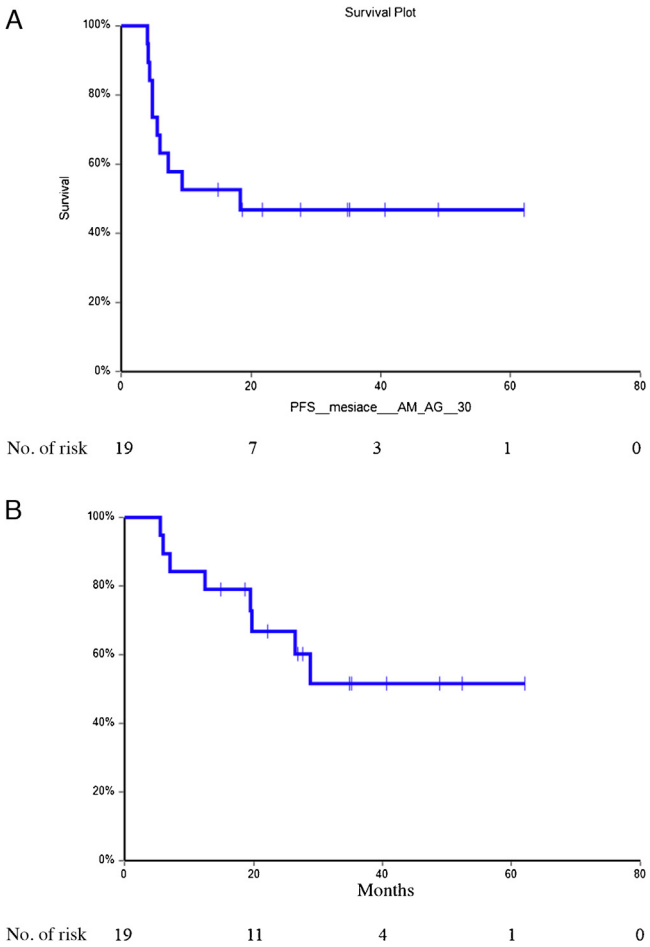


**Fig. 1 – Kaplan-Meier estimates of (A) progression-free survival (median PFS = 9.4 mo, 95% CI 5.5–18.4 mo) and (B) overall survival (median OS = 28.8 mo, 95% CI 19.7–28.8 mo). CI = confidence interval; OS = overall survival; PFS = progression-free survival.**

### 3.3. *Adverse events*

A total of 76 cycles of TIP were administered; all patients received four treatment cycles. The median (range) relative dose intensities of paclitaxel, ifosfamide, and cisplatin were 1.00 (0.96–1.02), 1.00 (0.82–1.02), and 1.00 (0.85–1.02), respectively. Hematological toxicity was the most common adverse event associated with study treatment (Table 3). Six (31.6%) patients experienced grade 3/4 neutropenia, thrombocytopenia and anemia were observed in three (15.8%) patients, while febrile neutropenia developed in two (10.5%) patients. Nonhematological grade 3/4 adverse events included syncope, non-neutropenic infections, fatigue, thrombosis paresthesia, and others (Table 3). In general, TIP was well tolerated, and no unexpected toxicity was observed.

### 3.4. *Prognostic value of plasma miR-371a-3p*

Twelve (63.2%) patients were positive for baseline miR-371a-3p, as defined previously [23]. The miR-371a-3p level correlated negatively with brain metastases (Pearson correlation coefficient [Pearson's $r$] = 0.53, $p = 0.03$) and positively with retroperitoneal lymph node involvement ($r = 0.68$, $p = 0.004$) as well as with risk of progression ($r = 0.42$, $p = 0.08$) and death ($r = 0.57$, $p = 0.01$). However, pretreatment miR-371a-3p dichotomized as positive versus negative was not prognostic for PFS (HR = 1.15, 95% CI 0.22–6.06, $p = 0.86$) or for OS (HR = 1.36, 95% CI 0.23–7.94, $p = 0.71$).

### 3.5. *Prognostic value of inflammation-based scores*

The pretreatment neutrophil to lymphocyte ratio (cutoff = 3.3 [25]) was not prognostic for PFS or OS (data not shown). Similarly, SII [24] was not associated with patient outcome; there was a trend for worse PFS (HR = 3.15, 95% CI 0.47–21.21, $p = 0.07$) and OS (HR = 3.65, 95% CI 0.50–26.65, $p = 0.06$) in patients with low SII (cut off = 1003 [24]).

## 4. Discussion

This phase II study failed to achieve its primary endpoint to improve CR rate in patients with an unfavorable STM

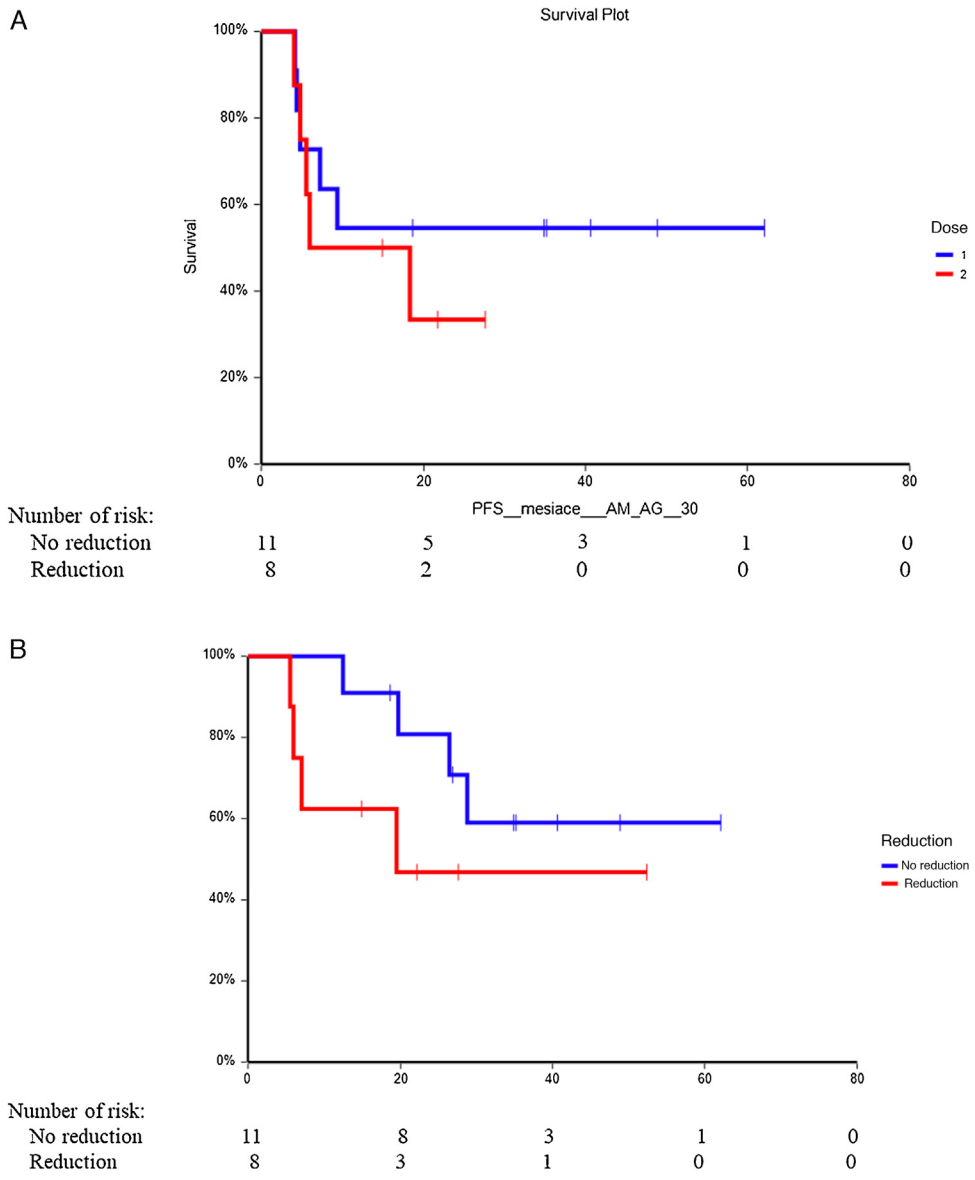


**Fig. 2 – (A) Kaplan-Meier estimates of progression-free survival according to the first-cycle of chemotherapy. Patients who received the full course of full-dose BEP during the first cycle had nonsignificantly better outcome than those who did not receive the full course of full-dose BEP: (A) hazard ratio [HR] = 0.51, 95% CI (0.14–1.90), $p = 0.27$ and (B) HR = 0.61 95% CI (0.09 – 4.06), $p = 0.56$. BEP = bleomycin, etoposide, and cisplatin.**

decline after the first cycle of chemotherapy by switching treatment regimen from BEP to TIP. Toxicity of this regimen was reasonable, with hematological toxicity as the main toxicity. Despite the fact that all patients received primary G-CSF prophylaxis, the incidence of febrile neutropenia was 10.5%. Estimated 2-yr PFS and OS were 46.8% and 69.8%, respectively. In previous trials in poor prognosis GCTs, estimated 2-yr PFS varies between 43% and 65% and OS varies between 61% and 87% [12,18,27,28], depending on the utilized regimen, with long-term survival of 67% according to the IGCCCG criteria [4]. In a clinical trial, most related to our study, which included similar patient population and utilized the same formula for calculating the unfavorable STM decline, the 3-yr PFS was 48% in patients treated with the unfavorable-BEP regimen. The dose-dense regimen that, in addition to BEP, utilizes paclitaxel, ifosfamide and oxaliplatin was able to improve PFS to 59% with an impact on OS, although nonstatistically significant [14,29]. Therefore, patient outcome in our trial is almost identical to that for BEP in this setting, suggesting that the hypothesis of substituting bleomycin and etoposide with paclitaxel and ifosfamide was not proved. Based on an indirect comparison with other trials, these data suggest that four courses of TIP are inferior to the dose-dense regimen evaluated in the GETUG-13 trial; even the toxicity, especially neuropathy, is lower. However, TIP could be an alternative to BEP in patients with contraindication of bleomycin and/or etoposide. Moreover, these data are consistent with previous

**Table 3 – Main grade 3 or 4 adverse events per patient according to NCI Common Terminology Criteria for Adverse Events version 4.03 classification (*N* = 19)**

| Variable | *N* | % |
|---|---|---|
| Any grade 3/4 toxicity | 9 | 47.4 |
| Neutropenia | 6 | 31.6 |
| Thrombocytopenia | 3 | 15.8 |
| Anemia | 3 | 15.8 |
| Febrile neutropenia | 2 | 10.5 |
| Syncope | 2 | 10.5 |
| Infection NOS | 1 | 5.3 |
| Fatigue | 1 | 5.3 |
| Paresthesia | 1 | 5.3 |
| Abdominal abscess | 1 | 5.3 |
| Tumor-duodenal fistula | 1 | 5.3 |
| Sepsis | 1 | 5.3 |
| Thrombosis | 1 | 5.3 |
| Insult on the finger of the hand | 1 | 5.3 |

NOS = not otherwise specified.

observations that suggest limited efficacy of new combinations and schedules of chemotherapy regimen in poor-risk GCTs compared with standard BEP. Better understanding of tumor biology and identification of new therapeutic targets are more promising approaches to improve patient outcome.

In this study, a total of eight (42.1%) patients did not receive the full course of full-dose BEP during the first cycle of chemotherapy due to very advanced disease. Compared with a previous trial [14], where the received dose was reduced by >20% in 4% of patients for cisplatin, 5% for etoposide, and 12% for bleomycin. This suggests more advanced disease, as expressed by very high βHCG in our trial; however, this had no impact on the long-term outcome, which is consistent with previous observations [14,20,30].

Numerous studies showed a prognostic value of SII in different types of cancers including GCTs [24,25,31–33]. However, in this study, SII was not associated with the outcome; surprisingly, there was a trend for worse outcome in patients with low pretreatment SII. The miR-371a-3p is an emerging new specific marker of GCTs, which showed clinical utility for GCTs in various clinical scenarios [23,34,35]. In this study, we observed that 12 (63.2%) patients were positive before administration of study treatment; however, this was not associated with the prognosis. In previous research, miR-371a-3p positivity was only 4% before the second cycle of therapy [23], suggesting much more advanced disease than in the majority of GCT patients. Therefore, so far we were not able to identify biomarkers informative enough to further stratify patients with unfavorable STMs after the first cycle of therapy.

## 5. Conclusions

In conclusion, treatment modification from the BEP to the TIP regimen in patients with an unfavorable STM decline after the first cycle of chemotherapy was not associated with improved outcome. While some countries adapted the GETUG-13 regimen as a standard of care in this patient population, based on improved outcome, four cycles of BEP are still considered the standard treatment option in this setting. However, TIP seems to be a reasonable approach in patients in whom bleomycin and etoposide cannot be administered. Further research should focus on better understanding of the biology of poor-prognosis GCTs and identification of new therapeutic targets.

***Author contributions*:** Michal Mego had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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